

Targeting Hypoxia-Inducible Factor (HIF) as a Therapeutic Strategy for CNS Disorders

Robert S. Freeman* and Maria Cecilia Barone

Department of Pharmacology and Physiology, University of Rochester School of Medicine, Rochester, NY 14642, USA

Abstract: Hypoxia occurs when oxygen availability drops below the levels necessary to maintain normal rates of metabolism. Because of its high metabolic activity, the brain is highly sensitive to hypoxia. Severe or prolonged oxygen deprivation in the brain contributes to the damage associated with stroke and a variety of other neuronal disorders. Conversely, the extreme hypoxic environment found in the core of many brain tumors supports the growth of the tumor and the survival of tumor cells. Normal cells exposed to transient or moderate hypoxia are generally able to adapt to the hypoxic conditions largely through activation of the hypoxia-inducible transcription factor HIF. HIF-regulated genes encode proteins involved in energy metabolism, cell survival, erythropoiesis, angiogenesis, and vasomotor regulation. In many instances of hypoxia or hypoxia and ischemia, the induction of HIF target genes may be beneficial. When these same insults occur in tissues that are normally poorly vascularized, such as the retina and the core of solid tumors, induction of the same HIF target genes can promote disease. Major new insights into the molecular mechanisms that regulate the oxygen-sensitivity of HIF, and in the development of compounds with which to manipulate HIF activity, are forcing serious consideration of HIF as a therapeutic target for diverse CNS disorders associated with hypoxia.

Keywords: EGLN, HIF prolyl hydroxylase, stroke, ischemia, cell survival, angiogenesis, transcription factor.

INTRODUCTION

Hypoxia occurs when the availability of oxygen to tissues drops below the levels necessary to maintain normal rates of metabolism. This can result from a reduction in blood flow, a decrease in oxygen partial pressure, or a decrease in the ability of cells to utilize oxygen for aerobic ATP production. The brain with its high oxygen demand is particularly vulnerable to hypoxia. The most obvious condition in which oxygen-deprivation contributes to brain damage is stroke. Heart failure, traumatic brain injury, brain cancer and subcortical vascular disease are other conditions that disrupt normal patterns of blood flow and oxygen delivery in the brain. Hypoxia may also contribute to neuronal dysfunction and cell death in major neurodegenerative diseases. Pathologic processes and toxins associated with Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis can produce a state analogous to chemical hypoxia by interfering with the mitochondrial electron transport chain, resulting in impaired oxygen utilization.

While severe and prolonged hypoxia causes cell death, tissue damage, and ultimately loss of life; transient or moderate hypoxia stimulates adaptive responses that allow cells and tissues to cope with reduced oxygen. These adaptive responses are critical for normal development and for coping with changing environmental and physiological conditions. Transient hypoxia can also produce therapeutic

benefits through a process known as preconditioning. In preconditioning, exposure to a brief period of hypoxia gives rise to a protective state that substantially reduces damage in the brain and other organs caused by a subsequent ischemic insult.

Whether oxygen deprivation results in an adaptive, protective, or detrimental outcome depends on the degree and duration of hypoxia and on the tissue that is affected. The oxygen content of tissues under normal physiologic conditions depends on their extent of vascularization and the metabolic activity of their constituent cells. In the brain, oxygen levels range from approximately 5% to less than 1%. This compares with oxygen levels of 21% in air, about 12% in arterial blood, and roughly 5% in venous blood. Despite substantial differences in oxygen content among brain regions and even among cells within a region, the mechanisms activated by various cells in response to hypoxia are surprisingly similar [1]. Less well understood is the nature of the cellular oxygen sensors and how it is that different types of cells can initiate similar responses at different oxygen tensions. These and related issues have been the topic of recent thorough reviews [2,3].

The cellular responses to hypoxia range from acute effects on ion channel function and membrane potential, to long lasting changes in gene expression. Gene expression during hypoxia is largely regulated by a family of transcription factors known collectively as hypoxia-inducible factor (HIF). HIF-regulated genes encode proteins involved in energy metabolism, cell survival, red blood cell production, blood vessel formation, and vasomotor regulation [4]. In most occurrences of ischemia, the induction of HIF target genes is beneficial; however, in tissues that are poorly vascularized, such as the retina and the core of solid tumors, induction of the same HIF target genes can promote disease [5].

