Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Human activated macrophages and hypoxia: a comprehensive review of the literature

Fattah Sotoodehnejadnematalahi [1]*, Bernard Burke [2]

[1] Department of Regenerative Biomedicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
[2] Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, LE1 9HN, UK

ARTICLE INFO

Article type:
Review article

Article history:
Received: Dec 19, 2013
Accepted: Jul 1, 2014

Keywords:
Hypoxia
Hypoxia inducible factors
Inflammation
Macrophages

ABSTRACT

Macrophages accumulate in poorly vascularised and hypoxic sites including solid tumours, wounds and sites of infection and inflammation where they can be exposed to low levels of oxygen for long periods. Up to date, different studies have shown that a number of transcription factors are activated by hypoxia which in turn activate a broad array of mitogenic, pro-invasive, pro-angiogenic, and pro-metastatic genes. On the other hand, macrophages respond to hypoxia by up-regulating several genes which are chief factors in angiogenesis and tumorigenesis. Therefore, in this review article we focus mainly on the role of macrophages during inflammation and discuss their response to hypoxia by regulating a diverse array of transcription factors. We also review the existing literatures on hypoxia and its cellular and molecular mechanism which mediates macrophages activation.

► *Please cite this paper as:*
Sotoodehnejadnematalahi F, Burke B. Human activated macrophages and hypoxia: a comprehensive review of the literature. Iran J Basic Med Sci 2014; 17:820-830.

Introduction

Large phagocytic mononuclear leukocytes represent a population of bone marrow-derived (myeloid) cells which are known as monocytes (1). Monocytes constitute ~5-10% of leukocytes in the peripheral blood, where they circulate for several days before populating tissues as macrophages, in the steady state or during inflammation (2, 3). At sites of injury or microbial invasion, monocytes express chemokine receptors such as CCR2 and chemoattractants such as MCP-1 (Monocyte Chemoattractant Protein 1, also called CCL2, which is a ligand for CCR2) which elicits increased recruitment of monocytes to peripheral sites where they differentiate into macrophages and contribute to host defence, tissue remodelling and repair (4-7). Macrophages are "professional" phagocytic cells which act as an early line of defence in the immune system by recognising and engulfing pathogens such as bacteria and viruses (8). Phagocytosis is believed to be involved in macrophage activation and it results in the release of cytokines such as IL-1 (Interleukin-1), IL-6 and TNF (Tumour Necrosis Factor) which promote inflammation (9-12).

The presence of areas of low oxygen tension (hypoxia) is a hallmark of many pathological tissues such as solid tumours (13, 14), wounds (15) and site of infection and inflammation (16). Cells of the monocyte/macrophage lineage are involved in all of the above pathologies (17-19). It has been known for some time that macrophages accumulate in poorly vascularised and hypoxic sites and respond rapidly to hypoxia by altering the expression of a wide range of their genes (20, 21).

The inflammatory macrophages

Inflammation is a response of a tissue to injury which could be a simple wound or a complex autoimmune inflammation such as rheumatoid arthritis (22). It has been shown that macrophages are major players in the inflammatory response and secrete pro-inflammatory and antimicrobial mediators (23, 24). For example, it has long been known that macrophages activated *in vitro* by interferon-γ (IFN-γ) followed by a microbial trigger, can increase production of pro-inflammatory cytokines such as TNF and interleukins including IL-1 and IL-6 (25). Also, innate activation of macrophages by ligation of TLRs such as TLR-4

*Corresponding author: Fattah Sotoodehnejadnematalahi. Department of Stem Cells and Developmental Biology, Royan Institute, Banihashem Sq., Banihashem St., Resalat highway, Tehran, Iran. Royan Regenerative Medicine and Cell Therapy Centre. Tel: +98-21-22306485; Fax: +98-21-22310406; email: Fattah212@gmail.com

with LPS (Lipopolysaccharide) is associated with microbicidal activity and production of other pro-inflammatory cytokines such as IFN-α and IFN-β (26, 27). Evidence to date suggests that macrophages-derived cytokines such as transforming growth factor-β (TGF-β), basic fibroblast growth factor and platelet-derived growth factor are important in tissue repair and remodelling (28, 29). In addition, it has also been shown that deactivation of macrophages, which is induced by presence of cytokines such as IL-10 or TGF-β, is associated with increased production of IL-4 which is an anti-inflammatory cytokine (25, 30).

Several studies have suggested that macrophages can be classified into two major groups, M1 and M2 (31-33). M1 macrophages are activated by IFN-γ, TNF (Tumour Necrosis Factor) or pathogen-associated molecular patterns such as LPS and can effectively destroy invading pathogens, tumour cells and foreign materials (25, 34). They act as antigen presenting cells and release pro-inflammatory cytokines such as TNF, IL-6, IL-1 and IL-12 and participate as inducer and effector cells in T helper 1 (Th1) responses (25, 34, 35). Accumulating evidence suggests that M2 macrophages, which result from culture in presence of IL-4, IL-13, IL-10 or TGF-β, can release anti-inflammatory cytokines, growth factors and mediators which are involved in wound repair and tissue remodelling and contribute as inducers in T helper 2 (Th2) responses (25, 34, 36, 37).

Overall, there are many stimuli which can push macrophages toward the activation phenotype. Hypoxia which often occurs in tumours and sites of infection can therefore activate macrophage expression of a broad range of genes including pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 (38-40).

Hypoxia

Molecular oxygen is essential for aerobic metabolism to maintain intracellular bioenergetics and to serve as an electron acceptor in many reactions (41). Ambient air is 21% O_2 (150 mm Hg) at sea level; however, most mammalian tissues have O_2 levels of 24-66 mm Hg (2%–9% O_2) (42). The term 'Hypoxia' describes low oxygen concentrations (43), which can affect and regulate many physiological and pathophysiological processes, including embryonic development (44) and wound healing (15). In biological systems, hypoxia usually occurs in pathological tissues including tumours, ischemic tissues, chronic obstructive pulmonary disease, atherosclerotic plaques (45, 46) and arthritic joints (47). It is known that a major obstacle to cell survival is reduction in oxygen availability, which is often confronted by cancer cells (48, 49). In general, rapid growth and abnormal angiogenesis at the site of the tumour, leads to insufficient blood supply and consequent depletion of oxygen. This eventually results in the formation of necrotic and hypoxic regions in the inner parts of the tumour (49, 50). Vaupel and Meyer in 2007 showed that O_2 concentrations within cancerous tissues are reduced compared to surrounding normal tissue, with severe hypoxia correlating with invasion, metastasis and patient death (51). The oxygen concentration in these pathological tissues ranged from 0 to 15mmHg (52). Hypoxia is also found in healthy tissues such as the spleen (oxygen levels as low as 0.5% or 3 mmHg) (53) and it is also a condition seen in embryogenesis in which hypoxia signalling is considered necessary for normal development (46).

