Eur Respir J 2007; 30: 364–372
DOI: 10.1183/09031936.00128706

REVIEW

The effects of hypoxia on the cells of the pulmonary vasculature

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ABSTRACT: Pulmonary hypertension is associated with remodelling of pulmonary vessels. Chronic hypoxia is a common cause of pulmonary hypertension and pulmonary vascular remodelling. Vascular remodelling is characterised largely by fibroblast, smooth muscle and endothelial cell proliferation, which results in lumen obliteration. Chronic hypoxia elicits expression of mitogens, growth factors and cytokines by fibroblasts and endothelial cells, and also the suppression of endothelial nitric oxide synthase. Although hypoxic pulmonary vascular remodelling is associated with medial hypertrophy, many *in vitro* studies have found that hypoxia does not lead to a direct increase in smooth muscle cell proliferation. This paradox is not well understood and this review aims to examine the various reasons why this might be so. The present authors reviewed data from *in vitro* studies and also considered whether hypoxia could act on adjacent cells such as fibroblasts and endothelial cells to trigger smooth muscle cell proliferation. It is possible that hypoxia is sensed by fibroblasts, endothelial cells, or both, and relayed to adjacent pulmonary artery smooth muscle cells by intercellular signalling, causing proliferation.

The present article reviews the data from *in vitro* studies of hypoxia on the three cellular components of the pulmonary vascular wall, namely endothelial cells, smooth muscle cells and fibroblasts.

KEYWORDS: Endothelial cells, fibroblast, hypoxia, proliferation, remodelling, smooth muscle cells

Pulmonary hypertension (PH) is a complication for those subjected to environmental hypoxia as a result of living at high altitude and for those suffering from chronic hypoxic lung diseases, such as chronic obstructive pulmonary disease (COPD), cystic fibrosis, bronchiectasis and asthma. PH is independently associated with increased morbidity and reduced survival in patients suffering from hypoxic lung disease [1], and is both accompanied and caused by pulmonary vascular remodelling [2]. The pulmonary vascular wall consists of three layers: adventitia, media and intima, whose cellular components are fibroblasts, smooth muscle cells (SMC) and endothelial cells (EC), respectively. The remodelling of pulmonary arteries is a complicated pathological process in which all three layers of the vascular wall are involved [3].

In the present article, the authors have reviewed the evidence from the many *in vitro* studies of hypoxia on pulmonary vascular cell proliferation and function but have concentrated on the controversy surrounding the reported effects of hypoxia on SMC proliferation.

VASCULAR REMODELLING AND PH IN RESPONSE TO HYPOXIA

Under normal conditions, the thickness of the vascular wall is maintained at an optimal level by a fine balance between proliferation and apoptosis of the resident cell types. If this balance is disturbed in favour of proliferation, the vascular wall thickens and eventually obliterates the vessel lumen, leading to increased resistance. This structural change of the vascular bed is termed vascular remodelling [3]. Pulmonary artery (PA) remodelling leads to an increase in pulmonary pressure resulting in further remodelling. Proliferation of adventitial fibroblasts increases within hours of hypoxic exposure [4], but, a few days after exposure (to hypoxia), thickening of the medial layer (hypertrophy and hyperplasia) begins to develop [5]. It is known that hypertrophy of SMC makes a greater contribution than hyperplasia in the larger, more

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Received:
October 03 2006
Accepted after revision:
March 01 2007

SUPPORT STATEMENT
O. Pak was supported by a European Respiratory Society Fellowship.

STATEMENT OF INTEREST
None declared.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

proximal arteries, whereas hyperplasia is more prevalent in the smaller resistance arteries [6, 7]. Furthermore, fibroblasts migrate into the medial layer and can transform into SMC [8]. EC also participate in hypoxic pulmonary remodelling by producing vasoconstrictive pro-proliferative factors (endothelin (ET)-1, angiotensin II, thromboxane A_2), and reducing the production of vasodilatory, anti-proliferative mediators (nitric oxide (NO) and prostaglandin-I_2). HOWELL *et al.* [9] showed that the volumes of intima and adventitia from lungs of hypoxic rats were increased 1.5-fold, and the volume of media was increased six-fold, compared with normoxic controls.

