

Hypoxia and chronic lung disease

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Received: 1 October 2007 / Revised: 23 October 2007 / Accepted: 24 October 2007 / Published online: 27 November 2007
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Abstract The lung is both the conduit for oxygen uptake and is also affected by hypoxia and hypoxia signaling. Decreased ventilatory drive, airway obstructive processes, intra-alveolar exudates, septal thickening by edema, inflammation, fibrosis, or damage to alveolar capillaries will all interpose a significant and potentially life-threatening barrier to proper oxygenation, therefore enhancing the alveolar/arterial pO_2 gradient. These processes result in decreased blood and tissue oxygenation. This review addresses the relationship of hypoxia with lung development and with lung diseases. We particularly focus on molecular mechanisms underlying hypoxia-driven physiological and pathophysiological lung processes, specifically in the infant lung, pulmonary hypertension, and chronic obstructive pulmonary disease.

Keywords Hypoxia · Lung · Pathology · HIF

Air, containing oxygen at approximately 21% partial pressure at sea level (140–150 mmHg), travels through up to 20 generations of airways by mass flow and then diffuses into the gas exchange units (alveoli) in the lung with a partial pressure of approximately 105–110 mmHg. The human lung contains approximately 480 million alveoli, which represent 64% of total lung structure. These alveoli are elegantly packed in only 1.3–2.6 L of total lung volume, providing a surface area of 120–150 m², equivalent to the dimensions of a “tennis court” dedicated for gas exchange. Although the molecular determinants of lung size are not known, oxygen diffusion constant and gas exchange surface area are roughly proportional to body weight and oxygen consumption in different species [1]. Physical hyperactivity and exposure to a cold environment or high altitude lead to increased oxygen diffusion capacity, in proportion to enhanced oxygen consumption [1].

Decreased ventilatory drive, airway obstructive processes, intraalveolar exudates, septal thickening by edema, inflammation, fibrosis, or damage to alveolar capillaries will all interpose a significant and potentially life-threatening barrier to proper oxygenation, therefore enhancing the alveolar/arterial pO_2 gradient. This review addresses the contribution of hypoxia to lung diseases and the potential molecular mechanisms involved in hypoxia-driven physiological and pathophysiological lung processes (Fig. 1), particularly in the infant lung, pulmonary hypertension, and chronic obstructive pulmonary disease. The fundamental concepts underlying hypoxia-induced gene expression and the molecular regulation of HIF(s) also apply to the lung, and they will be cited in relation to their demonstrated role in lung physiology or pathophysiology.

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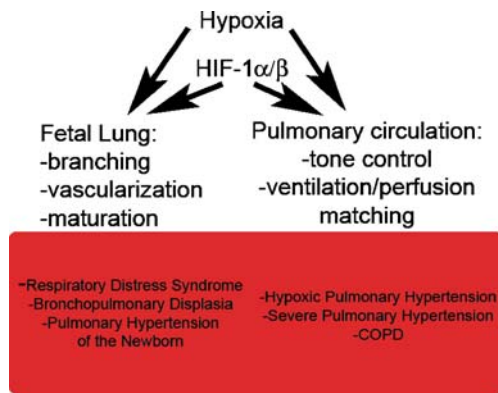


Fig. 1 Summary of effects of hypoxia and/or HIF-1/2 α in lung homeostasis and disease (*box*)

Hypoxia signaling in the lung

Hypoxia-inducible factor-HIF-1 α and HIF-2 α probably regulate the expression of most of hypoxia-dependent and many hypoxia-independent genes involved lung homeostasis and disease. Hypoxia triggers several additional signaling mechanisms, many of which interface with HIF-dependent signaling. Under hypoxia, the reduction of energy production has a major negative impact on the translational rate of proteins, as hypoxia and HIF-1 α inhibit mTOR signaling, a critical signaling pathway involved in the induction of cell growth and protein synthesis [2].