The role of hypoxic microenvironment in the pathogenesis and progression of human cancer was first proposed by Gray *et al* when intratumoral hypoxia was correlated with reduced efficiency of radiation therapy (54), and later on was discussed by other groups (55, 56). Hypoxia has also been shown to be linked to increased mutation rates (57), tumour invasion (58) and metastasis (59).

Genomic tools, including DNA microarrays, have enabled study of the global gene expression of many different cells and tissues under hypoxic stress (21, 60, 61) and more than 100 genes have been shown to be up-regulated by hypoxia. For example, hypoxia induces erythropoietin (EPO) (62), angiogenic cytokines such as vascular endothelial growth factor (VEGF) (63) and basic fibroblast growth factor (bFGF) which are required for adaption of the whole organism to general hypoxia by enhancing blood oxygen-carrying capacity and oxygen delivery (64). Also, hypoxic up-regulation of glucose transporter-1 (GLUT-1) which facilitates the transport of glucose across the plasma membranes of mammalian cells, has been detected in a variety of malignant tissues (65, 66).

It is well known that a variety of signalling pathways are activated by hypoxia (67, 68). Among these, the activation of the transcription factor hypoxia-inducible factor 1 (HIF-1) is a key element responsible for embryogenesis and up-regulation of numerous hypoxia inducible genes (69, 70). HIF-1-mediated gene expression allows an organism to respond to hypoxia by increasing oxygen delivery or adapting to decreased oxygen availability (71). Such targets for HIF-1, play critical roles in glycolysis, oxygen homeostasis, tissue remodelling, fat metabolism, angiogenesis, erythropoiesis and proliferation (72, 73).

Macrophages in hypoxia

It has been known for some time that macrophages are recruited and retained in poorly vascularised, hypoxic and necrotic sites including breast (74, 75) and ovarian carcinomas (76), wounds (77), atherosclerotic plaques (78) and arthritic joints

(79). In addition, It has been reported that chemoattractants such as colony stimulating factor 1 (CSF-1), MCP-1, VEGF and endothelin 1 recruit peripheral monocytes to tumour regions which are characterised by extremely low levels of oxygen and trigger differentiation into tumour associated macrophages (TAMs) (80). Several studies have shown that TAMs release a variety of enzymes and cytokines which promote tumour invasion, angiogenesis and metastasis, such as epidermal growth factor (EGF) and VEGF (19, 32, 81-83,).

A study by Burke *et al* (2003) showed that certain genes are up-regulated by macrophages under hypoxic conditions. They used cDNA array hybridization to determine the effect of hypoxia on mRNA of 1185 genes in primary human monocyte-derived macrophages (HMDM). This study showed hypoxia induced mRNA up-regulation of the enzyme matrix metalloproteinase-7 (MMP-7), neuromedin B receptor and DNA-binding protein inhibitor (Id2) as well as known hypoxia inducible genes such as VEGF and GLUT-1 (21). Another cDNA array research by White *et al* (2004) also revealed more than 30 mRNA pro-angiogenic genes which were up-regulated by hypoxia in primary macrophages. Among these genes, apart from VEGF, the best characterized ones were fibroblast growth factor 2 (FGF2), IL-8, macrophage migration inhibitory factor (MIF) and cyclooxygenase- 2 (COX-2) (84). In addition, it has been demonstrated that hypoxic macrophages up-regulate a number of transcription factors, such as HIF-1, which in turn up-regulate a broad array of genes including VEGF and GLUT-1 whose products promote tumour growth and angiogenesis (21, 85- 87).

Hypoxia-responsive transcription factors

Hypoxia activates a diverse array of transcription factors such as activator protein-1 (AP-1) (88, 89), cAMP-response element binding protein (CREB) (90, 91), specific protein 1 (SP-1) (92-94) and most importantly HIF-1 (95), which in turn activates a broad array of mitogenic, pro-invasive, pro-angiogenic, and pro-metastatic genes (96, 97). Since the discovery of HIF-1 by the Semenza lab in the early 1990s, it has been recognised as the central importance and described as the "master regulator" of the transcriptional response to hypoxia (96).

Hypoxia-Inducible Factors (HIFs)

There are two main types of HIF, HIF-1 and HIF-2 (98, 99) which are the predominant transcription factors mediating the effects of hypoxia on gene expression (100, 101). HIF-1,the most ubiquitously expressed and best characterised member of the family is recognised as a master regulator of hypoxic signalling whose activation has been shown to regulate the expression of over 70 genes at the transcriptional level (102).

Both HIF-1 and 2 are heterodimeric molecules consisting of α and β subunits which belong to a family of basic helix-loop-helix proteins (103). The HIF-β subunit, known as ARNT (Aryl hydrocarbon Receptor Nuclear Translocator) is found in the nucleus in both normoxia and hypoxia (98), whereas the α subunit is constitutively produced but is subjected to rapid degradation in the presence of oxygen (with a half-life of less than five minutes), only being stable in the absence of oxygen (i.e. hypoxia) (102). The HIF-1α subunit contains an oxygen-dependent degradation domain (ODD) and two transactivation domains (TAD) which are required for transcriptional activation activity of HIF-1, being capable of binding to two transcriptional co-activators, CREB binding protein (CBP) and p300 (104-107). In normoxia, specific proline residues at positions 402 and/or 564 in the ODD of the HIF-α subunit are hydroxylated by prolyl hydroxylase enzymes (PHD) (108, 109). PHD is a family composed of prolyl 4-hydroxylases (PHD1-4) which require iron (Fe (II)), 2-oxoglutarate, O_2 and ascorbate as substrates; their activity is reduced in hypoxia due to the limitation of O_2 concentration (110, 111). Hydroxylation of HIF-1α acts as a signal for recognition by the tumour suppressor VHL (von Hippel–Lindau protein), leading to ubiquitination and proteasomal degradation (Figure 1) (112-114).

In addition to prolyl hydroxylation by PHDs, another oxygen-dependent modification occurs in the transactivation domains of HIF-α subunit. It is dependent on the presence of an asparagine hydroxylase enzyme known as factor inhibiting HIF (FIH) (115, 116). In this oxygen-dependent regulatory mechanism, FIH blocks the interaction between HIF-α with p300 and CBP by hydroxylating an asparagine residue at position 803, thus inhibiting the activity of the HIF-1α transactivation domain (Figure 1) (117, 118).