It is accepted that hypoxia is a cause of pulmonary vascular cell proliferation and vascular remodelling, but the mechanisms remain unclear. *In vitro* studies have demonstrated that hypoxia has direct effects on cell proliferation in some, but not all, cell preparations [10–12]. Hypoxia is able to increase cell proliferation by inhibition (production and/or release) of antimitogenic factors (*e.g.* NO and prostacyclin) and by increasing the production and/or release of different mitogenic stimuli (*e.g.* 5-hydroxytryptamine, ET-1, platelet-derived growth factor (PDGF) and vascular endothelial-derived growth factor (VEGF)) and inflammatory mediators (*e.g.* interleukin (IL)-6, IL-8 and monocyte chemoattractant factor-1) from SMC, fibroblasts, EC and platelets [13–18]. Furthermore, hypoxic exposure leads to increased production of extracellular matrix components [19].

Several possible pathways have been implicated in the cellular response to hypoxia. For example, in SMC, hypoxia dramatically increases the level of Ca^{2+} in the cytoplasm [20]. Increased Ca^{2+} levels lead to activation of Ca^{2+}/calmodulin and mitogen-activated proein kinases (MAPKs) and expression of the early response gene *c-fos* [21, 22]. An elevated Ca^{2+} level in SMC has been shown to modulate proliferation and growth [22]. Another example is the Rho kinase pathway, which phosphorylates the myosin phosphatase target subunit-1 of smooth muscle myosin phosphatase leading to the inhibition of its activity [23]. This inhibition of smooth muscle myosin phosphatase activity leads to Ca^{2+} sensitisation of smooth muscle causing contraction, gene expression and increased proliferation [23].

These signalling processes may be different for different cell types. It is known that acute hypoxic exposure leads to early proliferation of pulmonary artery fibroblasts (PAF) and this proliferation appears to be dependent on the tumour suppressor protein (p38) MAPK [10–12] (fig. 1). p38 MAPK is among the key mechanisms that transmit signals from the cell surface to the nucleus, and belongs to the Ras/extracellular-signal-regulated kinase signalling pathway [24, 25]. p38 MAPK can directly influence gene transcription with a growing number of transcription factors known to be direct targets of p38 (activating transcription factor (ATF)-1, ATF-2, ATF-6, myocyte factor (MEF)2C, MEF-A, signalling lymphocytic activation molecule associated protein-1A and others) [26]. Another important target of p38 MAPK is the tumour suppressor protein itself, p53 [27]. A link has also been established between p38 MAPK and hypoxia inducible factor (HIF)-1α, the key transcription factor in the biochemical response to hypoxia. There was a reduction in HIF-1α expression in human PAF (HPAF) cells grown in conditions of acute hypoxia if the cells were pre-incubated with SB203580, a specific p38 MAPK inhibitor [28]. The relationship between p38 MAPK and HIF-1α is an attractive explanation for the role of p38 MAPK in hypoxia-mediated HPAF proliferation as HIF-1α is known to be responsible for the upregulation of hypoxia-sensitive gene products [26]. The mechanism of this relationship is unclear at present; HIF-1α may be a downstream effector of p38 MAPK or p38 MAPK may contribute towards HIF-1α stability.

Reduction of apoptosis also plays an important role in the remodelling of the pulmonary vasculature [29, 30]. A characteristic of human pulmonary arterial hypertension (PAH) and experimental PAH in rodents is loss of voltage-gated K^+ channel (Kv) current [31] due to a decreased expression of certain Kv channels [32–34]. Chronic Kv downregulation precipitates hypertrophy and hyperplasia of SMC and prevents removal of cells, by reducing apoptosis rates [30].

