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Hypoxia, Angiogenesis and Atherogenesis

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Abstract

The balance between vascular oxygen supply and metabolic demand for oxygen within the vasculature is tightly regulated. An imbalance leads to hypoxia and a consequential cascade of cellular signals that attempt to offset the effects of hypoxia. Hypoxia is invariably associated with atherosclerosis, wound repair, inflammation and vascular disease. There is now substantial evidence that hypoxia plays an essential role in angiogenesis as well as plaque angiogenesis. It controls the metabolism, and responses of many of the cell types found within the developing plaque and whether the plaque will evolve into a stable or unstable phenotype. Hypoxia is characterized in molecular terms by the stabilization of hypoxia-inducible factor (HIF)-1 α , a subunit of the heterodimeric nuclear transcriptional factor HIF-1 and a master regulator of oxygen homeostasis. The expression of HIF-1 is localized to perivascular tissues, inflammatory macrophages and smooth muscle cells where it regulates several genes that are important to vascular function including vascular endothelial growth factor, nitric oxide synthase, endothelin-1 and erythropoietin. This chapter summarizes the effects of hypoxia on the functions of cells involved in angiogenesis as well as atherogenesis (plaque angiogenesis) and the evidence for its potential importance from experimental models and clinical studies.

Keywords: hypoxia, HIF-1, proliferation, atherosclerosis, plaque formation, blood vessel

1. Introduction

The circulatory system develops early in mammalian embryogenesis. An oxygen supply is essential for normal tissue function, development and homeostasis. The vascular network within the cardiovascular system is essential for the delivery of oxygen, nutrients and other molecules to the tissues of the body [1]. Oxygen availability serves as an important regulator of the cardiovascular system. Oxygen balance may be perturbed if there is reduced oxygen diffusion, or increased oxygen consumption that may be a consequence of rapid cellular divi-

sion during embryonic development, by tumour growth, or by vasculature dysfunction due to vessel occlusion or rupture [2].

Hypoxia is defined by a reduced oxygen tension relative to those normally extant within a particular tissue. It has multiple impacts on the vascular system and cell function [3]. The effects of moderate hypoxia (3–5% O₂) are usually reversible and are usually accompanied by adaptive physiological responses in the cells. A lower oxygen tension (0–1% O₂) contributes to the pathophysiology of tumour progression and cell apoptosis [4] and is a feature of conditions that include cancer, ischemic heart disease, peripheral artery disease, wound healing and neovascular retinopathy. Hypoxia promotes vessel growth by stimulating an upregulation of multiple proangiogenic pathways that mediate key aspects of endothelial, stromal and vascular support cell biology. The role of hypoxia in human disease is now becoming increasingly clear [5] including the association between hypoxia and endothelial dysfunction that affects several cellular processes and signal transduction.

Hypoxia can occur in several ways: (1) hypoxic hypoxia is caused by an insufficient oxygen concentration in the air in the lungs, which may occur during sleep apnea, when the diffusion of oxygen to the blood is reduced, or at high altitude; (2) hypoxemic hypoxia occurs when the blood has reduced transport capacity as seen in carbon monoxide poisoning when haemoglobin cannot carry oxygen at its normal concentrations; (3) stagnant hypoxia results when the cardiac output does not match the demands of the body and the flow is not sufficient to deliver enough oxygenated blood to the tissue and (4) histotoxic hypoxia occurs when cells cannot utilize the available oxygen, for example following cyanide poisoning when oxygen cannot be used to produce ATP as the mitochondrial electron transport is inhibited.

Chronic tissue hypoxia (an oxygen tension of 2–3% for a prolonged period of time) may cause uncontrolled proliferation of cells. When physiological oxygen concentrations are restored, the increased blood flow supplies excessive oxygen; this may then lead to increased free-radical generation, tissue damage and concomitant activation of stress-response genes; a condition known as ‘reoxygenation injury’. In these circumstances, normal cells/tissues may not survive; but tumour cells are still able to proliferate despite the hypoxic milieu, as they have developed genetic and adaptive changes leading to resistance to hypoxia [6].

Hypoxia plays important roles in normal human physiology and development. For example, it is integral to normal embryonic development. Whatever the cause, or the severity of hypoxia, it leads to an induction of adaptive responses within the endothelial and vascular smooth muscle cells through the activation of genes that participate in angiogenesis, cell proliferation/survival and in glucose and iron metabolism [7].

In healthy vascular tissue, vascular smooth muscle cells (SMCs) and endothelial cells (ECs) proliferate at very low levels. However, SMCs and ECs can be stimulated to re-enter the cell cycle in response to several physiological and pathological stimuli. Hypoxia is considered an important stimulus of SMC and EC proliferation and is found in atherosclerotic lesions and rapidly growing tumours [4].

