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Review

Pulmonary hypertension: updating a mysterious disease

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1. Introduction

The patient with pulmonary hypertension still mystifies even the most astute of physicians. Pulmonary hypertension can be the result of multiple and diverse etiologies, and these include congenital heart defects, chronic lung disease, hepatic disorders, especially with portal hypertension, and autoimmune disease, particularly scleroderma. It can also arise as an unexplained condition. In the case of a congenital heart defect, the high flow and pressure are thought to be significant etiologically. If the primary etiology is treated in a timely fashion (e.g., repair of a congenital heart defect in early infancy, or improvement in a pulmonary condition), there is a good chance that pulmonary hypertension will regress, whereas, if the primary etiology is unknown, the disease will usually be rapidly progressive and fatal in outcome. The following mini-review will explore some of what we have learned about the pathophysiology of pulmonary hypertension and how common mechanisms at the cellular level may in fact lend themselves to novel therapeutic strategies directed at patients with secondary, as well as unexplained, pulmonary hypertension.

2. Developing a hypothesis: the first clue

Studies by our laboratory and others in the 1970's and early 1980's had focused on refining the assessment of pulmonary hypertension in patients with congenital heart defects by hemodynamic criteria, lung biopsy morphometry and wedge angiography (reviewed in [1]). We correlated increased pulmonary flow, pressure, and resistance with abnormal muscularization of peripheral normally non-muscular arteries, medial hypertrophy of proximal muscular arteries, and reduced arterial concentration, respectively, and with progressively abrupt arterial tapering on wedge angiography. The early developmental structural abnormalities precede neointimal formation, occlusion, and the plexiform lesions described by Heath and Edwards [2] which correlate with progressively severe hemodynamic dysfunction and abnormal arteriography. The evolution of structural abnormalities in the pulmonary arteries and the correlation with hemodynamic evidence of increased pulmonary artery pressure and resistance were investigated through animal models in which pulmonary hypertension could be reliably produced, such as rats exposed to hypoxia [3], or rats injected with the toxin, monocrotaline [4]. A variety of vasodilating agents were used to try to reduce pulmonary artery pressure and alleviate structural abnormalities but without direct evidence of a specific pathophysiology. Important observations did, however, emerge. For example, it was shown that hemodynamic changes could induce structural abnormalities, but that the latter could also arise in the absence of an initiating hemodynamic derangement. Endothelial related factors and endothelial injury appeared to be of primary importance [4] in inducing pulmonary arterial changes and there was abnormal upregulation of extracellular matrix genes which reflected a less well differentiated state of muscle [5]. There were also biologic and genetic features which pointed to immune/inflammatory mediation [6].

It was clear that making major inroads into the pathophysiology of pulmonary hypertension would require fundamental studies to understand processes of cellular and molecular dysregulation. The difficulty was finding the

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right clue to know exactly where to begin. For our laboratory, the clue came as a result of the intense scrutiny of ultrastructural changes in pulmonary arteries on lung biopsy specimens [7]. Our initial focus was on endothelial perturbations, given the vast information on endothelial derived dilators and constrictors. We described both structural and functional abnormalities in the endothelium. On scanning electron microscopy, the normal endothelium which appeared as a 'corduroy pattern' changed to a gnarled, cable-like structure in the hypertensive vessels. This was associated with alterations on transmission electron microscopy, namely an increased density of microfilament bundles and an increase in the density of rough endoplasmic reticulum. These structural abnormalities correlated with functional derangements such as increased endothelial production of von Willebrand factor (vWF) [8,9].

Coincident with the structurally-altered endothelium was an even more striking abnormality in the subendothelium. The internal elastic lamina, which normally separates the endothelial from the underlying smooth muscle cells, appeared to have broken down, suggesting that increased activity of an enzyme which could degrade elastin, as well as other matrix proteins, might be stimulating the remodeling process. We envisioned and gained evidence to support the scenario reflected in Fig. 1.

We reasoned that a variety of pulmonary hypertension-producing stimuli, such as the mechanical forces of high flow and pressure in the setting of a congenital heart defect, the vasoconstricting influence of hypoxia, or indomethacin [10], or a toxin, take as their first casualty the endothelial cell. The structural and functional alterations in the endothelium which ensue result in loss of barrier function and in the release from the endothelium or in the penetration into the subendothelium from the serum of a factor which stimulates smooth muscle cell (SMC) production and release of endogenous vascular elastase (EVE) [11]. The idea that EVE could come from SMC was predicated on observations made by other investigators [12] who first demonstrated activity of a serine elastase in cultured SMC and in atherosclerotic tissues. EVE is a powerful enzyme which, by virtue of its ability to degrade elastin, will also degrade proteoglycans which serve as storage sites for growth factors—i.e., transforming growth factor (TGF)β and basic fibroblast growth factor (bFGF) [13,14]. Increased expression of TGFβ has been observed in experimental models of pulmonary hypertension (sheep after chronic air embolization) [15] and lungs of transplant recipients [16].