It is important to note that there are instances where remodelling of pulmonary resistance vessels occurs in the absence of significant PH. For example, in humans with COPD, marked thickening of the walls of the pulmonary arteries has been observed in the absence of PH [35]. In chronically infected rat lungs where neither PH nor right ventricular hypertrophy was observed, pulmonary vessel walls were thickened [36, 37]. One possible explanation for these findings is that this thickening occurred in an outward direction such that it did not lead to reduction of the vessel lumen. This type of outward remodelling, called compensatory enlargement, has been well described in the systemic circulation [38, 39].

MODELS UTILISED TO STUDY PULMONARY VASCULAR REMODELLING

A number of models have been used to investigate the mechanisms underlying pulmonary vascular remodelling, of which hypoxia and monocrotaline are the most widely used. Hypoxia is a more physiological model than monocrotaline-induced PH for the study of pulmonary vascular remodelling. Monocrotaline-induced PH does not occur in nature whereas hypoxia is a pathological stimulus leading to the development of PH at high altitude or as a consequence of hypoxic lung disease at sea level.

Other, less commonly used, models of PH include systemic-to-pulmonary artery shunts and VEGF receptor-2 blockade by SU5416, which is a cause of pulmonary arterial endothelial cell death [2, 40, 41].

Monocrotaline

Monocrotaline (pyrrolizidine alkaloid) is a plant-derived toxin (from plants of the *Crotalaria* species). When injected into rats as a single *i.p.* dose it causes endothelial cell injury and subsequently a massive mononuclear infiltration into the perivascular regions of arterioles and muscular arteries. These animals develop severe PH in the 2–3 weeks following monocrotaline exposure [42], leading to the development of right ventricular hypertrophy progressing to right ventricular failure [43]. Although typical plexiform lesions are not normally found in monocrotaline-induced PH, it is used as a standard model for both secondary and primary PH [44].

