Cell. Mol. Life Sci. 65 (2008) 1133 – 1149
1420-682X/08/071133-17
DOI 10.1007/s00018-008-7472-0

Cellular and Molecular Life Sciences

Review

The magic of the hypoxia-signaling cascade

E. Benizri[a,†], A. Ginouvès[a,†] and E. Berra[b,*]

[a] ISDBC, CNRS UMR 6543, University of Nice, Centre Antoine Lacassagne, 33 Avenue Valombrose, 06189, Nice (France)
[b] Cell Biology and Stem Cells Unit, CICbioGUNE, Technology Park of Bizkaia-Building 801A, 48160 Derio (Spain), Fax: +34-944-061-324, e-mail: eberra@cicbiogune.es

Received 11 October 2007; received after revision 21 November 2007; accepted 23 November 2007
Online First 19 January 2008

Abstract. All organisms respond to changes in their environment by activating complex signaling cascades. The "hypoxia-signaling cascade" is activated in response to low oxygen availability and this activation is central to maintaining oxygen homeostasis and hence to survival. By regulating the transcriptional complex hypoxia-inducible factor, hypoxia is associated with several physiopathological processes. Several strategies, based on the targeting of the hypoxia-signaling cascade, have been developed to treat these pathologies. Our review summarize different aspects of the hypoxic pathway.

Keywords. Oxygen homeostasis, hypoxia, HIF signaling, physiopathology, therapy.

Introduction

In 1774, the scientist Joseph Priestley realized that "a mouse kept in a jar cannot survive in the absence of air renewal". Without knowing it, he had just discovered the oxygen molecule (O_2) and highlighted its absolute requirement for survival. All organisms rapidly respond to changes in oxygen availability. This response, triggered by many oxygen-sensing systems located at different levels in the organism, activates complex signaling networks, which culminate in the control of gene expression.

The signaling pathways activated by low oxygen availability, or hypoxia, allow cell adaptation and survival in the hypoxic environment. Among them, activation of the transcriptional complex hypoxia-inducible factor (HIF) contributes significantly to this adaptative response and constitutes a major regulator of oxygen homeostasis [1]. Since its discovery, the HIF cascade has been extensively studied and its role appears clearly evident in both physiological and pathological processes such as embryogenesis, tissue repair, ischemia and cancer.

This review summarizes our current knowledge of the HIF signaling cascade, its implication in physiopathology and the different therapeutic strategies developed so far to target this pathway.

The hypoxia inducible factor

HIF is a transcriptional factor composed of the constitutively expressed HIF1β subunit and one of the three HIFα subunits (HIF1α, HIF2α, HIF3α) [2–4]. Among them, HIF1α is the most extensively studied isoform. O_2-dependent HIFα regulation is essentially post-transcriptional and involves both regulation of its stability and its activity by a mechanism that is inherently O_2 dependent: the hydroxylation of proline (Pro) or asparagine (Asn) residues [5–7]. Indeed, hydroxylation of the Asn^{803} (in the

† These authors contributed equally to this work.
* Corresponding author.

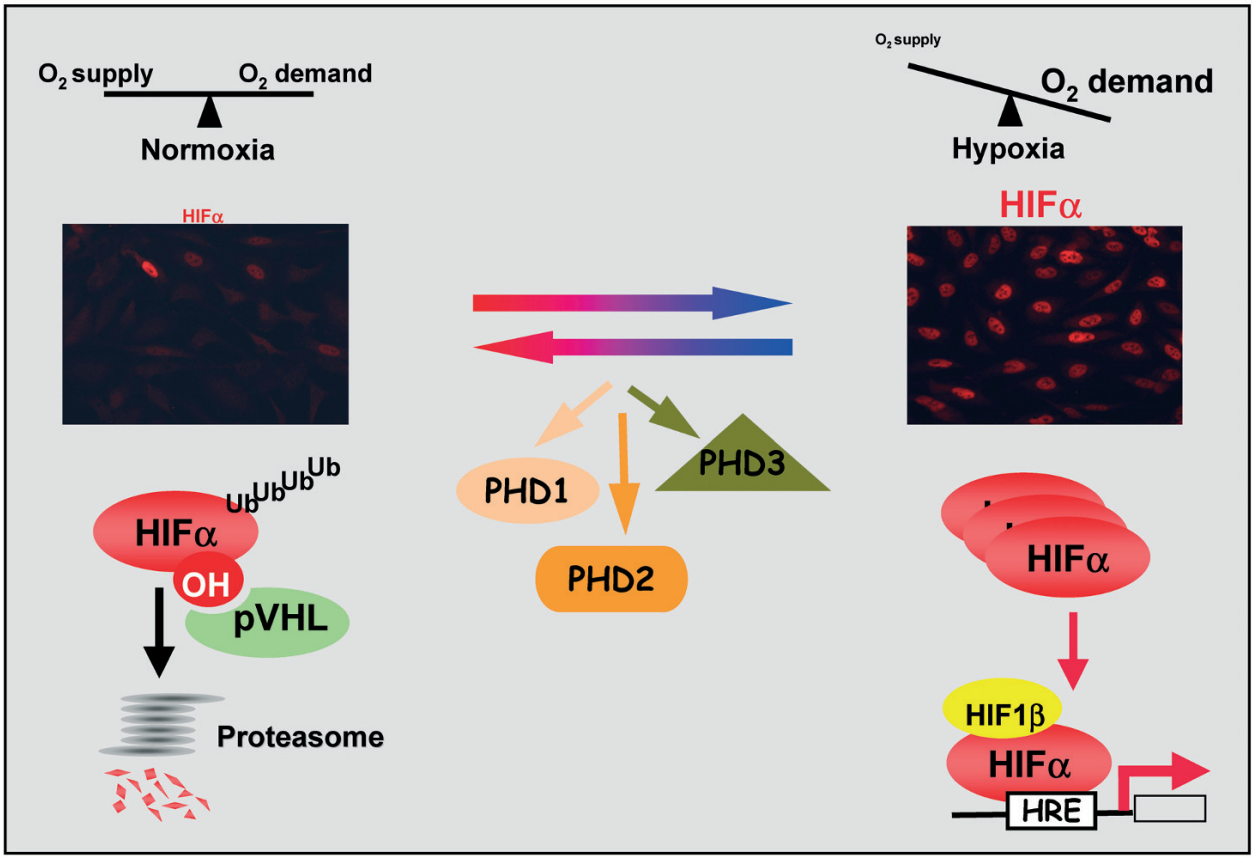

Figure 1. O_2-dependent regulation of hypoxia-inducible factor (HIF)α stability: Low oxygen availability stops the otherwise permanent HIFα degradation pathway, which implicates the proteasome and the product of the tumor suppressor gene von Hippel-Lindau (pVHL). The degradation pathway is triggered by hydroxylation of two proline residues within the oxygen-dependent degradation domain (ODDD) of the HIFα subunit by the prolyl hydroxylase domain-containing proteins (PHDs). These enzymes, acting as O_2 sensors, play a major role in the regulation of O_2 homeostasis and thus survival. Immunofluorescence inserts show the expression of HIF1α in well-oxygenated cells (normoxia) or upon low O_2 availability (hypoxia).

human HIF1α), within the C-terminal transactivation domain (C-TAD) by factor inhibiting HIF (FIH), inhibits the binding of the p300/CBP co-activators, and hence the transcriptional activity of the protein [7, 8]. Moreover, an oxygen-dependent degradation domain (ODDD) is involved in the O_2-dependent regulation of HIFα proteasomal degradation, which constitutes the limiting step in HIFα regulation, and consequently HIF activation [9, 10].

In well-oxygenated cells, HIFα is an exceptionally short-lived protein (half-life <5 min) and steady-state levels are very low [9]. In contrast, reduced O_2 availability accumulates HIFα by relaxing its ubiquitin-proteasome degradation. More precisely, ubiquitination and proteasomal degradation of HIFα require pVHL, the product of the von Hippel-Lindau tumor suppressor gene, which functions as an ubiquitin E3 ligase [11, 12]. The hydroxylation of two conserved proline residues (Pro^{402} and Pro^{564}, in the human HIF1α sequence), contained within the ODDD, triggers pVHL binding and thus HIF1α proteasome targeting [5, 6]. Stabilization of HIFα is due to disruption of the pVHL/HIFα interaction under hypoxic conditions (Fig. 1). Two independent groups identified the enzymes catalyzing the hydroxylation reaction: the HIF prolyl hydroxylases or prolyl hydroxylase domain-containing proteins (PHDs) [13, 14]. These enzymes belong to the superfamily of the Fe(II) and 2-oxoglutarate-dependent dioxygenases. They need the O_2 as a co-substrate, providing the molecular basis for their O_2-sensing function (*in vitro* K_m values for O_2= 230–250 μM) [15]. In addition, PHDs use Fe(II) and ascorbate as co-factors [16]. Fe(II) is critical for activating O_2 and as a template for the orderly binding of reactants. The ascorbate is necessary for maximal prolyl hydroxylase activity because it reduces the Fe(III) into Fe(II), and thus reactivates the enzymes.

Epstein et al. [14] identified three human and mouse *phd* genes (*phd1*, *phd2*, and *phd3*). The three isoforms are ubiquitously expressed, albeit at different levels [17, 18]. Fused to a green fluorescent protein (GFP),

PHD1 appears exclusively located in the nucleus, PHD2 mainly in the cytoplasm, and PHD3 in both compartments [19]. The contribution of each PHD to HIFα regulation depends on their relative abundance [20]. Nevertheless, we have shown that PHD2 is the rate-limiting enzyme that sets the low steady-state levels of HIF1α in normoxia, whereas PHD1 and PHD3 contribute to HIF1α regulation only upon a chronic hypoxia [21]. Furthermore, the expression and the activity of PHDs are tightly regulated at both transcriptional and post-transcriptional level [16, 22]. Initially identified as the regulator of the *epo* gene expression, HIF was further shown to control many hypoxia-target genes [22]. Indeed, by promoting the transcription of all these genes, HIF helps to maintain O_2 homeostasis. Among them, the vascular endothelial growth factor (VEGF) is essential for angiogenesis, inducible nitric oxide synthase (iNOS) and Heme Oxygenase 1 (HO1) favor vasodilatation, tyrosine hydroxylase (TH) regulates respiratory frequency, the glucose transporter GLUT-1 is involved in the anaerobic glycolysis and EPO is essential for erythropoiesis.

The activation of the HIF complex can be of major importance in certain physiological and pathological situations.

Physiology of the HIF cascade

Embryonic development

Hypoxia constitutes an important feature of the embryonic development by promoting the establishment and the differentiation of the vascular and hematopoietic systems. Indeed, as early as 1 week after fertilization, the nutrients and O_2 supplied by passive diffusion are not sufficient for the growing embryo and hypoxic gradients are subsequently established. This process, by promoting the expression of HIF-dependent genes such as *epo* and *vegf*, is absolutely required since mice deficient in these genes die during the embryo development as a consequence of severe hematopoietic and vascular defects [23–25]. Indeed, the phenotypic features of the knockout mice for *hif1α*, *hif2α*, *hif1β*, and *phds* agree with the central role of the HIF signaling pathway in the establishment of key physiological systems during the embryonic development.

HIF1α. In normal embryos, HIF1α expression increases between embryonic day (E) 8.5 and E9.5. HIF1α knockout mice stop their development at E8.5 and die at E10.5 because of vascular deficiencies and cardiac and neuronal abnormalities [26–28]. The vascular reduction in $hif1\alpha^{-/-}$ embryos is associated with a decrease in VEGF production. However, these vascular abnormalities occur at E9.25, whereas in $vegf^{-/+}$ embryos similar defects appear earlier, at E8.5 [23, 24]. It seems that $hif1\alpha^{-/-}$ mice produce enough VEGF to initiate the vasculogenesis and to form the vascular plexus but the following formation and maturation of the vasculature established from this plexus (angiogenesis) cannot occur in the absence of HIF1α expression.

