Taking It Off: New Insights into the Role of Tyrosine Phosphorylation–dependent Pathways in the Pathogenesis of Pulmonary Fibrosis

In this issue of the Journal, Tzouvelekis and colleagues (pp. 500–514) report that the SH2 domain–containing tyrosine phosphatase, SHP2, exerts a physiologically important antifibrotic effect in lung myofibroblasts (1). They observed that SHP2 expression was diminished in myofibroblasts isolated from patients with idiopathic pulmonary fibrosis (IPF) and, conversely, that enhanced expression of SHP2 or expression of constitutively active SHP2 attenuated fibrogenic responses in cultured cells and animal models of pulmonary fibrosis. The authors suggest that augmentation of SHP2 expression and/or activity should be considered as a novel therapeutic strategy for IPF.

IPF is characterized by progressive diffuse fibrosis of the lung leading to distortion of lung architecture and loss of function (2). The clinical course of IPF is relentlessly progressive and ultimately fatal, with a median survival of 2.5 to 3.5 years (2, 3). Although recent trials have demonstrated that pirfenidone (4) and nintedanib (5) slow the decline in lung function in IPF, new therapeutic approaches that not only slow the progression of fibrosis but also promote the resolution of established fibrosis are needed.

The mechanisms driving pulmonary fibrosis remain incompletely understood, and both genetic and environmental factors are important (6). IPF is a disease of aging, likely reflecting the cumulative effect of age-related genetic alterations that impede lung repair after repeated injury. Release of cytokines induces myofibroblast accumulation and deposition of excess extracellular matrix (ECM), culminating in diffuse lung fibrosis. IPF myofibroblasts exhibit profibrotic behavior in vitro, indicating the durability of these phenotypic alterations (7).

A diverse array of cells and mediators drive pulmonary fibrosis (8). Fibroblasts and myofibroblasts are responsible for secreting the excessive and disorganized (ECM) proteins present in the fibrotic lung and contracting the “scar” tissue, resulting in restrictive lung physiology (2). Surprisingly, the origin of these excessive fibroblasts and myofibroblasts remains uncertain (8). Local proliferation, transition of epithelial cells or pericytes to fibroblasts (termed mesenchymal transition), and influx from bone marrow–derived fibrocytes are all possible sources of myofibroblast accumulation. Alveolar and airway epithelial cells may contribute to fibrogenesis by production of profibrotic mediators in response to repeated injury. Lung macrophages, via secretion of soluble fibrogenic factors and/or modulation of the immune response, may also contribute to pulmonary fibrosis. Vascular cells, including endothelial cells and pericytes, may also contribute to the fibrotic process. Finally, fibrogenic growth factors, including transforming growth factor-α and -β, platelet-derived growth factor (PDGF), fibroblast growth factor, vascular endothelial growth factor, and connective tissue growth factor, participate in the pathogenesis of pulmonary fibrosis (2). From this brief discussion, it is apparent that fibrogenesis is a complex process indeed.

Signaling pathways involving reversible tyrosine phosphorylation regulate cellular responses to growth factors that contribute to organ fibrosis (9). In fact, nintedanib, a triple tyrosine kinase inhibitor (PDGF-R, fibroblast growth factor-R, and vascular endothelial growth factor-R), is effective in treating IPF (5). Tyrosine phosphorylation is tightly regulated by the opposing actions of protein tyrosine kinases and phosphatases (PTPs). Compared with protein tyrosine kinases, much less is known about the role of PTPs in fibrogenesis. Genetic deletion of SHP2 in alveolar epithelial cells results in spontaneous pulmonary fibrosis in mice, possibly related to dysregulated surfactant homeostasis (10); similarly, genetic deletion of SHP2 in macrophages enhanced bleomycin-induced pulmonary fibrosis in mice, possibly related to dysregulated surfactant homeostasis (10). We have reported that mice genetically deficient in PTPα are protected from experimental pulmonary fibrosis (12).

In the current article, the authors focus on the role of SHP2 in fibrogenesis (1). Interestingly, the Noonan syndrome, a genetic syndrome characterized by short stature, dysmorphic facial features, and cardiac abnormalities, is caused by gain-of-function mutations in SHP2 (PTPN11) in approximately half of cases (13). Aside from pulmonary lymphangiectasis, pulmonary abnormalities are not known to be part of this syndrome, although the prediction would be that patients with the Noonan syndrome would be protected from pulmonary fibrosis.

An important but unresolved question is what mechanisms are responsible for the antifibrotic effects of SHP2. One possibility is that SHP2 can bind to and down-regulate profibrotic tyrosine kinase growth factor receptors, such as the PDGF or transforming growth factor-β receptors, by dephosphorylating the receptor and inactivating their kinase activity (14). This could lead to fibroblast apoptosis, diminish cell motility, prevent...

Supported by funding from the National Institutes of Health grants ES023932 and HL132950 (G.P.D.).
fibroblast-to-myofibroblast transition, or decrease production of ECM proteins, all processes that could reduce fibrogenesis. It is also unclear how SHP2 is down-regulated specifically in myofibroblasts. Does this reflect epigenetic control of gene expression or post-translational modification of SHP2 protein? Although in vivo conditional SHP2 knockouts have been generated in macrophages and alveolar epithelial cells, no fibroblast-specific gene-targeted mice have been studied in preclinical models of pulmonary fibrosis. As augmentation of fibrosis has been noted with alveolar epithelial cell and macrophage deletion of SHP2, perhaps the mechanisms also rely on cross-talk between various cell types.

