Paracrine Proliferative Signaling by Senescent Cells in World Health Organization Group 3 Pulmonary Hypertension
Age Corrupting Youth?

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The term “pulmonary hypertension” (PH) is a simple and arguably simplistic acknowledgement that the mean resting pulmonary artery pressure (PAP) is greater than 25 mm Hg. It does not define pathophysiology or direct therapy and does not predict prognosis. More clinically useful is the World Health Organization classification system, which acknowledges 5 PH groups, each somewhat homogenous in terms of pathophysiology, lung histology, and prognosis.1 Group 1 PH is a collection of syndromes characterized by marked pulmonary arterial obstruction, including patients with idiopathic and familial pulmonary arterial hypertension, connective tissue diseases, congenital heart diseases, or hemoglobinopathies. Group 2 PH is associated with left heart disease (valvular and ventricular). Group 3 PH (cor pulmonale), relevant to this Editorial, is associated with lung diseases, such as chronic obstructive pulmonary disease (COPD), and with chronic hypoxia. Groups 4 and 5 PH are associated with chronic thromboembolic disease or miscellaneous systemic conditions, such as sarcoidosis, respectively. PH-specific therapies (L-type calcium channel blockers, prostanoids, phosphodiesterase 5 inhibitors, and endothelin antagonists) are approved only for use in group 1 PH. Because groups 2 and 3 PH are much more prevalent than group 1 PH, there is value in understanding their pathophysiology in hopes of developing rational therapies. Group 3 PH portends a much more favorable outcome than group 1 PH, and its activation results from physiological, hypoxia-induced changes in redox signaling as well as pathological mechanisms that may be shared with group 1 PH, including epigenetic silencing of superoxide dismutase 26 and activation of the endoplasmic reticulum stress protein, NOGO.7 Reinforcing the proliferative diathesis in group 3 PH is an impairment of apoptosis that is manifest in downregulation of the oxygen-sensitive potassium channel Kv1.5 and de novo expression of the antiapoptotic protein survivin (see Reference 4 for review).4 Metabolic and proliferative changes in the lung in group 3 PH are significantly tied to activation of transcription factors, notably HIF-1α and NFAT (see Reference 4 for review).4 Mice that are haploinsufficient for HIF-1α are significantly protected from chronic hypoxic PH.5 HIF-1α is a central feature of group 3 PH, and its activation results from physiological, hypoxia-induced changes in redox signaling as well as pathological mechanisms that may be shared with group 1 PH, including epigenetic silencing of superoxide dismutase 26 and activation of the endoplasmic reticulum stress protein, NOGO.7 Reinforcing the proliferative diathesis in group 3 PH is an impairment of apoptosis that is manifest in downregulation of the oxygen-sensitive potassium channel Kv1.5 and de novo expression of the antiapoptotic protein survivin (see Reference 4 for review).4

On this background emerges the report of Noureddine et al.5 They offer an additional theory for how PASMC proliferation might be stimulated. Noureddine et al5 discuss findings from 124 patients with COPD who underwent right heart catheterization and telomere length measurement (in circulating white blood cells). The majority of these patients have been previously reported by this group in publications that suggest that interleukin (IL)-6 levels are elevated and contribute to group 3 PH and that telomeres are shortened in leukocytes from COPD patients, consistent with increased cellular aging in COPD.9,10 They have previously concluded that leukocyte telomere shortening is correlated with the patient’s age as well as PaO2 and PaCO2.10 What is new in the current report is the demonstration of senescence and a related paracrine proliferative diathesis in PASMC explanted from a new cohort of 14 COPD patients during lung resection for localized tumors. These COPD patients were compared with 13 patients with a history of smoking but without COPD, who underwent surgery. Some of the COPD patients had group 3 PH, although the severity of the PH was mild. The pulmonary vasculature was assessed by quantitative histology. Immunohistochemical markers of proliferation (Ki67)
Cell senescence was originally described in cells that had ceased proliferating and was thought to be a part of the natural aging process. In human cells, the arrest is triggered by telomere shortening, although the exact relationship between telomere shortening and senescence signaling is ill defined. In addition to replicative telomere shortening, cellular senescence can also be induced through reactive oxygen species or other stressors, which in turn upregulate cell cycle inhibitors such as p16 (p16INK4A) and p21(p21cip1/WAF). Senescent cells do not divide even after exposure to mitogenic factors and develop a phenotypic flat morphology with increased β-galactosidase activity. These senescent cells are not innocent bystanders but rather have paracrine effects secreting factors with mitogenic, angiogenic, and antiapoptotic effects. It is in this way that Noureddine et al in this issue of Circulation Research postulate that senescent cells fulfill the Shakespearean role of “never-resting time” leading the surrounding cells to an unhealthy, irreversible “hideous winter” of vascular remodeling and pulmonary hypertension (Figure). Although this is a novel approach to understanding the hyperproliferative state in PH, this interesting study has several caveats and limitations.