The proliferation of ECs is pivotal to the formation of new micro-vessels and is important during organ development in embryogenesis and tumour growth, and also contributes to

diabetic retinopathy, psoriasis, rheumatoid arthritis and atherosclerosis. Abnormal SMC proliferation contributes to atherosclerosis, intimal hyperplasia after angioplasty and graft atherosclerosis after coronary transplantation [8, 9].

2. Consequences of hypoxia

Most cells are able to survive under hypoxic conditions through the transcriptional activation of a series of genes. The oxygen-sensitive transcriptional activator, hypoxia-inducible factor-1 (HIF-1) is the key transcriptional mediator of the hypoxic response and master regulator of O₂ homeostasis. It orchestrates the profound changes in cellular transcription that accompanies hypoxia by controlling the expression of numerous angiogenic, metabolic and cell cycle genes. Accordingly, the HIF pathway is currently viewed as a master regulator of angiogenesis [5].

HIF-1 is normally only found in hypoxic cells. It is a heterodimer that is composed of an O₂-regulated HIF-1 α subunit and a constitutively expressed HIF-1 β subunit [10]. In the α -subunit, there is an oxygen-dependent degradation (ODD) domain, where the 4-hydroxyproline formation is catalysed by proline-hydroxylase-2 (PHD-2). This leads to its ubiquitination by the von Hippel-Lindau E3 ubiquitin ligase (VHL) and subsequent proteasomal degradation under normoxic cellular conditions. This prevents the formation of a functional HIF dimer [11]. Since PHDs require oxygen for their catalytic activity, and function as cellular oxygen sensors, HIF degradation only occurs under normoxic conditions. Factor inhibiting HIF-1 (FIH) protein, which hydroxylates HIF-1, also contributes to HIF-1 inactivation in normoxic conditions, and thereby prevents the interaction of this subunit with the two transcriptional co-activators of HIF-1: p300 and CREB-binding protein (CBP) which are essential for HIF-1 transcription. Expression and stabilization of the HIF-1 complex is also regulated through feedback inhibition, as PHD-2 itself is activated by HIF-1 [12].

Under hypoxic conditions, HIF-1 protein is stable and active as the hydroxylase, VHL proteins, and FIH are all inhibited by a lack of oxygen. HIF-1 is then able to interact with its co-activators and can dimerize with its constitutively expressed β -subunit [12]. Once stabilized, the HIF-1 protein can bind to the regulatory regions of its target genes, inducing their expression; these target genes include VEGF (vascular endothelial growth factor) [13], erythropoietin [14] and nitric oxide synthase (NOS) [15, 16] and other proangiogenic factors such as PlGF (placental growth factor), or angiopoietins [12] (**Figure 1**).

It has been proposed that the induction of a pseudo-hypoxic response by inhibiting HIF prolyl 4-hydroxylases may provide a novel therapeutic target in the treatment of hypoxia-associated diseases [17].

Several small molecules, such as dimethyloxalyl glycine [18], Roxadustat (FG-4592) [19] and ZYAN1 [20], have been developed to inhibit prolyl hydroxylase domain-containing (PHD) enzymes, and cause HIF activation [21]. These agents have been applied to the treatment of renal anaemia in which there is a deficiency of erythropoietin [22, 23]. The administration of

