Review

Oxygen-regulated transcription factors and their role in pulmonary disease

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Abstract

The transcription factors nuclear factor interleukin-6 (NF-IL6), early growth response-1 (EGR-1) and hypoxia-inducible factor-1 (HIF-1) have important roles in the molecular pathophysiology of hypoxia-associated pulmonary disease. NF-IL6 controls the production of interleukin (IL)-6 in vascular endothelial cells, which may have anti-inflammatory activity by counteracting effects of IL-1 and IL-8. EGR-1 controls the production of tissue factor by macrophages, which triggers fibrin deposition in the pulmonary vasculature. HIF-1 activates the expression of the vasoconstrictor endothelin-1 in vascular endothelial cells. Angiotensin II induces HIF-1 expression and hypertrophy of pulmonary arterial smooth muscle cells. HIF-1 might therefore have multiple roles in the pathogenesis of pulmonary vascular remodeling.

Keywords: EGR-1, HIF-1, NF-IL6, vascular remodeling

Introduction

Chronic lung disease is a major cause of morbidity and mortality in western populations. It is the fourth leading cause of death in the USA, accounting for 5% of all deaths annually. Although many patients with chronic obstructive lung disease die from infections, the development of alveolar hypoxia is a complication that is also associated with increased mortality. Recent studies have begun to delineate the molecular responses to hypoxia within the lung and its vasculature. An understanding of the molecular pathophysiology of hypoxia-associated pulmonary disease might lead to strategies for the prevention and/or treatment of life-threatening complications. The pathophysiology of chronic diseases must ultimately be understood in terms of changes in gene expression that are mediated by transcription factors. The transcription of several dozen target genes is known to be induced in hypoxic cells (Table 1). Similarly, the activities of several transcription factors are induced in mammalian cells subjected to hypoxia, including activator protein-1 (AP-1), early growth response-1 (EGR-1), hypoxia-inducible factor-1 (HIF-1), high-mobility group (HMG) I(Y), nuclear factor interleukin-6 (NF-IL6), and NF-κB, although the mechanisms by which hypoxia is sensed and the signal is transduced remain enigmatic [1]. This review focuses on the transcription factors EGR-1, HIF-1, and NF-IL6, which are important in the pathophysiology of hypoxic lung disease.

AP-1 = activator protein-1; ECs = endothelial cells; EGR-1 = early growth response-1; ET-1 = endothelin-1; HIF-1 = hypoxia-inducible factor-1; HMG = high-mobility group; IL = interleukin; NF-IL6 = nuclear factor interleukin-6; PAS = Per/ARNT/Sim; SMC = smooth muscle cell; VEGF = vascular endothelial growth factor.

Table 1

Hypoxia-inducible gene expression

Gene product	Transcription factor*
Adenylate kinase 3	HIF-1
α_{1B}-Adrenergic receptor	HIF-1
Adrenomedullin	HIF-1
Aldolase A	HIF-1
Aldolase C	HIF-1
Carbonic anhydrase-9	HIF-1
Ceruloplasmin	HIF-1
Cyclooxygenase-2	HMG I(Y), NF-κB
Endothelin-1	HIF-1
Enolase-1	HIF-1
Erythropoietin	HIF-1
GADD153	Not determined
Glucose transporter-1	HIF-1
Glucose transporter-3	HIF-1
Glyceraldehyde-3-phosphate dehydrogenase	HIF-1
Heme oxygenase-1	AP-1, HIF-1
Hexokinase-1	HIF-1
Hexokinase-2	HIF-1
Insulin-like growth factor-2 (IGF-2)	HIF-1
IGF-binding protein-1	HIF-1
IGF-binding protein-2	HIF-1
IGF-binding protein-3	HIF-1
Interleukin-6	NF-IL6
Lactate dehydrogenase A	HIF-1
Nitric oxide synthase-2	HIF-1
NIP3	HIF-1
Ornithine decarboxylase	Not determined
p21	HIF-1
p27	Not determined
p35srj	HIF-1
Phosphofructokinase L	HIF-1
Phosphoglycerate kinase-1	HIF-1
Plasminogen activator inhibitor-1	HIF-1
Prolyl-4-hydroxylase α(I)	HIF-1
Pyruvate kinase M	HIF-1
Tissue factor	EGR-1
Transferrin	HIF-1
Transferrin receptor	HIF-1
Transforming growth factor β_3	HIF-1
Triosephosphate isomerase	HIF-1
Tyrosine hydroxylase	AP-1
Vascular endothelial growth factor (VEGF)	HIF-1
VEGF receptor FLT-1	HIF-1

*See [12] for literature citations.

