STAT3 signaling in pulmonary arterial hypertension

Roxane Paulin1,* Jolyane Meloche2 and Sébastien Bonnet2

1Vascular Biology Research Group; Department of Medicine; University of Alberta; Edmonton, AB Canada; 2Pulmonary Hypertension Group; CRIUCPQ; Department of Medicine; Laval University, Quebec City, QC Canada

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Abbreviations: ALK1, activin-like kinase 1; Ang-II, angiotensin II; BMPR2, bone morphogenetic type II receptor; CASMC, carotid artery smooth muscle cells; ChIP/PCR, chromatin immunoprecipitation associated with PCR; CML, Nε-carboxymethyl lysine; DHEA, dehydroepiandrosterone; DC, dendritic cell; ER, endoplasmic reticulum; EPC, endothelial progenitor cells; ETC, electron transport chain; eNOS, endothelium nitric oxide synthase; GPCR, G-protein coupled receptor; GRB2, growth factor receptor-bound protein 2; HIF-1α, hypoxia inducible factor 1α; IL-6, interleukin-6; INFγ, interferon γ; IRS-1/2, insulin-receptor substrate-1/2; JIP, c-Jun amino-terminal kinase-interacting protein; KLF5, Krüppel-like factor 5; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T-cells; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPARγ, peroxisome proliferator-activated receptor gamma; PTB, phosphotyrosine-binding; PVR, pulmonary vascular resistance; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; SH2, Src homology 2 domain; SHC, Src homology 2 domain-containing; SHP2, Src homology 2 domain-containing phosphatase 2; SMAD, mothers against decapentaplegic homolog; SOCS, suppressor of cytokine signaling; SRC, v-Src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; STAT, signal transducer and activator of transcription; TRPM3, transient receptor potential melastatin 3; TNFα, tumor necrosis factor α; ΔΨm, mitochondrial membrane potential

Pulmonary artery hypertension (PAH) is a proliferative disorder associated with enhanced pulmonary artery smooth muscle cell proliferation and suppressed apoptosis. The sustainability of this phenotype requires the activation of pro-survival transcription factor like the signal transducers and activators of transcription-3 (STAT3). Using multidisciplinary and translational approaches, we and others have demonstrated that STAT3 activation in both human and experimental models of PAH accounts for the modulation of the expression of several proteins already known as implicated in PAH pathogenesis, as well as for signal transduction to other transcription factors. Furthermore, recent data demonstrated that STAT3 could be therapeutically targeted in different animal models and some molecules are actually in clinical trials for cancer or PAH treatment.

Introduction

Pulmonary arterial hypertension (PAH) is a disease affecting the pulmonary vasculature, characterized by vasoconstricted and remodeled pulmonary arteries, increasing pulmonary vascular resistance (PVR) and pulmonary pressure. This is associated with a primary, compensatory right ventricular hypertrophy which rapidly will turn into right heart failure. Current approved therapies, despite improving quality of life remain insufficient to reverse PAH and improve survival; mortality rates are still unacceptably high (10% within 1 year, 35% within 3 years). It is well recognize that the pathogenesis of PAH has fundamental similarities with cancer as pulmonary artery smooth muscle cells (PASMCs) adopt a pro-proliferative, pro-survival, invasive phenotype (Table 1). Also, as in cancer, there is an environment of persistent inflammation. In some patients, there is a major genetic predisposition in the form of heterozygous mutations in BMPR-II (bone morphogenetic type II receptor) leading to an impaired function of SMAD (mothers against decapentaplegic homolog) pathway and an increased p38/MAPK (mitogen-activated protein kinase) activation. Mutations have also been detected in the ALK1 (activin-like kinase 1) gene and polymorphisms have been found in the sequence coding for the serotonin transporter 5-HTT and the gene KCNA5 coding for the potassium channel Kv1.5, leading to consider genomic instability as a part of PAH development. Plexiform lesions are complex vascular structures observed in idiopathic forms of PAH, and resemble neo-plastic disorders with an abnormal and “quasi malignant” endothelial cell growth. Oncogenic pathways, like p21 and p27, and the lack of tumor suppressor, like p53 have been implicated in PAH etiology as well. The metabolic switch from glucose oxidation to
glycolysis seen in cancer has also been described in PAH, \(^{24,25}\) with for origin the inhibition of the mitochondrial gate-keeping enzyme the pyruvate dehydrogenase (PDH). This is associated with mitochondrial suppression, \(^{25-27}\) hyperpolarization of the mitochondrial membrane potential (\(\Delta \Psi_m\)) and the inhibition of mitochondrial reactive oxygen species (ROS) generation, both being implicated in apoptosis suppression. \(^{26,28-30}\) Another important feature that PAH shares with cancer.