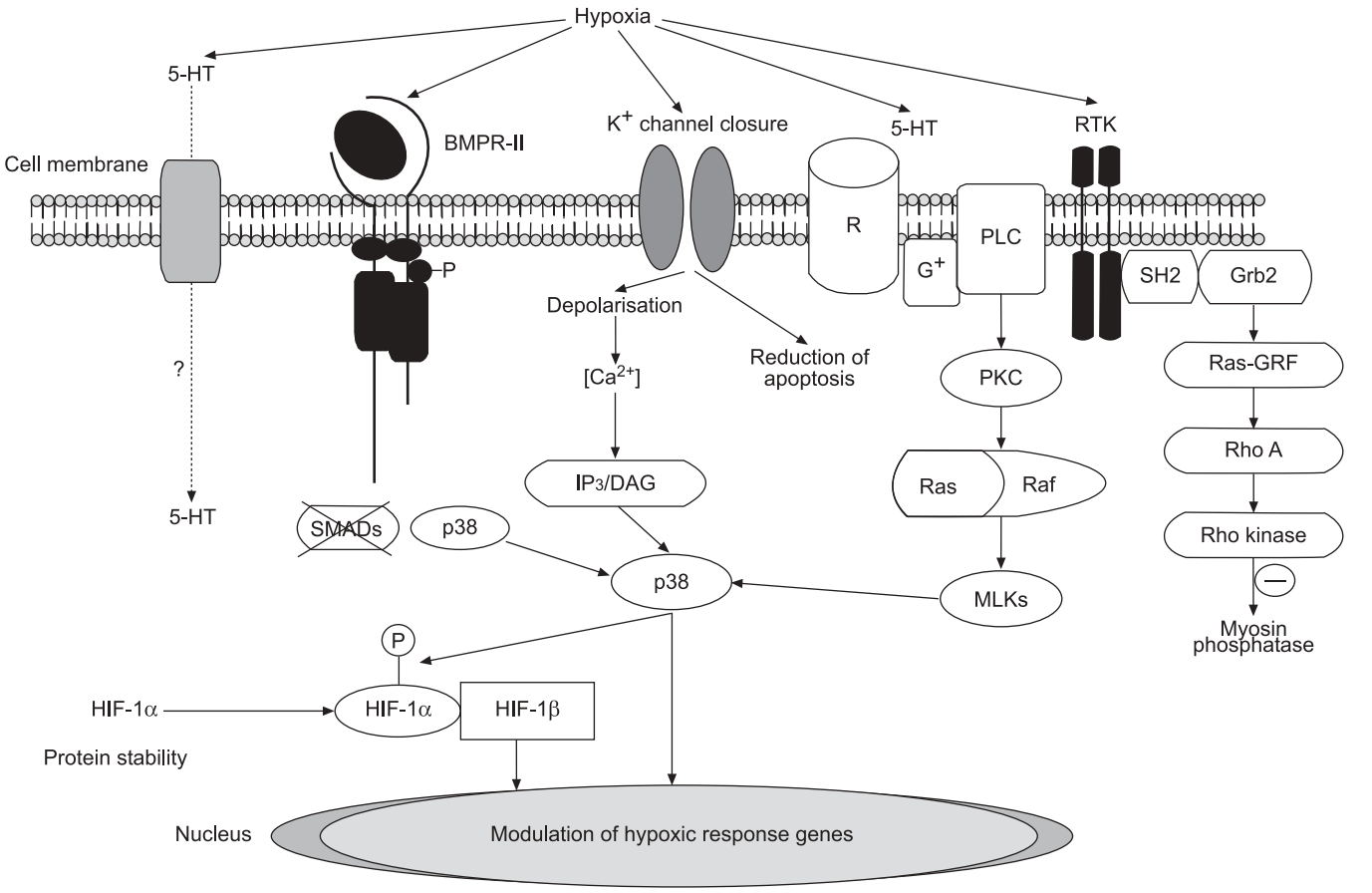

FIGURE 1. A schematic diagram illustrating the hypoxic signalling pathways that lead to vascular remodelling of the pulmonary artery. 5-HT: 5-hydroxytryptamine; BMPR-II: bone morphogenetic protein type II receptor; RTK; receptor tyrosine kinase; P: phosphate; R: receptor; G: g-protein; PLC: phospholipase C; SH: Src homolgy; Grb: growth factor receptor-bound protein; GRF: guanine nucleotide releasing factor; PKC: protein kinase C; IP3: inositol 1, 4, 5 trisphosphate; DAG: diacylglycerol; MLK: mixed lineage kinase; HIF: hypoxia-inducible factor.

Hypoxia

Hypoxia is the most commonly used model in the study of pulmonary vascular remodelling. The effect of acute hypoxia on vascular remodelling is generally studied by the culture of vascular cells. Cells are usually exposed to normobaric hypoxia (0–10% oxygen), for 4–24 h, with measurement of cell proliferation or the release of mitogenic factors [11].

Chronic hypoxia is usually studied *in vivo* in animals exposed to hypoxic conditions for days to weeks. In the most commonly used model, rats are exposed to normobaric (10% oxygen) or hypobaric (320 mmHg or 42.6 kPa) hypoxia for 2–3 weeks, typically leading to a 50% increase in mean PA pressure (PAP), and a doubling in weight of the right ventricle [45].

Acute hypoxia is a stimulus for pulmonary vasoconstriction [3, 46], a physiological process where regional hypoxia serves as an important regulatory mechanism in redirecting blood flow to better-oxygenated lung regions. Chronic global hypoxia, however, is a cause of PH. Pulmonary haemodynamic measurements performed in children and young adults resident at high altitude (partial pressure of oxygen in inspired air falls with increasing terrestrial elevation above sea level), show persistence of elevated PAP [47, 48]. Histological examination of pulmonary vessels in high-altitude residents who died from causes other than chronic pulmonary sickness shows typical patterns of pulmonary hypertension [49, 50]. Other causes of chronic hypoxia are the pathological conditions and diseases leading to alveolar hypoxia, including COPD, cystic fibrosis, bronchiectasis and asthma. Early studies demonstrate that 6% of patients with COPD develop cor pulmonale each year [51]. Mean PAP in patients with COPD increases slowly (0.28 mmHg·yr^{-1}) with 25% of all patients having a resting PAP of >20 mmHg [52]. Although these pressures are not high by the standards of idiopathic PH, these pressures are much higher during sleep, exacerbations of the lung disease or on exercise. Extrapolation of these data suggests that a significant number of patients with COPD will develop PH over the course of their disease and will have increased morbidity and mortality as a result.

