The role of hypoxia in pulmonary vascular diseases: a perspective

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Pulmonary hypertension is defined clinically as a mean pulmonary artery pressure of greater than 25 mmHg. From a clinical perspective, it matters whether the underlying lung vascular abnormalities are progressive and affect the performance of the right cardiac ventricle (94). Hypoxia (i.e., a decreased oxygenation of tissues) causes under most circumstances pulmonary vasoconstriction (96). A disease component attributable to chronic hypoxic pulmonary vasoconstriction is conventionally accepted and assigned a pathobiological role in many chronic lung diseases, including interstitial pulmonary fibrosis, acute respiratory distress syndrome and chronic obstructive pulmonary disease (15, 25, 101). Understandably, the degree of CHPH (27, 97), whereas cattle on the other end and sheep on one end of the spectrum develop only a mild degree of CHPH (78).

In most mammals chronic hypoxia causes chronic pulmonary hypertension (CHPH), and the severity of CPHH is remarkably species dependent. Mouse, hamster, guinea pig, and sheep on one end of the spectrum develop only a mild degree of CPHH (27, 97), whereas cattle on the other end develop severe CPHH (88). Early investigators of mechanisms of CPHH already wondered whether, in addition to pulmonary arterial smooth muscle constriction, hypoxia affected the remainder of the lung tissue. They also asked whether lung tissue hypoxia, independent of vasoconstriction, contributes to the development of chronic lung vascular disease and to CHPH.
result of a faulty tumor microvessel design or in the lung because of mechanical obstruction or compression of small vessels, the impairment of cell oxygenation generates a hypoxic stress response. In tumors, in the chronically sick and remodeled lung, and in anemia, the systemic arterial blood partial pressure of oxygen is normal whereas the tissue microenvironment is hypoxic. It is possible that in a variety of lung pathologies this concept of tumor hypoxia may be applied to the lung. In normal subjects breathing normobaric air, lung tissue hypoxia due to a reduced pulmonary perfusion is unlikely to occur, because a reduction in oxygen delivery by pulmonary arteries (carrying mixed venous blood with low oxygen content) would be readily compensated by direct alveolar diffusion of oxygen. However, in many different disease states, the existence is likely of microregions within the lung exhibiting local tissue hypoxia due to diffusion limitation or ventilation perfusion mismatch (20, 21, 60).

Hypoxia and Inflammation

Following the breakthrough discovery of the oxygen tension-dependent homoeostatic master “regulator hypoxia-inducible factor 1α” (HIF-1α) (98) that has provided fundamental and important information, we have on our way to unravel the complex “biochemistry” of hypoxia (71). Not only can we understand the molecular response of cells to hypoxia, HIF-1α importantly also connects hypoxia and inflammation (26, 54, 56, 66, 87). Indeed, hypoxia and inflammation are inseparably intertwined. “The interest concerning the relationship between hypoxia, an admitted primary trigger for many forms of PH [pulmonary hypertension], and inflammation/inflammatory infiltrates is obvious: frequent observation of the latter pattern in PH might be an argument in favor of a canonical pathophysiologic pathway and against a simple epiphenomenon in pulmonary arterial hypertension” (24). Figure 1 only shows a partial list of the genes that are expressed by the binding of the HIF-1α protein to hypoxia response elements contained in the sequence of a large number of genes. An expression map of HIF-dependent genes in endothelial cells has been published (46), but HIF-1α and HIF-2α are not the only transcriptional regulators that are active during hypoxia; for example, PGC-1α is another important transcription factor during hypoxia (63).

The first data indicating that CHPH and inflammation are related was the discovery of lung mast cell hyperplasia in chronically hypoxic rats (38). Lung mast cell hyperplasia also occurs in human idiopathic pulmonary arterial hypertension (PAH) (29), but it still remains unclear today whether resident mast cells multiply around pulmonary hypertensive vessels, or whether the mast cell accumulation is the consequence of a hypoxia- or lung injury-triggered release of mast cells from the bone marrow. Lung mast cell hyperplasia in pulmonary hypertensive lungs provided the initial background and rationale for the search for mediators of hypoxic pulmonary vasoconstriction and mediators of inflammation in the pulmonary hypertensive lung. Indeed, leukotrienes and platelet-activating factor levels were found to be elevated in the lung during hypoxia (48, 61). More recently, mast cells are viewed as repositories of vasoactive factors and growth factors; they also store and release metalloproteases (47).

