JAK/STAT Pathway in Pulmonary Hypertension

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Pulmonary hypertension is defined as a group of diseases characterized by a progressive increase in pulmonary vascular resistance (PVR), which leads to right ventricular failure and premature death. There are multiple clinical manifestations that can be grouped into five different types. Pulmonary artery remodeling is a common feature in pulmonary hypertension (PH) characterized by endothelial dysfunction and smooth muscle pulmonary artery cell proliferation. The current treatments for PH are limited to vasodilatory agents that do not stop the progression of the disease. Therefore, there is a need for new agents that inhibit pulmonary artery remodeling targeting the main genetic, molecular, and cellular processes involved in PH. Chronic inflammation contributes to pulmonary artery remodeling and PH, among other vascular disorders, and many inflammatory mediators signal through the JAK/STAT pathway. Recent evidence indicates that the JAK/STAT pathway is overactivated in the pulmonary arteries of patients with PH of different types. In addition, different profibrotic cytokines such as IL-6, IL-13, and IL-11 and growth factors such as PDGF, VEGF, and TGFβ1 are activators of the JAK/STAT pathway and inducers of pulmonary remodeling, thus participating in the development of PH. The understanding of the participation and modulation of the JAK/STAT pathway in PH could be an attractive strategy for developing future treatments.

pulmonary hypertension (PH) Janus kinase 2 (JAK2) signal transducer and activator of transcription 3 (STAT3)

1. Introduction

Pulmonary hypertension (PH) is defined as a group of diseases characterized by a progressive increase in pulmonary vascular resistance (PVR), which leads to right ventricular failure and premature death [1]. The term pulmonary hypertension is defined as a mean pulmonary artery pressure (mPAP) greater than 25 mmHg, measured at rest according to the guidelines issued by the European Society of Cardiology (SEC) and by the European Respiratory Society (SER) [2].

The clinical classification of PH is intended to categorize multiple clinical conditions into five groups according to their clinical presentation, pathological manifestations, hemodynamic characteristics, and treatment strategies [2]. The clinical classification may be updated when new data regarding the above features become available or when additional clinical entities are considered.

Group 1 is known as pulmonary arterial hypertension (PAH), which may be idiopathic or caused by human immunodeficiency virus, liver disease, or congenital heart disease (PAH-CHD). Group 2 PH is related to the left side of the heart. Long-term high blood pressure and mitral valve disease are also associated with group 2 PH. Group 3 PH refers to chronic lung diseases (PH-CLD) and/or hypoxemia. Group 4 PH is due to chronic thrombotic and/or embolic disease. Group 5 includes various blood disorders, systemic disorders, metabolic disorders, and other conditions such as kidney disease [3].

Janus kinase type 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) have been reported to participate in processes directly related to pulmonary artery remodeling such as smooth muscle proliferation, endothelial dysfunction, and inflammation. These cellular and molecular process are common in several vascular diseases such as vasculitis, atherosclerosis, and PH [4][5].

PH comprises multiple molecular pathways that lead to vasoconstriction, the remodeling of the pulmonary arteries, and increased pulmonary vascular resistance. Furthermore, the JAK/STAT pathway has been reported to be overactivated in the pulmonary arteries of patients with PH. There are a few reviews on the JAK/STAT pathway in PH [6]. Therefore, this review aims to analyze, in depth, the expression, activation, and molecular and cellular effects of JAK/STAT activation in PH, as well as the current development of new targeted therapies for PH.

2. JAK/STAT Pathway and Cellular and Molecular Dysregulation in Pulmonary Hypertension

Complex vascular remodeling processes are the substrate and hallmark of pulmonary hypertension. As described above, JAK/STAT are activated in response to cytokines, growth factors, or vascular contractile agonists such as ET-1 and Ang II. The secretion of these factors is altered in PH. In the early stages of the disease, HPAECs are injured, which alters their function as a barrier. On the other hand, PASMCs are in direct contact with these factors, thus enhancing pathways of growth, resistance to apoptosis, and migration.