Hypoxia-induced PH is characterised by a marked increase in pulmonary vascular resistance due to both vasoconstriction and remodelling. Muscular arteries in rats exposed to chronic hypoxia double their thickness, and distal extension of pulmonary artery smooth muscle cells (PASMC) into normally nonmuscular arteries can be observed [6]. Numerous investigations of human and animal models have shown that chronic hypoxia is a trigger of PA remodelling [3, 53]. It is generally

believed that the contribution of vasoconstriction is greatest early in the disease process and that structural remodelling of the pulmonary vascular bed becomes progressively more important with time. The concept that structural change is an important determinant of increased resistance and pressure in chronic PH is supported by observations that, after prolonged exposure to hypoxia, acute re-exposure to normal or even high levels of inspired oxygen are ineffective in reducing PAP.

EFFECTS OF HYPOXIA ON VASCULAR CELLS AND PULMONARY VASCULAR REMODELLING

Effects of hypoxia on EC and PA remodelling

The EC layer forms a permeable barrier between circulating blood cells and the underlying vascular tissue, which is composed of fibroblasts and SMC. As such, it is in a unique position to respond to circulating factors, and serves as a signal integrator and transducer to modulate events in the vasculature *via* paracrine effects. EC and SMC appear to cooperate intricately in various physiological events, including the control of vascular tone and cellular growth [54].

Effect of acute hypoxia on EC

There is little information in the literature about the influence of acute hypoxia on pulmonary EC proliferation. During acute hypoxic exposure, EC division slows but does not arrest; progression through the G-to-S transition point and/or progression from the S-to-G2/Mitosis phase of the cell cycle is altered with an increased proportion of EC in the S phase [55].

Effect of chronic hypoxia on EC

EC proliferation is increased by chronic hypoxia. In the chronically hypoxic rat model of PH, there is an increase in the number of EC in both the main PA and in the small muscular arteries [9, 53]. In hypoxic neonatal calves, the endothelial proliferative index is enhanced after 14 days' exposure to 8% oxygen [56]. In primary PH (idiopathic PAH), this EC proliferation leads to the formation of plexiform lesions [57, 58]. It is not known whether hypoxia-induced EC proliferation may lead to similar lesions, but other stimuli such as mechanical stretch or shear stress are also likely to be important.

Effects of hypoxia on SMC and PA remodelling

Medial thickening is the main determinant of pulmonary vascular resistance. Pre-capillary segments of the pulmonary vascular bed contribute the majority of pulmonary vascular resistance. It therefore follows that small changes in tone and/or structure in this area can lead to a large elevation of PAP. These vessels are normally only partially muscularised, although hypoxic pulmonary vascular remodelling leads to enhanced muscularisation [53], hence these vessels are a key feature of hypoxic pulmonary vascular remodelling.

Effect of acute hypoxia on SMC

There is much contradiction within the scientific literature about the influence of acute hypoxia on cultures of PASMC *in vitro*. It is unclear whether hypoxia has direct mitogenic or comitogenic effects on PASMC, whether hypoxia induces

TABLE 1 Influence of acute hypoxia on pulmonary artery smooth muscle cell (PASMC) proliferation