*Address correspondence to the author at the Department of Pharmacology and Physiology, University of Rochester School of Medicine, 601 Elmwood Avenue, Rochester, NY 14642, USA; Tel: (585) 273-489; Fax: (585) 273-2652; E-mail: Robert_Freeman@urmc.rochester.edu

Additionally, a few HIF target genes have been directly implicated in promoting cell death [6]. Thus activation of HIF may be protective or detrimental depending on the type of cell in addition to other (mostly unknown) factors.

In this review, we discuss the rationale for considering HIF as a target for therapies directed at alleviating brain disorders associated with hypoxia. Therapies aimed at increasing HIF activity could be beneficial for conditions associated with a failure to adapt to hypoxia, such as ischemic brain injury caused by stroke. Conversely, inhibiting HIF function might be useful for reducing growth of brain tumors and for blocking ischemia/reperfusion injury in the retina.

HIF and the Hypoxic Response

HIF is a heterodimeric / transcription factor conserved from nematodes to man. In humans, HIF consists of three different subunits (HIF-1, HIF-2, and HIF-3) and a single subunit, known as the aryl hydrocarbon receptor nuclear translocator (ARNT) or simply HIF-1. Both and subunits belong to the basic helix-loop-helix (bHLH)-containing PER-ARNT-SIM (PAS) domain family of transcription factors. Each subunit contains two PAS domains that mediate the / protein-protein interaction. HIF-1 is a nuclear protein whereas HIF-1 (the best characterized HIF-1 isoform) shuttles between the cytoplasm and nucleus. Moreover, while HIF-1 is constitutively present in all tissues and is not influenced by oxygen tension, the protein levels of HIF-1 are tightly linked to oxygen tension [7,8]. During hypoxia HIF-1 protein stability increases dramatically. Over the last three years, the mechanisms that underlie hypoxia-induced stabilization of

HIF-1 have been the focus of intense scrutiny.

Accumulation of HIF-1 in hypoxic cells is followed by its entry into the nucleus, where it binds HIF-1. Nuclear HIF-1 complexes bind to specific cis-regulatory sequences called hypoxia response elements (HREs), first identified in the promoter of the erythropoietin (EPO) gene [9,10]. To transactivate gene expression, HIF-1 must first associate with the transcriptional co-activators CREB-binding protein (CBP) and p300 [11]. HIF target genes are involved in virtually all aspects of hypoxic adaptation at both the cellular and systemic levels (Fig. 1). The importance of HIF-1 as a transactivator of hypoxia-dependent gene expression has been confirmed by disrupting the HIF-1 gene in mice and embryonic stem cells [12-14].

HIF in Development and Disease

An increasing body of evidence suggests that HIF is essential for ischemia induced angiogenesis, vascularization and growth of tumors, and as a mediator of hypoxic preconditioning [3]. HIF activation has been implicated in neovascularization of the retina—a major cause of blindness [15]; familial polycythemia—a condition caused by overproduction of red blood cells [16]; and von Hippel Lindau (VHL) disease—an inherited disorder that predisposes afflicted individuals to highly angiogenic tumors [17].

In normal tissues that become ischemic, such as the brain during stroke, enhancing HIF activity might increase cell survival through the expression of anti-apoptotic proteins, antioxidant enzymes and trophic factors. HIF activation in ischemic tissues could also increase oxygen delivery by