HIF2α. $hif2\alpha^{-/-}$ mice also present a high embryonic lethality between E9.5 and E16.5 [29–32]. HIF2α seems to be required for the remodeling of the vascular network in the embryo and the heart function due to the catecholamines produced by the Zuckerland organ [30, 32]. Two independent laboratories successfully obtained live $hif2\alpha^{-/-}$ embryos [29, 31]. However, the respiratory, cardiac and hematopoietic functions of these mice were strongly affected. In addition, the replacement of HIF1α by HIF2α expression in knockout mice cannot rescue embryo survival [33]. Thus, in spite of the high sequence similarities, HIF1α and HIF2α have distinct and non-redundant functions that are essential, at least, for the embryo development.

HIF1β. The invalidation of HIF1β in mice also promotes a delayed embryo development together with vascular and placenta formation abnormalities leading to death at E10.5 [34, 35].

PHDs. The invalidation of the PHDs isoforms in mice highlighted specific functions for each PHD in the embryonic development [36, 37]. Indeed, *phd2* knockout promotes severe defects in the placenta and heart formation, leading to embryonic lethality between E12.5 and E14.5, whereas $phd1^{-/-}$ and $phd3^{-/-}$ mice are viable and apparently normal. Hence, PHD1 and PHD3 expression cannot rescue the $phd2^{-/-}$ phenotype. Accordingly to our previous report showing the key and unique role of PHD2 in the HIF1α stability regulation *in cellulo*, these results highlight the critical role of PHD2 during embryonic development.

Postnatal life

Hypoxia, by activating the HIF transcription factor, controls the expression of several genes involved in the regulation of essential cellular processes during postnatal life.

Growth. The development of Drosophila flies in hypoxic conditions is normal but the size of the adults is smaller, due to a reduced cell growth [37]. In Drosophila, the Cdk4/CycD signaling pathway regulates cell growth. In Cdk4/CycD mutants whose

growth is impaired, the ectopic expression of the dmPHD (named Fatiga) is sufficient to rescue the normal phenotype [38, 39]. Cdk4/CycD induces the ribosomal protein mRpL12 that is required for cell growth stimulation and, in the case of mRpL12 mutants, in spite of normal Fatiga protein level, Fatiga activity is blocked promoting HIF activity [40]. The role of Fatiga involves the dmHIF1α (named Sima) since its mutants are able to rescue the phenotype observed in those of Fatiga [41].

Proliferation. The activation of the angiogenic process and the anaerobic glycolysis *via* HIF stimulate cellular proliferation. However, Carmeliet et al. [26] reported that proliferation of tumors derived from ES HIF1$\alpha^{-/-}$ cells is more important that those from ES HIF1$\alpha^{+/+}$ cells. This discrepancy could be due in part to an increased p21 expression that promotes cell cycle arrest in hypoxia [42].

Differentiation. Hypoxia, in general, helps to maintain cells in an undifferentiated state. Indeed, hypoxia inhibits the differentiation of preadipocyte fibroblasts into adipocytes and myoblasts into myocytes [43, 44]. Nevertheless, some studies have shown that it can promote the differentiation of certain cell types [45, 46].
Recently, a link has been established between the HIF signaling and the Notch signaling, implicated in the differentiation of many stem cells; this link could explain, at least partially, the mechanism of dedifferentiation under hypoxia [47]. Indeed, under hypoxia, HIF1α interacts with the intracellular domain (ICD) of Notch and so promotes the recruitment of HIF on the Notch-induced genes promoters and their expression. Furthermore, the Notch ligand DII4 is up-regulated under hypoxia [48]. In endothelial progenitor cells, the hypoxic inductions of the arterial factors DII4 and hey2 repress the expression of the venous regulator COUP-TFII and block the differentiation in veins of these cells. In addition, as a negative feedback loop, the factors hey1 and hey2 are able to repress HIF1α transcriptional activity.
HIF-target genes can also regulate the functions of stem cells under hypoxia in a direct way. Indeed, during the embryonic development, the hypoxic induction of HIF2α controls the expression of the Oct-4 transcription factor, which is essential to maintain the hematopoietic stem cells in an undifferentiated state and to regulate the embryonic stem cell differentiation [33]. Oct-4 is also expressed in several cancer cell lines and is induced under hypoxia in a renal carcinoma cell line strongly expressing HIF2α [49].
In addition, two recent studies have also revealed some cross talk between HIF and the transforming growth factor β (TGF-β) and Wnt-dependent signaling pathways [50, 51].

Migration. The ability of the leukocytes to migrate is enhanced by the HIF-dependent induction of CD11b/CD18, a member of the integrins superfamily [52, 53]. Hypoxia also favors the migration of dermal fibroblasts and keratinocytes [54, 55].

Death. The mechanisms regulating cell death under limited oxygenation conditions are complex. Indeed, not only the O_2 level, but also the degree of activation of certain oncogenes and nutrient deprivation determinate the future behavior of the cells [56].
In general, drastic oxygen conditions (0.1 % O_2 or anoxia) triggers cell death through the activation of pro-apoptotic proteins, like Bax, cytochrome c release, and caspases activation [57]. More precisely, this cell death is mediated by HIF. Indeed, HIF1 promotes the expression of the pro-apoptotic protein BNIP3, a member of the Bcl-2 family (Bcl2/adenovirus E1B 19Kda interacting protein 3), as well as the expression of NIX, homologous to BNIP3 [58]. The absence of HIF1 prevents cells from up-regulating BNIP3 and reduces the apoptotic process induced by anoxia [59]. Anoxia also activates the JNK pathway (c-Jun NH_2-terminal kinase), which can promote the apoptotic process [60].
Contrary to anoxia, moderate hypoxia ($\geq$1 % O_2) does not affect cell proliferation and cell survival [61]. This cell survival is ensured by the PI3K/Akt activation, which inhibits the pro-apoptotic protein, Bad and favors NF-κB activity [62, 63]. In a same way, Degenhardt et al. [56] have recently shown that cells are able to survive by inducing the autophagic process.
Hypoxia can also favor apoptotic resistance. In renal epithelial cells, Dong Z et al. [64] reported a better resistance of cells under hypoxia to a pro-apoptotic treatment such as staurosporine. In these cells, the hypoxic induction of the apoptotic inhibitory protein IAP-2 prevents Bax mitochondrial translocation.
All these cellular functions regulated by the hypoxic signaling pathway are integrated in several biological processes such as inflammation or wound healing.

Inflammation. Low O_2 and glucose levels often characterize the environment of inflammatory sites such as the cutaneous infection, arthritis, or the necrotic regions of solid tumors [65–68]. At these sites, the myeloid cells have to evolve in spite of reduced oxygenation.
Hypoxia profoundly affects many properties of the myeloid cells: phagocytosis capacity, migration, adhesion, cytokines secretion, *etc.* More precisely, functional inactivation of the HIF1-dependent signaling

pathway has been shown to inhibit cell aggregation, motility, invasion and the capacity of cells to finally destroy the pathogen bacteria after their phagocytosis [69].

In addition, hypoxia promotes the secretion of inflammatory chemokines that attract neutrophils and monocytes. Furthermore, the macrophages-secreted cytokines interleukin 1 (IL-1) or tumor necrosis factor-α (TNF-α) favor the expression and DNA binding of HIF1α *via* the MAPK and PI3K pathways [70]. They also promote the production of reactive oxygen species, and thus contribute to HIF1α protein stabilization [71].

Finally, hypoxia, by inhibiting PHDs, increases also the IKKβ activity that is involved in the inflammatory process by activating the NF-κB pathway [72].

Wound healing. Hypoxic regions are also present around the wound healing sites. The O_2 reduction in the wounded tissue is due in part to the modification of the vascular network but also, in a large part, to the O_2 consumption of the cells that strongly proliferate and are metabolically over-activated. These low-oxygenated areas contribute to the stimulation of the angiogenic process and tissue repair by the induction of several genes, such as *tgfβ1*, *pro-collagen α1*, *vegf*, and *pdgf* (platelet-derived growth factor) [73]. Hypoxia around the wound promotes also the motility of the skin cells such as keratinocytes and favors wound closure by activating re-epithelialization [55]. Recently, Li et al. [74] have shown that hypoxia, by inducing HSP90α secretion in the extracellular environment, promotes dermal fibroblasts migration. More precisely, HSP90α, whose secretion is induced by HIF1 appears sufficient to increase fibroblasts motility.

Pathology of the HIF cascade

HIF activity is involved in several pathologies. In the case of ischemia or amyotrophic lateral sclerosis (ALS), the insufficient activation of HIF, in spite of reduced oxygenation, promotes the progression of the disease, whereas in Alzheimer's disease (AD), cancer or pre-eclampsia, the sustained and strong activation of the pathway forms part of the basis of these pathologies.

Neurological diseases

Mounting evidence shows a crucial role for hypoxia in neurological disorders such as ALS, Parkinson disease, AD or schizophrenia [75]. However, the implication of the HIF transcription factor has only been shown for the ALS and AD.

ALS. This is a late-onset progressive neurodegenerative disease affecting the motor neurons of the brain and the spinal cord [76]. Denervation of the respiratory muscles and diaphragm is generally the fatal event. The lifetime risk is at about 1 in 1000. Even now, 10 years after the approval of Riluzole, there is no effective therapy, although a few drugs are presently in Phase I, II and III clinical trials.

In mice, ALS can be mimicked by the deletion of the *vegf* HRE sequence [77]. Indeed, this disorder seems to be caused by a reduced blood perfusion of the neurons. Moreover, in ALS-affected rats, the direct administration of VEGF in the brain extends the motor neurons survival, protects the neuro-muscular junctions and finally favors the lifespan of these animals. These data highlighted the role of the HIF-dependent VEGF induction on motor neurons survival. Furthermore, Subramanian et al. [78] have recently identified a mutation in the gene coding for angiogenin (another hypoxia-induced protein involved in angiogenesis) in patients showing sporadic and familial ALS.

AD. An important feature of the AD is the formation of extracellular senile plaques in the brain, the major components of which are small peptides called β-amyloid (Aβ) derived from β-amyloid precursor protein (APP) [79, 80]. APP is sequentially cleaved first by the β-secretase (β-site amyloid precursor protein cleaving enzyme, BACE) and then by the γ-secretase complex to generate Aβ. The molecular mechanism of this sporadic disease is still unknown. However, a reduced cerebral perfusion is a classical feature and one of the major contributors for the AD pathogenesis development. Ischemia or stroke gives rise to hypoxic conditions, which greatly increase the incidence of AD. Indeed, activation of the HIF1 signaling pathway could favor AD development at different levels: (i) APP, the expression of which is up-regulated in the post-ischemic brain [81]; (ii) the isoform 1 of the BACE β-secretase (BACE 1), which contains a functional HRE sequence [82]; (iii) the *aph-1a* promoter gene, which is a key component of the γ-secretase complex, and also contains an HRE site [83]. Thus, the hypoxic inductions of APP, BACE 1 and aph-1 would promote the production and the deposition of Aβ and consequently affect the learning and memory capacities, as observed in AD patients.

Pre-eclampsia

Pre-eclampsia is a human pregnancy-specific disorder affecting 2–10 % of pregnancies. It is a leading cause of prenatal morbidity and mortality for the fetus and the mother, and the only intervention that effectively reverses the syndrome is giving birth. During the early

stages of pregnancy, the cytotrophoblasts play a major role in the placentation process: the differentiation of the proliferative cytotrophoblasts into invasive cytotrophoblasts is absolutely required for this process [84]. In the case of pre-eclampsia, it does not occur and the cytotrophoblasts are blocked in their proliferative state. Consequently, the utero-placental circulation is impaired and promotes a placental ischemia. This defect in adequate vascular network formation can be rescued by the use of HIF1α antisense [85]. Thus, sustained activation of HIF, by inhibiting cytotrophoblasts differentiation, promotes the development of the disease.

Ischemia

Several diseases, such as cerebral, cardiac or vascular ischemia, occur when cells do not adapt correctly to a reduced oxygenation [1]. Atherosclerosis leads to a decrease in oxygen tissue perfusion and thus to the formation of a hypoxic area. Although HIF is activated in these hypoxic regions, its activation is not sufficient to restore oxygen homeostasis. In coronary arteries, atherosclerosis lesions cause a cardiac ischemia due to the decreased delivery of oxygen and nutrients to the heart and can lead to a myocardium infarction.