A reliable model for the study of CPH is the rat and several hundreds of experimental data have been reported during the last 50 years. It can be shown that within 24 h of exposure to simulated altitude (hypobaric hypoxia) inflammatory cells accumulate in the lungs and a simple readout of this event is the increased activity of lung tissue myeloperoxidase. But is lung inflammation causally related to CHPH or merely an associated event? This question was addressed by Ono et al. (56), who demonstrated that an anti-inflammatory drug that has no effect whatsoever on the pulmonary artery pressure prevented the lung inflammation that develops after hypoxia exposure and the development of CHPH in rats. Whereas the pathogenetic concept of CHPH had initially been summarized as “there is no CHPH without vasoconstriction,” the concept “there is no CHPH without inflammation” was new (56). Subsequently, investigators have moved to the molecular level. FIZZ1 (also known as RELMα or hypoxia-induced mitogenic factor HIMF) has been shown to be released during hypoxia from lung macrophages. HIMF is proinflammatory and proangiogenic via vascular endothelial growth factor (VEGF) and its receptor VEGFR2 (102); in the mouse HIMF also recruits bone marrow-derived mesenchymal cells to the lung (4).

Stenmark et al. (81) in their review discussed hypoxia and leukocytes and highlighted the recruitment of leukocytes and fibrocytes to the lung vessels and examined the systemic vasa vasorum as the port of entry. Perivascular pulmonary arterial inflammation has been examined in the chronically hypoxia calves and the fibroblasts have been characterized as proinflammatory, highly expressing interleukin-1 (IL-1), interleukin 6 (IL-6), the chemotactic cytokine regulated and normal T cell expressed and secreted (RANTES), stromal derived factor 1 (SDF-1) and its receptor chemokine receptor type 4 (CXCR4) (42), osteopontin, and the integrin receptor α6β3 (5). In Fig. 2, the focus is on the bone marrow activation. It is of interest that

Table 1. Hypoxic stress responses and the cancer paradigm (17, 19, 45)

| Proliferation and survival of stem cells (MYC, p53, AKT) |
| Macrophage migration and cytokine production |
| Glycolysis (GLUT 1, PGK1) |
| Angiogenesis (VEGF, PDGF) |
| Inflammatory cell infiltration (SDF1, CXCR4) |
| HIF-1α-dependent expression of p53, Bcl-2, p21 in stem cells |
hypoxia can induce Toll-like receptor (TLR) 2 and TLR6 in a HIF-1α-dependent fashion (40) and that activation of these TLRs activates NF-κB (90, 91). Also shown in the figure are the reciprocal activations between NF-κB and HIF-1α (90, 91).

A spillover of chemotactic factors like VEGF, leukotriene B4 (LTB4), FIZZ1, and others, released from the hypoxic lung tissue, may complement direct effects of hypoxia on the bone marrow. One result is mobilization of bone marrow-derived cells (likely including mast cells, mesenchymal precursor cells megakaryocytes, and dendritic cells) and their chemotaxis to the lung. Thus hypoxic/hypoxemic inflammation is clearly a systemic response.

IL-6 has recently received attention as a mediator of CHPH. IL-6 mRNA and protein levels were increased in chronically hypoxic mice. IL-6−/− mice showed less lung tissue inflammation, but the degree of CHPH was only moderately reduced in IL-6 knockout (KO) mice (69). Lung-specific IL-6 overexpression in mice increased the muscularization of small pulmonary arteries and the right ventricular systolic pressure in normoxic mice, indicating that, at least in mice, IL-6 contributes to lung vascular remodeling. However, when lung-specific IL-6 overexpressing mice were exposed to chronic hypoxia, severe CHF and pulmonary vascular lumen obliteration were observed (80). These two reports taken together suggest that IL-6 is increased in chronically hypoxic lung tissue but not necessary for the development of CHPH. The data also suggest that the combination of excessive IL-6 lung levels together with chronic hypoxia changes the mode of lung vessel remodeling toward angioproliferation. An elegant recent study by Brock et al. (14) connected IL-6 overexpression with decreased expression of the BMPR2 protein which is mutated in patients with familial idiopathic PAH (9), and the authors suggest that IL-6 signaling involves the overexpression of cluster microRNA miR-17–92, as shown in Figure 3A, where the alternative mechanism proposed by Steiner et al. (80) is also depicted (Fig. 3B). Clearly, increased IL-6 expression per se is insufficient to cause lung vascular occlusions, and Steiner et al. show that the second hit of chronic hypoxia is necessary. This raises the question: what is it exactly that chronic hypoxia sets in motion in the setting of elevated IL-6? One scenario is that chronic hypoxia mobilizes bone marrow cells, which “attempt to repair” lung vessels that have a low level expression of the lung vessel-protective BMPR2 protein.

