



The role of hypoxia-inducible factor 1 in atherosclerosis

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ABSTRACT

Atherosclerosis is by far the most frequent underlying cause of coronary artery disease, carotid artery disease and peripheral arterial disease, and is associated with high morbidity and mortality. Hypoxic areas are known to be present in human atherosclerotic lesions, and lesion progression is associated with the formation of lipid-loaded macrophages, increased local inflammation and angiogenesis. The key regulator of hypoxia, hypoxia-inducible factor 1 (HIF-1), plays a key role in the progression of atherosclerosis by initiating and promoting the formation of foam cells, endothelial cell dysfunction, apoptosis, increasing inflammation and angiogenesis. The objective of this review is to summarise the pathological role of HIF-1 in the progression of atherosclerosis.

Disruption of oxygen homeostasis has been postulated to contribute to atherosclerosis. As the atherosclerotic lesion develops, the arterial wall thickness increases and oxygen diffusion into the intima is markedly reduced. When cellular oxygen availability decreases, the transcription factor hypoxia-inducible factor 1 (HIF-1) plays a central role in hypoxic cellular adaptation. In addition, HIF-1 contributes to dysfunctions in various components of atherosclerosis, and increased local inflammation and angiogenesis (figure 1). The objective of this review is thus to provide insights into the accumulation mechanisms of HIF-1 and its roles in atherosclerosis, which may provide more efficient approaches in treating the disease.

HYPOXIA IS A FEATURE OF ATHEROSCLEROSIS

Evidence of hypoxia in atherosclerosis is supported by the in-vivo detection of hypoxia in macrophage regions in human atherosclerosis.¹ Hypoxia may promote lesion progression by promoting lipid accumulation, increased inflammation and angiogenesis.²

Early in atherogenesis, the atherogenic lipoproteins are cleared from the intima by the scavenging macrophages, giving rise to the accumulation of foam cell formation.² Hypoxia increases the formation of lipid droplets in macrophages and promotes increased secretion of inflammatory mediators, and lipid droplets may play a role in mediating the inflammatory response. Several articles have also suggested that hypoxia in the deep layer of plaque could induce angiogenesis by activating certain angiogenic proteins.^{3, 4}

MOLECULAR FEATURES OF HIF-1

HIF-1 is a ubiquitously expressed heterodimeric transcription factor that mediates adaptive responses to hypoxia in all nucleated cells of

metazoan organisms. HIF-1 consists of the rate-limiting factor HIF-1 α and the constitutively expressed HIF-1 β .^{5, 6} These proteins belong to the basic helix-loop-helix per-aryl hydrocarbon nuclear translocator Sim protein family.⁶ Interactions between the basic helix-loop-helix per-aryl hydrocarbon nuclear translocator Sim domains from the two subunits mediate their dimerisation, and individual basic regions of the two subunits then make contact with the hypoxia response element (HRE; 5'-RCGTG-3') DNA sequence.

HIF-1 β is constitutively expressed in the nucleus and its activity is not affected by hypoxia,⁷ whereas the HIF-1 α subunit has a short half-life (5 min) and is highly regulated by oxygen.⁸ The modification of HIF-1 α occurs within several domains such as the HRE DNA sequence two transactivation (stimulation of transcription) domains, N-terminal and C-terminal, and an oxygen-dependent degradation domain that mediates oxygen-regulated stability.⁹