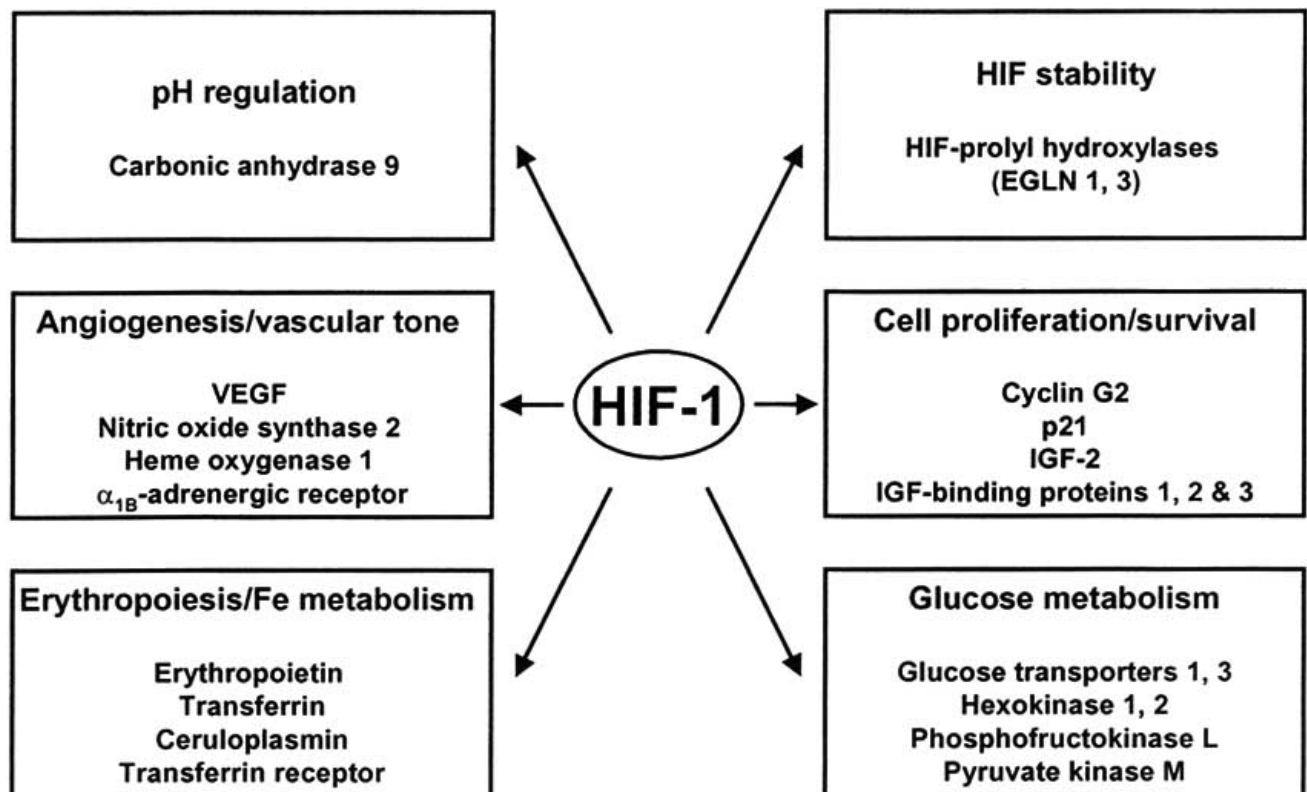


Fig. (1). A representative list of HIF target genes.

promoting erythropoiesis and angiogenesis. In brain tumors and in ischemic injury of the retina, blocking hypoxia-induced HIF activation might promote cell death and prevent new blood vessel growth—outcomes that could be beneficial under these conditions.

In VHL disease the oxygen-sensitive pathway responsible for destabilizing HIF-1 is disrupted. This results in the accumulation of active HIF in the nucleus and induction of HIF target genes. Tumors typically associated with VHL disease include CNS and retinal hemangioblastomas, pheochromocytomas, pancreatic neuroendocrine tumors, and renal clear cell carcinomas [18]. Overexpression of HIF-1 and HIF-1 target genes is detected in many human cancers including those that afflict the brain, such as glioblastomas, oligodendrogliomas, and anaplastic astrocytomas. HIF-1 overexpression is also correlated with increased mortality in various tumors including oligodendrogliomas. Additionally, HIF-1 overexpression has been associated with increased resistance of tumors to existing therapies. In tumors with high basal HIF-1, overexpression of HIF-1 target genes contributes to the increased reliance of tumor cells on anaerobic ATP production (i.e., the Warburg effect), the infiltration of new blood vessels into tumors, and the enhanced survival of cells in the core of tumors [18].