It should not be surprising that one of a variety of systemic effects of hypoxia is the modulation of immune responses [see Nicolls and Voelkel (54) and Voelkel et al. (94) for reviews of this topic]. The hypoxia-induced HIF-1α target VEGF (95) facilitates mobilization of cells from the bone marrow and affects the behavior of B and T lymphocytes (41). As such, VEGF firmly links inflammation and angiogenesis. Hypoxia can be immunosuppressive, inhibit T cell proliferation, and affect regulatory T cell function (100), and hypoxia upregulates HIF-1α mRNA in macrophages (79). Inflammatory cells that have migrated into the hypoxic lungs teleologically speaking may be reacting to activated or injured lung cells, in particular to lung vascular endothelial cells. Activated lung endothelial cells express surface adhesion receptors and participate in inflammatory cell-cell interactions. Here is just one example: endothelial cell production of IL-1 can lead to upregulated expression of HIF-1α, amplifying the effect of tissue hypoxia (Fig. 1). In conclusion, both professional inflammatory cells, like polymorphonuclear leukocytes and macrophages, and nonprofessional inflammatory cells, like endothelial cells and fibroblasts, become part of an inflammatory network during CHPH development. Although it may be a convenient concept, it is not yet clear whether HIF-1α expressed in hypoxic lungs (104) is central to CHPH, because so far experiments have only been conducted with HIF-1α+/− mice (105). Although partial HIF-1α deficiency reduces CHPH and polycythemia, the effects of partial HIF-1α deficiency on
inflammation are unknown, and conditional HIF-1α KO mice experiments are still lacking.

**Hypoxia, ROS Generation, and Sphingolipid Metabolism**

Hypoxia stress is associated with the cellular production of reactive oxygen species (ROS) and nitrogen species (92). Superoxide anion, generated via activation of NADPH oxidoreductases (NOX) (23), in particular NOX4 in endothelial cells (73), and H2O2 have been proposed as regulators of hypoxic pulmonary vasomotor tone regulation (3, 34). Again, HIF-1α plays an important role: HIF-1α mediates the expression of NOX-2 and ROS activate HIF-1α (106). Treatment of rats with n-acetylcysteine (NAC) diminishes the severity of CPH (31); whether NAC also affects hypoxic pulmonary inflammation is unknown.

The sphingolipids sphingosine-1-phosphate (S1P) and ceramide are now increasingly recognized as important regulators of barrier function, apoptosis, and chemotaxis in the context of chronic lung diseases (89). Exogenously administered S1P causes pulmonary vasoconstriction (57), which in the isolated perfused mouse lung depends on Rho-kinase activation (84); however, it is unknown whether S1P levels are increased in the chronically hypoxic lung, or whether chronic hypoxia generates a S1P-ceramide imbalance. S1P is catalyzed via sphingosine kinase by phosphorylation of sphingosine, and sphingosine kinase 1 expression is controlled by HIF-1α (76). Cell sources of S1P are red blood cells, platelets, and endothelial cells (36). S1P not only affects endothelial cell barrier function and is vasoactive, it is also cardioprotective (36). In pulmonary artery smooth muscle cells chronic hypoxia increased sphingosine kinase 1 gene expression (2) and S1P and ceramides indeed could play a role in the development of CPH.