Studies in our laboratory have shown that EVE releases bFGF in an active form that stimulates SMC proliferation. Interestingly, bFGF also stimulates the production of the matrix glycoprotein, tenascin (TN) [17], which optimizes the mitogenic response to bFGF and, in fact, permits the response to epidermal growth factor (EGF). An increase in SMC hypertrophy and in the synthesis of connective tissue proteins, which also contribute to thickening of the artery, is likely the response to released TGF-β, although we have not directly investigated this. The process of SMC migration also appears to depend, at least in experimental animals, on the continued activity of elastase. We have shown that elastin peptides stimulate the production of the matrix glycoprotein, fibronectin [18], which changes SMC from a contractile to a migratory phenotype [19,20].

Evidence in support of this hypothesis came first from experimental animals in which increased elastin turnover and high serine elastase activity were detected in the pulmonary arteries [21] early after exposure to a pulmonary-hypertension-inducing stimulus and prior to the

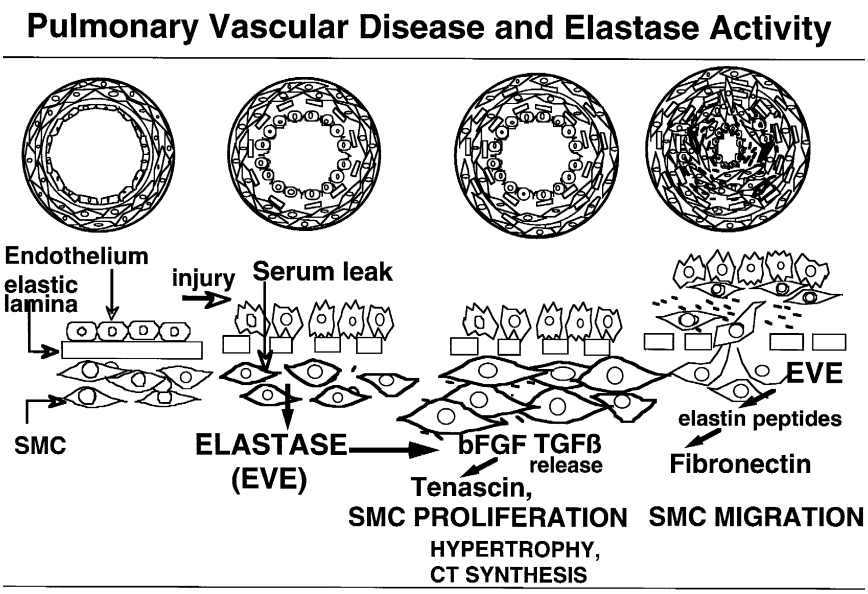

Fig. 1. Schema of the pathophysiology of pulmonary hypertension.

development of structural changes. A further increase in pulmonary artery elastase activity was documented with progression of pulmonary hypertension but not when there was potential for regression. A cause-and-effect relationship was further documented in studies in which a variety of elastase inhibitors were found to prevent or attenuate both the development and progression of pulmonary hypertension and vascular changes [22,23]. Further studies showed that elastase produced in association with pulmonary hypertension was a 20 kDa enzyme related to the serine proteinase, adipsin, and localized largely to the SMC of the vessel wall [24].

The exact relationship between the gene for adipsin and for endogenous vascular elastase (EVE) is still under study. We have, however, gained further understanding of how serum factors could in fact induce elastase production [11]. Smooth muscle cells in culture degrade radiolabeled elastin when stimulated with serum or endothelial-conditioned medium. Curiously, this is coupled to increased adhesion of the radiolabeled elastin to the cell surface. In fact, pretreating elastin or cells with serum or with endothelial-conditioned medium is as effective as serum alone in inducing elastase activity and appears to accelerate the process.

The serum or endothelial factor seems to serve as a bridge between elastin and the cell surface and engagement of a receptor results in an intracellular signaling mechanism which involves protein tyrosine phosphorylation and transcription of mRNA. One of the transcription factors involved may be AML-1 (unpublished). There is a DNA recognition site for AML-1 in the promoter of neutrophil elastase, suggesting that it might be the transcription factor for EVE.