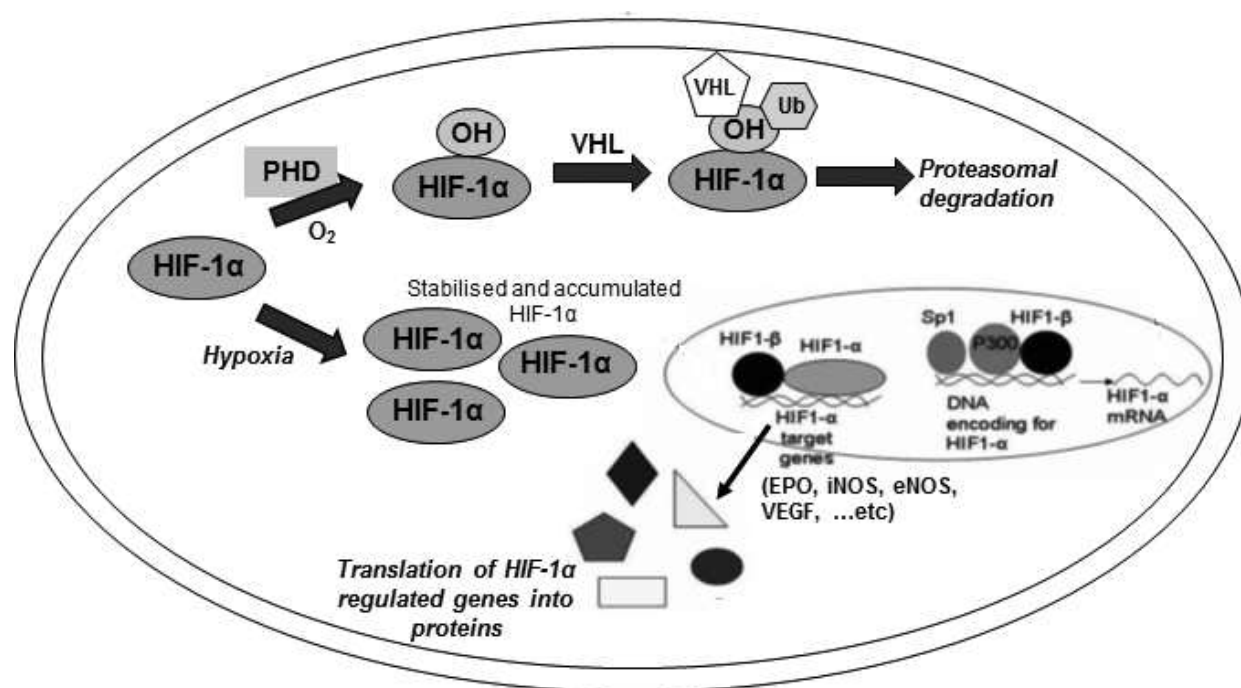


Figure 1. Regulation of the hypoxia-inducible transcription factor (HIF-1 α) pathway. Under normal oxygen tensions (normoxia), prolyl hydroxylase (PHD) enzymes, von Hippel-Lindau protein (pVHL), the ubiquitin ligase complex (Ub) and factor inhibiting HIF-1 (FIH) are active leading to HIF-1 α proteasomal degradation. Under hypoxic conditions, PHD, pVHL and Ub are not active leading to its cytoplasmic accumulation of HIF-1 α . The HIF-1 α gene is transcribed in the nucleus with the help of specificity protein (Sp) 1, P300, and HIF-1 β leading to transcription of HIF target genes such as EPO, NOS and VEGF.

these compounds is associated with an improved iron profile and an increase of endogenous erythropoietin production to near the physiological range. The clinical trials currently underway aim to address whether PHD enzyme inhibitors will improve clinical end-points, including cardiovascular events [24]. PHD inhibitors have been reported to reduce blood pressure [22] and plasma cholesterol concentrations [19]. Hence, there is a good reason to believe that some PHD inhibitors will reduce cardiovascular endpoints in patients with renal disease. Whether they will benefit a broader category of patients with high risk of cardiovascular disease is difficult to predict.

Hydroxylase activity can be also rescued by mutating specific regions, or by adding cobalt ions to the cell, the latter of which presumably compete for iron-binding sites. Some hydroxylases in the prolyl family can be selectively inhibited by Adriamycin *in vitro*. Cobalt (II) and nickel (II) ions increase HIF-1 activity in cells, presumably because these ions displace iron from the active sites of 2-oxo-glutarate (2OG) hydroxylases [12].

It has been shown that HIF-1 α can be regulated by non-hypoxic stimuli such as lipopolysaccharides (LPS), thrombin and angiotensin II (Ang II) [25]. Hormones such as angiotensin II and platelet-derived growth factor stimulate the HIF pathway by increasing HIF-1 α protein levels through production of reactive oxygen species (ROS) within the cell. Although the exact mechanism for this is unclear, it appears to be entirely distinct from the hypoxia pathways.

Thrombin and other growth factors appear to increase angiogenesis through HIF-1 α protein agonist mechanisms. Insulin similarly activates HIF-1 α through the action of multiple protein kinases necessary for expression and function. p53 is responsible for promoting ubiquitination of HIF-1 α , and may be another possible target for enhancing HIF-1. Homozygous deletion of the p53 gene has been found to cause HIF-1 activation [26]. Gene therapy may eventually be used to increase HIF-1 levels and relieve complications of ischemia. For example, delivery of a stabilized, recombinant form of HIF-1 α through adeno-associated virus (AAV) in order to overexpress HIF-1 has been shown to result in significantly increased capillary density in skeletal muscle [27]. While gene therapy approaches aimed at the process and effects of angiogenesis continue to be developed and studied, higher levels of success in pre-clinical trials currently are being sought before clinical applications are pursued. Amongst the remaining obstacles in using gene therapy for this purpose is the effective mode of delivery [12]. Inhibition of PHD2 using siRNA has been shown to decrease cardiac infarction size in murine models [28, 29].

In addition to HIF-1 α , there are two other members of HIF superfamily that have been described: HIF-2 and HIF-3 [30]. Both are important regulators of the hypoxia response with similar actions as HIF-1 [31] and lead to the transcriptional activation of target genes in hypoxia [32]. However, Eubank et al demonstrated opposing roles for the HIFs in tumour angiogenesis, with HIF-1 exhibiting proangiogenic properties that act through its effects on VEGF secretion, whereas HIF-2 exhibits anti-angiogenic activity by inducing the production of the endogenous angiogenesis inhibitor, sVEGFR-1 [33]. HIF-3 α has complementary functions, rather than redundant to HIF-1 α induction in protection against hypoxic damage in alveolar epithelial cells in protection against hypoxic damage in alveolar epithelial cells [34].