Cancer

Tumor progression is characterized by an anarchic proliferation of the cancer cells. This proliferation is so major that the tumor is rapidly deprived of oxygen and nutrients, leading to the appearance of hypoxic regions. This process occurs in most solid tumors. At the cellular level, intra-tumoral hypoxia is associated to several phenomena that ensure survival and growth of the cancer cells. Tumor hypoxia promotes a radio- and chemo-resistant environment [86, 87]. Hypoxia stimulates the angiogenic process to increase tumor perfusion, switches tumor cells metabolism, and keeps their proliferative rate constant by increasing the transport of glucose and the glycolytic pathway to maintain a suitable ATP production [22]. Furthermore, hypoxia deregulates the pro- and anti-apoptotic balance to favor cell survival and also increases the invasive properties of cancer cells by up-regulating different genes. In all these processes induced by hypoxia, the HIF transcription factor plays a decisive role.

HIFα expression in tumors

HIF1α is overexpressed in many different types of human cancers [88]. Its expression is associated to an aggressive phenotype and is a marker for a poor prognosis for many types of tumors, such as prostate tumor, oropharyngeal, esophageal or head and neck tumors, lung cancer, ovarian cancer and breast cancer [89, 90].

HIF2α is more frequently overexpressed in hepatic primary or metastatic cancer and in the macrophages associated to the tumor (tumor-associated macrophages, TAMs) whose invading rate is correlated to the tumor progression and to a poor prognosis [91, 92]. The topography of HIFα subunit expression in tumors is usually focal and mainly restricted around hypoxic/necrotic areas, even if there is not always a strict co-localization of HIFα and the hypoxic regions (Figs. 2, 3). Nevertheless, in certain tumors, such as in clear cell renal carcinoma (RCCs), mutation in gene coding for the pVHL protein leads to a constitutive stabilization of HIF1α and/or HIF2α [12]. In paragangliomas with a mutation in the gene for the succinate dehydrogenase kinase, HIF1α levels are also up-regulated due to the PHD inhibition [93]. More recently, in endometrial tumor, mutation in the *phd2* gene has also been reported and shown to over-activate HIF1 [94].

HIFα contribution to tumor progression

As previously mentioned, hypoxia-induced HIF activation controls the expression of several genes involved in many aspects of cancer progression.

Tumor angiogenesis. The angiogenic switch that represents one of the major events in tumor progression is triggered by hypoxia and HIF by inducing the expression of VEGF and its receptors (Flt1, Tie2). These results agree with findings of *in vivo* experiments by Carmeliet et al. [26]: HIF1$\alpha^{-/-}$ ES-derived tumors are smaller and poorly vascularized compared to those from HIF1$\alpha^{+/+}$ ES cells. In contrast, Ryan et al. [28] showed that HIF1α inactivation affects tumor growth but has no impact on vascular density.

Furthermore, it seems that the role of HIF is strongly dependent on the tumor environment [95]. HIF1$\alpha^{-/-}$ astrocytes injected subcutaneously induce some poorly perfused and very necrotic tumors, whereas the same astrocytes injected into the brain give rise to highly vascularized tumors.

Tumor invasion. Several genes that induce extracellular matrix remodeling and basal membrane digestion are regulated by hypoxia and HIF, such as *vimentin*, *fibronectin*, *keratin 14*, *18*, *19*, *matrix metalloproteinase 2*, *cathepsin D,* and *urokinase plasminogen activator receptor.* In parallel, HIF1α stabilization triggers the lose of E-cadherin expression and promotes epithelial-mesenchymal transition [96]. Indeed, HIF1 induces the expression of the lysyl oxidase, which regulates the SNAIL transcription factor whose activation represses the E-cadherin expression [97–99]. Moreover, several genes favoring cancer

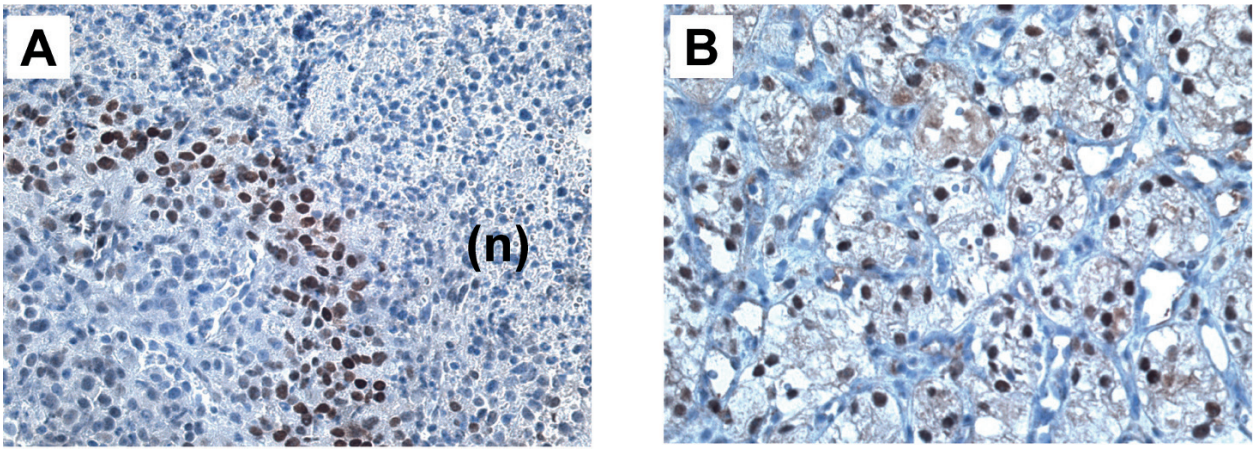

Figure 2. HIF1α expression in tumors. (*A*) HIF1α shows nuclear staining surrounding necrotic areas (n) in a sarcoma xenograft model; (*B*) HIF1α is constitutively expressed in a human renal clear cell carcinoma (RCC) because of the inactivating *vhl* mutation.

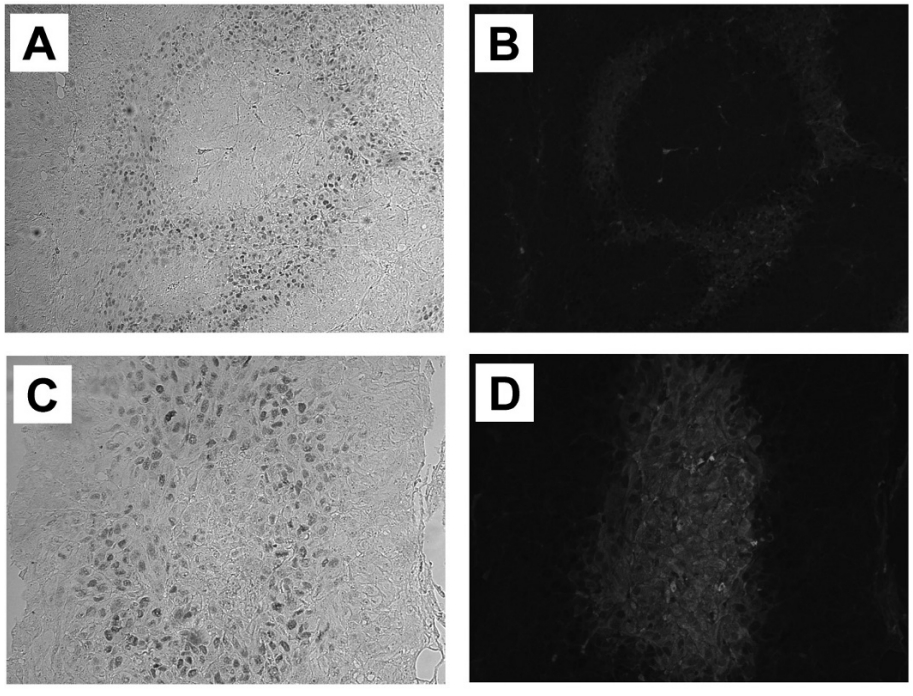

Figure 3. Staining of HIF1α protein and hypoxic regions in human tumors. Xenograft tumor of human melanoma: HIF1α staining (*A*) co-localizes with the hypoxic marker, pimonidazole (*B*). Xenograft tumor of human breast carcinoma: HIF1α staining (*C*) is not perfectly correlated to the hypoxic marker staining, pimonidazole (*D*).

cells migration, and metastatic properties are also regulated by the activation of the HIF complex: the proto-oncogene c-MET, the receptor CXCR4 and its unique chemokine ligand stromal cell-derived factor1 (SDF1 also called CXCL12).

Metabolic adaptation. HIF stimulates the expression of the glucose transporters and the glycolytic enzymes to assure the ATP delivery inside the tumor. Because the anaerobic glycolysis leads to an acidification of the tumoral microenvironment, tumor cells also induce, *via* HIF, the expression of exchangers such as the Na^+/H^+ exchanger (NHE1) or transporters such as the monocarboxylate transporter (MCT4) to maintain the pH homeostasis [100, 101]. In addition, tumor cells up-regulate the expression of carbonic anhydrases CAIX and CAXII, which are linked to the extracellular membrane and catalyze the conversion of CO_2 in HCO_3^- [102, 103]. The bicarbonates are then driven into the cells by the Cl^-/HCO_3^- exchangers and restore the intracellular compartment pH assuring the adaptation of these cells to the acidic environment.

Hypoxia and HIF cascade as therapeutic targets

The role of hypoxia and HIF in several pathologies is clearly established, particularly in cancer and cardiovascular diseases. For this reason, their modulation

might be a good strategy for treating hypoxic tumors or ischemia-related pathologies.

Cancer

In this section, we present an overview of the most relevant approaches established to improve survival based on the targeting of hypoxia and/or the HIF cascade.

Hypoxia as a therapeutic target in cancer

Tumor hypoxia has been exploited as a way to activate some drug compounds.

Hypoxia-activated drugs. Some anti-cancer agents (N-oxides, quinones, nitro-aromatics) have been explored as drugs that exploit the hypoxia itself. The most widely studied of these compounds is tirapazamine [104]. This 'hypoxic cytotoxin' is bioreductively activated in hypoxic cells and potentiates the cytotoxicity of radiation and chemotherapy, in particular that of platinum and taxanes family [105–107]. Tirapazamine has been studied in a Phase III clinical trial in combination with *cis*-platin and paclitaxel for non-small-cell lung cancer [108]. However, the association of this hypoxia-activated drug to the chemotherapy did not improved response rates and survival. These data are in contradiction with another study of head and neck cancer where the adjunction of tirapazamine increases the 3-year failure-free survival rate [109]. Consequently, other evaluations and studies are justified.

In addition, the capacity of anaerobic bacteria to proliferate under hypoxic conditions has been exploited as a tool for hypoxia-targeted anticancer therapy. In this context, the bacteria *Clostridium acetobutylicum* has been modified to overexpress cytosine deaminase, which is able to metabolize the 5-fluorocytosine into the toxic 5-fluorouracile (5-FU). Following the administration of these bacteria to rhabdomyosarcoma-bearing rats, cytosine deaminase is detected in the tumor, suggesting that this original approach might be useful [110].

Therapeutic failure is associated with hypoxic tumors and thus several approaches have been explored to reduce tissue hypoxia. The most obvious strategy to enhance radio- and chemo-sensitivity is the administration of high-pressure oxygen. In addition, the development of sensitizers is another method to potentiate the effect of classical therapies.

Erythropoietin. The administration of erythropoietin (Epo, the glycoprotein hormone endogenously secreted by renal fibroblasts under hypoxic conditions *via* an HIF-dependent mechanism) is used to increase tumor oxygenation [111]. Recombinant human Epo (rHuEpo) is indeed a crucial therapeutic tool in cancer therapy for patients suffering from anemia. Many studies have shown that correction of anemia, and consequently of hypoxia, is a prognostic factor of disease control and survival. Thus, in preclinical and clinical studies, rHuEpo may improve radio- and chemo-sensitivity of solid tumor possibly by increasing tumoral oxygenation [112, 113].

Hyperbaric oxygen therapy. Hyperbaric oxygen (HBO) therapy involves the administration of pure oxygen at a pressure superior than 1 atmosphere. Experimental studies suggest that hypoxia reduces the radio-sensitivity of cells as they require three times more radiation [114]. By increasing the oxygen tension of hypoxic cells, HBO may sensitize radiation therapy. However, it is known that reoxygenation of hypoxic tumor cells may stimulate tumor growth. Nevertheless, Feldmeier's group elegantly concluded, from clinical and preclinical studies, that intermittent HBO therapy has no stimulatory effect on tumor growth and dissemination [115]. Furthermore, intermittent HBO therapy increased the radio-curability of several cancers. In particular, it improved local tumor control and reduced mortality in head and neck cancer [116].