Recent evidence indicates that hypoxia induces the synthesis of microRNAs (MIRs), which add additional levels of complexity in the regulation of gene expression under hypoxia [3, 4]. MIRs, a group of small RNAs with approximately 22 nucleotides in length, have important roles in the regulation of gene expression. MIRs posttranscriptionally regulate gene expression by forming perfect base pairing with sequences in the 3' untranslated region (3'UTR) of genes, resulting in enhanced mRNA degradation, while imperfect matching may lead to repressed or inefficient mRNA translation. More than 500 MIRs have been identified in the mammalian genome so far. In a recent survey of MIRs induced or downregulated by hypoxia, several of hypoxia MIRs shared HIF-binding sequences in regulatory regions [4]. Of note, some of these MIRs protect against apoptosis [4]. A set of MIRs modulate the hypoxic expression of vascular endothelial growth factor (VEGF) expression and other angiogenic factors [5]. Other studies have shown that hypoxia-responsive transcription factors such as NF- κ B and p53 induce MIRs [6]. Although the investigation of hypoxic MIRs has been restricted to screenings in cell cultures and in silico comparison with tumor data sets [4], the list of MIRs present in the hypoxic lung has not been compiled thus far. Nevertheless, the first evidence that MIRs may participate in lung diseases was provided by the

report that MIR-155 might regulate inflammatory cell responses, such as in asthma [7].

During hypoxia, EGR-1, a zinc-finger transcription factor expressed in monocytes, leads to the production of tissue factor and fibrin deposition in the pulmonary vasculature [8]. In mice lung subjected to hypoxia, EGR-1 and tissue factor are coproduced in bronchial and vascular smooth muscle cells and alveolar macrophages [9]. Thus, hypoxia-induced EGR-1 activity accelerates pulmonary vascular thrombosis.

Hypoxia, lung development and growth, and respiratory distress syndrome

The lung develops in relative hypoxic conditions in utero as the fetus is exposed to approximately 25% of ambient oxygen levels [10]. In line with the evidence that the hypoxic fetal environment stimulates embryonic development [11], organogenesis, and organ-specific vascularization [12], the effect of hypoxia on lung development seems to reside on the stimulation of branching morphogenesis and the coordinated growth and development of a closely integrated pulmonary arterial blood supply. In vitro studies have shown that fetal oxygen tension enhances epithelial and vascular branching of mouse lung and maintains lung morphogenesis in rats [13]. Terminal branching of trachea is regulated by local oxygen tension, as hypoxia stimulates Bnl FGF, a growth factor that induces tracheal sprouting, while high oxygen tension suppresses terminal branching [14].

The expression of VEGF, a potent inducer of vasculogenesis in the lung, relies largely on HIF-1/2 α [15]. Indeed, human fetal lung explants cultured at physiological oxygen tension levels (i.e., about 40 mmHg) have increased VEGF compared with explants cultured at normoxic condition [16, 17]. Fetal VEGF is involved in lung development, as inhibition of VEGF receptor signaling impairs airway and blood vessel branching in lung explants [18].

Loss of function experiments in rodents highlighted the critical role of molecular control of hypoxia and particularly of HIF in lung growth and maturation. Deletion of HIF-1 α decreases branching morphogenesis and vascularization [19]. Deletion of HIF-2 α delays lung maturation in a VEGF-dependent manner, leading to pulmonary distress and fatality in knockdown pups. Both the alveolar epithelial and vascular systems show signs of significant immaturity, which resembles the lung complications of prematurity in humans [20].

Proper lung development requires a tight regulation of expression levels of HIF(s) and hypoxia-inducible genes, such as VEGF. Chemical stabilization of HIF-1 α due to prolyl-4-hydroxylase (PHD) inhibition with CoCl₂ or dexferrioxamine interrupts airway and vascular branching

in cultured fetal lung explants or causes hypervascularity with the nonspecific PHD inhibition with dimethyloxalylglycine [18]. Moreover, lung overexpression of VEGF through fetal development also leads to a hypervascular lung [21].