In addition to its involvement in various pathological conditions, HIF is also critical for normal development. Disrupting the HIF-1 gene in mice leads to defects in blood vessel and heart development that result in death by embryonic day 11 [12,14]. Mice lacking HIF-2 have defects in heart rate regulation and lung maturation and die around the 16th day of embryonic development [19,20]. The fact that disrupting either HIF-1 or HIF-2 results in embryonic lethality indicates that these closely related proteins have non-redundant functions during development. The different phenotypes of the HIF-1 and HIF-2 knockouts probably reflect the induction of distinct target genes or tissue-specific differences in their expression, or both [21,22]. Recently, a number of laboratories have generated mice with tissue-specific deletions of the HIF-1 locus. In one of these studies, mice with selective loss of HIF-1 in neuronal cells exhibited severe hydrocephalus, vascular regression, memory impairment, and a marked reduction in cortical neuron numbers [23]. The latter effect was apparently related to an increase in apoptosis during embryogenesis. Thus HIF-1 may exert a survival-promoting effect on neurons or their precursors during development.

Based on the various pathophysiological roles of HIF and its central role in regulating transcription-dependent adaptations to low oxygen during development and in the adult, it is not hard to believe that modulating HIF activity could have therapeutic benefits for a variety of diseases.

Regulation of HIF Stability

Transcription and translation of HIF-1 subunits is generally insensitive to oxygen. HIF-1 stability, on the other hand, is closely tied to oxygen tension. When oxygen is not limiting (i.e., normoxia), HIF-1 is rapidly degraded with a half-life of just a few minutes. Destabilization of HIF-1 is mediated through its oxygen-dependent degradation domain (ODD). The ODD targets HIF-1 for

polyubiquitination by an E3 ubiquitin-ligase complex comprised of the VHL tumor suppressor protein (pVHL), elongins B and C, cullin 2, and ring-box 1 (Rbx1). Polyubiquitination in turn tags HIF-1 for degradation by the 26S proteasome (Fig. 2). The oxygen-sensitive step in this process involves the binding of pVHL to the ODD of HIF-1 [17,24]. In the absence of sufficient oxygen or, in the case of VHL disease, when critical residues in pVHL are mutated, the affinity of HIF-1 for pVHL is greatly reduced [25,26]. Thus, during hypoxia HIF-1 escapes ubiquitination and is able to translocate to the nucleus where it transactivates the expression of hypoxia-response genes.

A major breakthrough came in 2001 with the demonstration that oxygen-dependent hydroxylation of a conserved proline (Pro⁵⁶⁴) in the ODD of HIF-1 was critical for mediating its interaction with pVHL [27-29]. Hydroxylation of Pro⁵⁶⁴ requires Fe(II) in addition to oxygen, explaining the ability of iron chelators such as desferrioxamine (DFO) to mimic hypoxia by stabilizing HIF-1. (For a discussion of iron chelators in neuroprotection and HIF stabilization, see [30]). Subsequent studies identified the enzymes responsible for hydroxylating Pro⁵⁶⁴ and a second proline (Pro⁴⁰²) at the opposite end of the ODD [31,32]. These studies not only described a family of HIF prolyl hydroxylases in humans, they also revealed that oxygen-dependent prolyl hydroxylation as a mechanism for regulating HIF stability is conserved throughout most of the animal kingdom.

Regulation of HIF by oxygen is not limited to proline hydroxylation and its effects on HIF-1 stability. Oxygen availability also influences the ability of HIF-1 to transactivate gene expression [33]. Under normoxic conditions, hydroxylation of an asparagine residue (Asn⁸⁰³) located in the C-terminal transactivating domain (C-TAD) of HIF-1 acts to inhibit its interaction with the transcriptional co-activators CBP and p300 [34]. As with proline hydroxylation, asparagine hydroxylation is greatly reduced by hypoxia. Thus, agents that prevent hydroxylation of specific proline and asparagine residues in HIF-1 are potentially useful tools for activating HIF-1.

Several other mechanisms for regulating HIF activity have been described, although their sensitivity to oxygen is generally not well defined. For example, the acetyltransferase ARD1 has been reported to acetylate a lysine residue in the ODD of HIF-1 and negatively influence HIF-1 stability [35]. Binding of HIF-1 to the p53 tumor suppressor protein has also been shown to decrease HIF-1 stability [36]. Another mechanism for inhibiting HIF-1 involves an alternatively spliced form of HIF-3, known as inhibitory PAS domain protein (IPAS), which may function as a dominant-negative inhibitor of HIF-1 [37,38]. Finally, growth factor-mediated activation of a signaling pathway involving phosphatidylinositol 3-kinase, Akt protein kinase, and mammalian target of rapamycin (mTOR) has been implicated in enhancing HIF-1 expression and activity under normoxic conditions [39].