As seen for radiotherapy, HBO therapy is able to sensitize chemotherapy by increasing tumor perfusion and cellular sensitivity. This has been demonstrated *in vitro* with 5-FU, doxorubicin, and taxol [117]. Thus, HBO therapy could be considered as adjuvant therapy in hypoxic tumors.

ARCON. Accelerated radiotherapy with carbogen and nicotinamide is another approach that makes it possible to circumvent the mechanisms of radio resistance. In this method, radiotherapy is associated with inhalation of hyperoxic gas to decrease diffusion-limited hypoxia and nicotinamide to decrease perfusion-limited hypoxia. In a breast xenograft model the radiation dose is decreased by 50% with ARCON [118]. However, the results obtained with ARCON in clinical studies have been disappointing. In non-small-cell lung cancer, there were no significant responses with ARCON [119]. Nevertheless, in bladder cancer, ARCON showed significant gains on local control, disease-free survival and overall survival compared with a group control [120].

Sensitizer agents. By simulating the action of oxygen, sensitizer agents compensate the low oxygen concentration and increase radiation-induced damage. The compounds most frequently used in clinical studies

Table 1. Summary of the most relevant anti-cancer therapeutic approaches targeting the HIF cascade developed so far.

Molecular screenings			
Target	Compound	Mechanism of action	Efficacy
HIF-targeting high-throughout	PX-478	?	Inhibition of tumor growth in animal models
	Topotecan	Topoisomerase-1 inhibitors	Inhibition of tumor growth in animal models
	ARC-111		Inhibition of HIF1α expression *in cellulo*
	Echinomycin	HIF DNA-binding activity	Disappointing results in clinical trials
	Chetomin	HIF / p300 binding	Inhibition of tumor growth in animal models
PHDs	Cyclosporine A	PHDs activators	Inhibition of HIF1α expression *in cellulo* (Doubts about future application because of the immunosuppressive effect)
	R59949		Inhibition of HIF1α expression *in cellulo*
Others	Rapamycin	PI3K/AKT/mTor pathway inhibitors	Disappointing results in clinical trials
	CCI-779		Used in clinic against Breast cancer
	Trastuzumab		Inhibition of tumor growth in animal models
	LY294002, Wortmannin		
	PD98059	MAPK pathway inhibitors	Inhibition of HIF1α expression *in cellulo*
	GL331		
	YC-1	?	Inhibition of tumor growth in animal models
	Geldanamycin	Hsp90 inhibitor	Inhibition of tumor growth in animal models
	Radicicol		
	2ME2	Microtubules	Inhibition of tumor growth in animal models

Gene therapy	
Strategies	Efficacy
Antisense therapy	Inhibition of tumor growth in animal models
Gene suicide	
RNA interference	

over the last few years were nitroimidazoles, but these again have had disappointing results. In carcinoma of cervix, Grigsby et al. [121] did not show any difference in a study comparing irradiation with or without misonidazole. On the other hand, in head and neck carcinomas, a randomized study showed encouraging results with nimorazole [122]. Indeed, in association with radiotherapy, nimorazole induced a better loco-regional control and cancer-related survival than the control group. In the same way, a meta-analysis suggested that radio-sensitization with misonidazole for astrocytomas increases 1-year survival by 8% [123].

HIF cascade as a therapeutic target in cancer

The inhibition of HIF1α expression impairs tumor growth when using HIF1α$^{-/-}$-derived ES cells [26]. Similarly, the expression of a dominant negative mutant of HIF1 (deprived of the two TAD domains) in PCI43 cells leads to the inhibition of tumor growth [124]. Inhibiting the hypoxia signaling cascade as a therapeutic approach to block tumor progression might target different processes: HIF1α protein stability, nuclear translocation, DNA-binding capacity or even the association with transcriptional repressors and/or co-activators. Several approaches have been developed using not only conventional strategies (as molecular screenings) but also more innovative strategies, such as RNA interference (Table 1).

HIF-targeting drug screening. High-throughout screenings have led to the discovery a certain number of efficient molecules targeting the HIF cascade:

- PX-478 is a drug developed by ProlX Pharmaceuticals. PX-478 suppresses HIF1α protein expression under normoxic and hypoxic condition, although its precise mechanism of action remains unclear. Moreover, PX-478 showed anti-tumor activity in a variety of xenograft animal models [125]. This effect correlates with a reduction of HIF1α protein level measured by immunohistochemistry. Furthermore, toxicological studies showed a moderated toxicity. To date, no clinical trial is being carried out with this promising drug.
- Topoisomerase-I inhibitors: Out of the 2000 agents tested by Rapisarda et al. [126], 4 were identified as HIF1 inhibitors. Three of these compounds are related to camptothecin, which inhibits topoisomerase-I activity. The best characterized in clinical study is NSC-609699 (topotecan). In a glioma xenograft model, topotecan inhibits tumor growth,

with a concomitant and dramatic reduction of the protein levels of HIF1α and HIF1-target gene products [127]. Recently, another topoisomerase I-inhibitor (ARC-111) has been tested *in cellulo*, showing inhibition of HIF1α expression upon hypoxia [128]. However, no clinical or preclinical studies are available at this moment using these drugs.

- Echinomycin is an antibiotic derived from quinoxaline family, which inhibits HIF1α DNA-binding activity [129]. This is not the only effect of this small molecule. This drug has been evaluated in numerous preclinical and clinical trials; however, with disappointing results [130, 131].
- Chetomin is small molecule that is able to disrupt HIF1 binding to the p300 co-activator. *In vivo*, this transcriptional inhibition reduces tumor growth by increasing the number of necrotic regions in treated xenograft tumors [132]. These effects seem to be specific for HIF1, since the expression of a p300-independent form of HIF1α in tumor cells rescues, at least partially, the phenotype induced by chetomin. This molecule is on standby for clinical evaluation.

PHDs activators. PHDs are clearly implicated in the regulation of HIF1α protein stability. The activation of these PHDs could be an interesting anti-HIF therapeutic strategy:

- Cyclosporin A is an immunosuppressive agent used in organ transplantation. Cyclosporin A also activates PHD activity [133], through this mechanism this drug inhibits hypoxia-induced HIF1α stabilization and HIF1-mediated cellular responses in glioma cells. However, these results observed *in cellulo* cannot be extrapolated to the *in vivo* situation. Indeed, because of its immunosuppressive functions, cyclosporin should not be used in cancer therapy where immunity is crucial.
- R59949 is a PHD activator. This agent is an inhibitor of diacylglycerol kinase [134]. *In cellulo*, R59949 inhibits HIF1α protein accumulation by stimulating PHD activity. This molecule offers a new possibility against cancer but has not yet been tested *in vivo.*

Indirect inhibitors of the HIF complex

Inhibitors of the PI3K/Akt/mTor cascade. Drugs interfering with the PI3K/Akt/mTor pathway can block the expression of HIF1α protein and HIF1-dependent gene products. Rapamycin inhibits HIF1α expression *in cellulo* [135]. An ester analogue of rapamycin, CCI-779, inhibits tumor growth in a rhabdomyosarcoma xenograft model [136]. This inhibition is related to the reduction of HIF1α and VEGF expression. CCI-779 has, however, shown contradictory results in numerous clinical studies [137, 138]. Other example is trastuzumab (herceptin). This humanized monoclonal antibody targets the human EGF receptor-2 (HER2) [139]. HER2 induces the expression of HIF1α and VEGF in breast cancer cells *via* the PI3K/AKT pathway [140]. Treatment of breast cancer cells with the HER2 inhibitor induces a dose-dependent inhibition of EGF levels but the impact on HIF1α still remains controversial [141]. LY294002 and wortmannin represent other tested agents that reduce HIF1α expression *in cellulo* [142]. *In vivo* and in clinical studies, these drugs reduce tumor growth but the impact on the HIF pathway has not yet been elucidated [143].

Inhibitors of the MAPK cascade. Some inhibitors have been developed, such as PD98059 that inhibits the transactivation ability of HIF1α but does not change the stabilization or DNA binding ability of HIF1α [144]. GL331 is a plant-derived MAPK inhibitor (the podophylotoxin). GL331 is known to block the ERK pathway and inhibit HIF1α expression in human lung cancer cells [145]. Nevertheless, there are no reported data on its use as an anticancer drug.

YC-1. YC-1 [3-(5'hydroxymethyl-2'-furyl)-1-benzylindazole] is another molecule that can inhibit HIF1α. Initially developed for circulatory disorders, YC-1 inhibits platelet aggregation and vascular contraction by activating soluble guanylyl cyclase [146]. The precise mechanism of HIF1α inhibition is not clear: NF-κB and Mdm2 may contribute to this inhibition. *In cellulo,* YC-1 inhibits HIF-1α accumulation at the post-translational level in hepatoma cells upon hypoxia [147]. *In vivo*, YC-1 reduces the tumor growth in five independent xenograft models, and inhibits the expression of HIF1α and HIF1-regulated genes [148]. Given that no serious toxicity was highlighted in mice, this drug could be a good candidate for a Phase I study.

Heat-shock protein 90 (Hsp90) inhibitors. Hsp90 is a chaperone protein required for the stability and/or maturation of numerous proteins, including key mediators of signal transduction and cell cycle control. Among these proteins, Hsp90 regulates HIF1α activation [149]. Moreover, *in vivo*, geldanamycin (an Hsp90 inhibitor) induces HIF1α protein degradation under normoxia or hypoxia [150]. In the same manner, radicicol is another Hsp90 inhibitor that inhibits DNA binding of the HIF1 complex and reduces hypoxia-

induced VEGF expression [151]. These compounds have shown preclinical efficacy in xenograft model and clinical trials [152]. Nevertheless, up to date, there is no reported data related to their impact on the HIF pathway.

Microtubule-disrupting agents (MDAs). Another interesting group of cytotoxic agents that target HIF1α are those that induce disruption of microtubule architecture. Among these drugs, 2-methoxyestradiol (2ME2) is an endogenous metabolite of estrogen that has an anti-angiogenic and anti-proliferative activity [153]. Recent data have shown that this anti-angiogenic effect is mediated through the inhibition of HIF1α expression in endothelial and cancer cells. Other agents that stabilize (taxol) or destabilize (vincristine) microtubules cause a similar effect. 2ME2 reduces tumor growth and angiogenesis *in vivo,* but the impact on HIF1α expression is still unclear [154].
These data are, however, in contradiction with the results reported by Jung et al. [155] showing that certain MDAs (vinblastine, colchicine or nocodazole) increase HIF1 activation through an NF-κB-dependent pathway. Further studies are necessary to clarify this point and validate the potential interest of these compounds.

Gene therapy

Antisense therapy. Several groups have evaluated the inhibition of HIF activity by antisense therapy. Sun et al. [156] were the first to show the efficacy of this treatment in a mouse lymphoma model. Intratumoral injection of a plasmid vector containing an HIF1α antisense led to the regression of small tumors (<1 mm) and slowed the growth of large tumors (>4 mm). This effect correlated to the inhibition of HIF1α and VEGF expression. The same team showed an excellent anti-tumor efficacy of the HIF1α antisense therapy in combination with pVHL overexpression in a glioma model [157]. Antisense therapy used in pancreatic cancer xenograft models similarly reduced tumor size and weight [158]. Accordingly, gene transfer techniques using antisense plasmids may provide future anti-cancer therapy but, to date, this anti-HIF1α strategy has not been tested in clinical trials.

Suicide gene therapy. This strategy is based on the administration into the tumor cells of a gene encoding for an enzyme that is able to transform a non-toxic pro-drug into a cytotoxic agent. HRE-controlled gene suicide therapy has shown interesting data *in cellulo* and *in vivo.* For example, an adenoviral vector under the control of an optimized HRE promoter has been used to express the gene encoding the human cytochrome P450 (CYP2B6) and shown to delay tumor growth upon treatment with cyclophosphamide [159]. Several teams have used similar strategies but, for the moment, no clinical data are available.

