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# Hypoxia inducible factor as a therapeutic target for atherosclerosis

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ABSTRACT

Atherosclerosis is a highly prevalent disease that can significantly increase the risk of major vascular events, such as myocardial or cerebral infarctions. The anoxemia theory states that a disparity between oxygen supply and demand contributes to atherosclerosis. Hypoxia inducible factor-1 (HIF-1) is a heterodimeric protein, part of the basic helix-loop-helix family and one of the main regulators of cellular responses in a low-oxygen environment. It plays a key role in the development of atherosclerosis through cell-specific responses, acting on endothelial cells, vascular smooth muscle cells (SMCs) and macrophages. Through the upregulation of VEGF, NO, ROS and PDGF, HIF-1 is able to cause endothelial cell dysfunction, proliferation, angiogenesis and inflammation. Activation of the NF-kB pathway in endothelial cells is an important contributor to inflammation and positively feedbacks to HIF-1. HIF-1 also plays a significant role in both the proliferation and migration of smooth muscle cells – two important features of atherosclerosis, while the formation of foam cells (lipid-laden macrophages) is also a critical step in atherosclerosis and mediated by HIF-1 through various mechanisms such as dysfunctional efflux pathways in macrophages. Overall, HIF-1 exerts its effect on the pathogenesis of atherosclerosis via a variety of molecular and cellular events in the process. In this review article, we examine the effects HIF-1 on vascular cells and macrophages in the development of atherosclerosis, highlighting the environmental cues and signalling pathways that control HIF-1 expression/activation within the vasculature. We will highlight the potential of HIF-1 as a therapeutic target on the disease development.

## 1. Introduction

Atherosclerosis is a chronic inflammatory disorder that affects the arteries in the body and has a high association with comorbidities and mortality (Gao, Chen, Zhou, & Fan, 2012). It is the primary cause of coronary artery disease and stroke in the developing world (Jaipersad, Lip, Silverman, & Shantsila, 2014) and most common cause of coronary artery disease and peripheral arterial disease worldwide (Sanchis-Gomar, Perez-Quilis, Leischik, & Lucia, 2016). The development of atherosclerosis is initiated by endothelial activation and involves lipid accumulation within the layers of the arterial wall, monocyte adherence, infiltration and the formation of a lipid core. Foam cell (lipid-laden macrophages) and smooth muscle cell proliferation are characteristic features of atherosclerosis. Monocytes migrate into the arterial intima where they proliferate and differentiate into macrophages. These macrophages proceed to take up oxidized LDL, forming foam cells. As a consequence of cell aggregation, initial lesions progress to advanced plaques. Production of reactive oxygen species (ROS) and ongoing inflammation leads to matrix degradation and a combination of cellular apoptosis and necrosis. This can cause eventual thinning of the fibrous cap on the plaque. Complications of atherosclerosis can include occlusion of the vascular lumen due to plaque growth or rupture of vessel wall and formation of a thrombus (Hansson, 2005).

As the plaque develops, the arterial wall thickens, significantly reducing oxygen diffusion into the intima. There is increasing evidence to suggest hypoxia and oxygen disruption in the pathogenesis of atherosclerosis. The anoxemia theory of atherosclerosis states that a mismatch between oxygen supply and demand in the arterial wall leads to the development of lesions and plaques (Björnheden, Levin, Evaldsson, & Wiklund, 1999). This is thought to occur by a number of potential mechanisms, such as promoting lipid accumulation, increasing inflammation and angiogenesis (Hultén & Levin, 2009). However, despite the plethora of research, money and time invested in this field, there has been limited development of successful preventative

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and therapeutic treatments (Duguid, 1946; Grotel, 1945). In order for such treatments to be generated, a greater understanding of the molecular events leading up to inflammation is required. One protein family of interest is hypoxia inducible factor (HIF).

HIF is a known vascular transcription factor. It is heterodimeric and consists of two main subunits, α and β, but also has three forms, HIF-1, HIF-2 and HIF-3. It has been shown that in low oxygen concentrations HIF functions to a greater extent than in normal or high oxygen conditions. Hypoxia, decreases the usual hydroxylation of HIF, limiting its proteolytic degradation. HIF enhances the compliance of the both cells and tissue in hypoxic states; it affects mitochondrial oxygen consumption, regulates mitochondrial autophagy, increases serum erythropoietin (a protein coding for increased production of red blood cells that carry oxygen to the tissues), and more crucially, controls angiogenesis. A hot topic, attracting interest in the past decade, is the role of HIF in the progression of vascular diseases and how changes in its expression levels affect the resolution of such conditions. In addition to the known link between HIF and oncogenesis, HIF is also associated to angiogenesis occurring in vascular diseases, such as angiogenesis after thrombosis and stenosis, or the weakened vessel formation in atherosclerosis, which increases the risk of plaque rupture or total arterial occlusion. Although it is known that HIF plays a role in the progression of atherosclerosis, it is important to note that in some cases HIF may play a beneficial role. As demonstrated by revascularization and collateral circulation in ischaemic brains; HIF appears to be primarily induced in the penumbra – the salvageable tissue post ischaemia (Shi, 2009). Similar evidence of revascularization has also been found in non-ischemic tissue, such as corneal revascularization, which may increase the viability of the cornea in degenerative conditions or abrasions (Kelly et al., 2003). This review aims to evaluate the role of HIF in atherosclerosis and to highlight its potential as a therapeutic target for treatment of atherosclerosis.