2.1. JAK/STAT Pathway and Vascular Remodeling

All forms of pulmonary hypertension are characterized by cellular and structural changes in the walls of pulmonary arteries. Our group explored the effects of JAK2 on pulmonary artery remodeling and studied the mesenchymal transition of HPAECs and HPASMCs. Incubating the HPAECs with TGFβ changed their endothelial phenotype to a mesenchymal/myofibroblast phenotype (EnMT), characterized by a loss of the endothelial markers VE-cadherin, VEGFR1, FVIII, and eNOS and an increase in the mesenchymal markers collagen type I and vimentin [7] (Figure 1). TGFβ1 also increased the expression of collagen type I and vimentin in HPASMCs and HPASMC proliferation. These effects were inhibited by siRNA-JAK2. Furthermore, the pulmonary arteries of IPF+PH showed coimmunostaining with α-SMA and JAK2/p-STAT3 in endothelial cells, suggesting that the endothelial cells had transformed into myofibroblasts [7]. Other studies proved that endothelial cells of intimal and plexiform lesions from

PAH patients are transformed into mesenchymal cells/myofibroblasts, thus contributing to PH [8]. Recently, our group found that fibroblasts isolated from IL-11-treated mice had an endothelial origin [9]. Accumulating evidence suggests that EnMT plays a pivotal role in the initiation and progression of this disease [8][10][11] and in pulmonary artery remodeling, which contributes to the progression of occlusive neointimal lesions in pulmonary arteries [8][12].

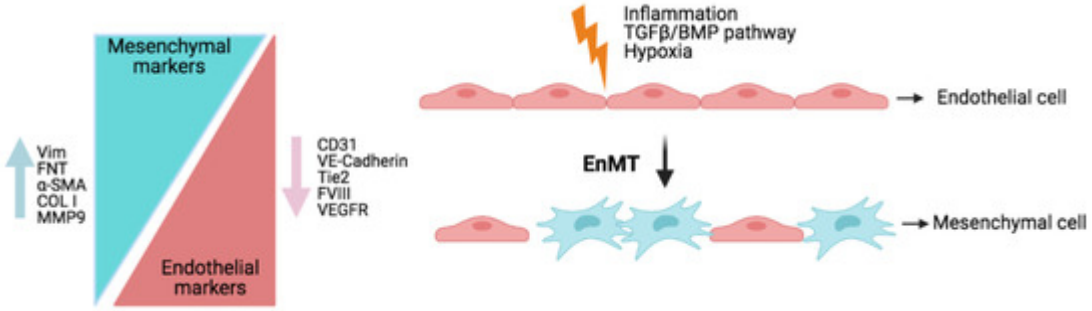

Figure 1. Endothelial-to-mesenchymal transition (EnMT). EnMT in PH is thought to be an important process contributing to vascular remodeling. Activated by hypoxia, inflammation, and TGF-β/BMP pathway signaling, pulmonary endothelial cells (HPAECs) undergo a cellular transition to a mesenchymal phenotype, in which they lose endothelial markers and gain mesenchymal markers. Abbreviations: COL I: collagen type 1; EnMT: transition from endothelial phenotype to a mesenchymal/myofibroblast phenotype; FNT: fibronectin; MMP9: matrix metallopeptidase 9; Vim: vimentin; α-SMA: alpha smooth muscle actin. Created with BioRender.

2.2. JAK/STAT Pathway, Proliferation, and Resistance to Apoptosis

PAH is a vascular disease characterized by pulmonary artery smooth muscle cell proliferation and pulmonary artery hypertrophy [13]. STAT3 inhibition prevents neointimal formation by inhibiting the proliferation and promoting the apoptosis of neointimal smooth muscle cells [14]. In cancer, STAT3 promotes the expression of the provirus integration site for Moloney murine leukemia virus' Pim1, a proto-oncogene serine/threonine-protein kinase. The development and progression of certain cancers due to increased cell proliferation and resistance to apoptosis have been related to the overexpression of Pim1 [15][16][17]. Paulin et al. showed that the treatment of healthy PASMCs with ET1, Ang II, and PDFG caused an increase in the PY705-STAT3/STAT3 ratio. Once activated, STAT3 increases Pim1 and nuclear factor of activated T cell (NFAT2) expression. They provide in vitro and in vivo evidence of the mechanisms by which Pim1 inhibition reverses PAH, which involves the inhibition of PASMC proliferation and decreasing Bcl-2, increasing apoptosis. They focused on PASMCs and not HPAECs because the latter are downregulated in established PAH, but Pim1 may also be implicated in endothelium-related vascular lesions, such as plexiform lesions [13]. The proliferation of HPAECs from iPAH was evaluated in the presence of VEGF and IL-15, and the authors demonstrated that there was greater proliferation than in control cells, especially in cells stimulated with VEGF. Moreover, cell proliferation was blocked by AG-490, a pharmacological inhibitor of the JAK/STAT3 signaling pathway. Finally, they observed that the increase in cell viability occurs in association with increased expression of the prosurvival factors Mcl-1, IL-15, and BcL-2 and persistent activation of the critical prosurvival STAT3 signal transduction pathway [18].