RNA interference. RNA interference (RNAi) is an alternative strategy to inhibit the HIF1 signaling pathway. This innovative technology is particularly interesting since RNAi triggers sequence-specific gene silencing. This approach is well controlled *in cellulo* but the conditions of administration are not yet well established *in vivo.* Mizuno et al. [160] reported the impact of HIF1α silencing on a xenograft model of pancreatic and hepatobiliary carcinoma cells stably transfected with a plasmid producing siHIF1α, and showed that this silencing significantly reduced tumor growth. More recently, Gillepsie et al. [161] showed, in a mouse model of glioma, the reduction of tumor growth and HIF1 activity following intratumoral injection of siHIF1α. All these results are very promising and suggest the efficacy of this strategy but, to date, no systemic administration has been evaluated.

Ischemic cardiovascular diseases

Many angiogenic factors (VEGF, FGF, PDGF) have been tested in preclinical studies, but in clinical practice the results are either modest or disappointing. Activating HIF, which induces angiogenesis *via* the expression of many angiogenic factors, might be more effective than targeting a single factor. Thus, the development of HIF stimulants may be helpful for the treatment of ischemic disease. However, all the strategies that improve systemic angiogenesis might induce side effects in patients with cancer, angioma, arthritis, retinopathies or atherosclerotic plaque progression. Consequently, we have to be very careful with the use of such agents. Two main strategies have been developed to activate HIF by either inhibiting the endogenous degradation pathway or by overexpressing the protein.

Inhibiting HIF degradation as a therapeutic approach in ischemia

PR39. This molecule was identified as a macrophage-derived peptide that induces HIF1α by inhibiting the proteasome [162]. In a pig model of chronic myocardial ischemia, the intracardial delivery of an adenoviral construction (Ad)-PR39 improves myocardial blood flow and collateral formation by a mechanism implicating HIF1α overexpression and induction of VEGF, VEGFR-1 and 2, syndecan and FGFR-1 [163].

Dibenzoylmethane. This product is a natural dietary compound and iron chelator, and inhibits HIF1α degradation and induces VEGF secretion under normoxia [164]. This agent might be of interest in ischemic disease but, to date, no clinical or preclinical data are available.

siPHDs. Another innovative strategy is the RNAi to silence PHD2 to increase HIF1 transcriptional activity. Indeed, intraperitoneal injection of siPHD2 in a mouse model of myocardial ischemia reperfusion injury induces up-regulation of cardiac iNOS expression [165]. This is translated clinically by a higher ventricular function and a lower infarct size than seen in the control group.

DMOG. Dimethyloxalylglycine (DMOG) is a small molecule that inhibits the oxoglutarate-dependent dioxygenase and thus induces HIF1α stabilization. In a mouse model of hind limb ischemia, administration of DMOG increased VEGF levels and capillary density [166]. Other PHDs inhibitors have shown similar results: P4-H, L-Mim, 3,4-DHB and S956711 [167].

Ectopic expression of HIF as a therapeutic approach in ischemia

A constitutively active form of HIF1α has been constructed associating the DNA-binding and dimerization domains from HIF1α and the transactivation domain from herpes simplex virus VP16 protein (HIF-1α/VP16). In preclinical studies, the administration of this strong constitutive transcriptional activator improved angiogenesis in a rabbit hind limb ischemia model (increasing the blood flow and the collateral vessel development) and in a rat myocardial infarction (reduction of the infarct size and increasing capillary density) [168].

Another recombinant replication-deficient adenovirus Ad2/HIF-1α/VP16 has been evaluated in a Phase I for critical limb ischemia patients with promising results and particularly with no major adverse effect [169].

AdCA5 is another replication-defective recombinant adenovirus encoding a constitutive form of HIF1α. In preclinical studies, ocular injection of AdCA5 in mice induced neovascularization of retinal vessels *via* the expression of multiple angiogenic factors [170]. In the same way, this construction injected in a rabbit model of limb ischemia stimulates the recovery of limb perfusion by increasing capillary density and luminal area of arteries [171].

All these results support our particular interest in the understanding of the molecular mechanisms underlying the hypoxia-signaling cascade to improve our basic knowledge and also to identify new prognostic and/or predictive clinical markers, as well as therapeutic targets against the pathologies in which hypoxia is implicated.

Acknowledgments. The authors have been supported by the Centre National de la Recherche Scientifique (CNRS), Ministère de l'Education, de la Recherche et de la Technologie, Ligue Nationale Contre le Cancer (Equipe labellisée), Cancéropôle PACA, and Association pour la Recherche sur le Cancer (Subvention no. 3693). E.B. is currently funded by the PN I+D SAF2007–64597, the ETORTEK Research Program 05–07 and the BIZKAIA XEDE Program from the Bizkaia County. We apologize to the many research groups whose work was not cited due to space constraints.

1 Semenza, G. L. (2000) HIF-1: Mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. 88, 1474–1480.
2 Ema, M., Taya, S., Yokotani, N., Sogawa, K., Matsuda, Y. and Fujii-Kuriyama, Y. (1997) 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 94, 4273–4278.
3 Gu, Y. Z., Moran, S. M., Hogenesch, J. B., Wartman, L. and Bradfield, C. A. (1998) Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. Gene Expr. 7, 205–213.
4 Wang, G. L., Jiang, B. H., Rue, E. A. and Semenza, G. L. (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc. Natl. Acad. Sci. USA 92, 5510–5514.
5 Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S. and Kaelin, W. G. Jr. (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: Implications for O_2 sensing. Science 292, 464–468.
6 Jaakkola, P., Mole, D. R., Tian, Y. M., Wilson, M. I., Gielbert, J., Gaskell, S. J., Kriegsheim, A., Hebestreit, H. F., Mukherji, M., Schofield, C. J., Maxwell, P. H., Pugh, C. W. and Ratcliffe, P. J. (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 292, 468–472.
7 Lando, D., Peet, D. J., Gorman, J. J., Whelan, D. A., Whitelaw, M. L. and Bruick, R. K. (2002) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev. 16, 1466–1471.
8 Mahon, P. C., Hirota, K. and Semenza, G. L. (2001) FIH-1: A novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev. 15, 2675–2686.
9 Huang, L. E., Gu, J., Schau, M. and Bunn, H. F. (1998) Regulation of hypoxia-inducible factor 1alpha is mediated by an O_2-dependent degradation domain *via* the ubiquitin-proteasome pathway. Proc. Natl. Acad. Sci. USA 95, 7987–7992.
10 Salceda, S. and Caro, J. (1997) Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J. Biol. Chem. 272, 22642–22647.
11 Kaelin, W. G. Jr. (2002) Molecular basis of the VHL hereditary cancer syndrome. Nat. Rev. Cancer 2, 673–682.
12 Maxwell, P. H., Wiesener, M. S., Chang, G. W., Clifford, S. C., Vaux, E. C., Cockman, M. E., Wykoff, C. C., Pugh, C. W., Maher, E. R. and Ratcliffe, P. J. (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399, 271–275.

13 Bruick, R. K. and McKnight, S. L. (2001) A conserved family of prolyl-4-hydroxylases that modify HIF. Science 294, 1337–1340.
14 Epstein, A. C., Gleadle, J. M., McNeill, L. A., Hewitson, K. S., O'Rourke, J., Mole, D. R., Mukherji, M., Metzen, E., Wilson, M. I., Dhanda, A., Tian, Y. M., Masson, N., Hamilton, D. L., Jaakkola, P., Barstead, R., Hodgkin, J., Maxwell, P. H., Pugh, C. W., Schofield, C. J. and Ratcliffe, P. J. (2001) *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 107, 43–54.
15 Hirsila, M., Koivunen, P., Gunzler, V., Kivirikko, K. I. and Myllyharju, J. (2003) Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. J. Biol. Chem. 278, 30772–30780.
16 Berra, E., Ginouves, A. and Pouyssegur, J. (2006) The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signaling. EMBO Rep. 7, 41–45.
17 Cioffi, C. L., Liu, X. Q., Kosinski, P. A., Garay, M. and Bowen, B. R. (2003) Differential regulation of HIF-1 alpha prolyl-4-hydroxylase genes by hypoxia in human cardiovascular cells. Biochem. Biophys. Res. Commun. 303, 947–953.
18 Lieb, M. E., Menzies, K., Moschella, M. C., Ni, R. and Taubman, M. B. (2002) Mammalian EGLN genes have distinct patterns of mRNA expression and regulation. Biochem. Cell Biol. 80, 421–426.
19 Metzen, E., Berchner-Pfannschmidt, U., Stengel, P., Marxsen, J. H., Stolze, I., Klinger, M., Huang, W. Q., Wotzlaw, C., Hellwig-Burgel, T., Jelkmann, W., Acker, H. and Fandrey, J. (2003) Intracellular localisation of human HIF-1 alpha hydroxylases: Implications for oxygen sensing. J. Cell Sci. 116, 1319–1326.
20 Appelhoff, R. J., Tian, Y. M., Raval, R. R., Turley, H., Harris, A. L., Pugh, C. W., Ratcliffe, P. J. and Gleadle, J. M. (2004) Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J. Biol. Chem. 279, 38458–38465.
21 Berra, E., Benizri, E., Ginouves, A., Volmat, V., Roux, D. and Pouyssegur, J. (2003) HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO J. 22, 4082–4090.
22 Semenza, G. L. (2003) Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer 3, 721–732.
23 Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., Declercq, C., Pawling, J., Moons, L., Collen, D., Risau, W. and Nagy, A. (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 380, 435–439.
24 Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O'Shea, K. S., Powell-Braxton, L., Hillan, K. J. and Moore, M. W. (1996) Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 380, 439–442.
25 Lin, C. S., Lim, S. K., D'Agati, V. and Costantini, F. (1996) Differential effects of an erythropoietin receptor gene disruption on primitive and definitive erythropoiesis. Genes Dev. 10, 154–164.
26 Carmeliet, P., Dor, Y., Herbert, J. M., Fukumura, D., Brusselmans, K., Dewerchin, M., Neeman, M., Bono, F., Abramovitch, R., Maxwell, P., Koch, C. J., Ratcliffe, P., Moons, L., Jain, R. K., Collen, D. and Keshert, E. (1998) Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 394, 485–490.
27 Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laughner, E., Wenger, R. H., Gassmann, M., Gearhart, J. D., Lawler, A. M., Yu, A. Y. and Semenza, G. L. (1998) Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 12, 149–162.
28 Ryan, H. E., Lo, J. and Johnson, R. S. (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J. 17, 3005–3015.
29 Compernolle, V., Brusselmans, K., Acker, T., Hoet, P., Tjwa, M., Beck, H., Plaisance, S., Dor, Y., Keshet, E., Lupu, F., Nemery, B., Dewerchin, M., Van Veldhoven, P., Plate, K., Moons, L., Collen, D. and Carmeliet, P. (2002) Loss of HIF-2alpha and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. Nat. Med. 8, 702–710.
30 Peng, J., Zhang, L., Drysdale, L. and Fong, G. H. (2000) The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. Proc. Natl. Acad. Sci. USA 97, 8386–8391.
31 Scortegagna, M., Ding, K., Oktay, Y., Gaur, A., Thurmond, F., Yan, L. J., Marck, B. T., Matsumoto, A. M., Shelton, J. M., Richardson, J. A., Bennett, M. J. and Garcia, J. A. (2003) Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice. Nat. Genet. 35, 331–340.
32 Tian, H., Hammer, R. E., Matsumoto, A. M., Russell, D. W. and McKnight, S. L. (1998) The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. Genes Dev. 12, 3320–3324.
33 Covello, K. L., Kehler, J., Yu, H., Gordan, J. D., Arsham, A. M., Hu, C. J., Labosky, P. A., Simon, M. C. and Keith, B. (2006) HIF-2alpha regulates Oct-4: Effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev. 20, 557–570.
34 Adelman, D. M., Gertsenstein, M., Nagy, A., Simon, M. C. and Maltepe, E. (2000) Placental cell fates are regulated *in vivo* by HIF-mediated hypoxia responses. Genes Dev. 14, 3191–203.
35 Maltepe, E. and Simon, M. C. (1998) Oxygen, genes, and development: An analysis of the role of hypoxic gene regulation during murine vascular development. J. Mol. Med. 76, 391–401.
36 Takeda, K., Ho, V. C., Takeda, H., Duan, L. J., Nagy, A. and Fong, G. H. (2006) Placental but not heart defects are associated with elevated hypoxia-inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. Mol. Cell. Biol. 26, 8336–8346.
37 Palos, L. A. and Blasko, G. (1979) Effect of hypoxia on the development of *Drosophila melanogaster* (Meigen). Aviat. Space Environ. Med. 50, 411–412.
38 Datar, S. A., Jacobs, H. W., de la Cruz, A. F., Lehner, C. F. and Edgar, B. A. (2000) The Drosophila cyclin D-Cdk4 complex promotes cellular growth. EMBO J. 19, 4543–4554.
39 Frei, C. and Edgar, B. A. (2004) Drosophila cyclin D/Cdk4 requires Hif-1 prolyl hydroxylase to drive cell growth. Dev. Cell 6, 241–251.
40 Frei, C., Galloni, M., Hafen, E. and Edgar, B. A. (2005) The Drosophila mitochondrial ribosomal protein mRpL12 is required for Cyclin D/Cdk4-driven growth. EMBO J. 24, 623–634.
41 Centanin, L., Ratcliffe, P. J. and Wappner, P. (2005) Reversion of lethality and growth defects in Fatiga oxygen-sensor mutant flies by loss of hypoxia-inducible factor-alpha/Sima. EMBO Rep. 6, 1070–1075.
42 Goda, N., Ryan, H. E., Khadivi, B., McNulty, W., Rickert, R. C. and Johnson, R. S. (2003) Hypoxia-inducible factor 1alpha is essential for cell cycle arrest during hypoxia. Mol. Cell. Biol. 23, 359–369.
43 Yun, Z., Lin, Q. and Giaccia, A. J. (2005) Adaptive myogenesis under hypoxia. Mol. Cell. Biol. 25, 3040–3055.
44 Yun, Z., Maecker, H. L., Johnson, R. S. and Giaccia, A. J. (2002) Inhibition of PPAR gamma 2 gene expression by the HIF-1-regulated gene DEC1/Stra13: A mechanism for regulation of adipogenesis by hypoxia. Dev. Cell 2, 331–341.
45 Morrison, S. J., Csete, M., Groves, A. K., Melega, W., Wold, B. and Anderson, D. J. (2000) Culture in reduced levels of oxygen promotes clonogenic sympathoadrenal differentiation by isolated neural crest stem cells. J. Neurosci. 20, 7370–7376.