The signal transducers and activators of transcription (STAT) protein family regulates diverse cellular processes including growth and survival, and is frequently deregulated in cancer and several other disorders. The family is composed of 7 isoforms (STAT1–4, 5A, 5B and 6) that are activated, phosphorylated in response to cytokines, growth factors or agonists. The role of STAT3 in PAH has been suggested in 2007\(^{31}\) and strengthened in the last couple of years, even leading to the conclusion that STAT3 activation might be an early event in PAH etiology, at the origin of several signaling cascades and that its role is critical in the sustainability of the pathologic phenotype. In this review, we will make a statement of the current knowledge regarding STAT3 implication in PAH as well as an overview of the speculated additional role that it could play and that might be the subject of future studies.

**STAT3 Upstream Signals**

STAT3 is a cytoplasmic latent transcription factor activated by phosphorylation on its tyrosine 705 residue (PY705) (allowing nuclear translocation and DNA binding of STAT3 after dimerization\(^{35}\)) in response to cytokines such as interleukin-6 (IL-6),\(^{36}\) growth factors such as platelet-derived growth factor (PDGF)\(^{36}\) and agonists such as endothelin-1 (ET1) and angiotensin II (AngII).\(^{37}\) The secretion of these factors is deregulated in PAH, their levels are increased in the serum of PAH patients.\(^{38-40}\) Following pulmonary artery endothelial cells (PAECs) injury occurring in the early stages of the disease, and alteration of their function as a barrier, PASMCs are in direct contact with these factors, thus enhancing pathways of growth, proliferation and resistance to apoptosis. A 2.8-, 3.2-, 2.5-, 2.8- and 1.9-fold increase in PY705-STAT3 has been described in healthy-PASMCs treated for 48 h with ET1, AngII, PDGF, IL-6 and TNF respectively\(^{35}\) suggesting the important role of STAT3 in these processes (Fig. 1). A second phosphorylation event occurs on the serine 727 residue (PS-727) of the STAT3 C-terminal activation domain. This phosphorylation seems to be necessary for maximal gene expression, since its mutation prevents STAT3 transcriptional functions;\(^{41}\) however, the exact role of this site of phosphorylation is still unclear.

Src and JAKs are among the proteins the most frequently involved in the transduction of the signal between the fixation of the agonist on the receptor and the phosphorylation of STATs.\(^{42,43}\) Some studies have mentioned the possible implication of JAKs proteins in PAH early in the decade, determining an increase in JAKs mRNA levels in rats with hypoxia-induced-PAH\(^{44}\) or through the beneficial effect of the JAK2 inhibitor AG490 in reversing PAECs proliferation rates.\(^{31}\) However, using either microarray or western blot, we failed to determine that JAK2 is upregulated/activated in PAH-PASMC compared with healthy PASMCs.\(^{30}\) At the opposite, we found an accurate 2-fold increase in both c-Src and Src-related protein SHP2 in PAH-PASMC compared with healthy PASMC (Fig. 1). These results have been reproduced in the hypoxia and monocrotaline experimental models of PAH as well.\(^{30}\) Recently, JAK2 has been found by microarray study to be expressed more highly in limited

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**Table 1. Cancer hallmarks shared with PAH**

| Cancer hallmarks\(^{32}\) | Evidence found in PAH |
|--------------------------|------------------------|
| Sustained proliferative signaling | • p21 and p27 implication\(^{19-23}\)  
| | • Src/STAT3/Pim1 axis activation\(^{18-30}\)  
| | • p38/MAPK activation\(^{12,13}\) |
| Evasion from growth suppressors | • p53 is protective against PAH\(^{19}\) and p53 expression is downregulated in monocrotaline model of PAH\(^{25}\)  
| | • impaired function of SMAD pathways\(^{9,10}\) |
| Active invasion and metastasis | None |
| Replicative immortality | None |
| Angiogenesis | Plexiform lesion\(^{6,18}\) |
| Resistance to cell death | • Bad phosphorylation\(^{13}\)  
| | • Increased Bcl2 expression\(^{26}\)  
| | • Mitochondrial membrane hyperpolarization\(^{14}\) |
| Avoided immune destruction | None |
| Tumor promoting inflammation | Increased level of pro-inflammatory cytokines\(^8\) |
| Genome instability and mutation | • Morphogenetic protein receptor 2 (BMPR2) mutation\(^{9,11}\)  
| | • Activin-like kinase 1 (ALK1) mutation |
| | • Polymorphisms found in the sequence coding for the serotonin transporter S-HTT and the gene KCNAs coding for the potassium channel Kv1.5\(^{17}\) |
| Deregulated cellular energetic | Warburg effect\(^{42,25}\) |
cutaneous systemic sclerosis (lcSSc) patients, especially when associated with PAH, than in controls and in idiopathic PAH where JAK2 levels were not affected. This suggests that JAK2 activation more likely occur in autoimmune disease-associated PAH. However, state of phosphorylation of JAK2 has not been measured in this study, poorly allowing any conclusion on whether or not JAK2 is activated. Nonetheless, both Src and JAK2 need to be considered with interest, since their relationship is complex and might be compensatory, the inhibition of one enhancing the activation of the second as seen in both cancer and VSMC diseases, leading to the development of mechanism of resistance to the treatment.