NF-IL6

NF-IL6 (also known as C/EBPβ, for CCAAT-enhancer-binding protein-β) is a basic-leucine-zipper transcription factor that activates *IL-6* gene transcription in hypoxic pulmonary vascular endothelial cells (ECs) [2]. The NF-IL6-binding site from the *IL-6* promoter was sufficient to direct the expression of a *lacZ* gene in the heart, kidney, and lung, but not the liver, of transgenic mice subjected to hypoxia or ischemia [3]. The basis for the tissue-specific induction of NF-IL6 expression in vascular ECs is not known. Because IL-6 is a cytokine with anti-inflammatory properties, it might counteract the effects of proinflammatory cytokines. For example, the expression of IL-1 and IL-8 are also induced in hypoxic ECs [4,5] and promote the expression of adherence molecules that recruit activated leukocytes to the vessel wall, leading to vascular leakage and/or thrombus formation [6•]. Thus, NF-IL6 might protect the integrity of the pulmonary vasculature under conditions of hypoxia or ischemia.

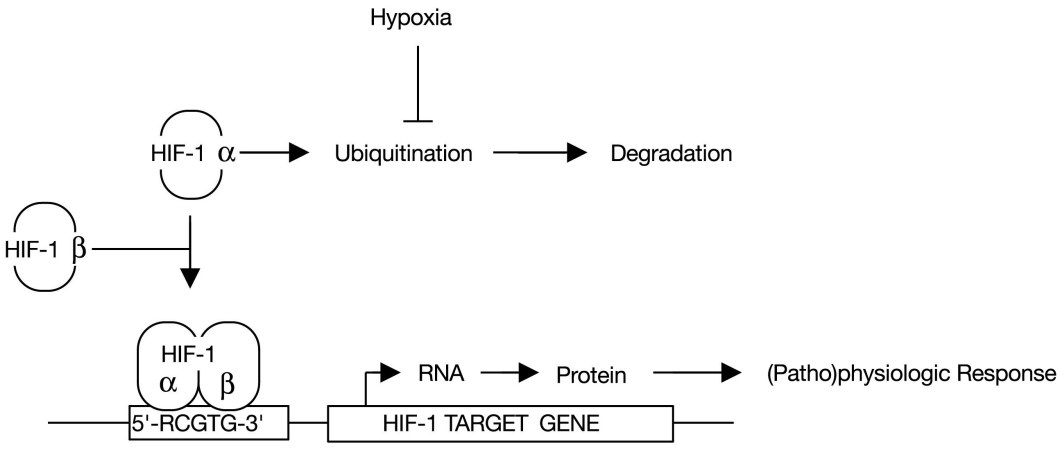
EGR-1

EGR-1 (also known as ZIF268) is a zinc-finger transcription factor that is expressed in hypoxic mononuclear phagocytes [7]. EGR-1-mediated production of tissue factor by monocytes leads to hypoxia-induced fibrin deposition in the pulmonary vasculature [8]. In the lungs of mice subjected to hypoxia, EGR-1 and tissue factor are co-induced in bronchial and vascular smooth muscle and alveolar macrophages [9]. When wild-type mice are exposed to an ambient O_2 concentration of 6% (reduced from the 21% O_2 in room air), EGR-1 and tissue factor expression are induced in the lung and marked vascular deposition of fibrin is observed, whereas none of these responses occur in knockout mice lacking expression of the gene encoding protein kinase C-β [10••]. The mechanism by which hypoxia activates protein kinase C-β activity in mononuclear phagocytes is unknown but this activity seems to be crucial for the induction of EGR-1 activity and tissue factor production. EGR-1 also mediates tissue factor production by endothelial cells in response to stimulation by vascular endothelial growth factor (VEGF) [11], the hypoxia-induced expression of which is mediated by HIF-1 (see below), indicating that hypoxia induces tissue factor expression by two different mechanisms that both involve EGR-1. Thus, in contrast to NF-IL6, hypoxia-induced EGR-1 activity promotes pulmonary vascular thrombosis.

