The hyperproliferative endothelial cell phenotype in idiopathic pulmonary arterial hypertension

Troy Stevens and Mark N. Gillespie

Department of Molecular and Cellular Pharmacology, Center for Lung Biology, University of South Alabama, Mobile, Alabama

IN THIS ISSUE OF AJP-LUNG, Masri and colleagues (11) report that pulmonary artery endothelial cells (PAECs) isolated from patients with idiopathic pulmonary arterial hypertension (IPAH) exhibit an unusual hyperproliferative potential, with decreased susceptibility to apoptosis. Interestingly, although the PAECs from IPAH patients grew rapidly when placed in Matrigel, they generated disorganized networks, suggesting a dysfunctional angiogenic potential. Signal transducer and activator of transcription 3 (STAT3) was constitutively phosphorylated (e.g., activated) in the hyperproliferative PAECs, and STAT3 inhibition reduced cell proliferation. Parallel immunohistochemistry studies resolved an increase in activated (phosphorylated) STAT3 in endothelial cells found within both plexiform and concentric lesions. Thus the authors conclude that endothelial cells isolated from IPAH patients possess a stable hyperproliferative, apoptosis-resistant phenotype.

This is now the third report from this group of investigators describing the function of PAECs isolated from IPAH patients (11, 21, 22), and a fingerprint for the unique behavior(s) of these cells is beginning to emerge. Whereas in the present manuscript we see that they are hyperproliferative and apoptosis resistant, earlier work showed that these PAECs possess increased arginase II activity with an accompanying decrease in nitric oxide biosynthesis (21) and have decreased oxygen consumption due to diminished mitochondrial complex IV activity, indicative of a mitochondrial defect (22). Indeed, PAECs from IPAH patients possess fewer mitochondria and lower mitochondrial DNA content, yet whole cell ATP concentrations are normal. This apparent disconnect between cellular ATP concentrations and mitochondrial function and numbers is explained by an approximate threefold increase in the rate of glycolysis. Such a “paradox” has been well established in hyperproliferative cancer cells, in the so-called Warburg effect, which is described as an increase in glycolysis in the presence of oxygen-aerobic glycolysis (18, 19). Thus, in this manner, PAECs isolated and cultured from IPAH patients possess “cancer-like” characteristics. Lee and colleagues (9) have previously suggested such as association with cancer in endothelial cells, or endothelial-like cells, found primarily with the plexiform lesion itself. Indeed, they have hypothesized that the plexiform lesion may result from the hyperproliferation of apoptosis-resistant endothelial cells, or cells that take on endothelial cell characteristics (16).

It is interesting, however, that aerobic glycolysis occurs in a variety of non-cancer cells (17). Indeed, the Warburg effect is observed in rapidly dividing cells, and thus the prominence of aerobic glycolysis per se is not indicative of a transformed cell phenotype. This issue, with all of its potential complexities, opens the door for us to question what may account for the emergence of a hyperproliferative, apoptosis-resistant endothelial cell phenotype exhibiting aerobic glycolysis in IPAH patients. We consider three possibilities. First, IPAH may select for a resident hyperproliferative, apoptosis-resistant cell population. Second, the vascular environment in this disease may induce a phenotype switch, with epigenetic modifications that are stable in cell culture. Third, somatic mutations or other genetic abnormalities may alter endothelial cell behavior.