Typically, growth factors like PDGF bind on receptor tyrosine kinases (RTK). These receptors are activated by ligand fixation and undergo dimerization, which juxtaposes the cytoplasmic tyrosine kinase domains, facilitating autophosphorylation on specific tyrosine residues, conformational changes and stabilization of the active kinase. Enzymes and adaptor/scaffolding proteins recognize phosphotyrosine residues, typically through Src homology-2 (SH2), such as phospholipase C-γ (PLC-γ), Janus associated kinases JAKs, v-Src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (Src), Src homology 2 domain-containing phosphatase 2 (Shp2) and growth factor receptor-bound protein 2 (Grb2) or phosphotyrosine-binding [PTB, such as insulin-receptor substrate-1/2 (IRS-1/2) or c-Jun N-terminal kinase-interacting protein (JIP)] domains, are phosphorylated and activated leading to the initiation of signal transduction pathways, STAT3 included. Interleukins acts through IL-receptors that are composed of two transmembrane glycoproteins chains, required for the generation of high-affinity IL-binding sites and signal-transduction. Signal transduction induces tyrosine phosphorylation, primarily of JAK2 and TYK2 (another member of the JAK family), which, in turn, phosphorylate and activate STATs. Other signal transduction molecules, including Src, SH2 and suppressor of cytokine signaling (SOCS) are also recruited, associated or not to JAK. ET1 and AngII exert their action through G-protein coupled receptors (GPCR), 7 transmembrane domain receptors that interact with a heterodimeric G-protein after ligand stimulation. G-proteins consist in a GDP/GTP-binding α-subunit and a βγ-subunit complex. Both Gα and Gβγ subunits can modulate downstream effectors activity: activation or inhibition of adenylate cyclase, activation of phospholipase C (PLC), activation of the small G protein RhoA GTPase, activation of the mitogen-activated protein kinase 1 (ERK). A JAK/Src-dependent STAT3 activation have been also reported after ET1 or

Figure 1. Spectrum of STAT3 implication in PAH. STAT3 is activated in response to cytokines, growth factors and agonists that are dysregulated in PAH; signal transduction trough their receptors (tyrosine kinase, G-protein-coupled, immunoglobin like or integrin), and involvement of SHP2 and Src. STAT3 activates a broad range of transcription factors and proteins, all implicated in the management of proliferation and resistance to apoptosis that lead to the development of PAH. By downregulating miR-204, STAT3 abolishes SHP2 repression, enhances Src activation and finally sustains its own activation. Several STAT3 inhibitors (in red) might be beneficial for the treatment of PAH. Some of them are in early phase clinical trial for the treatment of cancer. This might as well facilitate their utilization for PAH treatment as data on their tolerability and efficiency will be available soon. DHEA is actually undergoing a phase 3 clinical trials for chronic obstructive pulmonary diseases and pulmonary hypertension.
AngII-dependent GPCR activation. This can be due to direct signal transduction from the GPCR as well as a reciprocal cross-communication between RTKs and GPCRs, since numerous GPCRs have been shown to usurp the signaling machinery of RTKs and that signal transduction by RTKs is mediated, at least in part by transactivation of GPCR.

TNF receptors are as well able to constitutively form a complex with endogenous c-Src and JAK2 and to use them to engage signaling cascades, activate transcription factors, and alter gene expression.