In hypoxia, PHD activity decreases and enables rapid accumulation of HIF-α in the nucleus where it dimerises with the HIF-β subunit and binds to hypoxia response elements (HREs) in the promoters of various genes (70, 119). The decrease in oxygen availability also impairs FIH which results in a decrease in HIF-α subunit asparagine hydroxylation, allowing increased recruitment of transcriptional co-activators (p300/CBP) which eventually leads to enhanced transcriptional activation of HIF target genes (Figure 1) (16, 115, 120) which are implicated in many different aspects of oxygen delivery and metabolism including vasodilatation (nitric oxide synthases), iron metabolism (transferrin) (121), glucose transporters (GLUT-1), angiogenesis (VEGF), enhanced blood oxygenation (erythropoietin) (122) and glycolysis (phosphoglycerate kinase)(95).

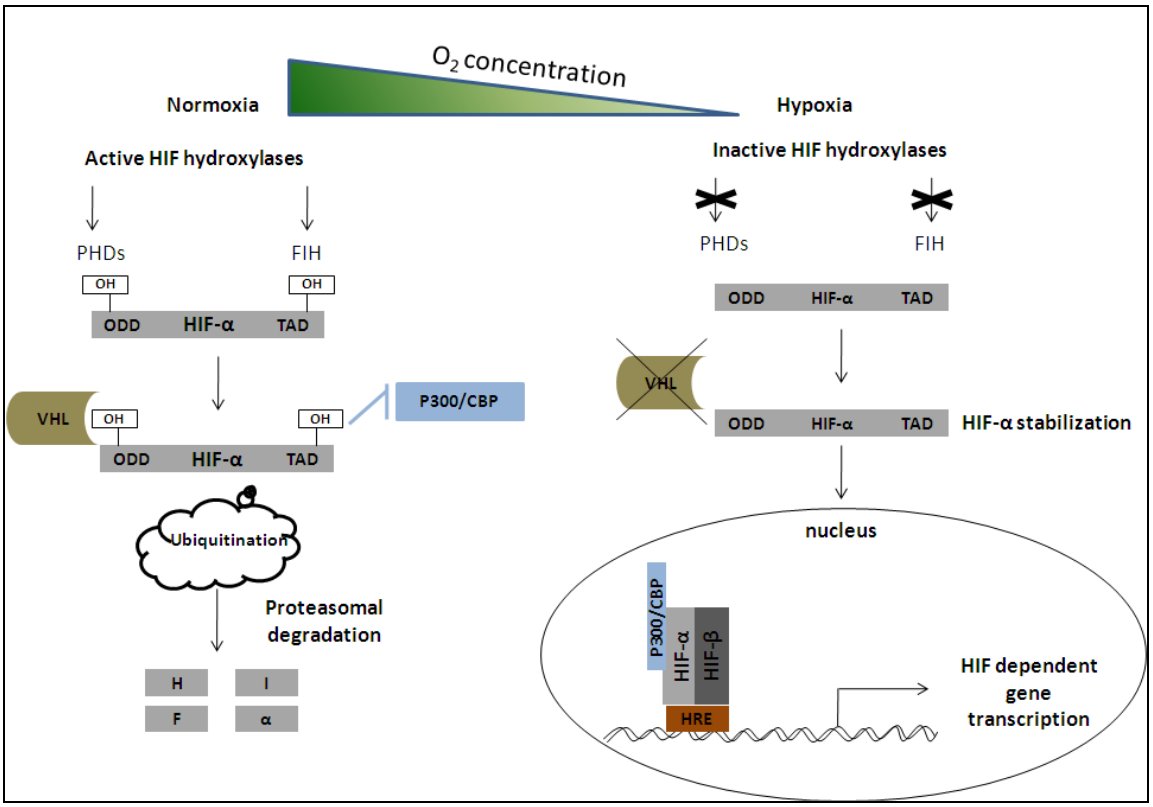

Figure 1. Regulation of HIF-1 in normoxia and hypoxia

In normoxia, the HIF-α subunit is first hydroxylated by PHD at two specific proline residues at positions 402 and/or 564 in the ODD region and then by FIH which blocks the interaction between HIF-α and p300 and CBP by hydroxylating an asparagine residue at position 803, thus inhibiting the activity of the HIF-1α transactivation domain. Both mechanisms then act as a signal for recognition by the tumour suppressor VHL leading to ubiquitination and proteasomal degradation. In hypoxia, reduced activity of PHD causes rapid accumulation of HIF-α in the nucleus where it dimerises with the HIF-β. This complex then binds to HREs in the promoters of various genes. Also, the activity of FIH enzyme will be impaired by the decrease in O_2 availability, leading to reduction of hydroxylation of HIF-α subunit asparagine. This mechanism allows increased recruitment of transcriptional co-activators (p300/CBP) which eventually leads to enhanced transcriptional activation of HIF target genes.

Hypoxia responsive elements (HREs)

Previous studies showed that HIF-1 binds to hypoxia responsive elements, a consensus sequence in the promoter of about 200 HIF target genes (among which around 100 genes have been confirmed) and initiates transcription by recruiting transcriptional co-activators such as p300/CBP (16, 97).

The minimal cis-regulatory element (CGTG) required for hypoxic induction of gene transcription was first identified by Semenza who also determined that this core HRE consensus sequence is required but is not sufficient for effective gene activation in response to hypoxia (95, 123). Analysis of 107 HIF-1 responsive genes showed that neighbouring nucleotides occur with non-random frequency, especially in the 5′ flanking bases, demonstrating that a fully functional HRE requires neighbouring DNA binding sites for additional transcription factors or co-activators, which may act to amplify the hypoxia response (16).

Role of HIF-1 in macrophages

As previously mentioned, macrophages are associated with a number of inflammatory sites such as atherosclerotic plaques (124), myocardial infarcts (125), rheumatoid arthritis (126), healing wounds (127), sites of bacterial infection and malignant tumours (20, 128, 129) in which hypoxia is present. In hypoxia, macrophages rely heavily on HIFs for energy production and activity, express HIF-1α protein abundantly and increase transcriptional activation of HIF target genes (21, 85). Unusually, macrophages are also significantly dependent on HIF-1 regulated genes for energy production in normoxia (130).

Some early studies using a rat alveolar macrophage-derived cell line and the human monocytic cell line (THP-1) reported that short term hypoxia did not increase HIF-1α mRNA, suggesting that HIF-1α is regulated by hypoxia by decreased protein stability (128, 131). However, our recent study showed increases in HIF-1α mRNA levels after long term hypoxia (5 days) in human primary macrophages and also we observed that this up-regulation is mediated by increased transcription rather than increased mRNA stability. Similar increases in HIF-1 mRNA in hypoxia have been reported by other groups in non-macrophage cell types but the subject is still somewhat controversial (132).