HIF Proline and Asparagine Hydroxylases

The enzymes that hydroxylate HIF-1 subunits are members of the Fe(II)- and 2-oxoglutarate (2OG)-dependent dioxygenase superfamily [31,40]. This large and diverse

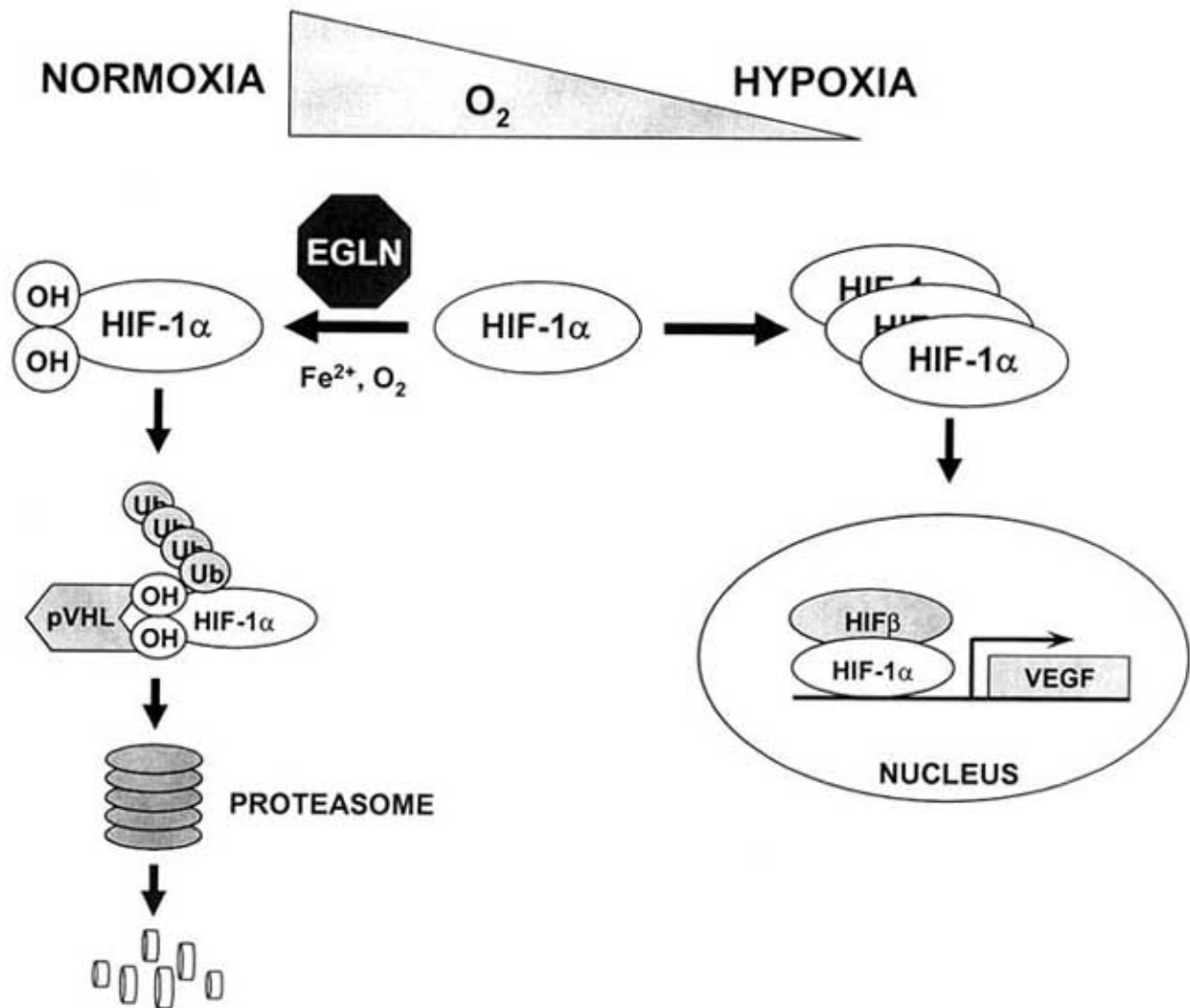


Fig. (2). A simplified scheme for the regulation of HIF stability by oxygen.

