Tyrosine Kinases and Endothelial Homeostasis in Pulmonary Arterial Hypertension: Too Hot to Handle?

The microvascular endothelial cell has established itself as one of the key players in the pathobiology of pulmonary arterial hypertension (PAH). In most conceptual drawings of the etiology of this deadly condition, endothelial homeostasis and (distorted) endothelial repair take center stage. The simplest version of the explanatory narrative of PAH goes from insult (e.g., infection, autoantibodies, drugs, and abnormal shear stress) via endothelial resilience (genetically determined [e.g., via BMPR2 (bone morphogenetic protein receptor type 2) expression]) to dysfunctional wound repair (1, 2). The result of this process is either microvascular rarefaction or obstructive remodeling, and both contribute to increased pulmonary vascular resistance and right heart failure. Along the way, cellular and molecular processes come to light that remind us of central mechanisms in the biology of cancer (3). To name a few, abnormal inflammatory responses, suppressed glucose oxidation, abnormal DNA repair, apoptosis resistance, and senescence create a quasimalignant state that reflects and may even contribute to ongoing vascular damage and repair. Given the biological similarities between PAH and cancer and recognizing the oncolytic potential of tyrosine kinase inhibitors, there has been much interest in the role of receptor and nonreceptor tyrosine kinases in PAH (4). Confusingly, preclinical and clinical evidence is available to suggest that tyrosine kinase inhibitors may both cause and reverse PAH (5–7). This paradox may partly be explained by the fact that damage to healthy endothelial cells may incite the development of PAH, whereas after a "quasimalignant phenotypic switch", PAH endothelial cells may become hyperproliferative and apoptosis-resistant. As such, the destruction of these phenotypically altered endothelial cells may confer a therapeutic benefit, at least in the short term.

In this issue of the *Journal*, Le Vely and colleagues (pp. 215–226) implicate a loss of c-Abl (c-Abelson) expression and activity in the endothelial dysfunction associated with PAH (8). At first sight, their findings may seem counterintuitive. Constitutive activation, not suppression, of the nonreceptor tyrosine kinase c-Abl is a hallmark of certain leukemias, and treatment with tyrosine kinase inhibitors has been successful in suppressing the abnormal proliferation that results from c-Abl activation (9, 10). If PAH is indeed a quasimalignant disease, wouldn't we have expected enhanced c-Abl activation in the cells and tissues of patients with PAH? Admittedly, the role of c-Abl in regulating the cell cycle, apoptosis, and proliferation in response to cellular stress is complicated and highly context-dependent, but the vast majority of studies predict high rates of cellular proliferation and apoptosis resistance after a loss of c-Abl activity (9, 10).

The report by Le Vely and colleagues quite clearly points in another direction, however. In a meticulous translational study, they found lower c-Abl and phosphorylated c-Abl concentrations in the endothelium of remodeled pulmonary vessels from patients with PAH and rats with two forms of experimental pulmonary hypertension. Cultured human PAH-endothelial cells displayed low c-Abl expression and activity. In control endothelial cells, both downregulation of c-Abl by RNA interference and c-Abl inhibition with dasatinib resulted in genomic instability and a failure to form tubes, while restoration of c-Abl in PAH-endothelial cells resulted in reduced DNA damage and apoptosis. Remarkably, the investigators stumbled on the existence of crosstalk between c-Abl and BMPR2. The manuscript is a fine example of translational research with careful use of human tissues and cells, experimental models, and mechanistic interventions. A great strength of the study is that the authors provide evidence for a novel signaling pathway in patient tissue, patient-derived cells, animals, and with mimicking experiments.

What does this study mean for our current understanding of PAH? Should we conclude that the paradigm of "quasimalignancy" is up for revision? Of course, a single study would be insufficient to counter a multitude of other studies demonstrating hyperproliferation and apoptosis resistance in the PAH lung. There are signals, however, that these features may paradoxically coexist with high rates of cellular death, particularly under stress, disturbed tube formation, and vascular rarefaction (11, 12). Alternatively, discrepant findings may be explained by the possibility that cellular proliferation and apoptosis may be more prominent in different stages of the disease. Perhaps after an initial trigger of endothelial apoptosis, early PAH is characterized by hyperproliferation, while late PAH is characterized by senescence and DNA damage (13). In this context, it is interesting to see that in the current study, enhancing c-Abl activity with the small molecule 5-[3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2,4-imidazolidinedione (DPH) rescued DNA damage and improved tube formation.