Although the oxygen-sensing mechanism involving oxygen-dependent hydroxylation of the HIF- α subunits is probably a universal mechanism in cells, and has been highly conserved during evolution, additional regulatory steps appear to determine which of the alternative subunits is induced [34]. One of the best studied hypoxic responses that will be discussed in this chapter is the induction of angiogenic factors and growth factors, which lead to the formation and growth of new blood vessels.

3. Hypoxia and angiogenesis

Blood vessels formation occurs through two basic mechanisms: (1) vasculogenesis represents de novo formation of blood vessels, and is derived from endothelial progenitors and (2) angiogenesis and arteriogenesis (formation of blood vessels from pre-existing blood vessels).

Angiogenesis is a tightly regulated multi-step process that begins when cells within a tissue respond to hypoxia. When tissues grow beyond the physiological oxygen diffusion limit, the relative hypoxia triggers expansion of vascular beds by inducing angiogenic factors in the cells of the vascular beds, which are physiologically oxygenated by simple diffusion of oxygen. Angiogenesis may be a physiological process, as in the case in embryonic development,

wound healing or vessel penetration into avascular regions. It may also be pathological, for example when it occurs during the formation of solid tumours, eye disease, chronic inflammatory disorders such as rheumatoid arthritis, psoriasis and periodontitis and atherosclerosis.

The regulation of angiogenesis (whether in physiological or pathological cases) by hypoxia is an important component of homeostatic mechanisms that link vascular oxygen supply to metabolic demand. An understanding of the processes involved in angiogenic, the role of the interacting proteins involved, and how all this is regulated by hypoxia through an ever-expanding number of pathways in multiple cell types may lead to the identification of novel therapies and modalities for ischemic vascular diseases as well as diseases characterized by excessive angiogenesis, such as rheumatoid arthritis, psoriasis, tumours, ischemic brain and heart attack [5, 6].

Angiogenesis in hypoxia is regulated by several pro- and anti-angiogenic factors [1]. HIF-1 has been established as the major inducer of angiogenesis [35]. It regulates the transcription of VEGF, a major regulator of angiogenesis which promotes endothelial cell migration towards the hypoxic area. During hypoxia, HIF-1 binds to the regulatory region of the VEGF gene, inducing its transcription and initiating its expression. VEGF is then secreted and binds to cognate receptor tyrosine kinases (VEGFR1 and VEGFR2) located on the surface of vascular endothelial cells triggering a cascade of intracellular signalling pathways that initiate angiogenesis [10]. These endothelial cells are recruited to form new blood vessels which ultimately supply the given area with oxygenated blood [12]. Interestingly, recent studies have shown that hypoxia influences additional aspects of angiogenesis, including vessel patterning, maturation and function [5].

Other factors such as angiopoietin-2/angiopoietin-1 [36, 37], angiopoietin receptor (Tie2) [38], platelet-derived growth factor (PDGF) [39], basic fibroblast growth factor (bFGF) [40] and monocyte chemoattractant protein 1 (MCP-1) [41] have also been reported to be responsible not only for increasing vascular permeability, endothelial sprouting, maintenance, differentiation and remodelling but also cell proliferation, migration, enhancement of endothelial assembly and lumen formation (**Figure 2**). In hypoxia, angiogenesis is also modulated by several factors that are secreted by leucocytes, which produce a high abundance of angiogenic factors, various interleukins such as TGF- β 1 and MCP-1 and proteinases [42]. Thus, hypoxia provides an important environmental stimulus not only for angiogenesis but also for related phenomena in the hypoxic or surrounding area, suggesting that hypoxia is more than simply a regulator of angiogenesis [6].

Angiogenesis may be detrimental when it is excessive. Therefore, angiogenic factors must be highly active but also be tightly regulated. Angiogenesis that is associated with pathological consequences may exhibit differences in the responsible molecular pathways in comparison to physiological angiogenesis. Mutations in oncogenes and tumour suppressor genes and disruptions in growth factor activity play an important role in tumour angiogenesis. The activation of the most prominent proangiogenic factor VEGF might be due to physiological stimuli such as hypoxia or inflammation or due to oncogene activation and tumour suppression function loss. Physiological angiogenesis that occurs during embryonic development or wound healing seems to be dependent on VEGF signalling, whereas tumour angiogenesis adopts