family is characterized by a core structural motif, comprised of a double-stranded α -helix, that is itself made up of 8 α -strands arranged in a 'jelly roll' topology. Situated within the jelly roll is a highly conserved 2-histidine-1-carboxylate (H-x-D/E...H) motif that binds Fe(II). Fe(II)/2OG dioxygenases utilize molecular oxygen (O_2) to oxidize their substrates. Fe(II) appears to be important for activating O_2 and for organizing the binding of reactants in the active site. During the HIF hydroxylation reaction, the enzyme must bind O_2 , 2-oxoglutarate, and the HIF substrate. Catalysis occurs when one oxygen atom is added to the peptidyl proline or asparagine in HIF while the other is used in a coupled reaction that decarboxylates 2-oxoglutarate to succinate [41].

In humans and other vertebrates, prolyl hydroxylases capable of modifying HIF-1 are encoded by three genes termed *egg-laying nine* (*EGLN*) 1, 2, and 3 because of their homology to the *egl-9* gene of *C. elegans* [31,42,43]. The *egl-9* gene was originally identified during a search for mutations causing defects in egg laying [44]. The proteins encoded by the *EGLN* genes have also been named HIF prolyl hydroxylases (HPH) and prolyl hydroxylase domain proteins (PHD), where *EGLN1* = HPH-2 = PHD2; *EGLN2* = HPH-3 = PHD1; and *EGLN3* = HPH-1 = PHD3. The

EGLN family is characterized by a highly conserved C-terminal domain, which among the Fe(II)/2OG dioxygenase is most similar to the catalytic domain of collagen prolyl-4-hydroxylase [31,32,40]. The N-terminal domains of the *EGLN* proteins are much less conserved, which probably accounts for their distinct subcellular localizations. Transfections with GFP-tagged proteins showed *EGLN1*-GFP mostly in the cytoplasm, *EGLN2*-GFP almost exclusively in the nucleus, and *EGLN3*-GFP distributed equally in the cytoplasm and nucleus [45]. An earlier study on the rat *EGLN3* protein, also known as SM-20 [46], revealed a mitochondrial targeting sequence in its N-terminus [47]. Surprisingly, the human and murine *EGLN3* genes appear to encode smaller proteins that lack the mitochondrial targeting sequence [42]. A potential caveat with all the localization studies to date is their reliance on over-expressed proteins. With this in mind, if these localization patterns hold true for the endogenous proteins, then it may indicate that different *EGLN* proteins function as hydroxylases for separate pools of cytoplasmic and nuclear HIF-1.

When over-expressed, each of the *EGLN* proteins can hydroxylate HIF-1 and reduce HIF-1 protein levels and activity [32,48]. Nevertheless, several observations indicate

that the function of the three EGLN proteins is not entirely redundant [49]. For example, all three EGLN proteins hydroxylate Pro⁵⁶⁴ in HIF-1 (and the analogous prolines in HIF-2 and HIF-3), but only EGLN1 and EGLN2 hydroxylate Pro⁴⁰² (as well as its equivalent in HIF-2) [50]. The significance of the more restrictive substrate specificity of EGLN3 is unknown.

EGLN mRNAs are expressed with unique although somewhat overlapping tissue specificity, raising the possibility that HIF regulation by the EGLNs is also tissue specific [46,51]. Interestingly, the mRNA expression of certain EGLN genes is also regulated by oxygen tension. Oxygen deprivation results in an increase in EGLN1 and EGLN3 mRNA levels whereas EGLN2 mRNA expression is unaffected [31,52,53]. The absence of hypoxia-induced EGLN2 expression is even more interesting in light of *in vitro* studies suggesting that EGLN2 is the least active isoform, at least with respect to hydroxylation of Pro⁵⁶⁴ [48]. Hypoxia-induced EGLN1 and EGLN3 expression almost certainly involves HIF, although canonical HREs have not yet been identified in their promoters. Nevertheless, hypoxia-dependent up-regulation of select EGLN proteins makes an attractive negative feed-back mechanism that could act to ensure the rapid removal of HIF- subunits when hypoxic cells are reoxygenated [54].