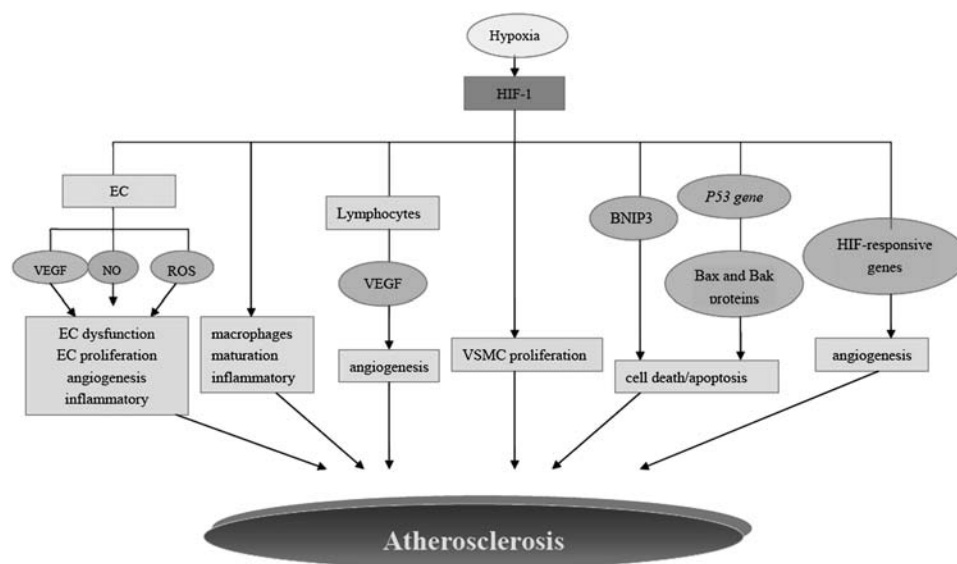
Under normoxic conditions, the HIF-1 α protein is degraded by ubiquitination and proteasomal degradation after hydroxylation of proline residues in the oxygen-dependent degradation domain. Under hypoxic conditions, the HIF-1 α protein accumulates and translocates to the nucleus where it heterodimerises with HIF-1 β to form an active transcription factor. The induction of HIF-1 by hypoxia takes place at the protein level, because HIF-1 α messenger RNA expression remains unchanged.

The genes regulated at the transcription level by HIF-1 are involved in a wide spectrum of cellular functional events, including angiogenesis, vascular reactivity and remodelling, vasomotor control, glucose and energy metabolism, cell proliferation and viability and nucleotide metabolism.¹⁰ Many of these functional processes are related to the pathogenesis of atherosclerosis. HIF-1 activates the expression of downstream genes by binding to a 50-base pair cis-acting HRE located in their enhancer and promoter regions.¹¹

HIF-1 AND ENDOTHELIAL CELLS IN ATHEROSCLEROSIS

Endothelial cells play a critical role in maintaining vascular homeostasis through their effects on vascular tone, recruitment of inflammatory cells, production of proinflammatory cytokines, maintenance of barrier and antithrombotic functions. Each of these homeostatic mechanisms is subject to perturbation when endothelium is exposed to hypoxia.¹² Hypoxia strongly affects the regulatory pathways of endothelial cells, leading to the activation of several transcription factors and to the release of cytokines and growth factors. Several

Figure 1 Main pathophysiological effects of HIF-1 in atherosclerosis. BNIP3, Bcl-2/E1B interacting protein; EC, endothelial cell; HIF, hypoxia-inducible factor; NO, nitric oxide; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.



studies have evaluated how HIF regulate functions of the hypoxic endothelial cells. The activation of HIF-1 allows the cells to survive by an adaptive modification of their energetic metabolism. On the other hand, HIF-1 induces expression of several angiogenic factors, including vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase, and platelet-derived growth factor. Enhanced expression of HIF-1 and VEGF, which colocalise to these same regions, provides strong evidence that endothelial cells proliferate and form vessels under hypoxic stimuli in the development of atherosclerosis.

Endothelial cell dysfunction plays an important role in the initiation and progression of atherosclerosis. Oxidative stress, overproduction of reactive oxygen species (ROS), plays a critical role in the pathogenesis of endothelial dysfunction.¹⁵ More recent evidence suggests that HIF-1 can be induced by free radicals, especially ROS. Analysis of events upstream of HIF-1 α suggests a crucial role for ROS generation upon hypoxia via mitochondria as a sensor mechanism of the oxygen level, and that ROS may be involved in stabilising HIF-1 α .¹⁴ ROS originating from the mitochondria has been attributed to triggering the increase of the transcription factor in human endothelial cells.^{15 16}