**Endothelial Cell-Mesenchymal Transition**

Endothelial-mesenchymal transition (EndMT) is part of a response-to-injury program that has been investigated in myocardial capillary endothelial cells of the mouse (108), in kidney fibrosis (107), and in cancer (59). Arciniegas et al. (7) discuss this cell phenotype switch in the context of chronic pulmonary hypertension. Endothelial cells, when exposed in culture to TGF-β, transdifferentiate into cells that express smooth muscle cell markers and insulin-like growth factor II, thrombin, and its receptor PAR1; as well, both Notch and NF-κB activation (6–8, 43, 55) have been associated with EndMT. Given the established link between hypoxia, HIF-1α, and NF-κB, one can postulate that chronic hypoxia promotes EndMT in lung vessels.

**Angioobliterative Remodeling in Severe Pulmonary Hypertension**

The so-far discussed hypoxia-induced disease-mechanistic components inflammation, EndMT, and immune system modulation interact in models of CPH, yet in animal models these mechanisms are insufficient to generate the lumen-obliterating pulmonary vasculopathy that characterizes severe forms of human PAH (93). We have used the term “angioobliterative” over the past years to distinguish between vessels with an open lumen and those that are closed such that there is no flow distal to the obliteration. Another implied meaning is “irreversibly closed,” in contrast to reversible muscularization of the pulmonary arteries after cessation of chronic hypoxic exposure. A second hit in addition to hypoxia is required. Knockout of the BMPR2 gene (44) or overexpression of IL-6 in mice (80), or induction of lung vascular endothelial cell apoptosis in rats together with chronic hypoxia (86), produce lumen obliteration in the lung vessels. BMPR2 protein expression is decreased in mice with CPH but by itself is insufficient to cause complex lung lesion formation (14). Although it is still unknown which factors precipitate angioproliferative pulmonary lesions in individual patients with severe PAH (77), it is highly probable that the partial or total lumen closure of peripheral small lung vessels renders the tissue distal to the vascular obstruction ischemic (and perhaps even hypoxic when oxygen diffusion from the alveoli is insufficient), and that, together with vasoconstriction (82), in these microareas the biology of ischemia-reperfusion does apply.

If the pathobiology of these lesions can indeed be modeled by applying the concept of quasi-malignant cell growth (62), then, as in tumors, we need to consider a role of the regional effects of hypoxia-ischemia. Hypoxia is a key factor in tumor progression; it regulates apoptosis [selection of populations that are resistant to apoptosis (28)], cell proliferation, and angiogenesis, and it impairs DNA repair pathways (13) and induces autophagy (67) and the anaerobic metabolic switch (70). Although most patients with idiopathic PAH are not hypoxicemic, unless they have developed an intracardial right-to-left shunt, lung tissue hypoxia-ischemia likely plays a role in the progressive worsening of the lung vascular disease. Another reason for lung tissue underperfusion and decreased peripheral lung tissue oxygenation is the pruning of the vascular tree, which can be demonstrated by pulmonary angiography in emphysema, in interstitial fibrosis, and in the angioobliterative forms of PAH. The coexistence of vessel loss and lumen-obliterating cell growth in the same lung remains mechanistically unexplained. In CPH we find vessel pruning (disappearance) and muscularized arterioles. Meyrick and Reid (49, 65) in their ultrastructural studies described the pulmonary vascular remodeling in CPH as a differentiation of pericytes and intermediate cell precursor cells into smooth muscle cells, a pathobiological concept that anticipated the discovery of vessel wall stem cells (10) and may complement the concept of EndMT.
Hypoxia Induces Epigenetic Modifications