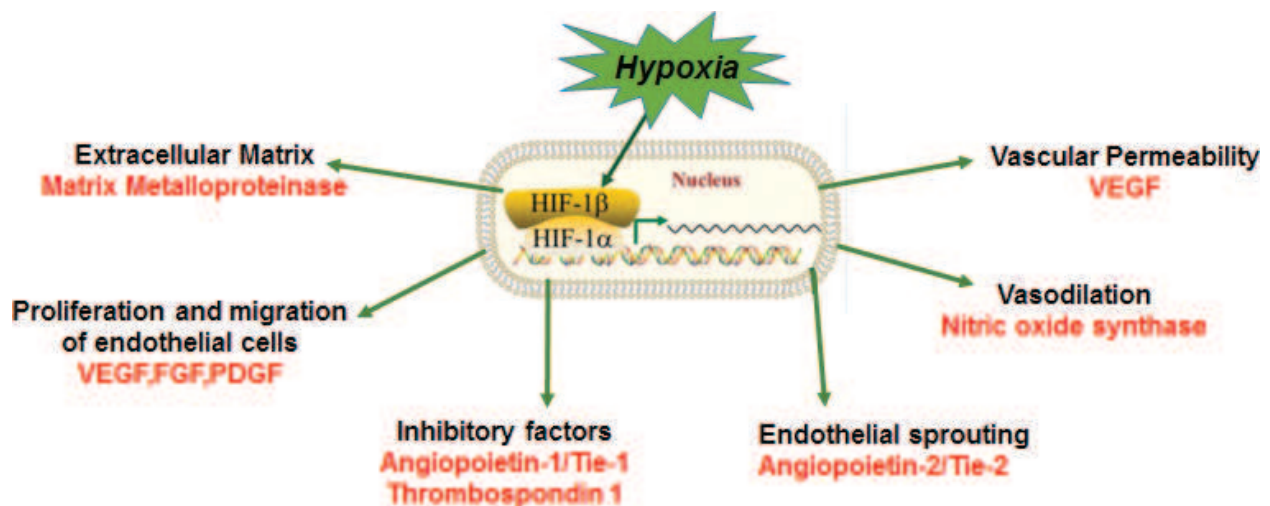


Figure 2. HIF-1 α regulates factors involved in developmental and pathological angiogenesis. HIF-1 α directly regulates genes involved in steps such as vasodilation, increased vascular permeability, extracellular matrix remodelling and proliferation.

the ability to shift its dependence from VEGF to other proangiogenic pathways, for example, through the recruitment of myeloid cells and the upregulation of alternative vascular growth factors (PlGF and FGF) [1].

The identification of alternative ways of inhibiting tumour growth by disrupting the growth-triggering mechanisms of increasing vascular supply through angiogenesis will depend on the understanding of how tumour cells develop their own vasculature. Other cofactors are essential to ensure maximum efficiency of the transcriptional machinery related to changes in oxygen availability within cells/tissues, and the roles of different HIFs in eliciting hypoxic responses seem to be more divergent as originally assumed. Chen et al. have shown new regulatory interactions of HIF-related mechanisms involving the interactions of basic HIFs, HIF-1 α and HIF-2 α with their regulatory binding proteins, histone deacetylase 7 (HDAC7) and translation initiation factor 6 (Int6), respectively [6]. Int6 induces HIF-2 degradation. In addition, silencing of *Int6* produces a potent, physiological induction of angiogenesis that may be useful in the treatment of diseases related to insufficient blood supply. The newly discovered binding proteins-HDAC7 for HIF-1 and Int6 for HIF-2 support the assumption that the 2 HIF isoforms play distinct roles in eliciting hypoxia-related responses. HIF-2 may be considered as one of the master switches for inducing angiogenic factors at least in some cell types [6].

The hypoxia/reoxygenation cycle leads to the formation of reactive oxygen species (ROS) that may subsequently regulate HIF-1 but in a rather complex manner. It has been suggested that ROS promote angiogenesis, either directly through stimulation of HIF-1 genes that are involved in stimulating angiogenesis, such as NOS and NADPH oxidase or through the generation of active oxidation products, including lipid peroxides. ROS are associated with the development of several chronic diseases that include atherosclerosis, type 2 diabetes mellitus, and cancer [43]. Although ROS have damaging effects on tissues, causing cell death at high concentrations, lesser degrees of oxidative stress may play a positive role during angiogene-

sis, or other pathophysiological processes. Angiogenesis induced by oxidative stress involves vascular endothelial growth factor (VEGF) signalling, although VEGF-independent pathways have also been identified [44].

The clinical importance of this biological process has become increasingly apparent over the last decade, and angiogenesis now represents a major focus for novel therapeutic approaches to the prevention and treatment of multiple diseases, most notably ischemic cardiovascular disease and cancer [10].

4. Atherosclerosis and plaque angiogenesis

Considering the important contributions of HIF-1 in angiogenesis, it may also be a promising target for treating ischaemic disease [1] and pressure-overload heart failure [45].

