The Cancer Paradigm of Severe Pulmonary Arterial Hypertension

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The plexiform lesions of severe pulmonary arterial hypertension (PAH) are similar in histologic appearance, whether the disease is idiopathic or secondary. Both forms of the disease show actively proliferating endothelial cells without evidence of apoptosis. Here, we discuss the pathobiology of the atypical, angioproliferative endothelial cells in severe PAH. The concept of the endothelial cell as a "quasi-malignant" cell provides a new framework for antiproliferative, antiangiogenic therapy in severe PAH.

Keywords: pulmonary arterial hypertension; plexiform lesions; angiogenesis; cancer

The World Health Organization classification distinguishes between idiopathic pulmonary arterial hypertension (IPAH) and forms of pulmonary arterial hypertension (PAH) that are associated with known causes—for example, congenital cardiac abnormalities, portopulmonary hypertension, HIV infection, or collagen vascular diseases (1) (Table 1). From a clinical perspective, the degree of pulmonary hypertension at the time of diagnosis is frequently severe—that is, the symptoms of dyspnea and fatigue are present when the disease is already at an advanced stage—and as a rule there is no early diagnosis. Although treatment of patients with severe PAH with epoprostenol or more recently developed oral agents has improved quality of life and exercise capacity (2, 3), this group of diseases has remained fatal whether due to progression of the pulmonary vascular disease or heart failure or both (4). The term "severe angioproliferative pulmonary hypertension" (SAPPH) (5–7) has been used to characterize a histologic hallmark of irreversible pulmonary vascular remodeling believed to be caused by angiogenic proliferation of endothelial cells. Both IPAH and many forms of associated PAH demonstrate complex, often glomeruloid appearing, lumen obliterating lung vascular lesions at sites of bifurcations (8). Whether these complex vascular lesions can fully explain the PAH is still debated by some investigators.

In this perspective, we review pathogenetic concepts of severe PAH, explain the term "complex vascular lesion," which could replace the previously used term "plexiform lesion," and propose a paradigm shift in an attempt to explain the pathobi-

ology of severe pulmonary hypertension with a neoplastic process of angiogenesis rather than the traditional pathophysiologic model, which has largely been based on the principles of pressure, flow, and shear stress (9, 10) (Figure 1).

PLEXIFORM OR COMPLEX VASCULAR LESIONS

The traditional pathogenetic concept of severe PAH was formulated by two pioneers of pulmonary vascular research, C. Wagenvoort and L. Reid (11, 12). The concept of chronic and sustained pulmonary vasoconstriction as the root cause of pulmonary vascular remodeling was largely derived from studies of rats that had been exposed to chronic hypoxia or treated with the alkaloid monocrotaline; both are models of PAH that can be successfully treated with a large variety of drugs (13–17). Although there are patients with severe PAH who have a reversible component to their PAH (i.e., vasoconstriction) (18), it has now been accepted that patients with a significant reversible PAH component at the time of diagnosis are a small minority (19). Without oversimplification, we recognize that the hypoxia and monocrotaline models are characterized by vasoconstriction and muscularized pulmonary arteries, and that the PAH can be treated. In contrast, in many forms of human PAH, pulmonary vasoconstriction is not very prominent (at least at the time of diagnosis) and, in addition to the muscularization, the pulmonary arteries are partially or completely obliterated by conglomerates of cells. We hypothesize that it is this vasoobliteration that makes the human forms of severe PAH not treatable with presently available drugs. The recognition of human obliterating endothelial cell proliferation (6) and of somatic endothelial cell gene mutations (20) has provided the underpinning for a shift toward a cellular growth concept of severe PAH. In fact, newer models of PAH have attempted to address this concern by creating models of pulmonary hypertension in which a neointima is formed that occludes the vascular lumen (21, 22). However, although these preclinical rodent models demonstrate intimal hyperplasia, it is less clear whether the lumen narrowing in these models is caused by endothelial cell growth. Although there may be some molecular features that can distinguish between IPAH and associated forms of severe PAH (20, 23), the plexiform (complex) vascular lesions contain endothelial cells, smooth muscle cells, lymphocytes, and mast cells, regardless of whether the disease is primary or associated with a known cause. In addition, the tumor suppressor proteins p27, peroxisome proliferator-activated receptor- γ , heme oxygenase, and caveolin are absent or reduced in expression in the plexiform lesions of both IPAH and associated forms of PAH (8, 24, 25). Although the pulmonary arterial smooth muscle cells and endothelial cells

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TABLE 1. 2003 REVISED NOMENCLATURE AND CLASSIFICATION OF PULMONARY HYPERTENSION