**Question 1: Is Telomere Shortening Related to PH or to Age and Oxidant Stress?**

The patients in this study, like those previously presented in patients with pulmonary fibrosis, are at risk of having category 3 PH. However, the majority of these COPD patients had normal PA pressures or at worst mild PH. The mean PAP in the cohort that underwent right heart catheterization was technically normal (24.6 mm Hg), and the average PVR was only slightly greater than normal (3.1 Wood Units). This raises the question of whether the telomeric shortening relates to PH or to the many other abnormalities in COPD. The authors acknowledge that telomere shortening in COPD and pulmonary fibrosis is not specific to the vasculature and in their prior study correlated with patient age and PO2. Moreover, in an elegant study of pulmonary fibrosis patients (which showed most had significant telomere shortening in leukocytes), there was no evidence of telomere shortening in the group 1 PH patients they used as control subjects. Because the PH is much more severe in group 1 versus group 3 PH, this argues against the PH itself being directly related to telomere shortening. Perhaps the age or worsened oxygenation in COPD patients has the more direct relationship to telomere length, with the mildly elevated PA pressures being a covariable. Unfortunately, there was not a
multivariate analysis showing that the relationship between telomere length and either PVR or proliferation was independent of age, PO₂, PCO₂, or quantified cigarette exposure. This is important because one of the strongest correlates of decreased telomerase even in the current study was age. The uncertainty about the directness of the link between proliferation and PH is compounded by the fact that a large part of the data (Table 1 from Reference 8) derives from leukocytes (suggesting senescence is a systemic rather than a lung-specific process).

**Question 2: Is Senescence Specific to PASMC in the Lung?**

The authors find that telomere shortening occurs in circulating leukocytes of COPD patients as well as in cultured PASMC. Furthermore, they also report that there is increased staining for the stress-induced senescence marker p16 in PASMC and lung endothelium, again highlighting that the process is not unique to PASMC. However, it is not clear how the senescence found in leukocytes and other cells affects PASMC proliferation to promote PH. The authors present evidence that it is the senescent PASMC in the vascular wall that drive the remodeling process (Figure).

**Question 3: Is There Precedent for PASMC Diversity in the Vasculature?**

Noureddine et al offer evidence that collections of senescent PASMC cause normal cells in the PA wall to proliferate by both paracrine and nonparacrine signaling.8 This idea has 2 central tenants: First, that there is diversity in the types of PASMC that populate the vascular wall, and second, that paracrine signaling can drive hyperplasia. There is a precedent for the existence of both radial and longitudinal diversity of PASMC within the arterial wall. Not only are their diverse populations of PASMC in the vasculature, but this diversity relates to parameters relevant to group 3 PH. There is a radial diversity of PASMC with populations of variable proliferative capacity as one proceeds from lumen to adventitia. Most of the proliferative response in PH occurs in meta-vinculin-negative PASMC.17 In addition, there is longitudinal diversity (in resistance versus conduit PAs) such that PASMC that mediate hypoxic pulmonary vasoconstriction are predominantly found in resistance PAs. This diversity reflects preferential expression of O₂-sensitive potassium channels and O₂-responsive mitochondria in PASMC from resistance versus conduit PAs.18,19

On the second count, there is precedent for senescent cells stimulating proliferation in bystander cells. In prostate cancer, senescent cells may stimulate hyperplasia of nearby normal epithelium.11 In contrast to this finding, enhancing cellular senescence has been exploited to regress several cancers, including lymphoma, osteosarcoma, and hepatocellular carcinoma.20 Indeed, there is a growing effort to counteract proliferation of cancer cells by enhancing oncogene-induced senescence, an intrinsic tumor-suppressive mechanism.21 Alimonti et al21 recently used a senescence response that is distinct from oncogene-induced senescence (PTEN loss–induced cellular senescence) to regress prostate cancer in a human xenograft model.

The proliferative effects of senescent SMC proliferation reported by Noureddine et al is also at odds with findings in the systemic vascular bed by Bennett et al.22 In a study of SMC in human atherosclerotic plaques, they found earlier senescence and slower rates of cell proliferation in SMC from atherosclerotic plaque.22 Thus, the relationship between telomerase, senescence, and proliferation is relevant to important diseases (COPD, cancer, and atherosclerosis), but the relationship is complex. More research is required before we will have confidence whether one should enhance or repress senescence in treating PH.

**Question 4: How Do the Many Previously Described Mechanisms of Excessive PASMC Proliferation Relate to This New Senescence Mechanism?**

In brief, the answer is, “we don’t know.” The field of PH research will be well served when there is more cross-testing of extant mechanisms because each is likely to be a part of the explanation, rather than the whole.

At the end of Sonnet 5, Shakespeare reassures the reader that age has not completely corrupted youth, and there is some solace to be found in the beauty of one’s offspring, which triumphs over the efforts of time such that “substance still lives sweet.” Noureddine et al describe increased presence of senescent cells in the pulmonary vasculature of patients with COPD with some pessimism, implicating them in the PASMC hypertrophy and elevated PA pressure. However, if we are to follow the cancer and atherosclerosis literature, perhaps all is not lost, and there may be benefit from enhancing cell senescence.

The Noureddine report should stimulate further study of senescent cells in PH now that their presence in the vascular wall has been revealed. Ultimately, we need to know whether we must eliminate old cells before they corrupt the youth or whether one should exploit the fatigue of the senescent cell to therapeutic benefit.

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None.

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