Recently, in human carotid artery smooth muscle cells (CASM), activation of the STAT3/Pim1/NFATc axis has been associated with signal transduction trough the receptor for advanced glycation end products (RAGE) after sensitization by the Nε-carboxymethyl lysine (CML). As RAGE has been previously implicated in PAH pathogenesis, especially by enhancing PAH-PASMCs migration, it is possible that STAT3 might also be activated by fixation of AGEs, or other ligands, on RAGE.

Hamodynamic forces that are increased by pressure overload, including shear stress and cyclic strain, have been recognized as important modulators of vascular cell morphology and function and are also upstream activators of STAT3. STAT3 activation has been determined in thoracic aorta embryonic A7r5A SMCs under cyclic strain (10% average strain at 60 cycles/min for various time periods). This mechanism is independent of JAK2 but Src-dependent for STAT3 tyrosine phosphorylation and p30/MAPK/ERK-dependent for STAT3 Serine 727 phosphorylation.

**STAT3 Plays Its Role of Signaling Hub in PAH**

The role of STAT3 in PAH, particularly with regard to the sustainability of the proliferative and anti-apoptotic phenotype, has been studied more in deep and several downstream STAT3 targets have been identified. The expression of Pim1 (provirus integration site for Moloney murine leukemia virus), a proto-oncogene encoding a serine/threonine protein kinase, has been described as a key downstream signal of STAT3 activation in PAH. Overexpression of Pim1 increases PASMCs proliferation within the remodeled PAs by promoting (1) bad phosphorylation/inhibition and mitochondrial hyperpolarization and (2) enhancing NFATc2 (nuclear factor of activated T-cells) activity. NFAT is a transcription factor that has been previously showed to account for the downregulation of K+ channels like Kv1.5, leading to cell depolarization, increased calcium levels and cell proliferation.

On the other hand NFATc2 activation also upregulates Bcl-2 leading to mitochondrial hyperpolarization and apoptosis resistance. Interestingly, several STAT binding sites have been identified within the promoter regions of NFATc1, NFATc2 and NFATc3 (the three major NFAT isoforms implicated in PAH) and Pim1. Chromatin immunoprecipitation associated with PCR (ChIP/PCR) experiments confirmed the interaction of STAT3 with the promoters of NFATc and Pim1 suggesting that STAT3 acts in an ingenious manner by increasing the expression of both the effector NFATc and its activator Pim1.

Survivin, a member of the inhibitor of apoptosis family of proteins, has been described as upregulated in PAH and playing a major role in PAH pathogenesis. STAT3 activation has been associated with cancer and systemic vascular remodeling and showed to cause an upregulation of Survivin mRNA levels. This has been shown in PAH recently, where STAT3 inhibition in PAH-PASMCs was associated with decreased Survivin mRNA levels. Survivin is another example of STAT3 downstream target, increasing further the role and impact of STAT3 activation in PAH. More recently it has been determined that this STAT3-dependent expression of Survivin is not direct but through activation of the transcription factor Krüppel-like factor 5 (KLF5). Once activated, this zinc-finger-type transcription factor promotes the upregulation of the cyclin B1 and triggers the expression of Survivin, and then contributes to PASMC proliferation and survival. Again, this suggests that STAT3 is a key mediator of PAH pathology as it activates a broad range of transcription factors and proteins, all implicated in the management of proliferation, and resistance to apoptosis that lead to the development of PAH.