46 Seagroves, T. N., Hadsell, D., McManaman, J., Palmer, C., Liao, D., McNulty, W., Welm, B., Wagner, K. U., Neville, M. and Johnson, R. S. (2003) HIF1alpha is a critical regulator of secretory differentiation and activation, but not vascular expansion, in the mouse mammary gland. Development 130, 1713–1724.
47 Gustafsson, M. V., Zheng, X., Pereira, T., Gradin, K., Jin, S., Lundkvist, J., Ruas, J. L., Poellinger, L., Lendahl, U. and Bondesson, M. (2005) Hypoxia requires notch signaling to maintain the undifferentiated cell state. Dev. Cell 9, 617–628.
48 Diez, H., Fischer, A., Winkler, A., Hu, C. J., Hatzopoulos, A. K., Breier, G. and Gessler, M. (2007) Hypoxia-mediated activation of Dll4-Notch-Hey2 signaling in endothelial progenitor cells and adoption of arterial cell fate. Exp. Cell Res. 313, 1–9.
49 Tai, M. H., Chang, C. C., Kiupel, M., Webster, J. D., Olson, L. K. and Trosko, J. E. (2005) Oct4 expression in adult human stem cells: Evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis 26, 495–502.
50 Kaidi, A., Williams, A. C. and Paraskeva, C. (2007) Interaction between beta-catenin and HIF-1 promotes cellular adaptation to hypoxia. Nat. Cell Biol. 9, 210–217.
51 McMahon, S., Charbonneau, M., Grandmont, S., Richard, D. E. and Dubois, C. M. (2006) Transforming growth factor beta1 induces hypoxia-inducible factor-1 stabilization through selective inhibition of PHD2 expression. J. Biol. Chem. 281, 24171–24181.
52 Kong, T., Eltzschig, H. K., Karhausen, J., Colgan, S. P. and Shelley, C. S. (2004) Leukocyte adhesion during hypoxia is mediated by HIF-1-dependent induction of beta2 integrin gene expression. Proc. Natl. Acad. Sci. USA 101, 10440–10445.
53 Scannell, G., Waxman, K., Vaziri, N. D., Zhang, J., Kaupke, C. J., Jalali, M. and Hecht, C. C. (1995) Hypoxia-induced alterations of neutrophil membrane receptors. J. Surg. Res. 59, 141–145.
54 Mogford, J. E., Tawil, N., Chen, A., Gies, D., Xia, Y. and Mustoe, T. A. (2002) Effect of age and hypoxia on TGFbeta1 receptor expression and signal transduction in human dermal fibroblasts: Impact on cell migration. J. Cell Physiol. 190, 259–265.
55 O'Toole, E. A., Marinkovich, M. P., Peavey, C. L., Amieva, M. R., Furthmayr, H., Mustoe, T. A. and Woodley, D. T. (1997) Hypoxia increases human keratinocyte motility on connective tissue. J. Clin. Invest. 100, 2881–2891.
56 Degenhardt, K., Mathew, R., Beaudoin, B., Bray, K., Anderson, D., Chen, G., Mukherjee, C., Shi, Y., Gelinas, C., Fan, Y., Nelson, D. A., Jin, S. and White, E. (2006) Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10, 51–64.
57 Saikumar, P., Dong, Z., Weinberg, J. M. and Venkatachalam, M. A. (1998) Mechanisms of cell death in hypoxia/reoxygenation injury. Oncogene 17, 3341–3349.
58 Bruick, R. K. (2000) Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc. Natl. Acad. Sci. USA 97, 9082–9087.
59 Kothari, S., Cizeau, J., McMillan-Ward, E., Israels, S. J., Bailes, M., Ens, K., Kirshenbaum, L. A. and Gibson, S. B. (2003) BNIP3 plays a role in hypoxic cell death in human epithelial cells that is inhibited by growth factors EGF and IGF. Oncogene 22, 4734–4744.
60 Zhou, G., Golden, T., Aragon, I. V. and Honkanen, R. E. (2004) Ser/Thr protein phosphatase 5 inactivates hypoxia-induced activation of an apoptosis signal-regulating kinase 1/MKK-4/JNK signaling cascade. J. Biol. Chem. 279, 46595–46605.
61 Santore, M. T., McClintock, D. S., Lee, V. Y., Budinger, G. R. and Chandel, N. S. (2002) Anoxia-induced apoptosis occurs through a mitochondria-dependent pathway in lung epithelial cells. Am. J. Physiol. Lung Cell Mol. Physiol. 282, L727–L734.
62 Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y. and Greenberg, M. E. (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91, 231–241.
63 Ozes, O. N., Mayo, L. D., Gustin, J. A., Pfeffer, S. R., Pfeffer, L. M. and Donner, D. B. (1999) NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature 401, 82–85.
64 Dong, Z., Wang, J. Z., Yu, F. and Venkatachalam, M. A. (2003) Apoptosis-resistance of hypoxic cells: Multiple factors involved and a role for IAP-2. Am. J. Pathol. 163, 663–671.
65 Arnold, F., West, D. C., Schofield, P. F. and Kumar, S. (1987) Angiogenic activity in human wound fluid. Int. J. Microcirc. Clin. Exp. 5, 381–386.
66 Denko, N. C. and Giaccia, A. J. (2001) Tumor hypoxia, the physiological link between Trousseau's syndrome (carcinoma-induced coagulopathy) and metastasis. Cancer Res. 61, 795–798.
67 Hockel, M. and Vaupel, P. (2001) Tumor hypoxia: Definitions and current clinical, biologic, and molecular aspects. J. Natl. Cancer Inst. 93, 266–276.
68 Mapp, P. I., Grootveld, M. C. and Blake, D. R. (1995) Hypoxia, oxidative stress and rheumatoid arthritis. Br. Med. Bull. 51, 419–436.
69 Cramer, T., Yamanishi, Y., Clausen, B. E., Forster, I., Pawlinski, R., Mackman, N., Haase, V. H., Jaenisch, R., Corr, M., Nizet, V., Firestein, G. S., Gerber, H. P., Ferrara, N. and Johnson, R. S. (2003) HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 112, 645–657.
70 Hellwig-Burgel, T., Rutkowski, K., Metzen, E., Fandrey, J. and Jelkmann, W. (1999) Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. Blood 94, 1561–1567.
71 Gerald, D., Berra, E., Frapart, Y. M., Chan, D. A., Giaccia, A. J., Mansuy, D., Pouyssegur, J., Yaniv, M. and Mechta-Grigoriou, F. (2004) JunD reduces tumor angiogenesis by protecting cells from oxidative stress. Cell 118, 781–794.
72 Cummins, E. P., Berra, E., Comerford, K. M., Ginouves, A., Fitzgerald, K. T., Seeballuck, F., Godson, C., Nielsen, J. E., Moynagh, P., Pouyssegur, J. and Taylor, C. T. (2006) Prolyl hydroxylase-1 negatively regulates IkappaB kinase-beta, giving insight into hypoxia-induced NFkappaB activity. Proc. Natl. Acad. Sci. USA 103, 18154–18159.
73 Tandara, A. A. and Mustoe, T. A. (2004) Oxygen in wound healing – More than a nutrient. World J. Surg. 28, 294–300.
74 Li, W., Li, Y., Guan, S., Fan, J., Cheng, C. F., Bright, A. M., Chinn, C., Chen, M. and Woodley, D. T. (2007) Extracellular heat shock protein-90alpha: Linking hypoxia to skin cell motility and wound healing. EMBO J. 26, 1221–1233.
75 Driscoll, M. and Gerstbrein, B. (2003) Dying for a cause: Invertebrate genetics takes on human neurodegeneration. Nat. Rev. Genet. 4, 181–194.
76 Brown, R. H. Jr. and Robberecht, W. (2001) Amyotrophic lateral sclerosis: Pathogenesis. Semin. Neurol. 21, 131–139.
77 Oosthuyse, B., Moons, L., Storkebaum, E., Beck, H., Nuyens, D., Brusselmans, K., Van Dorpe, J., Hellings, P., Gorselink, M., Heymans, S., Theilmeier, G., Dewerchin, M., Laudenbach, V., Vermylen, P., Raat, H., Acker, T., Vleminckx, V., Van Den Bosch, L., Cashman, N., Fujisawa, H., Drost, M. R., Sciot, R., Bruyninckx, F., Hicklin, D. J., Ince, C., Gressens, P., Lupu, F., Plate, K. H., Robberecht, W., Herbert, J. M., Collen, D. and Carmeliet, P. (2001) Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat. Genet. 28, 131–138.
78 Subramanian, V. and Feng, Y. (2007) A new role for angiogenin in neurite growth and pathfinding: Implications for amyotrophic lateral sclerosis. Hum. Mol. Genet. 16, 1445–1453.
79 Selkoe, D. J., Yamazaki, T., Citron, M., Podlisny, M. B., Koo, E. H., Teplow, D. B. and Haass, C. (1996) The role of APP processing and trafficking pathways in the formation of amyloid beta-protein. Ann. N. Y. Acad. Sci. 777, 57–64.
80 Grammas, P., Samany, P. G. and Thirumangalakudi, L. (2006) Thrombin and inflammatory proteins are elevated in Alz-