Atherosclerosis causes clinical disease through the occlusion of the arteries as a result of excessive build-up of plaque within the artery wall resulting from the accumulation of cholesterol, fatty material and extracellular matrix. This causes obstruction in the blood flow to the myocardium (coronary heart disease), brain (ischemic stroke) or lower extremities (peripheral vascular). The most common of these manifestations is coronary heart disease that includes stable angina pectoris and the acute coronary syndromes [46].

Coronary heart disease (CHD) is a major cause of mortality globally (1 in every 6 deaths annually). An estimated £2bn per annum is used to treat CHD and its co-morbidities [47]. Arterial injury plays a key role in the initiation and progression of CHD [48]. Treatments for CHD range from lifestyle changes and non-invasive medical therapies to pharmacological therapies and open surgical interventions. Despite the widespread use of drugs such as statins, there remains a significant proportion of individuals for whom response to therapy is sub-optimal, and who develop atherosclerosis [49, 50].

Atherosclerosis is a lipoprotein-driven disease affecting medium and large arteries that leads to plaque formation at specific sites of the arterial tree through intimal inflammation, necrosis, fibrosis and calcification. It is a chronic inflammatory process that involves increased oxidative stress, endothelial damage, and smooth muscle cell proliferation and migration. It is associated with several established risk factors, including hypertension, hyperglycaemia, ageing and dyslipidaemia [51]. It is important to control the factors involved in the progression of atherosclerosis because advanced atherosclerotic lesions are prone to rupture, leading to disability or death. Plaque at risk of rupture has been a major focus of research [52]. There is an emerging need for new therapies to stabilize atherosclerotic lesions. Further understanding of the effects of hypoxia in atherosclerotic lesions could indicate potential therapeutic targets [53, 54]. The presence of hypoxia in human carotid atherosclerotic lesions correlates with angiogenesis. Hypoxia plays a key role in the progression and development of advanced lesions by promoting lipid accumulation, increased inflammation, ATP depletion and angiogenesis. A recent study has convincingly demonstrated the presence of hypoxia in macrophage-rich regions of advanced human carotid atherosclerotic lesions [53].

4.1. Evidence for hypoxia within atherosclerotic plaque

Hypoxia in atherosclerotic plaques is now widely recognized, because of the use of specific probes in imaging studies [4]. Imaging plaque hypoxia could provide a means of assessing putative culprit lesions that are at risk of rupture, and are consequentially liable to adverse outcomes.

Hypoxia has been consistently found in atherosclerotic plaques *in vivo* in humans and animal models using different biomarkers [55]. The immunologically identifiable hypoxia marker, 7-(4''-(2-nitroimidazole-1-yl)-butyl)-theophylline (NITP), has been used to assess hypoxia in three murine models *in vivo*. NITP can bind to cells under low-oxygen conditions [56, 57].

Other non-invasive imaging techniques have also been applied, which directly target plaque hypoxia, and these techniques are now being further validated in human studies. The metabolic marker F-fluorodeoxyglucose (FDG) has been used to detect human atherosclerosis *in vivo* and may also serve as an indirect marker of plaque hypoxia as the enhanced glucose uptake in anaerobic metabolism results in an increased uptake of the labelled FDG [58]. F-18-fluoromisonidazole positron emission tomographic (PET) has been used for the *in vivo* assessment of hypoxia in advanced aortic atherosclerosis in rabbits where hypoxia has been found to be predominantly confined to the macrophage-rich regions within the atheromatous core, whereas the macrophages close to the lumen were hypoxia negative [47]. This was then related to hypoxia assessed by *ex vivo* tissue staining using pimonidazole, and immuno-staining for macrophages (RAM-11), new vessels (CD31) and hypoxia-inducible factor-1 α . ^{18}F -fluoromisonidazole (^{18}F -FMISO), a cell permeable 2-nitroimidazole derivative that is reduced *in vivo* by nitroreductases, regardless of the intracellular oxygen concentration, has been one of the leading radiotracers for imaging hypoxia [47]. In human studies, this imaging approach has been coupled with quantitative polymerase chain reaction (qPCR) and immune-staining of plaques tissues recovered by carotid endarterectomy to determine the gene expression of HIF-1 α and cluster of differentiation 68 (CD68, a marker of inflammation). HIF-1 α and CD68 expression were both found to be significantly correlated with F-FDG-uptake, indicating an association between the presence of hypoxia, inflammation and increased glucose metabolism *in vivo* [59].

Imaging plaque biomarkers such as CRP, interleukins 6, 10 and 18, soluble CD40 ligand, P- and E-selectin, NT-proBNP, fibrinogen and cystatin C show great potential in the prediction and improvement for vascular patients [60].