An increased level of HIF-1α protein in activated macrophages was first demonstrated by Hollander *et al* in 2001 (126) in inflamed joints of patients suffering from rheumatoid arthritis and later by Talks *et al* in 2000 (133) in tumour sections and Burke *et al* in 2002 (85) in isolated hypoxic human primary macrophages *in vitro*. Also, other studies showed increased levels of HIF-1α in inflammatory cells of healing wounds and suggested that this could be due to a release of inflammatory cytokines such as TNF-α which can strongly increase HIF-1α protein levels in cells after injury, leading to increased expression of HIF-1 responsive genes such as VEGF which regulate the process of tissue repair (134). Other groups have also investigated HIF-1 activity during differentiation of monocytes to macrophages (135). It was shown by Oda *et al* in 2006 that both HIF-1α and HIF-1β protein levels increase markedly during the differentiation of monocytes to macrophages in the monocytic cell line (THP-1) and in monocytes from human peripheral blood (136). They suggested that activation of protein kinase C (PKC) and mitogen-activated protein kinase (MAPK)-signalling pathways are responsible for this increase in HIF-1 gene expression (136).

Non-hypoxic up-regulation of HIF-1

Despite the name, numerous studies have now shown that HIF-1α can be induced by a variety of stimuli in addition to hypoxia. The key studies in this field are reviewed below.

Lipopolysaccharide (LPS)

LPS is a component of the cell wall of Gram-negative bacteria (137). It binds to the CD14 and TLR4 cell surface receptors of monocyte/macrophages (138, 139) leading to activation of a number of genes that are often associated with hypoxia, many of which are believed to be up-regulated independently of HIF-1 (140-143). Several studies have shown that LPS treated macrophages up-regulate genes such as VEGF, GLUT-1 and iNOS (inducible nitric oxide synthase) which are known to be regulated by HIF-1 (128, 131). In contrast to hypoxia, which is generally considered not to up-regulate HIF-1α mRNA, LPS has been shown to stimulate HIF-1α expression at transcriptional level under normoxia in alveolar-derived rat macrophages and human primary macrophages through a NF-κB site in the promoter of the HIF-1α gene (128, 131). It was shown that LPS increases HIF-1α protein expression in a time and dose-dependent manner which in turn modulates hypoxic gene activation (128). Also, an induced HIF-1α mRNA and protein expression in differentiated THP-1 cells treated with LPS under normoxia has been reported (131). This study, using RNAi against MAPK and also a specific inhibitor of this pathway, showed down-regulation of LPS-induced HIF-1α mRNA and protein in THP-1 cells suggesting a role for the MAPK pathway in LPS-dependent HIF-1α induction (131).

Phosphoinositide (PI) 3-kinase signalling

PI3-Kinase activities have been found in all types of eukaryotic cells and are linked to a diverse set of major functions of the cell, including cell growth, proliferation, motility, differentiation and survival (144-146). PI3-kinase phosphorylates the hydroxyl group at position 3 of the inositol ring of phosphatidylinositol (147). PI3-kinase has been the focus of intense study as increasing evidence suggests a key role for PI3-kinase pathway in many human diseases including allergy, inflammation, heart disease and cancer (148, 149). An interesting mechanism was proposed via which the normoxic activation of PI3-kinase could increase the rate of HIF-1α translation in vascular smooth muscle cells (VSMC) (150). It has been previously reported that activation of PI3-kinase by growth factors and hormones leads to the recruitment and activation of a downstream effector of PI3-kinase, known as the mammalian target of rapamycin (mTOR) (151, 152). mTOR activation results in increased phosphorylation and inactivation of 4E-binding protein 1 (4E-BP), the eukaryotic translation initiation factor, and activation of p70-S6 kinase 1 which leads to increased protein synthesis (151, 153). Inactivation of 4E-BP and activation of p70S6K has been shown to increase translation of HIF-1α mRNA through the 5' untranslated region (5'UTR) (150, 154). This is believed to be the main mechanism responsible for HIF-1α induction through the PI3-kinase dependent pathway, resulting in increased VEGF expression in vascular smooth muscle cells and human tumour cell lines (155, 156).

Cobalt ($CoCl_2$) stabilisation of HIF-1α

It has been demonstrated that $CoCl_2$ induces hypoxia-regulated genes by stabilizing HIF-1α in

normoxia (112). As outlined before, hydroxylation of the proline residues, which reside in the oxygen-dependent degradation domain of HIF-1α, by prolyl hydroxylase is one of the key mechanisms that mediate the binding of VHL with HIF-1α which eventually leads to proteasomal degradation of HIF-1α (109, 111). In a study, it has been suggested that iron is a critical factor for the activity of PHD as these enzymes have an iron-binding centre (110). In addition, this study suggested that $CoCl_2$ may act as a competitor for iron, inactivating PHD by binding and engaging an iron-binding site in the proline hydroxylase. Due to this enzymatic inhibition, HIF-α is not targeted for proteasomal degradation (110). Beside the inactivation of PHD by $CoCl_2$, another mechanism via which HIF-α could be stabilized by cobalt, has been proposed (157). In this process, cobalt stabilizes HIF-1α protein by direct binding to the ODD in HIF-1α, thereby preventing the interaction between HIF-1α and VHL protein and subsequently inhibiting proteasomal degradation which results in HIF-1α stabilization (157).

Desferrioxamine (DFO) stabilization of HIF-1α

Since the introduction of DFO in the 1960s, it has been widely used as a chelating agent to bind free iron in the bloodstream and removing excess iron from the body (158). Several studies have demonstrated that normoxic cells treated with DFO induced HIF-1 target genes such as EPO (159), VEGF (160) and GLUT-1 (161) by inducing the accumulation of HIF-1 protein. An early study demonstrated that DFO disrupts pVHL–HIF-α complex formation which is required for ubiquitination and proteasomal degradation of HIF-1α in normoxia (112). It has been demonstrated that DFO inhibits hydroxylation of HIF-1α by chelating the iron required for the activity of PHD enzyme (157). Therefore, due to inhibition of HIF-α hydroxylation, the pVHL–HIF-α complex formation is inhibited causing HIF-α stabilization which results in induction of HIF-1 target genes (162-164).

Conclusion

In this review article, we provided evidence which show hypoxic activated human macrophages could regulate broad array of angiogenesis and tumorigenesis genes. In addition, further ground working experiments suggested that high-level transcription of such genes in hypoxia appears to occur via a HIF-1 dependent mechanism which can be activated by hypoxia and DFO in addition to CoCl2. A better understanding of how hypoxic regulated genes are influenced by hypoxia in human macrophages will hopefully be helpful for the development of future therapies for a range of different diseases such as vascular disorders like atherosclerosis, where hypoxia-induced genes accumulation plays a key role in disease development. As macrophages have been shown to accumulate in the areas with low oxygen tension where hypoxic regulated genes are up-regulated, the knowledge of how such genes promoter is induced by hypoxia by elucidation of the hypoxia responsive elements could be an additional advantage for future tumour gene therapy whereby a therapeutic gene could be engineered to be regulated by the hypoxia responsive promoter. Macrophages transfected with this construct could be used in the delivery of the therapeutic gene to radiotherapy and chemotherapy resistant hypoxic tumour sites where the gene would be locally induced. In addition, hypoxic up-regulation of such genes by human macrophages which are recruited and retained in hypoxic and necrotic sites could be a potential prognostic factor in patients with malignant tumours.