As a corollary of these data, HIF-1 α may also regulate fetal to neonatal transition and postnatal lung growth and repair as suggested by the fact that, in the absence of hypoxia, normal postnatal and post-pneumectomized regenerative lungs have increased HIF-1 α expression for months after surgery [22].

Impairment of lung growth is a major factor limiting survival of preterm babies. Immature pulmonary surfactant protein predisposes preterm babies to develop respiratory distress syndrome (RDS), characterized by decreased lung compliance and impaired gas exchange leading to hypoxia. Oxygen therapy to treat RDS exposes these infants to oxidative injury, which may impair the expression of growth factors (i.e., VEGF) involved in vascular and alveolar development, therefore leading to bronchopulmonary dysplasia (BPD) [23]. The potential role of the HIF/VEGF axis in fetal lung maturation lung was demonstrated in HIF-2 α knockout mice that are born with lung immaturity and show a phenotype similar to respiratory distress syndrome (RDS). RDS predisposes to BPD, whose pathogenesis resembles, to some extent, that of retinopathy of prematurity, in which high oxygen levels lead to disruption of VEGF-maintenance of blood vessels and endothelial cell apoptosis, resulting in organ damage. In fact, the expression of HIF-1 α , HIF-2 α , and downstream angiogenic growth factors (VEGF, PECAM-1, Flt-1, and Tie-2) are decreased in animal models of RDS or BPD [24–27]. Several of adolescent and adult BPD patients exhibit an emphysematous phenotype, potentially related to disruption of alveolar maintenance (see emphysema, below). The findings that PHDs control HIF degradation under normoxia and PHD activity is increased with increased oxygen availability [28] have offered new opportunities to develop therapies for BPD, as HIF-1 α stabilization with PHD inhibitors improves lung growth and oxygenation [29].

Hypoxia, pulmonary circulation, and pulmonary hypertension

Overall, the pulmonary vasculature comprises approximately one-third of estimated total number of 1 to 6×10^{13} endothelial cells in the human body (covering an area equivalent to $1,200$ – $2,500$ m²). The pulmonary endothelium actively participates in hypoxia-induced responses, integrating sensing and effector functions that transmit changes in blood flow and vascular pressure. Injury or dysfunction of endothelium induced by hypoxia leads to increased perme-

ability, heightened vascular smooth muscle tone, enhanced thrombotic state, and cell proliferation, all of which participate in the process of tissue remodeling.

Acute decreases in inspired (alveolar) oxygen to levels below 12% of normal (approximately equivalent to an altitude of 18,000 ft) induce reversible constriction of vascular pulmonary smooth muscle cells (PASMCs), a unique property of pre-acinar pulmonary arteries present across most mammals, reptiles, birds, and fish [30–32]. This contraction constitutes hypoxic pulmonary vasoconstriction (HPV), i.e., a rise of 4–8 mmHg in pulmonary arterial pressures with decreased inspired oxygen. Experimentally, hypoxia triggers a biphasic pressure response, an early one followed by vasorelaxation and a persistent second wave of contraction; this second stage may represent the true basis of HPV. Teleologically, HPV serves to allow for matching between ventilation and perfusion, diverting blood flow from hypoxic to better aerated alveoli. The multidetector-row computer tomography (MR-CT) has permitted functional lung studies, such as the documentation of HPV in real time by imaging regional pulmonary blood flow and ventilation [33]. Interestingly, bacterial infection or lipopolysaccharide inhibit HPV, which can be visualized and documented by MR-CT (Fig. 2, based on [33]). Oxygen sensing in PASMCs has been attributed to: (1) mitochondrial respiration, (2) mitochondria-dependent decreases in reactive oxygen species (ROS), (3) mitochondria-dependent increases in ROS, (4) mitochondria membrane depolarization, (5) decreased ATP levels with generation of cyclic ADP-ribose, (6) NADPH oxidase, (7) small GTPase RhoA-mediated sensitization to intracellular Ca⁺², and (8) cytochrome *P450*-mediated activation of hemoxygenase-2 [32].