4.2. The development of a hypoxic environment within the atherosclerotic plaque

Hypoxia has been identified as a potential factor in the formation of vulnerable plaque, and it is clear that decreased oxygen plays a role in the development of plaque angiogenesis leading to plaque destabilization [61]. There have been a number of hypotheses of atherogenesis (plaque angiogenesis) proposing that an imbalance between the demand for and supply of oxygen in the arterial wall is a key factor in the development of atherosclerosis [2, 62].

During atherogenesis, the intima (the innermost layer of the artery wall) may thicken by the accumulation of cells and matrix, and the diffusion of oxygen can then become impaired. The vasa vasorum, forming the network of small blood vessels, are vulnerable to hypoxia espe-

cially at the site of arterial branching as they are end arteries and the blood flow is reduced in this region. It has been hypothesized that hypoxia within the vasa vasorum is due to reduced blood flow and consequent endothelial dysfunction, local inflammation and permeation of large particles such as microbes, LDL-lipoprotein and fatty acids which are transformed by macrophages into foam cells [63, 64], which may be an initiating factor in atherosclerosis [65]. Therefore, the micro-environment within the atherosclerotic plaque is thought to be an important determinant of whether a plaque progresses, and the likelihood of clinical complications. Recent reports provide substantial evidence that there are regions within the plaque in which hypoxia can be identified [46].

In addition to being a marker of hypoxia, HIF-1 α may directly enhance atherogenesis through several mechanisms, including smooth muscle cell proliferation and migration, new vessel formation (angiogenesis) and altered lipid metabolism [66]. The effects of HIF-1 α on macrophage biology and subsequent promotion of atherogenesis has been studied in mice. HIF-1 α expression in macrophages affects their intrinsic inflammatory profile and promotes the development of atherosclerosis [67]. Hence, HIF-1 α may play 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 [68].

It has been also proposed that the state of hypoxia, present in the atherosclerotic plaques of mice deficient in apolipoprotein E (ApoE^{-/-} mice), may promote lipid synthesis, and reduce cholesterol efflux through the ATP-binding cassette transporter (ABCA1) pathway: processes that are known to be mediated by HIF-1 α [55]. Hypoxia has also been reported to increase the formation of lipid droplets in macrophages to promote the secretion of inflammatory mediators, and atherosclerotic lesion progression by exacerbating ATP depletion and lactate accumulation in this model of atherosclerosis [53].

Several HIF-responsive genes have been found to be upregulated in atherosclerosis, such as VEGF, endothelin-1 and matrix-metalloproteinase-2 [69]. Hypoxia has the potential to fundamentally change the function, metabolism and responses of many of the cell types found within the developing atherosclerotic plaque, and this may in turn determine whether the plaque evolves into a stable or unstable phenotype. It is likely that this is mediated through effects on angiogenesis, extracellular matrix elaboration and lipoprotein metabolism. The hypoxic milieu in the atherosclerotic plaque may therefore also have implications for the putative therapeutic interventions for atherosclerosis. However, most *in vitro* studies have been conducted under normoxic conditions. The effects observed under these conditions may not accurately reflect those extant within the plaque [69].

The role of HIF-1 in atherosclerosis is not univocal. Silencing of HIF-1 α in macrophages reduces proinflammatory factors and increases macrophage apoptosis. Hyperlipidaemia impairs angiogenesis in an HIF-1b and nuclear factor (NF)- κ B-dependent manner. Specific knockdown of HIF-1 α in endothelial cells reduces atherosclerosis through reduced monocyte recruitment [26], whereas knockdown in antigen-presenting cells results in aggravation of atherosclerosis through T-cell polarization [70]. There is another non-lipid-driven mechanism by which alternative macrophages present in human atherosclerosis M(Hb) promote plaque neoangiogenesis and microvessel incompetence through an HIF-1 α /VEGF-A-dependent pathway [71].

HIF-1 α has also been implicated in the pathogenesis of in-stent restenosis following coronary revascularisation, stroke, peripheral artery disease, aortic aneurysm formation and pulmonary artery hypertension [72], and also appears to be involved in the calcification of blood vessels, which often accompanies atherosclerosis [73]. Despite being an intracellular transcription factor, HIF-1 could be possibly released into the circulation from damaged cells, similar to other transcriptional factors such as NF- κ B and p53 [73-75].

4.3. Other atherogenic mechanisms of hypoxia

Although plaque angiogenesis is a physiological response that facilitates the increased oxygen demand in the plaque, it can have adverse effects by facilitating intra-plaque haemorrhage (IPH) and the influx of inflammatory mediators. IPH as a result of immature plaque neovessels is associated with subsequent ischemic events. Inflammatory cell, endothelial cell and pericyte interactions can provide insight into the biological mechanisms of plaque angiogenesis [70].