First author [Ref.]	Acute hypoxia stimulates PASMC proliferation				Acute hypoxia decreases or does not influence PASMC proliferation			
	O_2 %	FCS %	Cell density on plates cells·cm^{-2}	Proliferation	O_2 %	FCS %	Cell density on plates cells·cm^{-2}	Proliferation
Frid [64]	3	10	~10000	↑ L1, L3R*	3	0.1	~10000	↔$^{\#}$
					3	10	~10000	↓ L2, L3S*
Cooper [65]	5	1, 2 and 5	5000	↑*	0	2	5000	↓
Yang [71]	0	10	3.4	↑*¶	0	10	6800	↓*
Lanner [60]	3	5	~5000	↑	3	0.1	~5000	↔
Benitz [62]	5–10	20	2500	↑				
Tamm [63]	3	5		↑*				
Ambalavanan [66]	1, 2, 3, 5, 7 and 10	10	10000	↑ 1, 5, 7, 10*				
Stotz [67]	5	1	4000	↑				
Lu [68]	2	5	2500	↑*				
Frank [69]	1			↑*				
Preston [70]	3	10, 0.1	2000–4000	↑*				
Hassoun [72]					0	10	~5000	↓
					3 and 10	10	~5000	↔
Dempsey [59]					3	0.1	~25000–50000	↔
Eddahibi [17]					0	0.2	~25000	↓*
Stiebellehner [61]					3	10	~25000	↓
					3	0.1	~25000	↔
Rose [73]					1	? (serum-free)		↓

FCS: foetal calf serum; L1: inner media, subendothelial layer; L3: outer media; R: rounded epithelioid cells; L2: middle media; S: spindle-shaped cells; ↑: increase of smooth muscle cells proliferation; ↓: decrease of proliferation; ↔: no difference. *: $p<0.05$, significant change; $^{\#}$: all cells; ¶: hypoxia-selected cells.

PASMC to produce an autocrine growth factor or whether hypoxia induces adjacent cells (EC or fibroblasts) to produce factor(s) that stimulate SMC proliferation. Many investigators have shown that, either acute hypoxia is not a direct stimulus for PASMC proliferation [59, 60], or it actually decreases PASMC proliferation [17, 61]. However, others have shown that acute hypoxia alone is an effective mitogenic stimulus for PASMC [62–70]. The present authors reviewed the current literature where investigators had studied the effect of acute hypoxia on PASMC proliferation (table 1).

There are several possible reasons for these contradictory findings.

Variation in the source of the PA cells chosen for study

It is known that hypertrophy of PASMC makes a greater contribution than hyperplasia in the larger, more proximal arteries, whereas hyperplasia is more important in the smaller resistance arteries [6, 7]. However, even experiments using explants from the same part of the pulmonary arterial tree have given conflicting results. For example, DEMPSEY *et al.* [59] found that hypoxia did not stimulate proximal PASMC proliferation, whereas AMBALAVAN *et al.* [66] showed that proximal PASMC proliferated at oxygen concentrations of 5–10%. STIEBELLEHNER *et al.* [61] found that hypoxia did not stimulate distal PASMC proliferation, whereas STOTZ *et al.* [67] showed a 5–10% increase in proliferation rates of pulmonary microvascular SMC in the face of acute hypoxia.

Phenotypic variations of SMC within the arterial media

Recent studies of the pulmonary circulation have demonstrated that morphologically, physiologically and immunohistochemically distinct SMC phenotypes exist within the arterial media of the PA [64]. FRID *et al.* [64] isolated four phenotypically unique subpopulations from the inner, middle, and outer compartments of the arterial media of the PA and showed that that cells from the inner medial subendothelial layer (L1) and those from the outer medial layer (L3R) exhibited a highly proliferative phenotype and, unlike traditional SMC, proliferated under hypoxic conditions. Conversely, cells from the middle medial (L2) and outer medial (L3S) layers had a decreased proliferative response under hypoxic conditions when compared with normoxia.

Severity of hypoxia

Investigators who found a positive correlation between acute hypoxia and PASMC proliferation tended to use moderate levels of hypoxia (1–5% oxygen) [62, 63, 65–69], whereas those who found that hypoxia caused a decrease in PASMC proliferation used severe hypoxia or even anoxia [17, 65, 71, 72].