High altitude pulmonary edema (HAPE) is characterized by shortness of breath that increases with altitude ascent without a period of acclimatization. It occurs in 0.1–15% individuals, pending individual susceptibility and preexisting cardiovascular diseases [34]. HAPE is associated with marked increases of pulmonary artery pressures caused by HPV, in excess of 35 to 40 mmHg. However, HPV is not uniform in the lung. Alveolar fluid leak with the potential complication of alveolar hemorrhage may result from a focally reduced HPV, leading to increased blood flow and disruption of the alveolar–capillary barrier. The main hypotheses to explain HAPE lie on excessive vasoconstriction, excessive inflammation due to hypoxia, or down-regulation of alveolar cell ion pumps that move sodium and fluid from alveoli into the blood stream [34]. As mice exposed to chronic hypoxia develop lung inflammation [35], it is conceivable that acute hypoxia may trigger acute lung inflammation. However, acute inflammation and HPV may not coexist, as inflammation caused by lipopolysaccharide prevents HPV (Fig. 2) [36].

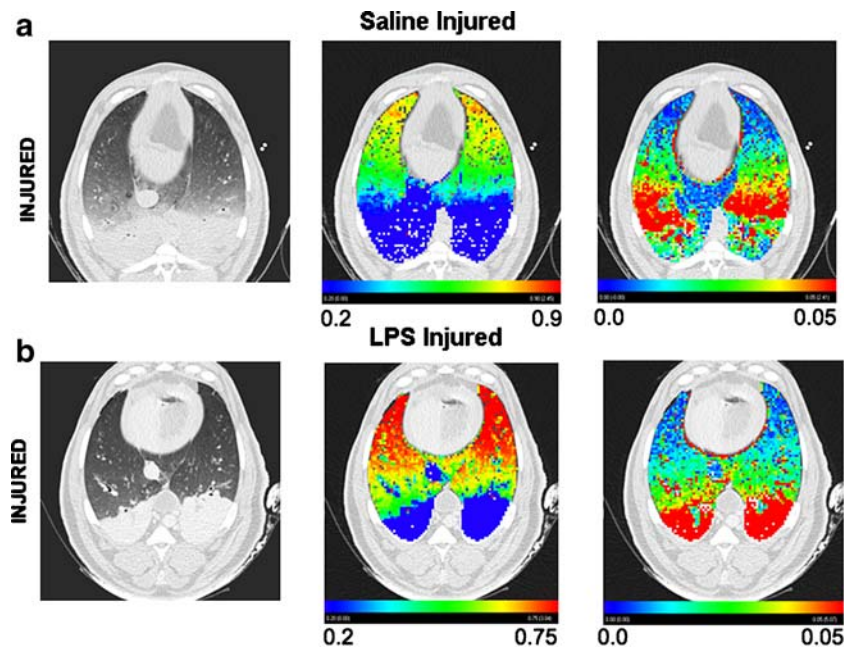


Fig. 2 Documentation of HPV by multidetector-row CT. **a** Saline-injured dog lung (in supine position, with nondependent areas at 12 o'clock and dependent regions at 6 o'clock) showing lung edema and atelectasis in the dependent areas (*whitened areas in the left upper panel*), with reduced aeration (digital quantification expressed as *blue pixels in upper middle panel*). This poorly aerated dependent region also shows markedly decreased blood flow (i.e., more *blue/green*

pixels in upper right panel) due to HPV. Normally, these dependent areas should receive most of the pulmonary blood flow. **b** LPS instillation causes loss of HPV, when the poorly aerated dependent flooded areas (*blue pixels in lower middle panel*) have preferential pulmonary blood flow (*red pixels in lower right panel*) (based on [33], reprinted under permission)