Regarding the first issue, we question whether IPAH “selects for” and enriches a hyperproliferative resident endothelial cell population. Although endothelium is typically quiescent in the native vessel wall, it slowly (1–3 years) replicates to renew senesced and aging cells, and to repair the vessel wall following injury. In 1976, Schwartz and Benditt (14) suggested that not all endothelial cells replicate equally well following injury. Indeed, they observed endothelial cell “niches” within the aortic wall that were enriched with replication-competent cells. More recently, Ingram and colleagues (6) have resolved progenitor cells from within a population of endothelial cells isolated from conduit vessels in the systemic circulation. In their work, aortic endothelial cells seeded at single cell density and grown for 2 wk in serum gave rise to colonies of varying size. Nearly 75% of aortic endothelial cells remained as single cells after 2 wk. Most of the remaining 25% of cells grew to small colonies, which ranged in size from 500 to 2,000 cells, and in only rare instances did single cells grow to colonies that were greater than 10,000 cells. When cells within the large colonies were replated in the single cell assay, they possessed an intrinsic capacity to replenish the entire hierarchy of growth potentials, whereas cells taken from low growth potential colonies never gave rise to fast growing cells. These findings suggest that endothelial cells isolated from the vessel wall are enriched with progenitor cells that rapidly proliferate and can renew the entire population. Considering the work of Masri and coworkers (11), it is tempting to speculate that IPAH selects for these high proliferative potential cells within the vessel wall, that is, hyperproliferative, apoptosis-resistant cells may reflect an increase in the number of resident progenitor cells, perhaps due to loss of more differentiated endothelial cells. Of course, we don’t yet know the answer to this query, and although fast-growing cells possess a high metabolic demand, we don’t yet know whether the progenitor cells described by Ingram’s group (6) exhibit the Warburg effect. Answers to both of these issues would shed important new insight into our understanding of the molecular and cellular basis of proliferating endothelial cells.

Regarding the second issue, we question whether the vascular environment in patients with IPAH initiates an epigenetic
change in all endothelial cells residing along the pulmonary artery. Epigenetic modifications are defined as being “beyond the gene” and commonly refer to DNA methylation, transcription factor acetylation, sumoylation, and histone reorganization (1, 3–5, 7, 8, 12, 13, 15, 20). These chemical modifications result in a change in cell phenotype that is stable through mitotic cell divisions. Thus, in the case of IPAH patients, an expected outcome would be unique cell attributes, such as hyperproliferation and apoptosis resistance, even when the cells are cultured and studied in vitro. At present, work has not been completed to determine whether endothelial cells from IPAH patients possess, for example, alterations in DNA methylation patterns that result in unique gene expression profiles. We already know from prior work by Xu and colleagues (21) that endothelial cells from pulmonary hypertension patients possess increased arginase II expression and decreased endothelial nitric oxide synthase expression; we don’t know whether any type of epigenetic modification contributes to this observation. The ability to procure the cells of interest now allows one to explore whether epigenetic modifications underlie the novel endothelial cell phenotype observed in patients with IPAH.

Regarding the third issue, we question whether somatic cell mutations contribute to the hyperproliferative, apoptosis-resistant phenotype. Indeed, the unusual endothelial cell phenotype could have arisen from a normal resident or itinerant lung cell population as a result of a genomic event, not a germline mutation of the sort incriminated in familial idiopathic pulmonary hypertension, but rather, something specific to effector cells in IPAH. For example, several years ago, Yeager and colleagues (23) reported the presence of a somatic mutation in the transforming growth factor (TGF)-β type II receptor specifically in plexiform lesions microdissected from lungs of patients with pulmonary hypertension. Along with somatic mutations, another possible genomic event that could contribute to the altered PAEC phenotype is accumulation of oxidative DNA “damage.” Several years ago, Bowers et al. (2) detected the common base oxidation product, 8-hydroxyguanine, in endothelial cells within plexiform lesions, although its location in specific DNA sequences was not determined. More recently, Lu et al. (10) reported that accumulation of oxidative DNA damage in gene promoters contributes to transcriptional downregulation in aging, thus raising the possibility that a similar mechanism may be operative in settings like IPAH. Access to endothelium from these patients allows one the opportunity to examine whether somatic events at the level of specific genes play a role in changing endothelial cell phenotype.

The present work by Masri and colleagues (11) has provided insight into the cellular adaptations that accompany IPAH. Presently, they describe a hyperproliferative, apoptosis-resistant endothelial cell that can be isolated from pulmonary arteries of IPAH patients. It will be of great interest to determine how these cells gain their hyperproliferative, apoptosis-resistant nature. We propose here that this behavior may reflect an endogenous cell phenotype that is enriched in patients with pulmonary hypertension (e.g., resident progenitor), a cell that has been pressured by the environment to take on new behaviors (e.g., epigenetic modification), a cell that exhibits somatic mutations (e.g., TGF-β type II receptor), or some combination of the three. Time will tell as to whether any of these proposed mechanisms contribute to the cell behaviors observed by Masri and coworkers (11). We will follow this emerging story with great interest, as these investigators and others continue to improve our understanding of the vascular defects that so critically define IPAH.

REFERENCES