heimer's disease microvessels: implications for disease pathogenesis. J. Alzheimers Dis. 9, 51–58.
81 Shi, J., Yang, S. H., Stubley, L., Day, A. L. and Simpkins, J. W. (2000) Hypoperfusion induces overexpression of beta-amyloid precursor protein mRNA in a focal ischemic rodent model. Brain Res. 853, 1–4.
82 Zhang, X., Zhou, K., Wang, R., Cui, J., Lipton, S. A., Liao, F. F., Xu, H. and Zhang, Y. W. (2007) Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. J. Biol. Chem. 282, 10873–10880.
83 Wang, R., Zhang, Y. W., Zhang, X., Liu, R., Zhang, X., Hong, S., Xia, K., Xia, J., Zhang, Z. and Xu, H. (2006) Transcriptional regulation of APH-1A and increased gamma-secretase cleavage of APP and Notch by HIF-1 and hypoxia. FASEB J. 20, 1275–1277.
84 Genbacev, O., Zhou, Y., Ludlow, J. W. and Fisher, S. J. (1997) Regulation of human placental development by oxygen tension. Science 277, 1669–1672.
85 Caniggia, I., Mostachfi, H., Winter, J., Gassmann, M., Lye, S. J., Kuliszewski, M. and Post, M. (2000) Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3) J. Clin. Invest. 105, 577–587.
86 Vaupel, P. and Mayer, A. (2005) Hypoxia and anemia: Effects on tumor biology and treatment resistance. Transfus. Clin. Biol. 12, 5–10.
87 Vaupel, P., Thews, O. and Hoeckel, M. (2001) Treatment resistance of solid tumors: Role of hypoxia and anemia. Med. Oncol. 18, 243–259.
88 Zhong, H., De Marzo, A. M., Laughner, E., Lim, M., Hilton, D. A., Zagzag, D., Buechler, P., Isaacs, W. B., Semenza, G. L. and Simons, J. W. (1999) Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res. 59, 5830–5835.
89 Trastour, C., Benizri, E., Ettore, F., Ramaioli, A., Chamorey, E., Pouyssegur, J. and Berra, E. (2007) HIF-1alpha and CA IX staining in invasive breast carcinomas: Prognosis and treatment outcome. Int. J. Cancer 120, 1451–1458.
90 Zhong, H., Semenza, G. L., Simons, J. W. and De Marzo, A. M. (2004) Up-regulation of hypoxia-inducible factor 1alpha is an early event in prostate carcinogenesis. Cancer Detect. Prev. 28, 88–93.
91 Leek, R. D., Talks, K. L., Pezzella, F., Turley, H., Campo, L., Brown, N. S., Bicknell, R., Taylor, M., Gatter, K. C. and Harris, A. L. (2002) 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. 62, 1326–1329.
92 Talks, K. L., Turley, H., Gatter, K. C., Maxwell, P. H., Pugh, C. W., Ratcliffe, P. J. and Harris, A. L. (2000) 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. 157, 411–421.
93 Selak, M. A., Armour, S. M., MacKenzie, E. D., Boulahbel, H., Watson, D. G., Mansfield, K. D., Pan, Y., Simon, M. C., Thompson, C. B. and Gottlieb, E. (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 7, 77–85.
94 Kato, H., Inoue, T., Asanoma, K., Nishimura, C., Matsuda, T. and Wake, N. (2006) Induction of human endometrial cancer cell senescence through modulation of HIF-1alpha activity by EGLN1. Int. J. Cancer 118, 1144–1153.
95 Blouw, B., Song, H., Tihan, T., Bosze, J., Ferrara, N., Gerber, H. P., Johnson, R. S. and Bergers, G. (2003) The hypoxic response of tumors is dependent on their microenvironment. Cancer Cell 4, 133–146.
96 Thiery, J. P. (2003) Epithelial-mesenchymal transitions in development and pathologies. Curr. Opin. Cell Biol. 15, 740–746.
97 Erler, J. T., Bennewith, K. L., Nicolau, M., Dornhofer, N., Kong, C., Le, Q. T., Chi, J. T., Jeffrey, S. S. and Giaccia, A. J. (2006) Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 440, 1222–1226.
98 Imai, T., Horiuchi, A., Wang, C., Oka, K., Ohira, S., Nikaido, T. and Konishi, I. (2003) Hypoxia attenuates the expression of E-cadherin *via* up-regulation of SNAIL in ovarian carcinoma cells. Am. J. Pathol. 163, 1437–1447.
99 Peinado, H., Del Carmen Iglesias-de la Cruz, M., Olmeda, D., Csiszar, K., Fong, K. S., Vega, S., Nieto, M. A., Cano, A. and Portillo, F. (2005) A molecular role for lysyl oxidase-like 2 enzyme in snail regulation and tumor progression. EMBO J. 24, 3446–3458.
100 Shimoda, L. A., Fallon, M., Pisarcik, S., Wang, J. and Semenza, G. L. (2006) HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes. Am. J. Physiol. Lung Cell Mol. Physiol. 291, L941–L949.
101 Ullah, M. S., Davies, A. J. and Halestrap, A. P. (2006) The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. J. Biol. Chem. 281, 9030–9037.
102 Romero, M. F. (2005) Molecular pathophysiology of SLC4 bicarbonate transporters. Curr. Opin. Nephrol. Hypertens. 14, 495–501.
103 Wykoff, C. C., Beasley, N. J., Watson, P. H., Turner, K. J., Pastorek, J., Sibtain, A., Wilson, G. D., Turley, H., Talks, K. L., Maxwell, P. H., Pugh, C. W., Ratcliffe, P. J. and Harris, A. L. (2000) Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res. 60, 7075–7083.
104 Laderoute, K., Wardman, P. and Rauth, A. M. (1988) Molecular mechanisms for the hypoxia-dependent activation of 3-amino-1,2,4-benzotriazine-1,4-dioxide (SR 4233) Biochem. Pharmacol. 37, 1487–1495.
105 Weitman, S., Mangold, G., Marty, J., Dexter, D., Hilsenbeck, S., Rake, J., Juniewicz, P. and Von Hoff, D. (1999) Evidence of enhanced *in vivo* activity using tirapazamine with paclitaxel and paraplatin regimens against the MV-522 human lung cancer xenograft. Cancer Chemother. Pharmacol. 43, 402–408.
106 Wouters, B. G., Wang, L. H. and Brown, J. M. (1999) Tirapazamine: A new drug producing tumor specific enhancement of platinum-based chemotherapy in non-small-cell lung cancer. Ann. Oncol. 10 Suppl 5, S29–S33.
107 Zeman, E. M., Hirst, V. K., Lemmon, M. J. and Brown, J. M. (1988) Enhancement of radiation-induced tumor cell killing by the hypoxic cell toxin SR 4233. Radiother. Oncol. 12, 209–218.
108 Williamson, S. K., Crowley, J. J., Lara, P. N., Jr., McCoy, J., Lau, D. H., Tucker, R. W., Mills, G. M. and Gandara, D. R. (2005) Phase III trial of paclitaxel plus carboplatin with or without tirapazamine in advanced non-small-cell lung cancer: Southwest Oncology Group Trial S0003. J. Clin. Oncol. 23, 9097–10104.
109 Rischin, D., Peters, L., Fisher, R., Macann, A., Denham, J., Poulsen, M., Jackson, M., Kenny, L., Penniment, M., Corry, J., Lamb, D. and McClure, B. (2005) Tirapazamine, cisplatin, and radiation *versus* fluorouracil, cisplatin, and radiation in patients with locally advanced head and neck cancer: A randomized phase II trial of the Trans-Tasman Radiation Oncology Group (TROG 98. 02). J. Clin. Oncol. 23, 79–87.
110 Theys, J., Landuyt, W., Nuyts, S., Van Mellaert, L., van Oosterom, A., Lambin, P. and Anne, J. (2001) Specific targeting of cytosine deaminase to solid tumors by engineered *Clostridium acetobutylicum.* Cancer Gene Ther. 8, 294–297.
111 Kelleher, D. K., Matthiensen, U., Thews, O. and Vaupel, P. (1995) Tumor oxygenation in anemic rats: Effects of erythropoietin treatment *versus* red blood cell transfusion. Acta Oncol. 34, 379–384.
112 Silver, D. F. and Piver, M. S. (1999) Effects of recombinant human erythropoietin on the antitumor effect of cisplatin in

SCID mice bearing human ovarian cancer: A possible oxygen effect. Gynecol. Oncol. 73, 280–284.
113 Stuben, G., Thews, O., Pottgen, C., Knuhmann, K., Vaupel, P. and Stuschke, M. (2001) Recombinant human erythropoietin increases the radiosensitivity of xenografted human tumours in anaemic nude mice. J. Cancer Res. Clin. Oncol. 127, 346–350.
114 Harrison, L. B., Chadha, M., Hill, R. J., Hu, K. and Shasha, D. (2002) Impact of tumor hypoxia and anemia on radiation therapy outcomes. Oncologist 7, 492–508.
115 Feldmeier, J. J., Heimbach, R. D., Davolt, D. A., Brakora, M. J., Sheffield, P. J. and Porter, A. T. (1994) Does hyperbaric oxygen have a cancer-causing or-promoting effect? A review of the pertinent literature. Undersea Hyperb. Med. 21, 467–475.
116 Bennett, M., Feldmeier, J., Smee, R. and Milross, C. (2005) Hyperbaric oxygenation for tumour sensitisation to radiotherapy. Cochrane Database Syst. Rev. CD005007.
117 Kalns, J., Krock, L. and Piepmeier, E., Jr. (1998) The effect of hyperbaric oxygen on growth and chemosensitivity of metastatic prostate cancer. Anticancer Res. 18, 363–367.
118 Rojas, A. (1992) ARCON: Accelerated radiotherapy with carbogen and nicotinamide. BJR Suppl 24, 174–178.
119 Bernier, J., Denekamp, J., Rojas, A., Trovo, M., Horiot, J. C., Hamers, H., Antognoni, P., Dahl, O., Richaud, P., Kaanders, J., van Glabbeke, M. and Pierart, M. (1999) ARCON: Accelerated radiotherapy with carbogen and nicotinamide in non small cell lung cancer: A phase I/II study by the EORTC. Radiother. Oncol. 52, 149–156.
120 Hoskin, P. J., Rojas, A. M., Phillips, H. and Saunders, M. I. (2005) Acute and late morbidity in the treatment of advanced bladder carcinoma with accelerated radiotherapy, carbogen, and nicotinamide. Cancer 103, 2287–2297.
121 Grigsby, P. W., Winter, K., Wasserman, T. H., Marcial, V., Rotman, M., Cooper, J., Keys, H., Asbell, S. O. and Phillips, T. L. (1999) Irradiation with or without misonidazole for patients with stages IIIB and IVA carcinoma of the cervix: Final results of RTOG 80-05. Radiation Therapy Oncology Group. Int. J. Radiat. Oncol. Biol. Phys. 44, 513–517.
122 Overgaard, J., Sand Hansen, H., Lindelov, B., Overgaard, M., Jorgensen, K., Rasmusson, B. and Berthelsen, A. (1991) Nimorazole as a hypoxic radiosensitizer in the treatment of supraglottic larynx and pharynx carcinoma. First report from the Danish Head and Neck Cancer Study (DAHANCA) protocol 5-85. Radiother. Oncol. 20 Suppl 1, 143–149.
123 Huncharek, M. (1998) Meta-analytic re-evaluation of misonidazole in the treatment of high grade astrocytoma. Anticancer Res. 18, 1935–1939.
124 Chen, J., Zhao, S., Nakada, K., Kuge, Y., Tamaki, N., Okada, F., Wang, J., Shindo, M., Higashino, F., Takeda, K., Asaka, M., Katoh, H., Sugiyama, T., Hosokawa, M. and Kobayashi, M. (2003) Dominant-negative hypoxia-inducible factor-1 alpha reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. Am. J. Pathol. 162, 1283–1291.
125 Welsh, S., Williams, R., Kirkpatrick, L., Paine-Murrieta, G. and Powis, G. (2004) Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1alpha. Mol. Cancer Ther. 3, 233–244.
126 Rapisarda, A., Uranchimeg, B., Scudiero, D. A., Selby, M., Sausville, E. A., Shoemaker, R. H. and Melillo, G. (2002) Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. Cancer Res. 62, 4316–4324.
127 Rapisarda, A., Uranchimeg, B., Sordet, O., Pommier, Y., Shoemaker, R. H. and Melillo, G. (2004) Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: Mechanism and therapeutic implications. Cancer Res. 64, 1475–1482.
128 Meng, F., Nguyen, X. T., Cai, X., Duan, J., Matteucci, M. and Hart, C. P. (2007) ARC-111 inhibits hypoxia-mediated hypoxia-inducible factor-1alpha accumulation. Anticancer Drugs 18, 435–445.
129 Kong, D., Park, E. J., Stephen, A. G., Calvani, M., Cardellina, J. H., Monks, A., Fisher, R. J., Shoemaker, R. H. and Melillo, G. (2005) Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res. 65, 9047–9055.
130 Chang, A. Y., Kim, K., Boucher, H., Bonomi, P., Stewart, J. A., Karp, D. D. and Blum, R. H. (1998) A randomized phase II trial of echinomycin, trimetrexate, and cisplatin plus etoposide in patients with metastatic nonsmall cell lung carcinoma: An Eastern Cooperative Oncology Group Study (E1587). Cancer 82, 292–300.
131 Muss, H. B., Blessing, J. A. and DuBeshter, B. (1993) Echinomycin in recurrent and metastatic endometrial carcinoma. A phase II trial of the Gynecologic Oncology Group. Am. J. Clin. Oncol. 16, 492–493.
132 Kung, A. L., Wang, S., Klco, J. M., Kaelin, W. G. and Livingston, D. M. (2000) Suppression of tumor growth through disruption of hypoxia-inducible transcription. Nat. Med. 6, 1335–1340.
133 D'Angelo, G., Duplan, E., Vigne, P. and Frelin, C. (2003) Cyclosporin A prevents the hypoxic adaptation by activating hypoxia-inducible factor-1alpha Pro-564 hydroxylation. J. Biol. Chem. 278, 15406–15411.
134 Temes, E., Martin-Puig, S., Acosta-Iborra, B., Castellanos, M. C., Feijoo-Cuaresma, M., Olmos, G., Aragones, J. and Landazuri, M. O. (2005) Activation of HIF-prolyl hydroxylases by R59949, an inhibitor of the diacylglycerol kinase. J. Biol. Chem. 280, 24238–24244.
135 Mayerhofer, M., Valent, P., Sperr, W. R., Griffin, J. D. and Sillaber, C. (2002) BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1alpha, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. Blood 100, 3767–3775.
136 Wan, X., Shen, N., Mendoza, A., Khanna, C. and Helman, L. J. (2006) CCI-779 inhibits rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/Hif-1alpha/VEGF signaling. Neoplasia 8, 394–401.
137 Duran, I., Kortmansky, J., Singh, D., Hirte, H., Kocha, W., Goss, G., Le, L., Oza, A., Nicklee, T., Ho, J., Birle, D., Pond, G. R., Arboine, D., Dancey, J., Aviel-Ronen, S., Tsao, M. S., Hedley, D. and Siu, L. L. (2006) A phase II clinical and pharmacodynamic study of temsirolimus in advanced neuroendocrine carcinomas. Br. J. Cancer 95, 1148–1154.
138 Hidalgo, M., Buckner, J. C., Erlichman, C., Pollack, M. S., Boni, J. P., Dukart, G., Marshall, B., Speicher, L., Moore, L. and Rowinsky, E. K. (2006) A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer. Clin. Cancer Res. 12, 5755–5763.
139 Hudis, C. A. (2007) Trastuzumab – Mechanism of action and use in clinical practice. N. Engl. J. Med. 357, 39–51.
140 Laughner, E., Taghavi, P., Chiles, K., Mahon, P. C. and Semenza, G. L. (2001) 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. 21, 3995–4004.
141 Yokoi, A., McCrudden, K. W., Huang, J., Kim, E. S., Soffer, S. Z., Frischer, J. S., Serur, A., New, T., Yuan, J., Mansukhani, M., O'Toole, K., Yamashiro, D. J. and Kandel, J. J. (2003) Blockade of her2/neu decreases VEGF expression but does not alter HIF-1 distribution in experimental Wilms tumor. Oncol. Rep. 10, 1271–1274.
142 Jiang, B. H., Jiang, G., Zheng, J. Z., Lu, Z., Hunter, T. and Vogt, P. K. (2001) Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth Differ. 12, 363–369.
143 Sourbier, C., Lindner, V., Lang, H., Agouni, A., Schordan, E., Danilin, S., Rothhut, S., Jacqmin, D., Helwig, J. J. and Massfelder, T. (2006) The phosphoinositide 3-kinase/Akt