References

1. Beekhuizen H, van Furth R. Monocyte adherence to human vascular endothelium. J Leukoc Biol 1993; 54:363-378.
2. Issekutz TB, Issekutz AC, Movat HZ. The *in vivo* quantitation and kinetics of monocyte migration into acute inflammatory tissue. Am J Pathol 1981; 103: 47-55.
3. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005; 5:953-964.
4. Kuziel WA, Morgan SJ, Dawson TC, Griffin S, Smithies O, Ley K, *et al*. Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. Proc Natl Acad Sci USA 1997; 94:12053-12058.
5. Mutsaers SE, Bishop JE, McGrouther G, Laurent GJ. Mechanisms of tissue repair: from wound healing to fibrosis. Int J Biochem Cell Biol 1997; 29:5-17.
6. Lu B, Rutledge B, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, *et al*. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. J Exp Med 1998; 187:601-608.
7. Smith PD, Ochsenbauer-Jambor C, Smythies LE. Intestinal macrophages: unique effector cells of the innate immune system. Immunol Rev 2005; 206:149-159.
8. Serbina NV, Jia T, Hohl TM, Pamer EG. Monocyte-mediated defense against microbial pathogens. Annu Rev Immunol 2008; 26:421-452.
9. Nathan CF. Secretory products of macrophages. J Clin Invest 1987; 79:319-326.
10. Uchimura E, Kodaira T, Kurosaka K, Yang D, Watanabe N. Interaction of phagocytes with apoptotic cells leads to production of pro-inflammatory cytokines. Biochem Biophys Res Commun 1997; 239:799-803.
11. Patel K, Bhaskaran M, Dani D, Reddy K, Singhal PC. Role of heme oxygenase-1 in morphine-modulated apoptosis and migration of macrophages. J Infect Dis 2003; 187:47-54.
12. Tripathi A, Sodhi A. Prolactin-induced production of cytokines in macrophages in vitro involves JAK/STAT and JNK MAPK pathways. Int Immunol 2008; 20: 327-336.

13. Hockel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996; 56:4509-4515.
14. Vaupel P, Thews O, Hoeckel M. Treatment resistance of solid tumors: role of hypoxia and anemia. Med Oncol 2001; 18:243-259.
15. Lee EY, Xia Y, Kim WS, Kim MH, Kim TH, Kim KJ, *et al*. Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF. Wound Repair Regen 2009; 17:540-547.
16. Wenger RH, Stiehl DP, Camenisch G. Integration of oxygen signaling at the consensus HRE. Sci STKE 2005; 2005:re12.
17. Lewis JS, Lee JA, Underwood JC, Harris AL, Lewis CE. Macrophage responses to hypoxia: relevance to disease mechanisms. J Leukoc Biol 1999; 66:889-900.
18. Griffiths L, Binley K, Iqball S, Kan O, Maxwell P, Ratcliffe P, *et al.* The macrophage - a novel system to deliver gene therapy to pathological hypoxia. Gene Ther 2000; 7:255-262.
19. Lewis JS, Landers RJ, Underwood JC, Harris AL, Lewis CE. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. J Pathol 2000; 192:150-158.
20. Murdoch C, Muthana M, Lewis CE. Hypoxia regulates macrophage functions in inflammation. J Immunol 2005; 175:6257-6263.
21. Burke B, Giannoudis A, Corke KP, Gill D, Wells M, Ziegler-Heitbrock L, *et al.* Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. Am J Pathol 2003; 163:1233-1243.
22. Schmid-Schonbein GW. Analysis of inflammation. Annu Rev Biomed Eng 2006; 8:93-131.
23. Gordon S. The role of the macrophage in immune regulation. Res Immunol 1998; 149:685-688.
24. Ma J, Chen T, Mandelin J, Ceponis A, Miller NE, Hukkanen M, Ma GF, *et al.* Regulation of macrophage activation. Cell Mol Life Sci 2003; 60:2334-2346.
25. Gordon S. Aernative activation of macrophages. Nat Rev Immunol 2003; 3:23-35.
26. Gao JJ, Filla MB, Fultz MJ, Vogel SN, Russell SW, Murphy, WJ. Autocrine/paracrine IFN-alphabeta mediates the lipopolysaccharide-induced activation of transcription factor Stat1alpha in mouse macrophages: pivotal role of Stat1alpha in induction of the inducible nitric oxide synthase gene. J Immunol 1998; 161:4803-4810.
27. Fujihara M, Muroi M, Tanamoto K, Suzuki T, Azuma H, Ikeda H. Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. Pharmacol Ther 2003; 100:171-194.
28. Nathan CF. Secretory products of macrophages. J Clin Invest 1987; 79:319-326.
29. Ricardo SD, van Goor H, Eddy AA. Macrophage diversity in renal injury and repair. J Clin Invest 2008; 118:3522-3530.
30. Goerdt S, Orfanos CE. Other functions, other genes: alternative activation of antigen-presenting cells. Immunity 1999; 10:137-142.
31. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. J Leukoc Biol 2006; 80:1298-1307.
32. Mantovani A, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev 2006; 25: 315-322.
33. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. Immunity 2010; 32:593-604.127.
34. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004; 25:677-686.
35. Van Ginderachter JA, Movahedi K, Hassanzadeh Ghassabeh G, Meerschaut S, Beschin A, Raes G, *et al.* Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. Immunobiology 2006; 211:487-501.
36. Benoit M, Desnues B, Mege JL. Macrophage polarization in bacterial infections. J Immunol 2008; 181:3733-3739.
37. Laskin DL. Macrophages and inflammatory mediators in chemical toxicity: a battle of forces. Chem Res Toxicol 2009; 22:1376-1385.
38. Ghezzi P, Dinarello CA, Bianchi M, Rosandich ME, Repine JE, White CW. Hypoxia increases production of interleukin-1 and tumor necrosis factor by human mononuclear cells. Cytokine 1991; 3:189-194.
39. Scannell G, Waxman K, Kaml GJ, Ioli G, Gatanaga T, Yamamoto R, *et al.* Hypoxia induces a human macrophage cell line to release tumor necrosis factor-alpha and its soluble receptors *in vitro*. J Surg Res 1993; 54:281-285
40. Albina JE, Henry WL, Jr, Mastrofrancesco B, Martin BA, Reichner JS. Macrophage activation by culture in an anoxic environment. J Immunol 1995; 155: 4391-4396.
41. Freeman BA. Oxygen: the air-borne nutrient that both sustains and threatens life. Nutrition 2000; 16:478-480
42. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 1989; 49:6449-6465
43. Semenza GL. Oxygen homeostasis. Wiley Interdiscip Rev Syst Biol Med 2010; 2:336-361.
44. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol 2008; 9:285-296.
45. Bjornheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall *in vivo*. Arterioscler Thromb Vasc Biol 1999; 19:870-876.
46. Brahimi-Horn C, Pouyssegur J. The role of the hypoxia-inducible factor in tumor metabolism growth and invasion. Bull Cancer 2006; 93:E73-80.
47. Stevens CR, Williams RB, Farrell AJ, Blake DR. Hypoxia and inflammatory synovitis: observations and speculation. Ann Rheum Dis 1991; 50:124-132
48. Sutherland RM. Tumor hypoxia and gene expression--implications for malignant progression and therapy. Acta Oncol 1998; 37: 567-574.