HIF-1 AND LEUCOCYTES IN ATHEROSCLEROSIS

The major risk factors of atherosclerosis induce the secretion of leucocyte-soluble adhesion molecules, which facilitate the attachment of monocytes to endothelial cells, and chemotactic factors, which in turn promote the monocytes' movement into the subintimal space. Monocytes differentiated into macrophages that then internalise large amounts of oxidised low-density lipoprotein forming cholesterol-laden macrophages called 'foam cells', which is an important process in atherosclerotic plaque development.¹⁷ In macrophages, both HIF-1 α and HIF-2 α expression are induced in response to hypoxia *in vitro*.^{18 19} Moreover, HIF-1 α appears to be required for macrophage maturation.^{20 21}

Lymphocytes are an important determinant in the development and progression of the atherosclerosis lesion. The pro-inflammatory response regulated by T-helper (Th) 1-mediated cytokine expression, mainly interferon- γ , was previously found to induce lipid and cell accumulation as well as apoptotic programmes within the plaque. In parallel, Th2 typical cytokines,

(ie, interleukin (IL)-10, IL-4) were shown to cross-regulate Th1 activity driving protective effects on lesion formation and stability.²² B lymphocytes and plasma cells are rare in the intimal plaque but may be abundantly present in adventitia next to advanced intimal disease.²³ Ben-Shoshan *et al*²² first evaluated the direct effects of HIF-1 α expression on the lymphocytic cytokine profile. The authors found a marked decrease in the transcription of interferon- γ accompanied by a parallel increase in IL-10 expression, and atherosclerotic plaque size and lipid core were significantly reduced in HIF-1 α -treated mice.

The role of polymorphonuclear leucocytes in progression of the atherosclerosis lesion has been discussed in detail by Soehnlein and Weber.²⁴ Polymorphonuclear leucocytes rely on glycolysis to generate ATP.²⁵ Studies of HIF-1 α -deficient neutrophils revealed that neutrophils require HIF-1 α to perform glycolysis.²⁶ Moreover, this HIF-mediated survival effect is also dependent on the activity of nuclear factor kappa B (NF- κ B). Migration of neutrophils involves a process of selectin-mediated rolling and β 2 integrin-mediated adhesion to endothelium.²⁷ It has been demonstrated that HIF-1 α regulates β 2 integrin (CD18 specifically) expression in these cells and thereby promotes neutrophil extravasation.²⁸

Dendritic cells (DC) are a heterogeneous set of antigen-presenting cells consisting of conventional DC, plasmacytoid DC, as well as inflammatory DC. DC form a network in the arterial intima of young healthy individuals.²⁹ The notion of the involvement of DC in atherosclerosis is further fuelled by the finding that the numbers of DC increase in advanced human plaques, and DC accumulation in lesions is associated with plaque growth and inflammation.³⁰ Recent work has revealed that hypoxia and HIF-1 α modulate DC maturation, activation and antigen-presenting functions.³¹ This DC activation was accompanied by HIF-1 α protein accumulation and enhanced glycolytic activity. Moreover, knockdown of HIF-1 α significantly reduced glucose uptake, inhibited maturation and led to an impaired capacity to stimulate allogeneic T cells.³¹

Mast cells are derived from CD34⁺CD117⁺ CD3⁻Fc ϵ R1⁻CD14⁻ progenitor cells.^{32–34} Recent studies demonstrated that activation of HIF-1 α in mast cells stimulates the expression of VEGF.^{35 36} From endothelial dysfunction to plaque rupture, VEGF directly and indirectly attracts inflammatory cells into the intima in various stages of atherogenesis.

HIF-1 AND SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS

Vascular smooth muscle cells (VSMC) are one of the major constituents of blood vessels. VSMC proliferation and migration that respond to vascular injury contribute to vessel narrowing and play an important role in the atherosclerotic process. It has been recognised that hypoxia is a stimulus to VSMC proliferation and migration, a process known as vascular remodelling.^{37–38} Several studies have demonstrated^{39–40} that HIF-1 α is essential to VSMC proliferation exposed to hypoxia.