HIF-1

Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric basic helix–loop–helix–Per/ARNT/Sim (PAS)-domain transcription factor composed of HIF-1α and HIF-1β subunits [12]. In contrast to NF-IL6 and EGR-1, HIF-1 is expressed in most, if not all, nucleated mammalian cells in response to hypoxia; several dozen target genes have been identified, including VEGF (Table 1). HIF-1β is expressed constitutively, whereas HIF-1α expression in the lung is

Figure 1



HIF-1-mediated gene expression in response to hypoxia. Under non-hypoxic conditions, the HIF-1α subunit is subject to ubiquitination and degradation, whereas under hypoxic conditions HIF-1α ubiquitination is inhibited, resulting in the accumulation of HIF-1α, which dimerizes with HIF-1β. The heterodimer recognizes the core binding-site sequence 5′-RCGTG-3′ (R, purine) found within the hypoxia response element of a target gene. Once bound, HIF-1 interacts with the transcription initiation complex (consisting of RNA polymerase II and associated factors) via coactivators including CBP, P300, SRC-1, and TIF-2 (see [12] for details and literature citations).

regulated by the inspired O_2 concentration [13] (Fig. 1). Homozygous-null knockout mice that completely lack HIF-1α expression die at mid-gestation owing to the failure of embryonic vascularization [14–16]. Mice heterozygous for the null allele (*Hif1a*$^{+/-}$), and thus partly deficient for HIF-1α expression, develop normally and are indistinguishable from their wild-type littermates under normoxic conditions. Wild-type mice exposed to 10% O_2 develop pulmonary hypertension in response to chronic hypoxia. Medial wall thickening in pulmonary arterioles results in increased pulmonary arterial pressure and right ventricular hypertrophy. The remodeling is progressive and if continued leads to cor pulmonale. In *Hif1a*$^{+/-}$ mice, the hypoxia-induced muscularization of pulmonary arterioles is significantly impaired, resulting in significantly less medial wall thickening, pulmonary artery hypertension, and right ventricular hypertrophy after 3 weeks at 10% O_2 [17••].

Although the pathophysiology of pulmonary hypertension is complex [18], a major component of this process is the elaboration of peptides, such as endothelin-1 (ET-1) and angiotensin II, that induce smooth muscle cell (SMC) contraction and hypertrophy. ET-1 expression is induced within the pulmonary vasculature of hypoxic rats [19], and ET_A-receptor antagonists prevent/reverse chronic hypoxia-induced pulmonary hypertension [20]. A HIF-1-binding site in the *ET-1* gene promoter is required for hypoxia-induced transcription [21•], suggesting that ET-1 mRNA expression by hypoxic pulmonary vascular ECs is mediated by HIF-1.

Expression of angiotensin-converting enzyme (ACE), which converts angiotensin I to angiotensin II, is also induced within the pulmonary vasculature of hypoxic rats [22]. The administration of captopril, an ACE inhibitor, or losartan, a type 1 angiotensin receptor antagonist, also attenuates the development of hypoxic pulmonary hypertension [23]. Angiotensin II, which induces vascular SMC hypertrophy, has recently been shown to induce HIF-1α expression [24••]. These results suggest that HIF-1 might be required for the angiotensin-induced hypertrophy of vascular SMCs in the hypoxic lung.

In addition to SMC hypertrophy, hypoxia is associated with increased pulmonary vascular tone, which also reduces luminal diameter. One determinant of vascular tone is the production by ECs of ET-1, a potent SMC vasoconstrictor. The activity of voltage-gated potassium (K_V) channels is another important determinant of SMC membrane potential and pulmonary vasomotor tone. Acute hypoxia inhibits K_V channel activity, resulting in the depolarization and vasoconstriction of pulmonary artery SMCs. Chronic hypoxia is associated with downregulation of $K_V1.2$ and $K_V1.5$ mRNA expression [25], but the involvement of HIF-1 or another hypoxia-induced transcription factor in this process has not yet been demonstrated.

Conclusion

NF-IL6, EGR-1, and HIF-1 mediate important physiological responses to chronic hypoxia in macrophages and pulmonary vascular ECs and SMCs. NF-IL6 might mediate adaptive anti-inflammatory responses, but definitive studies with knockout mice have not been performed. In contrast, analysis of *Egr1*$^{-/-}$ and *Hif1a*$^{+/-}$ mice has provided definitive evidence that EGR-1 and HIF-1 mediate pathophysiologic responses to chronic hypoxia, suggesting that pharmacologic inhibition of these factors might be

useful in the prevention or treatment of hypoxia-induced pulmonary vascular pathology.

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