Migration, invasion and motility of PASMCs in PAH have not been extensively studied. It is well characterized that PDGF is a potent inducer of PASMCs migration, at least by activation of the ERK/MAPK axis. PASMCs stimulated by BMP2 or S100A4 have also increased level of migration due to activation of BMPR2 and RAGE receptors. Several studies have demonstrated that hypoxia stimulates PASMCs migration, in a manner that is not dependent of Hif-1α. Finally, it is also suggested that PASMCs migration is regulated by sphingosine-1 via ROCK activation. STAT3 has been described to be implicated in SMCs invasion. Indeed, cellular invasiveness has been determined as dependent on the balance between two opposing forces: the proinvasive oncogenes Src-STAT3 and the anti-invasive tumor suppressors p53-PTEN. STAT3 is a required downstream effector of Src to induce podosome structures and related invasive phenotypes. STAT3 promotes Src phenotypes through the suppression of p53 and the p53-inducible protein caldesmon, a known podosome antagonist. In contrast, enhanced p53 attenuates STAT3 function and Src-induced podosome formation by upregulating the tumor suppressor phosphatase PTEN. PKCδ seems to also have a critical role in smooth muscle cells motility as PKCδ-deficient SMCs showed an abnormal cytoskeleton structure and decreased migration in response to mechanical stress (60 cycles/min and 5, 15 or 20% elongation). The protein kinase C delta (PKCδ) has been found increased in expression in PAs of chronic hypoxia-induced PAH rats. Among the 13 PKC members, PKCδ is the only PKC member that is a substrate for protein tyrosine kinases. For example, growth factors and Src kinases have been shown to induce tyrosine phosphorylation of PKC. Moreover, PKCδ is able to interact with the SH2 domain and part of the adjacent C-terminal transactivation domain of STAT3 to significantly enhance the interaction of STAT3 and the IL-6 receptor subunit glycoprotein (gp) 130, which is the initial step for STAT3 activation by IL-6. These findings suggest strongly that PKCδ is implicated at several levels in the transduction of the signal upstream to STAT3.
Finally, in patients with pulmonary arterial hypertension, reduced bioavailability of nitric oxide (NO) has been observed and associated with reduced pulmonary levels of the endothelium NO synthase (eNOS).\textsuperscript{93} Interestingly, a PKCδ/STAT3 axis has been implicated in the downregulation of eNOS expression and subsequent decrease in NO generation.\textsuperscript{94} Indeed, PKCδ activation following ET-1 stimulation in ovine fetal PAECs has been associated with STAT3 phosphorylation. This, increased the binding of STAT3 on the eNOS promoter resulting in inhibition of eNOS expression.\textsuperscript{95} Thus, PKCδ/STAT3 pathway might be at least in part responsible for the downregulation of the NO signaling in PAH.

**Reciprocal Regulations between STATs and miRNA in PAH**

Active interactions between STATs and miRNAs have been described in pathways driving immune cells maturation and cancer, with the presence of reciprocal regulations between STATs and miRNA.\textsuperscript{95} The inappropriate STAT3 activation seen in PAH has been linked with miRNA expression. mir-204, which is located within the intron 6 of the human transient receptor potential melanotrans 3 (TRPM3), has been shown negatively regulated, like TRPM3, by STAT3 binding on the promoter.\textsuperscript{96} mir-204 has been previously described as tumor suppressor in cancer\textsuperscript{97} as its downregulation induces PDGF-dependent proliferation.\textsuperscript{98} The identified direct target of mir-204 in PAH is SHP2, a cytoplasmic tyrosine phosphatase implicated in Src activation.\textsuperscript{98,99} Thus, by downregulating mir-204, STAT3 abolishes SHP2 repression, enhances Src activation and finally sustains its own activation (Fig. 1). The understanding of this feedback mechanism is of great therapeutic interest since it could explain that targeting STAT3 signaling upstream to Src activation would only have a short impact, as the loop is still active. In PAH, therapeutic interventions restoring mir-204 expression should be an interesting strategy equivalent with Src and STAT3 inhibitors.

Brock and colleagues demonstrated that in a model of HPAECs stimulated with IL-6, STAT3 activation results in STAT3 binding on the promoter region of the miR-17/92 gene (C13orf25), enhancing transcription of the gene and expression of the miRNA and BMPR2 downregulation.\textsuperscript{100} Indeed, by computational analysis, several miRNAs encoded by the cluster miR-17/92 have been identified to directly target BMPR2. Src is also an interacting partner of the BMPR2 C terminus domain. BMPR2 mutation resulting in a truncated BMPR-II C-terminal domain is able to increase c-Src phosphorylation in response to BMP-2.\textsuperscript{101} This suggests that the effect of STAT3 on BMPR2 and subsequently on Src might be another mechanism built to maintain its own activation (Fig. 1).