Pulmonary arterial hypertension (PAH)
1. Idiopathic (IPAH)
2. Familial (FPAH)
3. Associated with
a. Collagen vascular disease
b. Congenital systemic to pulmonary shunts (large, small, repaired or nonrepaired)
c. Portal hypertension
d. HIV infection
e. Drugs and toxins
f. Other (glycogen storage disease, Gaucher’s disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy)
4. Associated with significant venous or capillary involvement
a. Pulmonary venoocclusive disease
b. Pulmonary capillary hemangiomatosis
Pulmonary venous hypertension
1. Left-sided atrial or ventricular heart disease
2. Left-sided valvular heart disease
Pulmonary hypertension associated with hypoxemia
1. Chronic obstructive pulmonary disease
2. Interstitial lung disease
3. Sleep-disordered breathing
4. Alveolar hypoventilation disorders
5. Chronic exposure to high altitude
Pulmonary hypertension due to chronic thrombotic and/or embolic disease
1. Thromboembolic obstruction of proximal pulmonary arteries
2. Thromboembolic obstruction of distal pulmonary arteries
3. Pulmonary embolism (tumor, parasites, foreign material)
Miscellaneous
Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosing mediastinitis)

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from patients with PAH have been studied *ex vivo* (26–28), the cells of the complex vascular lesions in humans have yet to be isolated and examined *ex vivo*.

THE CELL(S) OF ORIGIN OF THE COMPLEX VASCULAR LESIONS

Whether there is indeed one simple cell type that “starts” the complex vascular lesion remains unclear; however, the pathobiological model of intraluminal angioproliferation (7, 29) is based on *endothelial* cell growth. This model or disease concept appreciates that, in severe PAH, the “law of the endothelial cell monolayer” has been broken in favor of a tumorlike intraluminal growth (Figure 2). What makes the endothelial cell attractive as

the cell that starts the complex lesion is that there are at least three possible sources of proliferating endothelial cells: (1) a stemlike endothelial cell that is apoptosis resistant and grows after neighboring intimal monolayer cells have been injured and have died (30), (2) a bone marrow–derived precursor cell (31), and (3) an endothelial cell derived via vascular endothelial growth factor (VEGF)–driven transdifferentiation of dendritic cells (32). Although the VEGF receptor blockade/chronic hypoxia model of severe PAH supports endothelial cell apoptosis as an initiating and important mechanism of pulmonary hypertension (33), and Sakao and colleagues demonstrated the emergence of a hyperproliferative abnormal endothelial cell phenotype *in vitro* after induction of normal endothelial cell apoptosis and exposure of the surviving endothelial cells to shear stress (29), we do not know whether bone marrow–derived precursors or dendritic cells contribute to the pool of proliferating endothelial cells of the plexiform lesions. Vascular smooth muscle cells within the complex vascular lesions could also be derived from endothelial cells via transdifferentiation (34).

THE CANCER PARADIGM

The answer to the question “Why is severe PAH so difficult to treat?” might be that the vascular lesion cells are abnormal cells. A cancer model for “primary pulmonary hypertension” was first suggested in 1998 (35). This hypothesis can now be put into the context provided by Hanahan and Weinberg in their classic paper (36) published in 2000 entitled the “Hallmarks of Cancer.” In this article, the authors frame the cancer paradigm by integrating a relatively small number of categorical, cancer-defining mechanisms. These are as follows: angiogenesis, evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, and limitless

Figure 1. Pathogenetic concepts of severe pulmonary arterial hypertension: a paradigm shift.

Figure 2. Hematoxylin-and-eosin-stained section from a lung of a patient with idiopathic pulmonary arterial hypertension demonstrating the intraluminal proliferation of endothelial cells that occurs in severe angioproliferative pulmonary hypertension. Arrows indicate the bland, flattened endothelial cells lining the lumen of newly formed vessels. The upper right inset shows a higher power view of the endothelial cells that comprise these lesions.

replicative potential. The complex vascular lesions of severe PAH are governed by some, but not all, of these traits. Thus, although the lesions are not cancer in the true sense of the word, they are certainly neoplasms in that there is a process of abnormal and uncontrolled growth of cells. The lesions are angiogenic (7), there are no apoptotic cells found in the lesions (24, 37, 38), and antiapoptotic proteins are expressed in the lesion cells (20). Impaired transforming growth factor (TGF)- β signaling may make the lesional endothelial cell insensitive to

the endothelial cell growth-controlling effects of TGF- β (39), and speculating further, abnormal endothelial cells with a hyperproliferative potential could be released from the complex vascular lesions and recirculate into the lung where they are then trapped within partially obliterated lung vessels. The proliferating endothelial cells of the complex vascular lesions appear to proceed “via a process formally analogous to Darwinian evolution, in which a succession of genetic changes, each conferring one or another growth advantage, leads to the progressive

Figure 3. A panel of plexiform lesions demonstrates the expression pattern of several markers of malignancy. (A) Expression of p16 in the central part of two plexiform lesions (in proximal portions of bifurcating vessels). (B) Highly expressed β -catenin. (C) Luminal endothelial cell expression of multiple drug resistance protein (MDRP) in a plexiform lesion. (D) Increased expression of survivin in central portion of plexiform lesion. (E) Dense expression of chemokine receptor (CXCR)-4 in a plexiform lesion. (F) Expression of epithelial growth factor receptor (EGFR)-3 in a plexiform lesion.

