‘Hypoxio-spondin’: thrombospondin and its emerging role in pulmonary hypertension

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In 1971, Baenziger et al.1 identified a 190 kDa membrane protein in intact platelets that rapidly (within <2 min) disappeared after treatment with thrombin, while a new 107 kDa protein emerged. They concluded that this transition represented hydrolysis of a thrombin-sensitive protein, which likely triggered the physiological effects of thrombin on platelets. Accordingly, the protein was named ‘thrombospondin’ (currently known as thrombospondin-1 or TSP1).2 Since then, TSP1 has emerged as a multifunctional protein playing important roles in numerous physiological and pathological processes, including thrombosis, angiogenesis, tumorigenesis, inflammation, apoptosis, and fibrosis.

Recently, TSP1 was linked to pulmonary hypertension (PH), a progressive disease of multiple origins characterized by increased pulmonary artery pressure (>25 mmHg at rest) and extensive lung vascular remodelling that ultimately leads to death due to decompensating right heart failure.3 Despite important advancements in the pharmacotherapy of PH, there is still no cure for this fatal disease. Notably, all of the TSP1 regulated processes listed above, including tumour-like monoclonal proliferation of endothelial cells,4 are implicated in PH pathogenesis. Indeed, the functional role of TSP1 in PH was recently evidenced in independent studies showing partial protection of mice deficient in TSP1 (tsp1−/−) from the classic hallmarks of hypoxia-induced PH, i.e. right ventricular pressure elevation and hypertrophy, medial wall thickening, and distal muscularization of pulmonary arterioles.5,6

A study by Labrousse-Arias et al.7 sheds new light onto the mechanisms driving TSP1 expression in hypoxic PH, and identifies potential downstream effectors. Using an elegant combination of in vitro and in vivo techniques, the authors show that hypoxia up-regulates TSP1 within 24 h in murine lungs, freshly isolated murine lung fibroblasts, and human pulmonary artery endothelial (PAEC) and smooth muscle cells (PASMC). TSP1 up-regulation in most cell types was paralleled by increases in hypoxia-inducible factor-2α (HIF-2α), and siRNA-mediated knockdown of HIF-2α prevented the hypoxia-induced increase in TSP1 in PAEC. Notably, of the tested lung vascular cell types, only murine PASMCs failed to show a significant hypoxia-induced increase in TSP1 mRNA, and concomitantly lacked induction of HIF-2α. The critical role of HIF-2α in up-regulation of TSP1 was corroborated in mice lacking the von Hippel-Lindau protein (vhl−/−). Since vhl is required for the rapid ubiquitination and proteasomal degradation of HIFs, vhl−/− mice offer a model of constitutive normoxic HIF activation. Accordingly, vhl−/− mice showed increased lung TSP1 expression; this effect was absent in mice lacking both vhl and hif2α, yet not in vhl−/−/hif2α−/− double knockout mice. Based on the data presented, a new regulatory axis emerges whereby TSP1 can be rapidly induced by hypoxia in a HIF-2α-dependent manner (Figure 1), a notion in line with previous preclinical and clinical data pointing towards a role for HIF-2α in PH.8–10

Despite clear evidence for its functional role, the actual cellular source of TSP1 in PH remains poorly understood. Comparing human control lungs vs. those from end-stage PH patients, Labrousse-Arias and colleagues noted up-regulation of TSP1 in fifth-order pulmonary arteries (paralleled by increased HIF-1α and HIF-2α) of PH lungs; yet surprisingly, TSP1 (and likewise, HIF-1α and HIF-2α) expression was lower in lung parenchyma of PH patients. This finding could indicate that increased TSP1 expression in PH is a predominant feature of large pulmonary arteries and not parenchymal pulmonary arterioles. However, PH is first and foremost a disease of the small blood vessels, and attenuation of hypoxia-induced PH in tsp1−/− was associated with reduced muscularization of precapillary arterioles.5,6 Whether this apparent discrepancy reflects temporal regulation during PH, with TSP1 contributing to remodelling in the small arterioles during early- but not late-stage disease, or differences in species or stimulus (hypoxia vs. PH patients), remains to be determined. To complicate matters, increased TSP1 expression does not necessarily reflect increased local production, but may result from reduced local uptake of TSP1 secreted locally or even remotely. Upon receptor-mediated binding, TSP1 is generally rapidly internalized and degraded11,12 but the extent to which hypoxia regulates these processes is unclear. Regulated TSP1 catabolism would also explain seemingly discordant results between mRNA and protein levels, such as in murine PASMCs where hypoxia failed to increase TSP1 mRNA levels significantly, yet more than doubled TSP1 protein expression. Finally, Labrousse-Arias...
and colleagues demonstrated hypoxia-induced TSP1 expression in pulmonary vascular cells in vitro; yet TSP1 is also prominently expressed and secreted from immune cells, such as macrophages or platelets, both of which have been implicated as important regulators and propagators of PH. Whether all of these cells contribute conjointly to TSP1 release during hypoxia in vivo or whether a specific cell type dominates remains to be addressed, i.e. using cell type-specific Cre-Lox recombination in mice.

Just like the potential sources of TSP1, its downstream targets and effects are multifaceted. Lungs of PH patients and chronically hypoxic mice express increased levels of the cognate TSP1 receptor CD47, activation of which disrupts its constitutive interaction with caveolin-1, and in turn promotes endothelial NO synthase uncoupling and superoxide formation in PAECs. Consolidating a key role of this TSP1-CD47 axis in PH, monocrotaline-treated rats were largely protected from the development of PH and lung vascular remodelling by blocking a anti-CD47 antibody. Apart from CD47, however, TSP1 binds a variety of other receptors, including CD36 (glycoprotein IV or IIIb), CD138 (syndecan 1), or integrins, and interacts with various proteases such as plasminogen, urokinase, thrombin, or elastase, many of which have been implicated in PH. For example, serum levels of syndecan 1 or soluble CD36 are commonly elevated in patients with SSc and are associated with a higher prevalence of elevated right ventricular and/or pulmonary artery systolic pressures. Remodelled pulmonary arteries also exhibit induction of uPA receptor, and a series of studies implicate elastase in PH, with elastase inhibitors effectively reversing experimental PH. Importantly, TSP1 also activates latent transforming growth factor-β (TGF-β), allowing recognition by its receptor complex, blockade of which attenuates PH in monocrotaline-treated rats. Without claiming completeness, this list highlights the multitude of potentially relevant (and putatively targetable) downstream receptors/effectors of TSP1 in PH.

In summary, this report offers compelling evidence suggesting that HIF-2α-dependent induction of TSP1 provides a novel pathway for HIF-mediated pulmonary vascular dysfunction. While its original identification as a thrombin-sensitive protein coined its name ‘thrombospondin’, its induction by hypoxia and role in PH may, in fact, lead us to think of TSP1 rather as the new ‘hypoxio-spondin’.

References