**STAT3 and Associated Therapies (Anti-Cancer and DHEA)**

There is evidence that dehydroepiandrosterone (DHEA) is an efficient STAT3 inhibitor in PAH (Fig. 1), confirming previous findings in the liver.\textsuperscript{102} The exact molecular mechanism by which DHEA decreases STAT3 activation remains to be established. Nonetheless, we have evidence that DHEA effects in vascular tissue is likely mediated through a plasma membrane receptor coupled to G protein.\textsuperscript{76} Other mechanisms cannot be ruled out, for example, DHEA has been shown to increase PPAR-γ mRNA levels.\textsuperscript{103} PPAR-γ could be implicated in the DHEA-dependent STAT3 inhibition as activation of the peroxisome proliferator-activated receptor gamma (PPAR-γ), a member of the nuclear receptor family which is downregulated in PAH,\textsuperscript{104} have an inhibitory effect on STAT3.\textsuperscript{105–107} A direct physical protein-protein interaction has been shown between PPAR-γ and activated STAT3, resulting in decreased transcriptional activity of STAT3. PPAR-γ agonist ciglitazone treatments in glialblastoma cell lines showed a decreased expression of phosphorylated form of STAT3 associated with an increased expression of STAT3 inhibitors like the suppressor of cytokine signaling (SOCS) 3 and the protein inhibitor of activated STAT3 (PIAS3).\textsuperscript{108} Considering that PPAR-γ agonists used in the treatment of PAH patients (rosiglitazone and pioglitazone) could be associated with adverse cardiovascular events,\textsuperscript{109} DHEA might be a smart alternative way to increase PPAR-γ and decrease STAT3 activation in PAH. DHEA is actually undergoing a phase 3 clinical trials for chronic obstructive pulmonary diseases and pulmonary hypertension.\textsuperscript{110}

Plumbagin is a natural vegetal organic compound known to block STAT3 in cancer cells. This molecule has been tested in PAH animal models, and has been shown to reverses experimental PAH through Src/STAT3/NFAT inhibition\textsuperscript{111} (Fig. 1). Nonetheless, little is known about human tolerance of the molecule and side effects, making its translation to clinical use unlikely.

Several STAT3 inhibitors are in early phase clinical trials for the treatment of diverse malignancies. STA-21 has completed phase I/II clinical trials for the treatment of psoriasis,\textsuperscript{112} but no results are publicly available yet. OPB-31121 (Fig. 1) is currently in phase I in patients with advanced solid tumors.\textsuperscript{59} Pyrimethamine is in phase II/I trials for the treatment of chronic lymphocytic leukemia and small lymphocytic leukemia.\textsuperscript{61} RTA 402 is an antioxidant inflammation modifier able to modulate NFkB, STAT3 and Nrf2 [nuclear factor (erythroid-derived 2)-like 2]. A phase I trial has been completed in 34 patients with solid tumors and lymphoid malignancies. RTA 402 is well tolerated up to 900 mg/day with prolonged exposure up to 12 mo. Data indicate appropriate modulation of the targets NFkB, STAT3 and Nrf2 and suggest clinical efficiency, including a partial response observed in a patient with thyroid cancer, and prolonged disease stabilization in nine patients, six of which lasted for 6–12 mo (3 melanoma, 2 renal cell, 1 medullary thyroid). The recruitment of patients with solid tumors and lymphoid malignancies for the phase II trials has begun.\textsuperscript{62}

**STAT3 Implication in Endothelial Cells, Right Ventricle and Inflammation**

Although these results are very promising for PAH, the impact of these drugs on PAEC, RV and inflammatory response are crucial.
Indeed, while triggering apoptosis in PASMCs may improve PAH, triggering apoptosis of remaining and unaltered PAECs might at the opposite worsen it. Another important factor to consider in PAH therapy is RV health since survival in PAH seems to be determined by the condition of the RV better than the degree of the afterload increase. Thus, PAH therapies, if not beneficial for the RV, must at least be not detrimental.

**Right ventricle.** Several evidences have shown that STAT3 plays a critical role in cardiac hypertrophy in response to stimuli like increased afterloads, infarct, ischemia and neurohormones. First of all, a transgenic conditional STAT3 overexpression in mice cardiomyocytes is sufficient to provoke a spontaneous ventricular hypertrophy, suggesting that STAT3 is critical for cardiomyocyte compensatory response. This response is followed by increase transcription of antioxidants proteins like SOD-2, and induction of anti-apoptotic and cyto-protective proteins like Bcl-xL and Hsp70. Cardiomyocytes appear to be more resistant to H2O2 induced-apoptosis when STAT3 expression is high. STAT3 is involved as well in response to mechanical stress. In a feline model of heart under pressure, STAT3 have been showed activated at 48 h in a manner at least in part dependent of Src activation. Src/STAT3 activation has not been particularly measured in RV hypertrophy but could be a mechanism implicated in the protective response of RV by compensation. The question of possible adverse effects of STAT3 inhibition is intriguing. Nonetheless, we believe that as soon as STAT3 inhibition is effective in the decrease of pulmonary pressure and resistance, the mechanisms of RV compensation would not be necessary anymore, STAT3 inhibition in RV being hence without consequences.