Chronic hypoxia causes pulmonary hypertension (PH), which consists of an increase in mean pulmonary artery pressures in excess of 25 mmHg at rest or 30 mmHg on exercise [37]. Hypoxia-induced PH is usually mild (i.e., less than 45 mmHg) and potentially reversible upon normalization of inspired oxygen levels. PH associated

with several lung diseases, particularly with sleep apnea and potentially with chronic obstructive pulmonary diseases (COPD), has been linked to chronic hypoxia. The experimental model of hypoxia-induced PH is based on exposure of rodents to 50% reduced oxygen levels for more than 2 weeks. This model is characterized by vascular cell

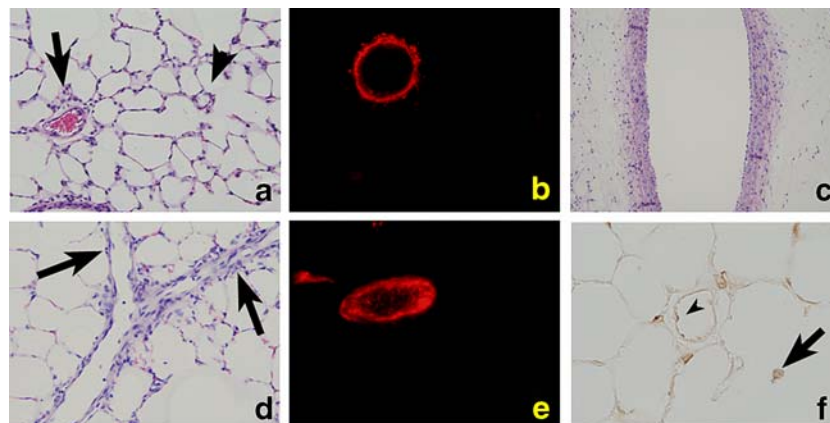


Fig. 3 Hypoxic pulmonary vascular remodeling. Images are from rats exposed to normoxia or chronic hypoxia (equivalent to 17,000 ft altitude above sea level) for 3 weeks. **a** Pulmonary artery of a rat under normoxia conditions. Note that there is a loss of medial smooth muscle cells in arteries less than approximately 30 μm in diameter (*arrowheads*), located in alveolar tissue distal to terminal bronchioles. The vascular media represents approximately 5–8% of the vessel diameter. **b** Immunofluorescence of a normal pulmonary artery stained with anti-smooth muscle cell α -actin antibody. **c** Large pulmonary

artery of rats exposed to chronic hypoxia, showing prominent medial layer. **d** Extension of medial muscular layer towards the lung periphery of a pulmonary artery sectioned longitudinally. **e** Immunofluorescence with an anti-smooth muscle α -actin antibody highlights increased medial thickness of a chronically hypoxic pulmonary artery. **f** Hydroxyprobe-based detection of cellular hypoxia, showing staining of endothelial cells (*arrowhead*) and of type II epithelial cells (*arrow*) in lungs exposed to chronic hypoxia

proliferation, involving initially endothelial cells, followed by PASMCs, and then adventitial fibroblasts [38]. Increased medial thickness becomes apparent with 2 weeks of chronic hypoxia exposure (Fig. 3). Hypoxia stimulates a wide array of potential mediators of PASMC growth, including platelet-derived growth factor, transforming growth factor β , and NADP oxidase subunit 4. Although there is no evidence that humans living in high altitude or suffering from lung diseases associated with chronic hypoxia are at increased risk of severe PH (i.e., pulmonary artery pressures in excess of 45 mmHg), investigation of the underlying mechanisms involving hypoxia sensing have offered new insights in the pathogenesis of the human disease.