On the other hand, it has been suggested that STAT3 regulates the expression of miR-204 [19]. miR-204 expression in PASMCs is downregulated in both human and rodent PAH. STAT3 activation suppresses miR-204 expression, which activates the Src kinase and NFAT. STAT3 also directly induces the expression of NFATc2. NFAT and SHP2 were required to maintain PAH-PASMC proliferation and resistance to apoptosis [20]. Caspase-1 also induced the proliferation of PASMCs through the caspase-1/IL-8/IL-6/STAT3 signaling pathway, causing PH in mice exposed to hypoxia [21].

2.3. JAK/STAT Pathway, Migration, and Angiogenesis

Migration and angiogenesis in cells implicated in PH have not been extensively studied. Studies have identified STAT3 as the central prosurvival molecular signaling pathway and the primary regulator of angiogenesis [18]. Migration was greater in iPAH cells than in the control in response to VEGF or FGF. Other studies have indicated that dominant-negative STAT3 abolishes VEGF-induced endothelial cell migration and suppresses VEGF-induced tube formation on collagen gels [22].

It is postulated that, in lungs with PH, HPAECs in plexiform lesions express proteins involved in angiogenesis, especially VEGF. VEGF stimulates the migration and proliferation of HPAECs [23]. However, concentric proliferative lesions, which occur proximally to the plexiform lesion and then evolve distally in a thin-walled dilated blood vessel, showed reduced expression of VEGF and VEGFR2 [24].

2.4. Imbalance in Vasoactive Mediators: Vasoconstriction

In patients with PH, the generation of vasodilatory mediators is reduced, and the generation of vasoconstrictor mediators is increased, which in turn increases the generation of reactive oxygen species and reactive nitrogen species, which play an important role in the development and/or progression of PH [25][26][27][28]. ROS modulate the effects and/or release of several vasoactive factors, such as ET-1 and prostacyclin, which can acutely influence vessel tone [29][30]. Furthermore, the inhibition of ROS production has been shown to attenuate hypoxic PH (HPH) in both rat and mouse models of chronic hypoxia [31].

Although several enzymes produce ROS, the most important is arguably NADPH oxidase, which plays a key role in the remodeling and vasoconstrictive aspects of PH [30]. Seven enzyme subtypes of Nox have been identified in a wide range of cell types, but only Nox1, Nox2, Nox4, and Nox5 are found in the pulmonary vasculature [29][32]. Nox4 is overexpressed in PASMCs, and its siRNA-mediated silencing reduces ROS levels and cell proliferation, suggesting that Nox4 mediates pulmonary vascular remodeling [25][27][33]. Ang II plays an important role in the development of hypertension and is one of the most important inducers of NADPH oxidase-dependent superoxide production in PASMCs [34] and in the entire vascular wall [35]. The effects of Ang II on NADPH oxidases are mediated mainly through the AT1R receptor [36]. Ang II also stimulates NADPH oxidases, which are involved in intracellular H_2O_2 production and mediate vascular hypertrophy [33][37]. Although ATR1 activation induces the JAK2/STAT3 pathway, there is no direct evidence of an interaction between JAK/STAT and the NADPH oxidase system.

The relationship between reactive nitrogen species and JAK/STAT is more direct. As we have mentioned previously, ET-1 overexpression promotes STAT3 phosphorylation, which leads to a decrease in NO and eNOS levels. To verify the involvement of STAT3 in this process, studies were carried out with dominant negative mutants of PKCδ/STAT3, where it was observed that an increase in STAT3 activity and decrease in eNOS promoter activity were inhibited [38]. This suggests that STAT3 antagonism could provide a novel therapeutic strategy for improving vascular homeostasis.

Serotonin (5-HT), Ang II, and ET-1 promote the vasoconstriction of the pulmonary artery. Previous reports have shown that the inhibition of JAK2 can reduce the contraction of the rat aortic ring induced by intracellular Ca^{2+} and 5-HT, Ang II, and ET-1 [39][40]. In addition, there is evidence of the role of JAK2 in the pulmonary vasoconstriction of small pulmonary arteries in control subjects and patients with PH-IPF. It is known that the vascular remodeling of the human pulmonary artery occurs in small resistant-type intrapulmonary vessels that are part of the pulmonary vascular bed, which are responsible for the pressure elevation observed in PH [41]. JAK2 inhibition with JSI-124 relaxed small pulmonary arteries precontracted using 5-HT in patients with PH-IPF. Moreover, JAK2 has been suggested to play a role in the maintenance of the basal tone of the pulmonary arteries because JSI-124 had direct relaxing effects on untreated basal pulmonary arteries. Electrophysiological experiments using the patch clamp technique demonstrated that JAK2 inhibits BK_{Ca} potassium currents and increases intracellular Ca^{2+} in PASMCs, thus contributing to pulmonary arteries' constriction [7].