The macrophage migration inhibitory factor (MIF) has recently emerged as a key factor in vascular remodelling and in the development and progression of atherosclerosis.^{41–44} Fu *et al*⁴⁵ reported that overexpression of HIF-1 α was able to upregulate MIF gene and protein expression under normoxia, whereas knockdown of HIF-1 α expression in human umbilical artery smooth muscle cells inhibited hypoxia-induced MIF expression.

HIF-1 AND CELL APOPTOSIS/DEATH IN ATHEROSCLEROSIS

During the progression of atherosclerosis, endothelial cells, macrophages and smooth muscle cells die by apoptosis or necrosis, which may lead to the formation of a destabilising lipid-rich core and a fragile and rupture-prone fibrous cap.⁴⁶ Furthermore, apoptosis contributes dramatically to the high tissue factor activity and thrombogenicity of the lipid-rich core.⁴⁷ HIF-1 α is involved in hypoxia-induced apoptosis. The detailed mechanisms have been elucidated by Greijer and van der Wall⁴⁸ and Giatromanolaki *et al*.⁴⁹

HIF-1 AND ANGIOGENESIS IN ATHEROSCLEROSIS

Because angiogenesis is a major consequence of hypoxic tissue, the presence of intraplaque angiogenesis suggests the existence of hypoxia in human atherosclerosis. Moreover, it has been suggested that hypoxia in the deep layer of plaque could induce angiogenesis by activating certain angiogenic proteins.^{3–4} HIF-1 is closely related to the angiogenic process.⁵⁰

A possible role for HIF in atherosclerosis is supported by the presence of intraplaque angiogenesis and the implication of several known HIF-responsive genes in atherosclerosis, such as VEGF,^{5–51} endothelin-1^{5–52} and matrix-metalloproteinase-2.^{5–53} HIF also plays a major role in mediating important alterations associated with atherogenesis and angiogenic activity of macrophages.¹ Under atherogenic conditions, the high expression of HIF-1 in macrophages promotes foam cell formation and atherosclerosis.⁵⁴

It is well established that HIF plays a major role in VEGF expression.⁵⁵ The accumulation of HIF-1 heterodimer complexes activates VEGF and genes necessary for cell metabolism in a low-oxygen environment. HIF-1 directly activates VEGF and VEGF receptor (VEGFR) transcription by binding to HRE. Deletion of HIF-1 α in endothelial cells disrupted an autocrine loop necessary for the hypoxic induction of both VEGFR-1 and VEGFR-2 by VEGF signalling.⁵⁶ A recent study⁵⁷ confirmed a pathological role of a VEGFR-1 ligand placental growth factor (PlGF) in atherosclerosis, whereby PlGF deficiency inhibited early-stage atherosclerosis and adventitial PlGF promoted intimal hyperplasia and vasa vasorum proliferation.

It is also shown that HIF-1 α induces angiogenesis by the expression of E26 transformation-specific 1 (Ets-1).^{58–59} VEGF and Ets-1 each enhance the expression of the other and cooperatively facilitate angiogenesis. Ets-1 also contributes to angiogenesis by the proliferation of endothelial cells and their conversion to the angiogenic phenotype.⁶⁰ Moreover, Ets-1

can accelerate plaque vulnerability by the expression of apoptosis-inducible ligands and proteinases including matrix metalloproteinases.^{61–62}

HIF-1 AND INFLAMMATION IN ATHEROSCLEROSIS

Atherosclerosis is a chronic inflammatory response in the walls of arteries, resulting from interactions between plasma lipoproteins, cellular components such as monocyte/macrophages, T lymphocytes, endothelial cells and smooth muscle cells as well as the extracellular matrix of the arteries.^{63–65} The above inflammatory response requires effector cells to adapt to stress induced by pro-inflammatory stimulation. HIF-1 transcription complex plays a pivotal role in cellular adaptation to inflammatory stress.⁶⁶ Atherosclerosis is in large part due to the accumulation of macrophages that take up oxidised low-density lipoproteins. In macrophages, HIF expressions are induced in response to hypoxia *in vitro*.^{18–19} Moreover, HIF-1 α appears to be required for macrophage maturation.^{20–21} Furthermore, overexpression of HIF-2 α in normoxic human macrophages leads to enhanced transcription of pro-angiogenic genes including VEGF, IL-8, platelet-derived growth factor β and angiotensin-like 4.⁶⁷