Pulmonary endothelial cell dysfunction, a leading concept of the pathogenesis of PH [39], may account for excessive pulmonary vascular cell growth and/or decreased cell death [40]. An extension of this paradigm is the evidence that the pulmonary vascular remodeling in severe PH involves elements of abnormal angiogenesis [40], and it resembles that seen in neoplastic cells [41]. HIF-1 α controls several features involved in angiogenesis and cancer cell growth, including mitochondria function, which have been validated recently in studies of experimental PH [42]. Mitochondria of PASMCs are different from their systemic counterpart, as they have lower respiratory rates, are more depolarized, have more manganese superoxide dismutase (MnSOD), and produce more hydrogen peroxide [43]. Under normoxic conditions, the electron transport in mitochondria results in a small production of superoxide radicals, in proportion to alveolar oxygen pressure. Superoxide, a potentially toxic reactive oxygen species (ROS), is converted to a hydrogen peroxide that acts as diffusible second messenger and subsequently regulates activity of redox-sensitive transcription factors, including HIF-1 α , and the activation and expression of voltage-gated K_v^+ channels (e.g., $K_v^+ 1.5$). There has been an intense debate whether hypoxic pulmonary vascular cells have increased or decreased oxidative stress. However, one view predicates that, within seconds of exposure to hypoxia, mitochondrial ROS production decreases, which inhibits oxygen-sensitive K_v^+ channels and causes membrane depolarization, activation of voltage-gated L-type calcium channels, and calcium influx, thereby initiating HPV [44]. If this redox oxygen sensor is impaired, disrupted mitochondria create a false “hypoxic signal”, chronically activating HIF-1 α under normoxic conditions. HIF-1 α activation downregulates oxygen-sensitive K_v^+ channels ($K_v^{+1.5}$) and initiates a cascade that causes PH. This mechanism has been postulated to lead not only to enhanced vasoconstriction, but also to resistance to cell death via hyperpolarization of mitochondria and enhanced survivin expression [45].

The involvement of HIF-1 α in PH was documented by the finding that heterozygous mice when exposed to

hypoxia (10% O_2 for 1 to 6 weeks) developed less polycythemia, right ventricular hypertrophy, pulmonary hypertension, pulmonary vascular remodeling, and weight loss than wild-type mice [46]. HIF-2 α , which is abundantly expressed in the lung, is also involved in PH, in that heterozygous mice are fully protected against PH and right ventricular hypertrophy caused by chronic hypoxia [47]. HIF-1 α regulates PASMC K_v^+ capacitance, K^+ current density, and membrane depolarization, which are decreased in HIF-1 α - $^{-/-}$ cells [48]. After exposure to chronic hypoxia, wild-type PASMCs increase the expression and activity of Na^+/H^+ exchanger (NHE), which results in increased intracellular pH and cell growth [49]. Recent reports indicate that hypoxic PASMCs have increased expression of canonical transient receptor potential (TRPC) channels 1 and 6 in a HIF-1 α -dependent manner [50].

There is experimental and clinical data that implicate serotonin in PH and lung hypoxic responses. Serotonin (5-HT) could affect pulmonary vascular remodeling, as it promotes vasoconstriction and vascular smooth muscle cell growth [51]. Hypoxia modifies plasma levels of serotonin, serotonin transporter activity, and expression of 5-HT $_{1B}$ and 5-HT $_{2B}$ receptors [52]. However, rather than 5-HT transporters, serotonin receptors might instead mediate hypoxic PH as demonstrated by the protection against hypoxic PH documented in mice lacking 5-HT $_{2B}$ receptors [53].

Under physiological conditions, endothelial cells remain quiescent and slow growing with only 1 dividing cell per 10,000 cells at any given time, which translates into a rate of one cellular division every 2 weeks. In severe pulmonary arterial hypertension (PAH), there is a unique and characteristic presence of plexiform lesions in the lumens of medium and small precapillary pulmonary arteries. These lesions show enhanced expression of VEGF, VEGF receptor, HIF-1 α , and HIF-1 β , which suggests that lesions may develop by a process of disordered angiogenesis [54]. Similar results were recently obtained with cultured endothelial cells isolated from patients with IPAH. These cells have greater proliferation rate and decreased apoptosis, higher level of phosphorylated STAT3 and increased expression of its downstream prosurvival target, Mcl-1 [55]. Moreover, endothelial cells from idiopathic PAH lungs have decreased mitochondria and use preferentially the glycolytic pathway to generate energy, properties in common to cancer cells [56]. Although the involvement of HIF in this phenotype and in disease severity has not yet been formally tested, it is conceivable that vascular cells have an altered hypoxia-sensing mechanism, predisposing them to increased growth potential and even genomic instability. In a model of severe PH in the rat, chronic hypoxia in association with chronic VEGF receptor inhibition with SU5416 may be critically involved in the initial endothelial cell death and selection of apoptosis-

resistant proliferating cells, which obliterate the pulmonary arteries [57].