To confirm that elastase could release growth factors in an active form, serum-treated elastin was used as a stimulus to induce the release of elastase from pulmonary vascular smooth muscle cells. In so doing we demonstrated that bFGF was released in an active form and in a concentration similar to that achieved by adding human leukocyte elastase to the cultures. We tested the effect of bFGF and confirmed its biological activity in stimulating smooth muscle cell growth [14]. Basic fibroblast growth factor also induces tenascin [17]. This molecule is regulated by a variety of cytokines and growth factors in addition to bFGF and also by mechanical forces, and is known to have functions related to cell differentiation and proliferation. While tenascin has been found in diseased vascular tissue, its role had remained obscure. We made the observation by immunohistochemistry that TN was expressed in pulmonary arteries in biopsy tissue from patients with congenital heart defects and that its expression correlated with the severity of the lesion. Moreover, TN co-localized with proliferating cells as judged by expression of epidermal growth factor and proliferating cell nuclear antigen. A similar relationship was seen in the evolution of experimental pulmonary vascular disease (PVD) [25].

A direct relationship between TN expression and proliferating smooth muscle cells was subsequently documented in cell culture. When smooth muscle cells were grown on collagen gels with or without TN, there was little influence on cell number, but when bFGF was added, the proliferative response was stimulated and, in fact, TN appeared to be a prerequisite for the mitogenic response to epidermal growth factor. The mechanism appears to involve a TN-mediated change in the cytoskeleton such that, when TN is engaged by its integrin, the $\alpha_v\beta_3$ molecule, actin filaments line up in a focal adhesion complex, EGF receptors are clustered, and addition of EGF results in rapid phosphorylation of the receptor and a nuclear signal necessary to send cells on their way to mitosis. These studies show that TN is a cell survival factor, and further work also indicates that withdrawal of endogenous TN results in apoptosis (unpublished).

Elastase activity may also be related to SMC migration. We had made the observations that neointimal formation in the fetal lamb ductus arteriosus was related to smooth muscle cell migration and that this was associated with poorly-assembled elastic fibers. Further in vitro studies showed that elastin peptides can convert SMC from contractile to migratory by upregulating their production of fibronectin. Recent studies by our group have shown that the mechanism of fibronectin upregulation is post-transcriptional and related to binding of the A + U consensus sequence in the 3′ untranslated region of the fibronectin mRNA by a microtubule-associated protein (unpublished).

These studies suggest that, by knowing more about the cellular and molecular mechanisms regulating the pathophysiology of pulmonary hypertension, there is the potential for novel therapeutic targets. That is, we may be able to prevent elastase activity by upregulating the endogenous inhibitor, elafin, by inhibiting the serum or endothelial factors involved in its induction, by preventing the interaction of the potential transcription factor, AML-1, with the DNA binding site on the promoter of the elastase gene, and by reducing the ability of the enzymes to release smooth muscle cell mitogens and to upregulate fibronectin.

3. Immune / inflammatory processes

There is still controversy as to the role of immune/inflammatory cells in the pathophysiology of pulmonary hypertension. Macrophages have been described in advanced lesions in the lungs of transplant recipients [26]. In addition, in studies of familial pulmonary hypertension, certain immunogenetic markers have been identified [27]. Still, a specific gene which might be a candidate for the development of unexplained pulmonary hypertension or even hyper-reactivity of the pulmonary circulation in response to known stimuli has not been identified. A genetic strain of rat, the ‘fawn hooded’, however, has been described in which high circulating levels of endothelin-1

correlate with heightened reactivity and structural remodeling in response to minimal hypoxia [28]. It is interesting that, despite the presence of advanced structural changes in unexplained pulmonary hypertension, progress has been made in the treatment through lung transplantation [29] and also through the continuous infusion of prostacyclin [30]. Despite lack of acute hemodynamic effect, continuous prostacyclin infusion has dramatically reduced symptoms and pulmonary artery resistance in some cases. These clinical studies suggest that inducing regression of advanced changes may be feasible.

4. Lessons for the systemic circulation

The exciting windfall from studies investigating mechanisms associated with the pathobiologic evolution of pulmonary hypertension is that we have gained new insights into the study of systemic vascular disease. For example, we have recently been successful in demonstrating not only that there is increased elastase activity in coronary arteries from experimental animals in which accelerated neointimal formation occurs after cardiac transplantation, but also that inhibition of that elastase activity with the specific elastase inhibitor, elafin, markedly reduces the incidence of the lesions from 70 to 30% of vessels, as well as their severity, and also appears to prevent much of the associated myocardial necrosis [31].

5. Conclusion

While considerable progress has been made, there is, to date, no cure for advanced pulmonary vascular disease. The technological revolution in biology will improve our understanding of the most fundamental cellular and molecular mechanisms and integration of these processes will surely lead to new ideas and new hope.

*The search for truth is in one way hard and in another way easy for no one can master it fully nor miss it fully but each adds a little to our knowledge of nature and from all things assembled there arises a certain grandeur.*Aristotle.

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