In addition to the well-recognized, but still on a mechanistic level incompletely understood, genetic underpinnings of CHPH (11, 103), there is now an increasing interest in epigenetic control mechanisms of PAH (39). Polymorphisms in the EPAS1 or HIF-2α gene appear to account for the blunted polycythemic response of Tibetan high-altitude dwellers (11). Whether these polymorphisms also explain blunted pulmonary vascular responses in Tibetan highlanders is not clear. Both methylation and acetylation of histones determine the extent and degree of gene expression, and both are subject to dietary and environmental regulation. For example, a decreased expression of histone deacetylase-2 (HDAC2) has been shown in the lung tissue samples from smokers with chronic obstructive pulmonary disease (32). Mizuno et al. (52) demonstrated recently that HDAC inhibition decreases the expression of HIF-1α and causes emphysema. In contrast, chronic hypoxia increases lung tissue expression of HDAC 1, 2, 3, and 5 and HDAC inhibition ameliorates CHPH (18, 109). However, contrasting data have been published by Charron et al. (22) suggesting that activation of HIF-1α by hypoxia decreases HDAC2 levels; of interest is also the report by Hezroni et al. (30), which suggests that HDAC inhibition promotes the pluripotency of embryonic stem cells. Another example of epigenetic regulation is the copper-dependent expression and trans-activating activity of HIF-1α. Copper depletion decreases HIF-1α expression in the lung tissue and the expression of the HIF-1α target genes VEGF and LOX (51) and causes emphysema. However, animals raised on a Cu²⁺-depleted diet exposed to chronic hypoxia demonstrate the typically observed degree of pulmonary vascular muscularization (12). Thus HDAC inhibition, but not copper depletion, inhibits lung vascular muscularization in the rat CHPH model, indicating that HDAC dependently expressed genes and proteins participate in the remodeling responses of the lung vessels during chronic hypoxia. Endogenously generated HDAC inhibitors or environmental factors that inhibit HDAC activity would be expected to modify CHPH. Figure 5 shows also that HDAC

degree of lung vessel closure in these latter models suffices to generate hypoxic/ischemic lung tissue regions distal to the occlusions is unknown, but likely. If so, tissue hypoxia could drive the continued maturation of lung vascular lesions that proceeds past the time of chronic hypoxia exposure of the animals (1).

Fig. 5. In this schematic increased histone deacetylase (HDAC) expression is depicted upstream of HIF-1α, whereas HDAC inhibition leads to decreased HIF-1α expression, but increased expression of p53, both of which affect the muscularization of pulmonary arterioles during chronic hypoxia.

Fig. 6. Lung histology and vascular lesions of rats treated with the HDAC inhibitor trichostatin A for 2 wk (daily intraperitoneal injection) following the development of severe angioobliterative pulmonary hypertension in the Sugen/chronic hypoxia model. Trichostatin A treatment did not affect the vascular lesions. Lumen occlusion, perivascular infiltrates, and emphysematous changes are present (trichrome stain).
inhibition affects the lung tissue expression of the “guardian of the genome, p53.” This transcription factor controls cell growth and death and a large number of mutations have been associated with cancer cell growth. Mizuno et al. (50) have shown that p53 KO mice develop enhanced pulmonary vascular remodeling (muscularization) after chronic hypoxia; thus one can postulate that the HDAC inhibition-induced p53 expression contributes to the diminished muscularization in the HDAC inhibited CPHP model (18). Remarkably, HDAC inhibition did not affect the angioobliteration that develops in the rat model of combined chronic hypoxia and VEGF receptor blockade (53), Fig. 6.

Synopsis

The experimental exploration of CPHP initially focused on the role and mechanism of hypoxic pulmonary vasoconstriction. Hypoxic vasoconstriction can be investigated in lung arterial rings (33); however, the study of lung vascular remodeling in CPHP requires the intact animal. The discoveries of HIF-1α and PGC-1α, a transcriptional regulator of mitochondrial metabolic programs (72), have opened the door to the understanding of the molecular mechanisms of hypoxic stress in normal and tumor tissues. Systemic hypoxic hypoxia evokes a complex system response that involves neuroendocrine and innate immune system activation, is associated with the production of ROS, and is modulated by epigenetic phenomena. Many different cell types contribute to hypoxic vascular remodeling, and transitions between cell types (EndMT) have also been described. Although chronic hypoxia determines the adaptation to high altitude and chronic intermittent hypoxia leads to sleep apnea-related pulmonary hypertension, ischemic microregions of the lung are likely to exist in many chronic lung diseases in which pulmonary arterioles are lost or obliterated. In these areas, HIF- and PGC-1α-driven gene and protein expression is likely to play a role in the manifestation of “hypoxic inflammation.”

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

N.F.V. conception and design of research; N.F.V. performed experiments; N.F.V., S.M., and H.J.B. edited and revised manuscript; N.F.V., S.M., and H.J.B. approved final version of manuscript.

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