The recruitment of T lymphocytes and proliferation and migration of smooth muscle and endothelial cells are essential for atherosclerotic plaque formation and development. During this process, a number of pro-inflammatory factors and cytokines, leukotrienes and chemokines are increased in expression, especially in lipid-loaded foam cells, such as IL8, tumour necrosis factor α (TNF α), interleukin (IL)-1, vascular cell adhesion molecule 1 (VCAM-1) and 15-lipoxygenase-2 (15-LOX-2). Moreover, macrophages are trapped in hypoxic areas of the lesion; however, the exact mechanisms have yet to be determined.

The majority of inflammatory cells contributing to early atherosclerosis probably enter the artery wall from the lumen [76, 77]. However, the vasa vasorum and associated microvessels may provide an alternate route by which leucocytes can enter the vascular wall [78]. As atherosclerosis progresses, angiogenic factors within the micro-environment of the plaque may stimulate new vessel formation. This combination of delicate new vessel network and inflammatory cells, that elaborate proteolytic enzymes, may contribute to intra-plaque haemorrhage and subsequent plaque rupture [79]. The involvement of vasa vasorum and intimal hyperplasia in the pathophysiology of atherosclerosis is supported by several experimental animal studies [80, 81].

Hypoxia may also induce macrophage migration inhibitory factor (MIF). MIF plays a critical role in the progression of atherosclerosis by several different mechanisms. These include the MIF-triggered arrest and chemotaxis of monocytes and T cells through its receptors CXCR2/4. Further, *in vivo* studies have shown that the blockade of MIF in mice with advanced atherosclerosis leads to plaque regression and reduced monocyte and T-cell content. Additionally, the neuronal signalling molecule Netrin-1 was recently shown to play an important role in macrophage retention in atherosclerotic plaques. Notably, netrin-1 expression has been shown to be regulated by hypoxia, but this may be tissue or disease specific [55].

Atherosclerotic lesion formation is associated with vessel wall thickening resulting in regional limited oxygen exchange. Vascular cells respond to hypoxic conditions with changes in cell metabolism, angiogenesis, apoptosis and inflammatory responses comparable to cells in tumours. Local hypoxic regions and hypoxic cells have been identified in human atherosclerotic lesions and in experimental models. Increased oxygen consumption by cells with a high

metabolic activity, such as macrophages, further depletes the oxygen availability, creating a hypoxic environment in the atherosclerotic lesion. In macrophages, hypoxia not only affects the metabolism and lipid uptake but also results in an increased inflammatory response characterized by increased IL-1 β and caspase-1 activation. Hypoxia also augments the thrombotic potential of atherosclerotic plaques through upregulation of tissue factor.

The identification of specific inflammatory markers pertaining to the arterial wall in atherosclerosis may be useful for both diagnosis and treatment. These include macrophage inhibiting factor (MIF), leucocytes and P-selectin. Purinergic signalling is involved in the control of vascular tone and remodelling. Endothelial cells release purines and pyrimidines in response to changes in blood flow (evoking shear stress) and hypoxia. They then act on P2Y, P2X and P1 receptors on endothelial cells leading to release of EDRF mediated by nitric oxide and prostaglandins and EDHF, resulting in vasodilatation. The therapeutic potential of purinergic compounds for the treatment of vascular diseases, including hypertension, ischaemia, atherosclerosis, migraine and coronary artery and diabetic vascular disease as well as vasospasm is discussed [82]. Modern therapeutic modalities involving endothelial progenitor cells therapy, angiotensin II type-2 (AT2R) and ATP-activated purinergic receptor therapy are notable to mention. Future drugs may be designed to target three signalling mechanisms of AT2R which are (a) activation of protein phosphatases resulting in protein dephosphorylation, (b) activation of bradykinin/nitric oxide/cyclic guanosine 3',5'-monophosphate pathway by vasodilation and (c) stimulation of phospholipase A(2) and release of arachidonic acid. Drugs may also be designed to act on ATP-activated purinergic receptor channel type P2X7 molecules which acts on cardiovascular system. Better understanding of the vascular inflammatory processes and the cells involved in the formation of plaques may prove to be beneficial for future diagnosis, clinical treatment and planning innovative novel anti-atherosclerotic drugs [83].

Systemic hypoxia that is, for example, associated with obstructive sleep apnoea (OSA) also promotes atherosclerosis. The processes by which it may do this include effects on lipid metabolism and efflux, inflammation, altered macrophage polarization and glucose metabolism [84].

5. Conclusion

Hypoxia is involved in several pathophysiological processes, including embryogenesis, angiogenesis and atherogenesis. HIF-1 appears to be an important mediator controlling cellular response to hypoxia. It also appears to be related to atherosclerotic progression and rupture. A better understanding of the mechanism involved in these processes may provide some novel therapeutic approaches to the treatment of cardiovascular disease.

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