**Endothelial cells.** Increased and sustained STAT3 phosphorylation has also been identified in the endothelium in plexiform lesions of idiopathic lung tissues as well as in PAECs isolated from idiopathic PAH human lungs, suggesting the implication of STAT3 in the proliferative and survival phenotype of PAEC within plexogenic lesions. Later, the downregulation of the caveolin-1 protein (known to initiate the coupling between integrins and the Ras-ERK pathway to promote cell cycle progression) has been identified as a mechanism promoting STAT3 activation. In PAH-PAECs and PAECs exposed to monocrotaline pyrrole, STAT3 is located in cytoplasmic vesicles like endosomes that might be involved in the transport of STAT3 to the nucleus. Caveolin-1 overexpression has been shown to increase the targeting of STAT3 to lysosomes, thus inhibiting STAT3 transcriptional activity. A marked cytoplasmic sequestration of activated PY-STAT3 has been also found in PAECs in the rat model of monocrotaline-induced PAH as well as in human plexiform lesions. Therefore, STAT3 inhibition might be beneficial as well to reverse plexiform lesion. It might be of particular interest to further study this aspect in the Hypoxia/ Sugen rat model or the overexpressing Mts1/S100A4 mice model, that both develop lesions that somewhat resemble human plexogenic arteriopathies.

However, STAT3 is not only implicated in promoting proliferation or survival, but also in enhancing angiogenesis. Another important therapeutic strategy for PAH consists in the inducing the re-endothelization of vessels (endothelial barrier recovery) and promoting the formation of new vessels by mobilizing endothelial progenitor cells. It is believed that endothelial progenitor cells (EPCs) constitute one aspect of the endothelial repair process by providing beneficial endothelial-derived factors, like HGF or FGF that mobilize and recruit Lin-c-kit+Sca-1+CD34+ progenitor cells from bone marrow into the injured organ. STAT3, along with ERK/MAPK and PI3K/Akt, is one of the predominant pathways activated in response to these factors, suggesting that STAT3 inhibition might counteract re-endothelization processes and worsen the disease. Importantly, PAECs isolated from idiopathic patients, despite persistent STAT3 activation, were found less able to produce organized networks in tube formation assays than control cells in response to angiogenic factors. This demonstrates that STAT3 is not the only player involved in the appropriate recruitment and migration of PAECs during the angiogenic response. Angiogenesis is a complex mechanism involving several other molecules; therefore STAT3 inhibition might not be that detrimental in the overall angiogenic function and it is critical to continue to increase the knowledge in this area.

**Inflammation.** Growing evidence demonstrate that inflammation plays a key role in triggering and maintaining pulmonary vascular remodeling in PAH. Indeed, different cell types such as chemokines, cytokines and antibodies play a role in the pathogenesis of PAH. The inflammatory milieu in PAH is composed of resident and recruited macrophages, dendritic cells, T and B cells and mast cells. B lymphocytes are probably the critical actors of inflammation by transforming into plasma cell auto-antibody, antigen presentation, cytokine production, differentiation of effectors T-cells and collaboration with DC. While regulatory lymphocyte CD4+ CD25 (Treg) activity is usually decreased in autoimmune disorder, increased circulating Treg and decreased CD8+ cytotoxic T cells have been reported in idiopathic PAH, controlling T and B cells activity and DC function. DCs are antigen-presenting cells responsible for the initiation of the inflammatory response. DCs have the ability to differentiate into other cell types including ECs, which might be critical for PAH pathogenesis. An increased number of DCs have been found in pulmonary vascular lesion in human and MCT-induced PAH. Immature dendritic cells have been found in pulmonary vascular remodeling, suggesting their involvement in PAH immunopathology.

Many studies in cancer support that STAT3 inhibition decreases immunosuppression in tumor microenvironment. Indeed, STAT3 activation inhibits the production of Th1-type cytokines such as IL-12 and IFN-γ, mediators critical for potent anti-tumor immune responses and that are also decreased in PAH. Furthermore, STAT3 regulates the release of IL-10, VEGF and IL-6, factors that prevent DCs maturation into antigen-presenting cells and thus alter the inflammation response. Thus the presence of activated STAT3 in PAH could explain the presence of immature DC in the pulmonary vasculature in PAH. Thus STAT3 impairs both adaptive and innate immune responses against tumor and its inhibition might have a beneficial impact on restoring normal inflammation processes in the lungs.
However, we cannot rule out the possibility that STAT3 inhibition may decrease T-cells activation, especially via the downregulation of the Pim-1/NFAT axis, which is shown to be implicated in PAH and inflammatory cells.\textsuperscript{26,28,135} Further studies would be needed to establish the role of STAT3 inhibition in inflammation processes in PAH and to determine if the overall effect of this therapy is beneficial.