HIF are also induced by inflammatory cytokines. Pro-inflammatory cytokines, tumour necrosis factor alpha and IL-1 β , have been shown to increase the accumulation and transcriptional activity of HIF-1 α . Tumour necrosis factor alpha-induced HIF-1 α stimulation requires NF- κ B at the level of HIF-1 α protein stabilisation without affecting its mRNA level. Similarly, IL-1 β acts on HIF-1 α protein stability by triggering NF- κ B activity and inhibiting von Hippel–Lindau-mediated protein degradation. The fact that HIF can be activated in response to inflammatory cytokines indicates that HIF may play an important role in inflammation.⁶⁸

HIF-1 EXPRESSION IN ATHEROSCLEROTIC PLAQUE

Vink *et al*⁶⁹ observed HIF-1 α expression in 49% of carotid and 60% of femoral endarterectomy specimens. HIF-1 α was found in macrophages, macrophage-derived foam cells and to a lesser extent in smooth muscle cells. HIF-1 α knockdown has also been shown to block the formation of lipid-loaded macrophages,⁵⁴ indicating a role for HIF-1 α in lipid accumulation. Furthermore, NF- κ B has been shown to control HIF-1 α expression *in vivo*.^{70–71} The potentially important cross-talk between HIF-1 α and NF- κ B in mediating responses to hypoxia is covered in several excellent reviews on this topic.^{71–72}

PHARMACOLOGICAL MANIPULATION OF HIF-1 α

As the central role of HIF-1 α in physiology and pathology, pharmacological approaches to exploit aspects of the HIF response therapeutically are emerging from basic science laboratories. HIF-1 expression is detected in atherosclerotic plaque and HIF-1-regulated genes have an impact on the progression of atherosclerosis. Disruption of the HIF-1 pathway might be effective in the treatment of atherosclerosis. HIF inhibitors decrease HIF mRNA or protein levels, inhibit DNA binding or decrease HIF-mediated transactivation.⁷³ Several small molecular inhibitors of the HIF-1 transcriptional activation pathway have also been identified and shown to decrease HIF-1 α levels, inhibit the expression of VEGF and other HIF-1 target genes, impair xenograft growth and vascularisation, and inhibit angiogenesis.⁷⁴ Several studies have demonstrated associations between HIF-1 and statins, simvastatin attenuated HIF-1 α expression in the coronary artery wall,⁷⁵ various statins inhibited HIF-1 binding to the HRE of the plasminogen activator

Take-home messages

- ▶ HIF-1 affects the regulatory pathways of endothelial cells and is essential to VSMC proliferation and macrophage maturation leading to atherosclerosis.
- ▶ HIF-1 α induces angiogenesis by the expression of VEGF and E26 transformation-specific 1 (Ets-1).
- ▶ HIF-1 plays a pivotal role in the chronic inflammatory stress in the walls of arteries leading to atherosclerosis.
- ▶ HIF-1 α is involved in hypoxia-induced apoptosis which may lead to the formation of a destabilizing lipid-rich core and a fragile and rupture-prone fibrous cap.
- ▶ Future studies on HIF-1 regulation and its role in atherosclerosis will assist in designing novel treatments in the retardation and stabilisation of atherosclerosis in humans.

inhibitor-1 gene in VSMC and endothelial cells,⁷⁶ atorvastatin inhibited hypoxia-induced HIF-1 activity⁷⁷ and fluvastatin accelerated HIF-1 α protein ubiquitination and proteasomal degradation.⁷⁸ The antagonistic regulation of HIF-1 expression by statins may be a potential therapeutic application for atherosclerotic diseases.

Hopefully, future studies on HIF-1 regulation and its role in atherosclerosis will elaborate the fundamental mechanism of this disorder, and will assist in designing novel treatments in the retardation and stabilisation of atherosclerosis in humans.

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