Although it is tempting to conclude that augmenting the function of SHP2 is a viable therapeutic approach for the treatment of IPF, it is premature to do so. Therapeutic intervention might involve genetic or pharmacological approaches with small-molecule SHP2 agonists. Gene therapy–based approaches using liposomal-plasmid DNA complexes or viral vectors have had some success with diseases such as cystic fibrosis (15). An important limitation to this approach relates to the ability to target gene therapy specifically to the pathological myofibroblasts, which reside in the interstitial compartment and thus may not be easily accessible via an airway-based delivery mechanism. Although the use of pharmacological enhancers of SHP2 is appealing, this approach is also potentially problematic because of the inherent nonspecificity of small molecules targeting protein tyrosine phosphatase activity, given the highly conserved structure of the catalytic domain of these phosphatases (16). Furthermore, if administered systemically or directly to the lung, small-molecule enhancers have the potential to influence the activity of SHP2 and other tyrosine phosphatases in other cell types, where they regulate key cellular functions and participate in cancer pathogenesis. Finally, there is the important and unfortunate fact that many (if not most) patients with IPF come to medical attention late in their clinical course, at a time when the inflammatory and early fibrotic processes are no longer active, and there is no evidence at present that augmenting SHP2 activity can resolve existing (mature) fibrosis.

In conclusion, the report by Tzouvelekis and colleagues demonstrates the antifibrotic role of SHP2 in myofibroblasts, key cells in the pathogenesis of IPF (1). Augmentation of SHP2 activity, either through the use of small-molecule activators or genetic modification, holds future promise for the treatment of this debilitating and life-threatening condition.

References

Mitochondrial Calcium Transport: A Potentially Prominent, Therapeutically Targetable Contributor to Pulmonary Arterial Hypertension Progression

In this issue of the Journal (pp. 515–529), Hong and colleagues (1) provide evidence indicating a key role for decreases in the function of the pulmonary arterial smooth muscle (PASM) mitochondrial calcium uniporter (MCU) complex in controlling many important aspects of the cancer cell–like metabolic profile associated with changes in mitochondrial function and proliferation seen in pulmonary arterial hypertension (PAH) (2, 3). Evidence is also provided for a depletion of MCU in pulmonary arteries from the rat monocrotaline pulmonary hypertension model and in PASM cells from humans with PAH. In addition, microRNAs miR-138 and miR-25 appear to be key down-regulators of MCU. The study provides novel mechanistic insights into multiple ionic, metabolic, and mitochondrial aspects of the role of these regulatory systems in the progression of PAH, including new potential therapeutic targeting of MCU expression through anti-miRs.

The roles for changes in intracellular calcium in controlling vascular contractile function and remodeling of pulmonary arteries that participate in the development and progression of pulmonary hypertension are well established (4). However, the role of mitochondrial calcium and the function of MCU in vascular physiology and pathophysiology remain rather poorly understood. A study (5) of mice depleted of MCU revealed an alteration in the phosphorylation of pyruvate dehydrogenase in skeletal muscle, which resembled a change in PAH extensively studied by Archer and colleagues (2) and by Sutendra and Michelakis (3). On the basis of their work, alterations in the phosphorylation of pyruvate dehydrogenase appear to be controlling a cancer cell–associated Warburg-type shift in energy metabolism from aerobic mitochondrial metabolism to glycolysis that appears to suppress apoptosis and promote PASM proliferation associated with vascular remodeling in PAH (2, 3).

The study by Hong and colleagues (1) demonstrates these changes in PASM from the monocrotaline-induced rat model of PAH. Decreases in MCU are associated with increased cytosolic calcium and decreased mitochondrial calcium in both the rat monocrotaline model and in PASM cells from humans with PAH. Multiple approaches modulating the expression and inhibition of MCU provide new evidence for its potentially normal role in attempting to attenuate increases in cytosolic calcium and to maintain mitochondrial calcium–regulated processes under conditions that potentially promote PAH development. These experiments also document how the function of MCU is lost by inhibition or depletion, and how this loss of MCU coordinates the promotion of processes linked to PASM migration, proliferation, and resistance to apoptosis through relationships shown in Figure 1. Moreover, conditions promoting increased or restored MCU function are demonstrated to reverse key aspects of these PAH-associated processes. Although these results clearly highlight an important role for the MCU transporter in controlling intracellular calcium and in the pathogenesis of PAH, observations in this study raise many additional questions, which remain to be investigated. For example, how does the function of MCU influence well-established roles for calcium in eliciting and/or maintaining PASM cell contraction, and in regulating mitotic and migratory aspects of PASM cells suggested in this and other (6) studies. Besides, intracellular calcium can accumulate in PASM cells through increased voltage-gated and receptor-operated cation entry.

**Figure 1.** Model showing how down-regulation of the mitochondrial calcium uniporter by microRNA (miR)-25 and miR-138 in pulmonary arterial hypertension (PAH) could alter mitochondrial and extramitochondrial calcium (cytosol) levels in ways that drive metabolic changes in pulmonary arterial smooth muscle cells potentially linked to processes contributing to PAH-associated vascular remodeling. PASM = pulmonary arterial smooth muscle cell; PDH = pyruvate dehydrogenase.