Before establishing c-Abl as a treatment target in PAH, however, a fuller understanding of the mechanism is required. The fact that c-Abl has been described as a driver of a number of endothelium-disruptive processes, including barrier disruption (14), apoptosis (15), and prothrombotic endothelial cell activation (16), argues against the swift implementation of a therapeutic strategy that puts c-Abl in overdrive. Future studies need to address a number of remaining questions. First, the study does not provide a conclusive mechanistic explanation for a loss of

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c-Abl in PAH, and an important question is whether the loss of c-Abl expression follows suppression at the RNA concentration or reflects increased protein degradation. Interestingly, Abl kinases are subject to ubiquitination and degradation by the ubiquitin E3 ligase c-Cbl (Casitas B lineage lymphoma) (17), processes upregulated during hypoxia (18). Hypoxia is also identified by the authors as a factor decreasing c-Abl expression. However, it is not clear whether hypoxia is an early phenomenon in PAH at all. Second, the link between DNA damage and c-Abl, a cytosolic nonreceptor tyrosine kinase, remains a black box. As the Abl family of kinases is among the group of best-preserved tyrosine kinases, it comes as no surprise that these kinases are involved in a variety of essential functions (17). Many of these may underly the involvement of c-Abl in DNA stability. c-Abl contains a combination of three nuclear localization motifs and a nuclear exit sequence, allowing for its shuttling to and from the nucleus. Partnering with DNA repair proteins in the nucleus, c-Abl may thus be a central regulator of DNA repair. In the cytosol, c-Abl may contribute indirectly to DNA repair by tyrosine phosphorylation of transcription factors (16) or the endocytic machinery, such as caveolin-1 (14). Phosphorylation of caveolin-1 may also explain the intriguing finding of restored BMPR2 expression after recovery of c-Abl. In the aggregate, the investigators show that in PAH endothelial cells, apoptosis, DNA damage, and BMPR2 expression group together around c-Abl, but firm cause and effect relationships are not established. Third, it remains difficult to pin down the exact action of the tyrosine kinase inhibitors involved. Although the use of interfering RNA provides a solid basis for the proposed mechanisms, it is unclear why relevant concentrations of imatinib, the patriarch of c-Abl inhibitors, does not mimic the effects of c-Abl siRNA. In addition, while dasatinib does inhibit c-Abl, it should be taken into account that the broad inhibitory spectrum of dasatinib may result in c-Abl–independent DNA damage.

What are this study's implications for the management of PAH now and in the future? The first important issue to consider is the potential harm that could be done when using drugs that lower c-Abl expression and activity. Some kinase inhibitors that are in clinical use have profound inhibitory effects, such as dasatinib (5). The study by Le Vely and colleagues adds to a better mechanistic understanding of pulmonary vascular toxicity of dasatinib. Many other kinase inhibitors have a similar inhibitory effect on c-Abl, and specific c-Abl inhibitors are being developed (e.g., for the treatment of Parkinson's disease [19]). These developments show that pharmacovigilance remains of utmost importance. And as mentioned above, there is a remaining concern with imatinib, a tyrosine kinase inhibitor that may very well affect c-Abl activity and is gaining renewed interest as a treatment for PAH (20). Second, could rescue of c-Abl be a viable direction for drug development? In their study, Le Vely and colleagues use the Abl tyrosine kinase activator DPH to effectively restore DNA damage in endothelial cells derived from patients with PAH. However, as shown above, overactivation of c-Abl may carry unwanted side effects. It is precisely this unpredictability of the ultimate effects of tyrosine kinases and their inhibitors that makes predictions about therapeutic potential very difficult. At this time of incomplete understanding, tyrosine kinase inhibitors or activators in PAH may still be too hot to handle. ■

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