49. Vaupel P, Mayer A, Hockel M. Tumor hypoxia and malignant progression. Methods Enzymol 2004; 381:335-354.
50. Griffiths L, Binley K, Iqball S, Kan O, Maxwell P, Ratcliffe P, *et al.* The macrophage - a novel system to deliver gene therapy to pathological hypoxia. Gene Ther 2000; 7:255-262.
51. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 2007; 26:225-239.
52. Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001; 93:266-276.
53. Caldwell CC, Kojima H, Lukashev D, Armstrong J, Farber M, Apasov SG. *et al.* Differential effects of physiologically relevant hypoxic conditions on T lymphocyte development and effector functions. J Immunol 2001; 167:6140-6149.
54. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 1953; 26:638-648.
55. Brizel DM, Dodge RK, Clough RW, Dewhirst MW. Oxygenation of head and neck cancer: changes during radiotherapy and impact on treatment outcome. Radiother Oncol 1999; 53:113-117.
56. Overgaard J. Hypoxic radiosensitization: adored and ignored. J Clin Oncol 2007; 25:4066-4074.
57. Yuan J, Glazer PM. Mutagenesis induced by the tumor microenvironment. Mutat Res 1998; 400:439-446.
58. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003; 3:347-361.
59. Subarsky P, Hill RP. The hypoxic tumour microenvironment and metastatic progression. Clin Exp Metastasis 2003; 20:237-250.
60. Greijer AE, van der Groep P, Kemming D, Shvarts A, Semenza GL, Meijer GA, *et al.* Up-regulation of gene expression by hypoxia is mediated predominantly by hypoxia-inducible factor 1 (HIF-1). J Pathol 2005; 206:291-304.
61. Weinmann M, Belka C, Guner D, Goecke B, Muller I, Bamberg M, *et al.* Array-based comparative gene expression analysis of tumor cells with increased apoptosis resistance after hypoxic selection. Oncogene 2005; 24:5914-5922.
62. Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci USA 1993; 90:4304-4308.
63. Liu Y, Cox SR, Morita T, Kourembanas S. Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer. Circ Res 1995; 77:638-643.
64. Egger M, Schgoer W, Beer AG, Jeschke J, Leierer J, Theurl M, *et al*. Hypoxia up-regulates the angiogenic cytokine secretoneurin via an HIF-1alpha- and basic FGF-dependent pathway in muscle cells. FASEB J 2007; 21:2906-2917.
65. Airley R, Loncaster J, Davidson S, Bromley M, Roberts S, Patterson A, *et al.* Glucose transporter glut-1 expression correlates with tumor hypoxia and predicts metastasis-free survival in advanced carcinoma of the cervix. Clin Cancer Res 2001; 7:928-934.
66. Macheda ML, Rogers S, Best JD. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J Cell Physiol 2005; 202:654-662.
67. Haddad JJ. Hypoxia and the regulation of mitogen-activated protein kinases: gene transcription and the assessment of potential pharmacologic therapeutic interventions. Int Immunopharmacol 2004; 4:1249-1285.
68. Benizri E, Ginouves A, Berra E. The magic of the hypoxia-signaling cascade. Cell Mol Life Sci 2008; 65:1133-1149.
69. Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, *et al*. Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha. EMBO J 1998; 17:6573-6586.
70. Sutter CH, Laughner E, Semenza GL. Hypoxia-inducible factor 1alpha protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations. Proc Natl Acad Sci USA 2000; 97:4748-4753.
71. Wenger RH. Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. FASEB J 2002; 16:1151-1162.
72. Semenza GL. Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. Trends Mol Med 2001; 7:345-350.
73. Hirota K. Hypoxia-inducible factor 1, a master transcription factor of cellular hypoxic gene expression. J Anesth 2002; 16:150-159.
74. Leek RD, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. Br J Cancer 1999; 79:991-995.
75. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 2002; 196:254-265.
76. Negus RP, Stamp GW, Hadley J, Balkwill FR. Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. Am J Pathol 1997; 150:1723-1734.
77. Crowther M, Brown NJ, Bishop ET, Lewis CE. Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors. J Leukoc Biol 2001; 70:478-490.
78. Husain T, Abbott CR, Scott DJ, Gough MJ. Macrophage accumulation within the cap of carotid atherosclerotic plaques is associated with the onset of cerebral ischemic events. J Vasc Surg 1999; 30:269-276.
79. Kinne RW, Brauer R, Stuhlmuller B, Palombo-Kinne E, Burmester GR. Macrophages in rheumatoid arthritis. Arthritis Res 2000; 2:189-202.
80. Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. Blood 2004; 104:2224-2234.