Figure 4. Plexiform lesions (*) from patients with severe pulmonary arterial hypertension. (A) Loss of Wnt7a can be seen in the endothelial cells of this lesion. Note the maintained Wnt7a expression in the surrounding lung parenchyma. (B) A similar loss of activated leukocyte adhesion molecule (ALCAM)-1 expression is seen in this lesion.

conversion of normal human cells” (36). The loss of the expression of the prostacyclin synthase gene, encoding the enzyme required to produce prostacyclin (40), and acquisition of survivin (38) can be seen in such a Darwinian context because one would expect that a cell that “wants” to grow

and divide would develop such a strategy. Prostacyclin is antiproliferative and survivin is antiapoptotic. We propose that several growth signaling pathways are deregulated in the lesion cells and we may already see the tip of the iceberg with the loss of expressed proteins of the TGF- β receptor pathway (Smads) (41), high expression of β -catenin (Figure 3), loss of a member of the Wnt pathway, Wnt7a (Figure 4), and loss of the activated leukocyte adhesion molecule ALCAM-1 (42) (Figure 4) and its binding partner N-cadherin, both of which are highly expressed in normal lung microvascular endothelial cells. As mentioned, plexiform lesions contain several different cell types, which, as in cancer tissue, “may have been conscripted to serve as active collaborators in the neoplastic agenda” (36).

Consistent with a neoplastic hypothesis of severe PAH, Masri and colleagues have characterized endothelial cells obtained from the pulmonary arteries of patients with IPAH as hyperproliferative and apoptosis resistant (27) and displaying a high glycolytic rate (43).

THE COMPLEX VASCULAR LESION: CANCER OR ANGIOGENIC NICHE

Elegant experiments by Sundberg and coworkers (44) and McDonald and colleagues (45) have illustrated the importance of VEGF and VEGF signaling for normal and tumor vessel growth and infection/inflammation-triggered angiogenesis. Inflammatory and immune system cells are present in complex vascular lesions (46) and there is high expression of hypoxia-inducible factor (HIF)-1 α , VEGF, VEGF receptors, and nitric oxide synthase—all signature genes and proteins of angiogenesis (7). Although these inflammatory cells may be locally derived, they could also represent cells of bone marrow origin. In fact, the angiogenic process in the lung may be driven by cells of bone marrow origin (47), such as megakaryocytes, c-kit⁺ cells (Figure 5), and even mast cells. Megakaryocytes and mast cells are very productive growth factor “factories” and protease repositories. Recent reports have shown that bone marrow-derived mesenchymal stem cells, injected intravenously or after marrow transplant in transgenic animals, can actually home to the lung and differentiate into resident cells (48, 49). The plexiform lesions could be regarded as a stem cell niche (50). Perhaps the synchronous appearance of these vascular lesions at specific locations in small vessel branch points (8) is more akin to the process of widespread metastases resulting in innumerable micronodules in a characteristic distribution (the “seed and soil hypothesis”) (51). The expression of p16 in plexiform lesion cells (Figure 3) is shared with the expression of p16 in pre-

Figure 5. Immunohistochemical stain for CD117 (c-kit). (A) CD117 cells are scattered throughout the vascular lesion. Mast cells can also stain for CD117. However, serial sections stained for mast cells showed no mast cells in this lesion (not shown) and rare mast cells in other lesions. (B) Hematoxylin-and-eosin-stained section of a plexiform lesion and surrounding lung parenchyma illustrates megakaryocytes (arrows) around the lesion. The inset shows the multilobulated appearance of these cells.

cancerous lesions of human endometrial adenocarcinoma (52) and colon cancer (53).