**Speculations**

**STAT3 implication in mitochondria/oxygen sensing.** A recent study described the potential implication of STAT3 in the mitochondrial dependent energy metabolism.\textsuperscript{136} Indeed, the mitochondrial protein GRIM-19 (gene associated with retinoid interferon-induced mortality), a component of the electron transport chain (ETC) complex I, is known to interact with STAT3 and inhibit its transcriptional activity.\textsuperscript{137-140} This interaction is associated with a mitochondrial STAT3 localization and seems to be critical for ETC function and energy production by glucose oxidation.\textsuperscript{136} This phenomenon is independent of either STAT3 binding to DNA, phosphorylation on the residue tyrosine 705 or dimerization, but is dependent on phosphorylation on the residue serine 727.

Another study also demonstrated that a transgenic STAT3 constitutive activation in primary mouse embryonic fibroblasts enhances a HIF-1α-dependent increase in glycolysis and a HIF-1α-independent reduction in mitochondrial respiration.\textsuperscript{141} In fact, H. Yu and co-authors have previously shown that a constitutive STAT3 activation (downstream of Src activation) is required for HIF-1α mRNA expression in response to hypoxia or growth factors in cancer, STAT3 acting directly at the level of HIF-1α promoter to enhance transcription.\textsuperscript{142} Thus, STAT3 might play an important role as a master metabolic regulator in STAT3-dependent human cancer cell lines, and in PAH.

**Possible role in NFκB activation.** Moreover, in several type of tumor, the constitutive activation of STAT3 is associated with an increased expression of STAT3 that accumulates in a non-phosphorylated form U-STAT3. U-STAT3 has been demonstrated as able to link a non-phosphorylated form of NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), U-NFκB to form a complex that accumulates in the nucleus and activates a selection of gene.\textsuperscript{143-145} Other studies using tyrosine 705 mutants showed in keeping results as these mutant can associate with NFκB and induce the expression of genes such as mras or met, which are likely to play an important role in cell transformation by STAT3.\textsuperscript{146-148} These findings increase the spectrum of the functions that STAT3 might have in PAH, as STAT3 can induce different transcriptional programs depending on which sites are phosphorylated.

**STAT5 and organelle integrity.** There is evidence that PAH pathogenesis is associated with dysfunction in organelle function and trafficking. Golgi dysfunctions have been first described in human idiopathic PAH and the HIV-macaque model of pulmonary hypertension, with an increase and cytoplasmic dispersal of the Golgi matrix protein/tether giantin and p115.\textsuperscript{149} ER stress has been also found with activation of the ER unfolded protein response, activation of the ER shape regulator Nogo, a tubular and bundled ER\textsuperscript{150} and disrupting the ER-mitochondrial unit.\textsuperscript{151} Interestingly, non-phosphorylated STAT5 has been recently reported unexpectedly associated with the Golgi and ER with the non-genomic function to preserve the structural and the functional integrity of these organelles. Indeed, STAT5 siRNA on endothelial cell result in cystis dilatation of the ER with accumulation of Nogo at the boundaries, dilatation and fragmentation of the Golgi, distortion of the nucleus and mitochondrial fragmentation. These data strongly suggest the involvement of STAT5-related ER/Golgi/mitochondrial dysfunctions in the disease pathogenesis.\textsuperscript{152}

**Conclusion**

PAH is a lethal disease for which treatments are limited. Over the past two years, STAT3 has been demonstrated as a key mediator of PAH pathology and thus has been revealed as a new therapeutic target. Pharmaceutical agents, such as DHEA and other STAT3 inhibitors like plumbagin, are able to reverse experimental PAH by restoring most of the molecular abnormalities seen in PAH-PASMCs: they decrease the expression/activation of Pim1, Survivin and NFAT, and upregulate BMPR2 and miR-204 (Fig. 1). All these downstream effects contribute to decrease PAH-PASMC proliferation and survival, making STAT3 an interesting therapeutic target for PAH treatment. Targeting STAT3 has the potential to not only inhibit cell proliferation, survival and motility but also immune escape and altered immunologic environment and we believe that STAT3 inhibition will result in positive outcome in PAH patients. Several STAT3 inhibitors have been designed for the cancer and are currently in clinical trials for cancer. Since they seem to be safe and well tolerated, their translation to bedside might be facilitated for PAH clinic. STAT3 inhibition opens hence new clinical avenues for PAH.

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