81. Goswami S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley FJ, *et al.* Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. Cancer Res 2005; 65:5278-5283.
82. Bingle L, Lewis CE, Corke KP, Reed MW, Brown NJ. Macrophages promote angiogenesis in human breast tumour spheroids *in vivo*. Br J Cancer 2006; 94:101-107.
83. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res 2006; 66: 05-612.
84. White JR, Harris RA, Lee SR, Craigon MH, Binley K, Price T, *et al*. Genetic amplification of the transcriptional response to hypoxia as a novel means of identifying regulators of angiogenesis. Genomics 2004; 83:1-8.
85. Burke B, Tang N, Corke KP, Tazzyman D, Ameri K, Wells M, *et al.* Expression of HIF-1alpha by human macrophages: implications for the use of macrophages in hypoxia-regulated cancer gene therapy. J Pathol 2002; 196:204-212.
86. Leek RD, Talks KL, Pezzella F, Turley H, Campo L, Brown NS, *et al*. Relation of hypoxia-inducible factor-2 alpha (HIF-2 alpha) expression in tumor-infiltrative macrophages to tumor angiogenesis and the oxidative thymidine phosphorylase pathway in Human breast cancer. Cancer Res 2002; 62:1326-1329.
87. Elbarghati L, Murdoch C, Lewis CE. Effects of hypoxia on transcription factor expression in human monocytes and macrophages. Immunobiology 2008; 213:899-908.
88. Bandyopadhyay RS, Phelan M, Faller DV. Hypoxia induces AP-1-regulated genes and AP-1 transcription factor binding in human endothelial and other cell types. Biochim Biophys Acta 1995; 1264: 72-78.
89. Fantozzi I, Zhang S, Platoshyn O, Remillard CV, Cowling RT, Yuan JX. Hypoxia increases AP-1 binding activity by enhancing capacitative Ca2+ entry in human pulmonary artery endothelial cells. Am J Physiol Lung Cell Mol Physiol 2003; 285:1233-1245.
90. Beitner-Johnson D, Millhorn DE. Hypoxia induces phosphorylation of the cyclic AMP response element-binding protein by a novel signaling mechanism. J Biol Chem 1998; 273:19834-19839.
91. Leonard MO, Howell K, Madden SF, Costello CM, Higgins DG, Taylor CT, *et al.* Hypoxia selectively activates the CREB family of transcription factors in the *in vivo* lung. Am J Respir Crit Care Med 2008; 178:977-983.
92. Xu Q, Ji YS, Schmedtje JF Jr. Sp1 increases expression of cyclooxygenase-2 in hypoxic vascular endothelium. Implications for the mechanisms of aortic aneurysm and heart failure. J Biol Chem 2000; 275:24583-24589.
93. Lee M, Bikram M, Oh S, Bull DA, Kim SW. Sp1-dependent regulation of the RTP801 promoter and its application to hypoxia-inducible VEGF plasmid for ischemic disease. Pharm Res 2004; 21:736-741.
94. Sanchez-Elsner T, Ramirez JR, Sanz-Rodriguez F, Varela E, Bernabeu C, Botella LM. A cross-talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SP1 and Smads. J Mol Biol 2004; 336:9-24.
95. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 1994; 269:23757-23763.
96. Semenza GL. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol 1999; 15:551-578.
97. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 2000; 88:1474-1480.
98. Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 1995; 270:1230-1237.
99. Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii-Kuriyama Y. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development. Proc Natl Acad Sci USA 1997; 94:4273-4278.
100. Semenza GL. HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol 2001; 13:167-171.
101. Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer. Nat Rev Cancer 2008; 8:967-975.
102. Semenza GL. Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology (Bethesda) 2004; 19:176-182.
103. Dery MA, Michaud MD,Richard DE. Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. Int J Biochem Cell Biol 2005; 37:535-540.
104. Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, *et al.* Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha. EMBO J 1998; 17:6573-6586.
105. Ema M, Hirota K, Mimura J, Abe H, Yodoi J, Sogawa K, *et al.* Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. EMBO J 1999; 18:1905-1914.
106. Jiang BH, Zheng JZ, Leung SW, Roe R, Semenza GL. Transactivation and inhibitory domains of hypoxia-inducible factor 1alpha. Modulation of transcriptional activity by oxygen tension. J Biol Chem 1997; 272:19253-19260.
107. Pugh CW, Ratcliffe PJ. The von Hippel-Lindau tumor suppressor, hypoxia-inducible factor-1 (HIF-1) degradation, and cancer pathogenesis. Semin Cancer Biol 2003; 13:83-89.
108. Semenza GL. Expression of hypoxia-inducible factor 1: mechanisms and consequences. Biochem Pharmacol 2000; 59:47-53.
109. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, *et al*. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 2001; 292:468-472.
110. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, *et al.* C. elegans EGL-9 and mammalian homologs define a family of

dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001; 107:43-54.
111. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, *et al.* HIF alpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 2001; 292:464-468.
112. Maxwell PH, Wiesener MS, Chang, GW, Clifford SC, Vaux EC., Cockman ME, *et al.* The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999; 399:271-275.
113. Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, Conaway JW. Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. Proc Natl Acad Sci USA 2000; 97:10430-10435.
114. Tanimoto K, Makino Y, Pereira T, Poellinger, L. Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. EMBO J 2000; 19:4298-4309.
115. Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev 2001; 15:2675-2686.
116. Cockman ME, Webb JD, Ratcliffe PJ. FIH-dependent asparaginyl hydroxylation of ankyrin repeat domain-containing proteins. Ann N Y Acad Sci 2009; 1177:9-18.
117. Hewitson KS, McNeill LA, Riordan MV, Tian YM, Bullock AN, Welford RW, *et al.* Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. J Biol Chem 2002; 277:26351-26355.
118. McNeill LA, Hewitson KS, Claridge TD, Seibel JF, Horsfall LE, Schofield CJ. Hypoxia-inducible factor asparaginyl hydroxylase (FIH-1) catalyses hydroxylation at the beta-carbon of asparagine-803. Biochem J 2002; 367:571-575.
119. Jewell UR, Kvietikova I, Scheid A, Bauer C, Wenger RH, Gassmann M. Induction of HIF-1alpha in response to hypoxia is instantaneous. FASEB J 2001; 15:1312-1314.
120. Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science 2002; 295:858-861.
121. Ratcliffe PJ, O'Rourke JF, Maxwell PH, Pugh CW. Oxygen sensing, hypoxia-inducible factor-1 and the regulation of mammalian gene expression. J Exp Biol 1998; 201:1153-1162.
122. Gleadle JM, Ratcliffe PJ. Induction of hypoxia-inducible factor-1, erythropoietin, vascular endothelial growth factor, and glucose transporter-1 by hypoxia: evidence against a regulatory role for Src kinase. Blood 1997; 89: 503-509.
123. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, *et al.* Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. J Biol Chem 1996; 271:32529-32537.
124. Bjornheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall *in vivo*. Arterioscler Thromb Vasc Biol 1999; 19:870-876.
125. Jurgensen JS, Rosenberger C, Wiesener MS, Warnecke C, Horstrup JH, Grafe M, *et al*. Persistent induction of HIF-1alpha and -2alpha in cardiomyocytes and stromal cells of ischemic myocardium. FASEB J 2004; 18:1415-1417.
126. Hollander AP, Corke KP, Freemont AJ, Lewis CE. Expression of hypoxia-inducible factor 1alpha by macrophages in the rheumatoid synovium: implications for targeting of therapeutic genes to the inflamed joint. Arthritis Rheum 2001; 44:1540-1544.
127. Stevens CR, Williams RB, Farrell AJ, Blake DR. Hypoxia and inflammatory synovitis: observations and speculation. Ann Rheum Dis 1991; 50:124-132.
128. Blouin CC, Page EL, Soucy GM, Richard DE. Hypoxic gene activation by lipopolysaccharide in macrophages: implication of hypoxia-inducible factor 1alpha. Blood 2004; 103:1124-1130.
129. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 2006; 441:437-443.
130. Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, *et al.* HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 2003; 112:645-657.
131. Frede S, Stockmann C, Freitag P, Fandrey J. Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-kappaB. Biochem J 2006; 396:517-527.
132. Staples, KJ, Sotoodehnejadnematalahi F, Pearson H, Frankenberger M, Francescut L, Ziegler-Heitbrock L, Burke B. Monocyte-derived macrophages matured under prolonged hypoxia transcriptionally up-regulate HIF-1alpha mRNA. Immunobiology 2011; 216:832-839
133. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, *et al.* The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000; 157:411-421.
134. Albina JE, Mastrofrancesco B, Vessella JA, Louis CA, Henry WL, Jr, Reichner JS. HIF-1 expression in healing wounds: HIF-1alpha induction in primary inflammatory cells by TNF-alpha. Am J Physiol Cell Physiol 2001; 281:C1971-1977.
135. Knowles HJ, Mole DR, Ratcliffe PJ, Harris AL. Normoxic stabilization of hypoxia-inducible factor-1alpha by modulation of the labile iron pool in differentiating U937 macrophages: effect of natural resistance-associated macrophage protein 1. Cancer Res 2006; 66:2600-2607.
136. Oda T, Hirota K, Nishi K, Takabuchi S, Oda S, Yamada H, *et al.* Activation of hypoxia-inducible factor 1 during macrophage differentiation. Am J Physiol Cell Physiol 006; 291:C104-113.
137. Hajjar AM, Ernst RK, Tsai JH, Wilson CB, Miller SI. Human Toll-like receptor 4 recognizes host-specific LPS modifications. Nat Immunol 2002; 3:354-359.
138. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, *et al.* Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive

to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J Immunol 1999; 162:3749-3752.
139. Moynagh, P.N. Toll-like receptor signalling pathways as key targets for mediating the anti-inflammatory and immunosuppressive effects of glucocorticoids. J Endocrinol 2003; 179: 139-144.
140. Pocock J, Gomez-Guerrero C, Harendza S, Ayoub M, Hernandez-Vargas P, Zahner G, *et al.* Differential activation of NF-kappa B, AP-1, and C/EBP in endotoxin-tolerant rats: mechanisms for *in vivo* regulation of glomerular RANTES/CCL5 expression. J Immunol 2003; 170:6280-6291.
141. Covert MW, Leung TH, Gaston JE, Baltimore D. Achieving stability of lipopolysaccharide-induced NF-kappaB activation. Science 2005; 309:1854-1857.
142. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006; 441:431-436.
143. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005; 5:749-759.
144. Rameh LE, Cantley LC. The role of phosphoinositide 3-kinase lipid products in cell function. J Biol Chem 1999; 274:8347-8350.
145. Fry MJ. Phosphoinositide 3-kinase signalling in breast cancer: how big a role might it play? Breast Cancer Res 2001; 3:304-312.
146. Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol 2001; 17:615-675.
147. Zvelebil MJ, MacDougall L, Leevers S, Volinia S, Vanhaesebroeck B, Gout I, *et al.* Structural and functional diversity of phosphoinositide 3-kinases. Philos Trans R Soc Lond B Biol Sci 1996; 351:217-223.
148. Stein RC. Prospects for phosphoinositide 3-kinase inhibition as a cancer treatment. Endocr Relat Cancer 2001; 8:237-248.
149. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 2009; 8:627-644.
150. Page EL, Robitaille GA, Pouyssegur J, Richard DE. Induction of hypoxia-inducible factor-1alpha by transcriptional and translational mechanisms. J Biol Chem 2002; 277:48403-48409.
151. Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. J Biol Chem 2002; 277:27975-27981.
152. Alam H, Maizels ET, Park Y, Ghaey S, Feiger ZJ, Chandel NS, *et al.* Follicle-stimulating hormone activation of hypoxia-inducible factor-1 by the phosphatidylinositol 3-kinase/AKT/Ras homolog enriched in brain (Rheb)/mammalian target of rapamycin (mTOR) pathway is necessary for induction of select protein markers of follicular differentiation. J Biol Chem 2004; 279:19431-19440.
153. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, *et al.* Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000; 60:1541-1545.
154. Richard DE, Berra E, Pouyssegur J. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. J Biol Chem 2000; 275:26765-26771.
155. Jiang BH, Jiang G, Zheng JZ, Lu Z, Hunter T, Vogt PK. Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth Differ 2001; 12:363-369.
156. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001; 21:3995-4004.
157. Yuan Y, Hilliard G, Ferguson T, Millhorn DE. Cobalt inhibits the interaction between hypoxia-inducible factor-alpha and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor-alpha. J Biol Chem 2003; 278:15911-15916.
158. Breuer W, Ermers MJ, Pootrakul P, Abramov A, Hershko C, Cabantchik ZI. Desferrioxamine-chelatable iron, a component of serum non-transferrin-bound iron, used for assessing chelation therapy. Blood 2001; 97:792-798.
159. Wang GL, Semenza GL. Desferrioxamine induces erythropoietin gene expression and hypoxia-inducible factor 1 DNA-binding activity: implications for models of hypoxia signal transduction. Blood 1993; 82:3610-3615.
160. Agani F, Semenza GL. Mersalyl is a novel inducer of vascular endothelial growth factor gene expression and hypoxia-inducible factor 1 activity. Mol Pharmacol 1998; 54:749-754.
161. Dongiovanni P, Valenti L, Ludovica Fracanzani A, Gatti S, Cairo G, Fargion S. Iron depletion by deferoxamine up-regulates glucose uptake and insulin signaling in hepatoma cells and in rat liver. Am J Pathol 2008; 172:738-747.
162. Mole DR, Maxwell PH, Pugh CW, Ratcliffe PJ. Regulation of HIF by the von Hippel-Lindau tumour suppressor: implications for cellular oxygen sensing. IUBMB Life 2001; 52:43-47.
163. Pham I, Uchida T, Planes C, Ware LB, Kaner R, Matthay MA, *et al.* Hypoxia upregulates VEGF expression in alveolar epithelial cells in vitro and *in vivo*. Am J Physiol Lung Cell Mol Physiol 2002; 283:L1133-1142.
164. Woo KJ, Lee TJ, Park JW, Kwon TK. Desferrioxamine, an iron chelator, enhances HIF-1alpha accumulation via cyclooxygenase-2 signaling pathway. Biochem Biophys Res Commun 2006; 343:8-14.