"CHANNELOPATHY" AND CANCER

Dysfunctional voltage-sensitive potassium channels (K_v) have been described in pulmonary artery smooth muscle cells from patients with IPAH (26) and impaired K^+ channel performance has been linked to pulmonary vasoconstriction, whereas vasorelaxation due to nitric acid and cyclic guanosine monophosphate has been linked to activation of Ca^{2+} -dependent K^+ channels (54). Subsequently, Ekhterae and coworkers demonstrated that Bcl-2, an antiapoptotic protein, inhibits pulmonary artery smooth muscle cell apoptosis by impairing the activity of K_v channels (55), whereas cytochrome c, a mitochondrial inducer of apoptosis, increases K^+ currents (56), and overexpression of the gene encoding K_v in pulmonary artery smooth muscle cells increases caspase-3 activity and induces apoptosis (57). McMurtry and coworkers used dichloroacetate (DCA), a pyruvate dehydrogenase inhibitor that increases K^+ currents in myocytes, to show restored $K_{v2.1}$ and reduced pulmonary hypertension in the chronic hypoxia rat model (58). The same group also showed that DCA induces vascular smooth muscle cell apoptosis. The role of K^+ channels in the control of cell proliferation has recently been reviewed (59); K^+ channel expression is being used as a tumor marker (60); and finally, normalization of the function of $K_{v1.5}$ in cancer cells can inhibit cancer cell growth (61). Thus, we conclude that K^+ channel abnormalities exist in the pulmonary vascular smooth muscle cells from patients with IPAH and that such abnormalities can contribute to deranged vasomotor tone and vascular cell growth control.

COMPLEX VASCULAR LESIONS AND IMMUNE CELLS

The association between connective tissue disorders and severe PAH has been long recognized and recently reviewed (5), and the presence of inflammatory cells, including T and B lymphocytes and macrophages, has been reported by several investigators. Recently, Perros and colleagues (46) found dendritic cells in and around the complex vascular lesions and Bonnet and coworkers (62) found NFATc2⁺ lymphocytes in the pulmonary artery walls of patients with severe PAH. Although the link between chronic inflammation, angiogenesis, and cancer development (63) has been firmly established, we still have difficulties determining the relationships, if any, between autoimmunity, cancer, and the angiogenesis of severe PAH. However, we remind the reader of the increased prevalence of thyroid cancer in patients with autoimmune thyroiditis, colon adenocarcinoma in patients with ulcerative colitis, hepatocellular carcinoma in patients with autoimmune hepatitis, and cholangiocarcinoma in patients with primary sclerosing cholangitis. Both dendritic cells and regulatory T cells have been associated with malignancies (64) and increased numbers of regulatory T cells have recently been reported in peripheral blood from patients with IPAH (65).

CONCLUSIONS

The cellular and molecular hypothesis developed in this perspective—namely, a neoplastic, angioproliferative disorder—likely more accurately reflects the clinical reality (short survival, no cure) of patients with severe PAH than a vasomotor tone (vasoconstriction and impaired vasodilation)—centered model. This neoplastic paradigm does not exclude pulmonary vasomotor tone abnormalities as participating during some phase of the pathogenesis, but more than 15 years after the introduction of

prostacyclin infusion therapy, it has become clear that vasodilators cannot cure the patients afflicted by this group of disorders.

Finally, can the bone morphogenic protein receptor II (BMPRII) mutations that have been found in patients with familial and nonfamilial forms of IPAH (66) be integrated into a cancer hypothesis of severe PAH? Indeed, there is a growing literature that associates bone morphogenic proteins (BMPs) and their receptors with cell growth control, even in cancers (67–69). Patients with PAH show altered growth response of pulmonary artery smooth muscle cells to BMPs (70), and BMP4 has been shown to inhibit proliferation and promote myocyte differentiation of fetal lung fibroblasts (71), thus implying that mutations in BMPRII could lead to cells resistant to effects of BMPs. Tenascin C is expressed in the complex vascular lesions (72) and tenascin has been shown to modulate the sensitivity of cells to fibroblast growth factor (FGF) and to BMP4 (72).

Although the reported gene mutations (66, 73) described in patients with IPAH have not been able to fully explain the pathobiology of severe PAH, one can hypothesize that the set of genes that account for the susceptibility to develop severe PAH are the genes normally responsible for maintaining the orderly, nonproliferative replacement of apoptotic lung microvascular endothelial cells (73). This orderly homeostatic repair may also rely on the participation of an intact immune system (74). The work by Masri and colleagues (27) showed that endothelial cells derived from patients with IPAH demonstrated a hyperproliferative, apoptosis-resistant phenotype that was characterized by persistent activation of the signal transducer and activator of transcription 3 (STAT3). STAT3 is a critical prosurvival molecular signaling pathway and a primary regulator of angiogenesis. Persistently activated STAT3 has also been identified as a key factor in virus-transformed cells and tumor cells, and can be activated by BMPRII mutations (75–77). In all likelihood, it is the aggregate of protein expression changes that characterize the lesional endothelial cells as phenotypically abnormal. And, because most of the tissue expression changes found in severe PAH are shared between IPAH and associated forms of PAH, there are likely multiple trigger factors and pathways that lead from an initial, postulated endothelial cell damage to the exuberant endothelial cell proliferation and obliterative vasculopathy. Further examination of these pathways may lead to more effective therapeutic strategies in this group of usually fatal diseases.

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