Mutations of bone morphogenetic protein receptor 2 (BMPR-2) are involved in approximately 60% of the familial cases of familial PAH. Up to the present day, it is not clear how the germ line mutation in a single copy of the gene causes IPAH. There is evidence that either a state of haploinsufficiency or dominant negative effects leads to enhanced growth of cultured IPAH PSMCs. Although BMPR-2 heterozygous mice have normal lungs and pulmonary circulation at baseline, they develop more pronounced PH (though of mild severity) under inflammatory conditions or under hypoxia [58].

Hypoxia and chronic obstructive pulmonary diseases

COPD is the fourth leading cause of morbidity and mortality in the United States alone [59]. This syndrome is mostly caused by chronic cigarette smoke inhalation and exposure to environmental pollutants. In COPD, progressive airflow limitation and destruction of the alveolar capillary network may lead to decreased oxygen transport and alveolar hypoxia.

The finding that blockade of VEGF receptor signaling causes rodent emphysema led to several studies that documented decreased VEGF and VEGF receptor expression in emphysematous lungs [60]. Whether the expression of HIF and other signaling molecules involved in hypoxia sensing is abnormal in emphysema remains unknown. There is recent evidence that cigarette smoke impairs HIF-1 α expression in ischemic limbs of mice, causing decreased revascularization [61]. Moreover, we found that RTP-801 or Redd-1, a negative regulator of mTOR signaling and hypoxia-responsive gene product, is induced by cigarette smoke. RTP801 knockout mice were significantly resistant to cigarette smoke-induced inflammation and emphysema [62]. About 60% of patients with COPD suffer from mild PH. Up to 5% of COPD patients have severe levels of PH with pulmonary artery pressures similar to that found in the systemic circulation [63]. PH in COPD is not fully explained by hypoxia alone, as it occurs in non-hypoxic patients and long-term oxygen therapy or nitric oxide inhalation do not reverse the pulmonary vascular changes. A direct toxic effect of cigarette smoke on pulmonary vasculature has been suggested to act in concert with a potential hypoxic effect observed in COPD [63].

Conclusions

Cellular signaling involved in hypoxia sensing participates in several physiological and pathophysiological processes

in the lung. Several of these effects can be traced to availability of oxygen itself, as long-term oxygen therapy is the only proven effective therapy that increases long-term survival for patients in COPD [64]. Hypoxia itself or hypoxia-induced signaling may underlie the pathogenesis of several life-threatening lung diseases, as it regulates the expression of several critical genes. Lessons learned in the role of hypoxia in tumor growth and organ biology will certainly apply to fundamental aspects of lung repair, cell injury, or inflammation. We are just the beginning of the systematic exploration of this paradigm in pulmonary diseases. These efforts will translate in a better understanding of hypoxia-driven cell signaling and of suitability of therapeutic targets aimed at several lung diseases, including lung immaturity, pulmonary hypertension, and COPD.

Acknowledgements The authors thank Shehzin Mozammel and Reda Girgis for providing the immunofluorescence images and Dr. Brett Simon for the documentation of MR-CT, Ms. Amy Richter for the hydroxyprobe staining, and Mrs. Kathleen Wolfe for the secretarial support.

Grant support This work has been supported by the CMREF Center for Tissue Processing Center of the Pulmonary Hypertension Breakthrough Initiative, P150 HL 084946-01 (Pathology Core and Project 5), and HL 66554.

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