2.5. JAK/STAT Pathway and Inflammation Associated with Pulmonary Hypertension

It is now recognized that perivascular inflammation is a common contributing factor in almost all forms of PH [42][43][44][45] because inflammatory cell infiltrates comprising T- and B-lymphocytes and macrophages have been identified. Upon endothelial cell injury, HPAECs become dysfunctional and alter their secretion of cytokines and other factors that regulate coagulation, thrombosis, and vascular tone [46]. However, the exact mechanisms by which inflammation might facilitate the progression of PH are still under investigation. Recent evidence has pointed to the role played by exogenous signaling molecules produced by inflammatory cells. The metabolite of the 15-lipoxygenase pathway, 15-HETE, upregulates several cytokines such as IL-6 and TNF-α, both of which have been implicated in PH [47][48]. IL-6 and IL-8 may be an important part of the initial response to injury, contributing to the process of vascular remodeling in PAH, and have been demonstrated to modulate HPAECs' and HPASMCs' function [49]. Graham et al. studied the role of the IL-6–STAT3–NFATc2 pathway and demonstrated that medial remodeling was decreased in IL-$6^{-/-}$ mice treated with hypoxia or *Schistosoma* [50].

We can conclude that the JAK/STAT pathway contributes to cell proliferation, differentiation, growth, and resistance to apoptosis. However, the participation of the JAK/STAT pathway in inflammation, the production of ROS, and angiogenesis has not been widely studied (Figure 2).

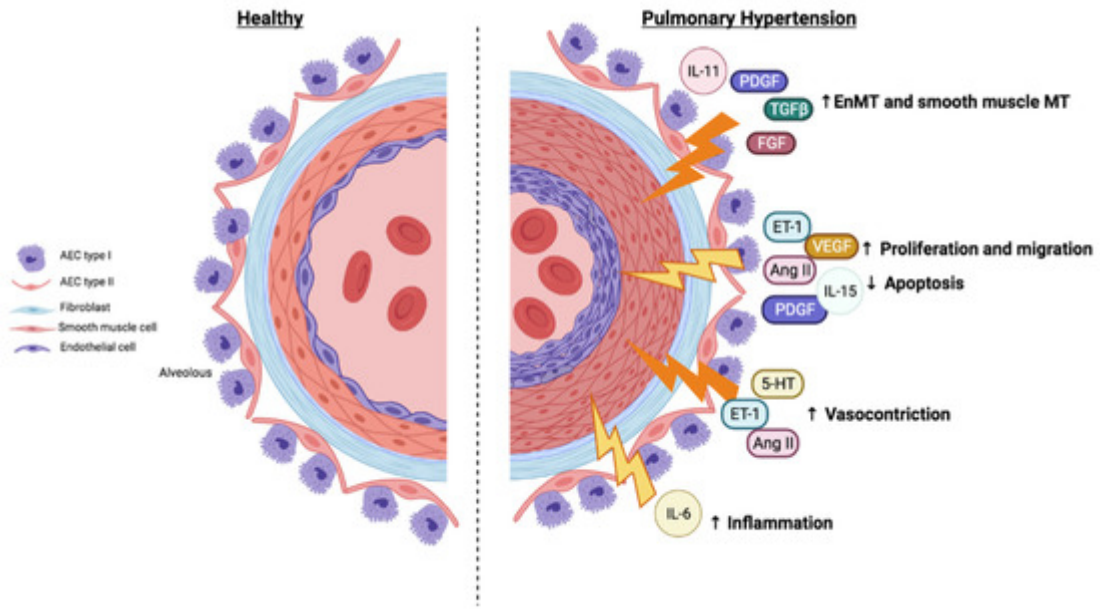

Figure 2. Cellular and molecular processes in pulmonary hypertension. PH is characterized by abnormal pulmonary artery remodeling, excessive pulmonary vasoconstriction, and processes that usually affect all vessel layers (intima, media, and adventitia), resulting in the loss of vascular cross-sectional area and, therefore, elevated pulmonary vascular resistance. The intimal changes include endothelial injury, endothelial and muscular cell proliferation, and the invasion of the intima by myofibroblasts, with enhanced matrix deposition and intimal fibrosis. These structural changes suggest a switch from a quiescent state to a proliferative, apoptosis-resistant cellular phenotype. These structural changes are triggered by the dysregulation of the expression and release of cytokines, growth factors, and vascular contractile agonists such as ET1 or Ang II. Abbreviations: AEC: alveolar epithelial cells; Ang II: angiotensin II; ET-1: endothelin 1; FGF: fibroblast growth factor; IL: interleukin; PDGF: platelet-derived growth factor; TGFβ: transforming growth factor-β; VEGF: vascular endothelial growth factor; 5-HT: serotonin. Created with BioRender.

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