In Defense of the Nucleus: NUDT1 and Oxidative DNA Damage in Pulmonary Arterial Hypertension

Oxidative stress is an imbalance that favors the production and accumulation of reactive oxygen species (ROS) over antioxidants, resulting in organelle dysfunction, protein damage, and apoptosis (1). Excess synthesis of ROS can result in cellular damage owing to the oxidation of vital biomolecules such as proteins, lipids, carbohydrates, and DNA. Oxidative damage to DNA can alter the chain integrity by inducing single- or double-strand breaks, cross-linking, or the formation of toxic DNA adducts (2, 3). Along with this, oxidative stress can also affect the efficacy of DNA damage repair mechanisms such as base excision repair or nonhomologous end joining, which could result in mutations and chromosomal instability that would propagate to daughter cells. In response to oxidative damage, compensatory responses such as apoptosis and autophagy are activated to allow time for repair or dispose of the damaged cell to protect the tissue. However, these responses can also go awry and create a chronic injury that serves as the pathological basis for several chronic lung diseases like pulmonary arterial hypertension (PAH), the present article's focus.

PAH is a cardiopulmonary disease characterized by obstructive vascular lesions that feature highly proliferative and apoptosis-resistant fibroblasts, pericytes, and endothelial and smooth muscle cells (4). Several studies have established that PAH cells switch from glucose oxidation to glycolysis as the primary source of cell energy. Oxidative stress is a significant aspect of this "metabolic switch," contributing to organelle damage and dysregulation of signaling pathways and gene expression (5). Given the link between oxidative stress and DNA damage, it is reasonable to speculate that ROS-induced damage to the DNA structure or the repair mechanisms could tip genetically susceptible cells to assume the PAH phenotype. For instance, pulmonary endothelial cells carrying BMPR2 and TopBP1 mutations exhibit more significant DNA damage in response to oxidating agents compared with healthy cells (6, 7). Thus, elucidating the mechanisms that protect the DNA against oxidative damage could provide a new paradigm to understand the role of oxidative stress in PAH and open exciting opportunities for therapeutic interventions.

In this issue of the *Journal*, Vitry and colleagues (pp. 614–627) present compelling evidence that establishes NUDT1 (Nudix hydrolase 1) as a key player in preventing oxidative DNA damage in pulmonary vascular cells (8). NUDT1 is an enzyme that neutralizes oxidized nucleotides (e.g., oxo-dGTP) formed via metabolism to prevent their incorporation into the DNA. Mutations that inactivate NUDT1 are associated with cancer, including hereditary conditions such as familial adenomatous polyposis. By applying sophisticated proteomics methods, the group showed that NUDT1 was elevated in PAH-derived pulmonary artery smooth muscle cells, remodeled right ventricles, and three experimental rat models. In this context, NUDT1 appears to serve a pathologic role that favors the PAH phenotype, as knockdown of NUDT1 resulted in the accumulation of oxidized nucleotides, DNA damage, and reduced proliferation. The mechanism promoting NUDT1 expression is dependent on the FOXM1 (forkhead box transcription factor), a transcription factor known to promote malignancy through activation of antioxidant responses, drug resistance, and angiogenesis (9, 10). Beyond cancer, the link between NUDT1 and FOXM1 also emphasizes the importance of FOXM1 to PAH pathogenesis demonstrated in other work by the same group. In addition to these mechanistic studies, the investigators invested effort to carry out preclinical studies using (S)-crizotinib, a NUDT1 selective inhibitor (11), in monocrotaline and Sugen/hypoxia rat models. As predicted from the *in vitro* work, rats treated with (S)-crizotinib exhibited improved pulmonary hemodynamics and reduced vascular remodeling and right ventricular function. It is worth pointing out that (S)-crizotinib was well tolerated by the control animals and did not induce cytotoxicity in healthy human cells. The authors conclude that NUDT1 is a significant player in PAH pathogenesis through its capacity to protect DNA against oxidative damage that could serve as a new therapeutic target in PAH.

Revisiting the role of oxidative stress in light of the study findings, we must now acknowledge that oxidative stress appears to be a double-edged sword in PAH. We are biased toward seeing oxidative stress as a pathological process to be stopped using antioxidants or agents that promote endogenous antioxidant pathways. In the case of NUDT1, increasing antioxidant activity could theoretically have led to a more aggressive PAH phenotype, although this was not the case in control cells overexpressing NUDT1. Although there is definite evidence that oxidative stress is involved in PAH pathogenesis (5, 12), we currently lack an understanding of how ROS production can lead to protective or damaging responses. This knowledge gap might explain why PAH clinical studies using promising drugs with antioxidant properties have yielded mixed results. The authors' systematic approach was critical in establishing the true nature of NUDT1 contribution and the potential benefit that oxidized nucleotides may offer in triggering abnormal cell removal. Nevertheless, the study did leave open questions that are of potential importance to grasping the true extent of NUDT1 in the pulmonary circulation. The lack of NUDT1 in pulmonary endothelial cells was surprising, considering that the formation of oxidized nucleotides is likely taking place in these cells and should produce pathological changes in cell behavior and biological responses. The association between NUDT1 and bioenergetics is intriguing because both glycolysis and glucose oxidation were affected by NUDT1 expression. Because the mitochondria and the endoplasmic reticulum are the primary sources of ROS in the cell, it will be interesting to explore whether NUDT1 may interact with proteins in these organelles or have a role in regulating the response of mitochondria DNA to oxidative stress.

Regardless of the limitations inherent to using cell and animal models, this work is significant because it opens an exciting new

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chapter in our understanding of Redox biology in PAH. Recognizing the double-edged nature of oxidative stress should make us more cautious when approaching the study of ROS and in determining the best way to translate our scientific findings into potential therapies. ■

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