pathway: A new target in human renal cell carcinoma therapy. Cancer Res. 66, 5130–5142.
144 Richard, D. E., Berra, E., Gothie, E., Roux, D. and Pouyssegur, J. (1999) p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. J. Biol. Chem. 274, 32631–32637.
145 Chang, H., Shyu, K. G., Lee, C. C., Tsai, S. C., Wang, B. W., Hsien Lee, Y. and Lin, S. (2003) GL331 inhibits HIF-1alpha expression in a lung cancer model. Biochem. Biophys. Res. Commun. 302, 95–100.
146 Tulis, D. A. (2004) Salutary properties of YC-1 in the cardiovascular and hematological systems. Curr. Med. Chem. Cardiovasc. Hematol. Agents 2, 343–359.
147 Chun, Y. S., Yeo, E. J., Choi, E., Teng, C. M., Bae, J. M., Kim, M. S. and Park, J. W. (2001) Inhibitory effect of YC-1 on the hypoxic induction of erythropoietin and vascular endothelial growth factor in Hep3B cells. Biochem. Pharmacol. 61, 947–954.
148 Yeo, E. J., Chun, Y. S., Cho, Y. S., Kim, J., Lee, J. C., Kim, M. S. and Park, J. W. (2003) YC-1: A potential anticancer drug targeting hypoxia-inducible factor 1. J. Natl. Cancer Inst. 95, 516–525.
149 Minet, E., Mottet, D., Michel, G., Roland, I., Raes, M., Remacle, J. and Michiels, C. (1999) Hypoxia-induced activation of HIF-1: Role of HIF-1alpha-Hsp90 interaction. FEBS Lett. 460, 251–256.
150 Mabjeesh, N. J., Post, D. E., Willard, M. T., Kaur, B., Van Meir, E. G., Simons, J. W. and Zhong, H. (2002) Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein *via* the proteosome pathway in prostate cancer cells. Cancer Res. 62, 2478–2482.
151 Hur, E., Kim, H. H., Choi, S. M., Kim, J. H., Yim, S., Kwon, H. J., Choi, Y., Kim, D. K., Lee, M. O. and Park, H. (2002) Reduction of hypoxia-induced transcription through the repression of hypoxia-inducible factor-1alpha/aryl hydrocarbon receptor nuclear translocator DNA binding by the 90-kDa heat-shock protein inhibitor radicicol. Mol. Pharmacol. 62, 975–982.
152 Sharp, S. and Workman, P. (2006) Inhibitors of the HSP90 molecular chaperone: Current status. Adv. Cancer Res. 95, 323–348.
153 Fotsis, T., Zhang, Y., Pepper, M. S., Adlercreutz, H., Montesano, R., Nawroth, P. P. and Schweigerer, L. (1994) The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. Nature 368, 237–239.
154 Ricker, J. L., Chen, Z., Yang, X. P., Pribluda, V. S., Swartz, G. M. and Van Waes, C. (2004) 2-methoxyestradiol inhibits hypoxia-inducible factor 1alpha, tumor growth, and angiogenesis and augments paclitaxel efficacy in head and neck squamous cell carcinoma. Clin. Cancer Res. 10, 8665–8673.
155 Jung, Y. J., Isaacs, J. S., Lee, S., Trepel, J. and Neckers, L. (2003) Microtubule disruption utilizes an NFkappa B-dependent pathway to stabilize HIF-1alpha protein. J. Biol. Chem. 278, 7445–7452.
156 Sun, X., Kanwar, J. R., Leung, E., Lehnert, K., Wang, D. and Krissansen, G. W. (2001) Gene transfer of antisense hypoxia inducible factor-1 alpha enhances the therapeutic efficacy of cancer immunotherapy. Gene Ther. 8, 638–645.
157 Sun, X., Liu, M., Wei, Y., Liu, F., Zhi, X., Xu, R. and Krissansen, G. W. (2006) Overexpression of von Hippel-Lindau tumor suppressor protein and antisense HIF-1alpha eradicates gliomas. Cancer Gene Ther. 13, 428–435.
158 Chang, Q., Qin, R., Huang, T., Gao, J. and Feng, Y. (2006) Effect of antisense hypoxia-inducible factor 1alpha on progression, metastasis, and chemosensitivity of pancreatic cancer. Pancreas 32, 297–305.
159 Binley, K., Askham, Z., Martin, L., Spearman, H., Day, D., Kingsman, S. and Naylor, S. (2003) Hypoxia-mediated tumour targeting. Gene Ther. 10, 540–549.
160 Mizuno, T., Nagao, M., Yamada, Y., Narikiyo, M., Ueno, M., Miyagishi, M., Taira, K. and Nakajima, Y. (2006) Small interfering RNA expression vector targeting hypoxia-inducible factor 1 alpha inhibits tumor growth in hepatobiliary and pancreatic cancers. Cancer Gene Ther. 13, 131–140.
161 Gillespie, D. L., Whang, K., Ragel, B. T., Flynn, J. R., Kelly, D. A. and Jensen, R. L. (2007) Silencing of hypoxia inducible factor-1alpha by RNA interference attenuates human glioma cell growth *in vivo*. Clin. Cancer Res. 13, 2441–2448.
162 Li, J., Post, M., Volk, R., Gao, Y., Li, M., Metais, C., Sato, K., Tsai, J., Aird, W., Rosenberg, R. D., Hampton, T. G., Sellke, F., Carmeliet, P. and Simons, M. (2000) PR39, a peptide regulator of angiogenesis. Nat. Med. 6, 49–55.
163 Post, M. J., Sato, K., Murakami, M., Bao, J., Tirziu, D., Pearlman, J. D. and Simons, M. (2006) Adenoviral PR39 improves blood flow and myocardial function in a pig model of chronic myocardial ischemia by enhancing collateral formation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, R494–R500.
164 Mabjeesh, N. J., Willard, M. T., Harris, W. B., Sun, H. Y., Wang, R., Zhong, H., Umbreit, J. N. and Simons, J. W. (2003) Dibenzoylmethane, a natural dietary compound, induces HIF-1 alpha and increases expression of VEGF. Biochem. Biophys. Res. Commun. 303, 279–286.
165 Natarajan, R., Salloum, F. N., Fisher, B. J., Ownby, E. D., Kukreja, R. C. and Fowler, A. A., 3rd. (2007) Activation of hypoxia-inducible factor-1 *via* prolyl-4 hydoxylase-2 gene silencing attenuates acute inflammatory responses in postischemic myocardium. Am. J. Physiol. Heart Circ. Physiol. 293, H1571–H1580.
166 Milkiewicz, M., Pugh, C. W. and Egginton, S. (2004) Inhibition of endogenous HIF inactivation induces angiogenesis in ischaemic skeletal muscles of mice. J. Physiol. 560, 21–26.
167 Warnecke, C., Griethe, W., Weidemann, A., Jurgensen, J. S., Willam, C., Bachmann, S., Ivashchenko, Y., Wagner, I., Frei, U., Wiesener, M. and Eckardt, K. U. (2003) Activation of the hypoxia-inducible factor-pathway and stimulation of angiogenesis by application of prolyl hydroxylase inhibitors. FASEB J. 17, 1186–1188.
168 Vincent, K. A., Shyu, K. G., Luo, Y., Magner, M., Tio, R. A., Jiang, C., Goldberg, M. A., Akita, G. Y., Gregory, R. J. and Isner, J. M. (2000) Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF-1alpha/VP16 hybrid transcription factor. Circulation 102, 2255–2261.
169 Rajagopalan, S., Olin, J., Deitcher, S., Pieczek, A., Laird, J., Grossman, P. M., Goldman, C. K., McEllin, K., Kelly, R. and Chronos, N. (2007) Use of a constitutively active hypoxia-inducible factor-1alpha transgene as a therapeutic strategy in no-option critical limb ischemia patients: Phase I dose-escalation experience. Circulation 115, 1234–1243.
170 Kelly, B. D., Hackett, S. F., Hirota, K., Oshima, Y., Cai, Z., Berg-Dixon, S., Rowan, A., Yan, Z., Campochiaro, P. A. and Semenza, G. L. (2003) Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. Circ. Res. 93, 1074–1081.
171 Patel, T. H., Kimura, H., Weiss, C. R., Semenza, G. L. and Hofmann, L. V. (2005) Constitutively active HIF-1alpha improves perfusion and arterial remodeling in an endovascular model of limb ischemia. Cardiovasc. Res. 68, 144–154.