Seeding density of SMC in cell culture plates

The current authors found that in all studies of acute hypoxia where increased proliferation was observed, PASMC were seeded at a density $\leqslant$5,000 cells·cm^{-2} [62, 65, 67, 68, 70] whereas in all studies where hypoxia caused a decrease in proliferation, cells were seeded at a density of $>$10,000 cells·cm^{-2} [17, 59, 61, 64]. It is likely that contact inhibition would appear earlier in experiments where cells were seeded at higher densities, and KUEHL *et al.* [74] showed a correlation between cell proliferation in response to hypoxia and seeding density.

Concentration of serum in the cell culture plates

In studies showing that acute hypoxia increased PASMC proliferation *in vitro*, the serum concentration used for stimulation of proliferation was usually $>$2% (nine out of 11 studies), whereas in those showing no change, or even a decrease in proliferation with hypoxia, the serum concentration used was often $<$2% (six out of nine studies) [62, 63, 65–69].

Effect of chronic hypoxia on SMC

Chronic hypoxia leads to proliferation of PASMC *in vivo*. Histological and morphological analysis of PAs of animals exposed to chronic hypoxia, and humans who died from high altitude PH, showed a significant thickening of the muscular layers [42, 75]. Chronic hypoxia also causes extension of SMC into normally nonmuscular arteries.

Effects of hypoxia on fibroblasts and PA remodelling

An increasing volume of experimental data supports the view that adventitial fibroblasts play an important role in pulmonary vascular remodelling. The vascular adventitia can act as a biological processing centre for the production, storage and release of key regulators of vessel wall function. In response to stress or injury (*e.g.* hypoxia), resident adventitial cells can be activated and reprogrammed to exhibit different functional and structural behaviours, which include proliferation, differentiation, upregulation of contractile and extracellular matrix proteins and release of factors that directly affect medial SMC tone and growth.

Effect of acute hypoxia on fibroblasts

Investigators have shown that acute hypoxia is a direct trigger for fibroblast proliferation *in vitro* in rat, bovine and human models in the presence or absence of exogenous mitogens [8]. Exposure to acute hypoxia leads to fibroblast proliferation and hypertrophy [10–12]. In these models, hypoxic proliferation of fibroblasts has been shown to last longer and exceed that of PAEC or SMC [76].

Effect of chronic hypoxia on fibroblasts

Proliferation of adventitial fibroblasts occurs before that of other cell types in animal models of chronic hypoxia [4]. In small pulmonary arteries, fibroblasts increase production of extracellular matrix proteins (type I collagen and elastin), which contributes to the narrowing of the vascular lumen [77]. In addition, there is an early and dramatic upregulation of collagen, fibronectin and tropoelastin mRNA followed by the subsequent deposition of these proteins [78].

Repair of injured tissue is an essential requirement for any living organism. PH is a haemodynamic stress, leading to injury of the arterial wall. SMC, EC and fibroblasts in the pulmonary vascular wall play specific roles in this response to this injury. Fibroblasts are in a unique position for this role, since they are less differentiated and have remarkable plasticity, allowing for rapid migration, proliferation, synthesis of connective tissue, contraction, cytokine production and, most importantly, transdifferentiation into other types of cells [8]. Hypoxia-induced changes in fibroblast proliferative and matrix-producing phenotypes are accompanied by the appearance of smooth muscle α-actin in tissues from pulmonary hypertensive subjects, suggesting that fibroblasts can be

transdifferentiated into myofibroblasts [8]. This transdifferentiation involves a complex network of microenvironmental factors and pathways in which extracellular matrix components along with growth factors, cytokines and adhesion molecules play a role. In rats exposed to hypoxia, pre-capillary vessels of a diameter of ~25 μm, which are normally devoid of SMC, begin to generate this cell type from adventitial fibroblasts within 24 h [79]. Light microscopy of nonmuscular arterioles after exposure to hypoxia shows that smooth muscle begins to form by day 2 at simulated altitude, the proportion of muscularised arterioles increasing along with increasing PAP [80]. Interestingly, these studies show that after return to normoxia, SMC persist in normally nonmuscularised arterioles, suggesting that smooth muscle cells may remain for long periods after exposure to hypoxia.