## 2. HIF family

HIFs are critical mediators of the cellular response to low oxygen. They belong to the Per-ARNT-SIM subfamily of the basic helix-loop-helix (bHLH PAS) protein family that can regulate cells in low oxygen environments. To date, three transcription factors have been discovered (HIF-1, 2, 3; Fig. 1). As HIF is a transcription factor, it determines angiogenesis and thus oxygen supply to tissues under hypoxic conditions, through the regulation of transcription of genes including Vascular Endothelial Growth Factor (VEGF) and Erythropoietin (EPO). VEGF is the major protein involved in promoting vessel formation throughout the organism life, whereas EPO controls erythropoiesis, the production of red blood cells. (Benizri, Ginouves, & Berra, 2008; Formenti et al., 2010; Smith, Robbins, & Ratcliffe, 2008; Wilkins, Abboud, Hancock, & Schofield, 2016).

The key regulator of hypoxia, HIF-1 is a ubiquitous transcription factor able to control the responses of nucleated cells in hypoxia. It is a heterodimer protein made up of HIF-1β (its activity is unaffected by hypoxia) and HIF-1α - the active subunit, that has a half-life of 5 min and is highly regulated by oxygen (Salceda & Caro, 1997). HIF-1α is expressed in all nucleated cells (Lim, Kiriakidis, Sandison, Paleolog, & Davies, 2013) and enables a rapid response to hypoxia. A significant amount of energy is devoted to the continuous turnover of HIF-1α in normoxic conditions. HIF-1α allows cells to adapt to low oxygen environments by activating genes that alter energy metabolism (Semenza, Roth, Fang, & Wang, 1994), cell proliferation (Carmeliet et al., 1998b), angiogenesis and vascular remodelling (Rose et al., 2002).

In non-hypoxic conditions, two specific proline residues on HIF-1α protein are hydroxylated in oxygen-dependent degradation domains by prolyl hydroxylase domain proteins (PHD). In addition to that, acetylation of a lysine residue allows for the binding of von Hippel-Lindau, which interacts with the elongin C protein. This facilitates the recruitment of an ubiquitin ligase complex, marking HIF-1α for proteasomal degradation. FIH-1 (Factor inhibiting HIF-1) is also able to inhibit HIF-1 through the hydroxylation of asparagine residue 803. This blocks the transactivation activity, thus enabling the negative regulation of HIF-1. During hypoxia, the oxygen dependent activity of PHDs and FIH-1 are inactivated. Oxygen is required as a substrate for the enzymes to hydroxylate HIF-1 proteins, but as it is unavailable, it causes HIF-1α to accumulate and translocate to the nucleus (Wang, Jiang, Rue, & Semenza, 1995). Here, it dimerises with the HIF-1ß subunit through a common helix-loop-helix bonding (Jiang, Semenza, Bauer, & Marti, 1996), to form an active transcription factor and proceeds to form a complex with the hypoxia-responsive element (HRE) of target genes.

HIF-2 is similarly made up of 2 subunits and structurally, is approximately 48% homologous to HIF-1. It therefore shares some properties with HIF-1α, such as binding with HIF-1ß. HIF-2α plays an important role in development of the embryonic heart and provides essential protection against heart failure via catecholamine homeostasis in utero (Sun, Pang, Shi, Huang, & Wang, 2015). Although HIF-1 is ubiquitous, HIF-2 appears to be cell type specific, including, but not limited to endothelial cells, cardiomyocytes, glial cells and hepatocytes (Wiesener et al., 2003). The involvement of HIF-2 in cancers through cell proliferation, angiogenesis, metastasis and resistance to chemotherapy, has been well established (Zhao, Du, Shen, Zheng, & Xu, 2015).

The role of HIF-3 is less understood. The gene is able to give rise to several variants of HIF-3α through the use of different promotor

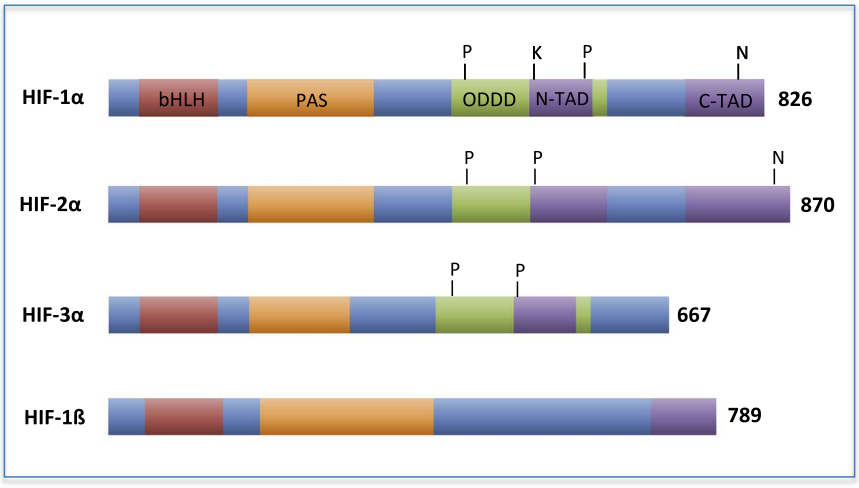


**Fig. 1.** The domain structure for HIF-1α, HIF-2α, HIF-3α and HIF-1β. The bHLH and PAS regions are required for the heterodimerisation of the α and ß subunits. The oxygen-dependent degradation domain (ODDD) region mediates the stability of the molecule through the hydroxylation of the two proline (P) residues and acetylation of lysine (K). Both HIF-2α and HIF- 3α are also regulated in a similar oxygen dependent manner through P and K residues. HIF-1α has two trans-activating domains (N-TAD and C-TAD), similar to HIF-2α, but HIF-3α and HIF-1β only have one TAD. The N terminus is a bHLH that allows for DNA binding, and the C terminus recruits transcriptional regulatory proteins. The number at the end represents the number of amino acids in the subunit.