The pulmonary vascular adventitia of neonatal calves has been found to contain multiple, and functionally distinct, subpopulations of fibroblasts [78]. Proliferation under hypoxic conditions is highly variable among these subpopulations, with some exhibiting more than a two-fold increase in DNA synthesis, while others show a decrease in DNA synthesis. These observations suggest that hypoxia specifically selects certain phenotypically and functionally distinct subpopulations of fibroblasts to act as stem cells for the vascular wall. Since each subpopulation of fibroblasts responds uniquely to hypoxia, they may serve special functions in response to injury. Thus, the adventitial fibroblasts residing in the vessel wall may be a critical regulator of vascular remodelling under hypoxic conditions.

INTERACTIONS BETWEEN FIBROBLASTS, SMC AND EC

Studies have shown that EC secrete mitogenic factors under hypoxic conditions that induce SMC proliferation. Bovine aortic EC have been shown to secrete several SMC growth factors, including PDGF, endothelial-derived growth factor, insulin like growth factor 1, fibroblast growth factor and IL-1 [13, 14, 81, 82]. It is known that EC produce PDGF-B in response to hypoxia [12], but SMC do not [66]. It has also been shown that increased ET-1 expression in pulmonary vascular EC appears to be transcriptionally mediated by hypoxia [13, 14]. The expression of such vasoactive agents as ET-1 and PDGF-B is dramatically increased in EC exposed to low oxygen tension [14] and, similarly, VEGF is induced in SMC. In addition to increasing the release of growth factors, hypoxia stimulates the production of some extracellular matrix proteins, such as thrombospondin-1 in human EC [83]. Thrombospondin-1 modulates SMC proliferation and migration and may be a negative regulator of angiogenesis [84]. Because of their localisation at the interface between blood and tissue, EC are responsible for the maintenance of vascular homeostasis. They fulfil a series of functions and constantly interact with circulating leukocytes and SMC present in the media. Any disturbance of their metabolism can thus lead to alterations of blood vessel function [85].

The addition of conditioned medium, obtained from pulmonary EC exposed to hypoxia, to cultures of quiescent PASMC has been shown to result in a significant increase in total cell number [86, 87]. Conversely, others have shown that hypoxic bovine PAEC release an inhibitor of PASMC growth isolated from the main PA of calves [72].

A strong proliferative response of PASMC was noted when SMC were co-incubated with adventitial fibroblasts from human and rat PA [73]. There is increasing experimental evidence demonstrating that fibroblasts exert significant paracrine effects on other cells. Fibroblasts are known to produce a wide array of cytokines, growth factors (transforming growth factor-β, epithelial growth factor, insulin-like growth factor, PDGF) and inflammatory mediators, which function as paracrine regulators of neighbouring cell (endothelial, SMC and epithelial) proliferation [88]. It has been established that matrix proteins such as collagen, fibronectin and proteoglycans play a prominent role in migration, proliferation and differentiation [19]. It is therefore possible that secretion of mitogenic factors by hypoxic fibroblasts results in proliferation of neighbouring PASMC.

SUMMARY

Acute hypoxia has direct proliferative effects on pulmonary artery fibroblasts but many *in vitro* studies have not shown this effect in pulmonary artery smooth muscle or endothelial cells. Quiescent pulmonary artery smooth muscle cells may require a priming step for acute hypoxia-induced proliferation. Acute hypoxia may decrease pulmonary artery endothelial cell proliferation, but hypoxic endothelial cells and adventitial fibroblasts can release factors that are mitogenic for smooth muscle cells, and moderate-acute hypoxia can enhance the proliferative effects of peptide growth factors and other growth stimulants. It is feasible that endothelial cells or fibroblasts may sense hypoxia and be a key determinant of pulmonary artery smooth muscle cell proliferation.

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