Another Fe(II)/2OG dioxygenase termed factor inhibiting HIF-1 (FIH1) is responsible for hydroxylating Asn⁸⁰³ in the C-TAD of HIF-1 [34,55]. As with HIF prolyl hydroxylation, asparagine hydroxylation is inhibited by hypoxia, resulting in de-repression of HIF-1 activity. FIH1 was originally identified as an interacting partner of HIF-1 and pVHL capable of blocking HIF-1 transactivating activity [56]. Protein interaction and protein structure studies have since revealed that asparagine hydroxylation by FIH1 sterically hinders the binding of HIF-1 to CBP/p300 transcriptional co-activators [57-59]. It is important to note that FIH1 and the EGLN proteins utilize the same cofactors and virtually the same chemistry to hydroxylate their substrates. Therefore, most current inhibitors of HIF prolyl hydroxylation will also inhibit FIH1-mediated asparagine hydroxylation [60].

HIF Hydroxylases as Oxygen Sensors

Downregulating EGLN activity with pharmacologic inhibitors, RNA interference, or through loss-of-function mutations in the *C. elegans* and *Drosophila*, confirmed that EGLN-dependent prolyl hydroxylation is the primary mechanism for destabilizing HIF under normoxic conditions. By themselves, these experiments do not reveal whether the EGLN (and FIH1) proteins are also important oxygen sensors. In other words, is the activity of the EGLN proteins, and consequently HIF-1 hydroxylation, directly related to oxygen availability over a physiologically relevant range of oxygen tensions? Initial studies suggested that oxygen availability is rate-limiting for hydroxylation of HIF-1 over the range of oxygen tensions likely to be experienced *in vivo* [31]. A recent *in vitro* study using crude preparations of recombinant EGLN proteins supports this conclusion [50]. The results from this study indicated that each of the three EGLN proteins has a relatively high KM for oxygen that is near the concentration of oxygen in room air.

Assuming a similar low oxygen-affinity for the EGLNs *in vivo*, then EGLN catalyzed reactions will almost certainly proceed at sub-maximal rates under physiologic oxygen levels, which can be an order of magnitude lower than in air.

A novel approach for examining the oxygen sensitivity of HIF prolyl hydroxylases involves the construction of fusion proteins containing a minimal portion of the ODD fused to a reporter gene such as luciferase or GFP. (A similar approach has been described for studying factors that influence Cdk2 and Skp2 regulation of p27 turnover [61]). Hydroxylation of the critical proline in the ODD portion of the fusion protein should trigger its binding to pVHL and its subsequent degradation by the proteasome. The predicted result is that luciferase activity or GFP fluorescence will be altered in a way that is inversely proportional to prolyl hydroxylase activity. Stable cell lines and transgenic mice expressing inverse HIF prolyl hydroxylase reporters could prove to be useful indicators of the effects of varying oxygen on HIF stability *in vivo*.

Therapeutic Targeting of HIF Prolyl Hydroxylases

Two recent reports involving the retina exemplify the potentially wide-ranging therapeutic benefits of targeting HIF. In the first report, induction of HIF-1 by hypoxia provided significant protection against light-induced retinal degeneration [62]. The protective effect was mediated by HIF-1-dependent up-regulation of EPO, a well-known survival factor for a variety of neurons [63]. The second study dealt with a model for hyperoxic retinopathy in mice hemizygous for HIF-2. In this paradigm, a partial reduction in HIF-2 protected the retina from vascular hypertrophy and retinal neovascularization [64]. The results from these studies highlight the opposing influences that HIF activity can have on diverse disease states.

HIF activates a large number of genes important for the ability of cells and tissues to adapt to an ischemic event. Because of this, treatments aimed at increasing HIF activity are predicted to be more beneficial than those that impact just one or two HIF target genes. Initial support for this idea came from a study in which delivery of a naked DNA encoding a constitutively active HIF-1 /VP16 hybrid transcription factor was found to promote angiogenesis and accelerate reperfusion in a rabbit model of hindlimb ischemic damage [65]. Additional evidence for the therapeutic potential of HIF came from experiments with transgenic mice expressing a stabilized, ODD-deleted form of HIF-1 in keratinocytes [66]. In contrast to mice overexpressing VEGF in keratinocytes, stabilization of HIF-1 resulted in peripheral hypervascularization without significant vascular leakiness or inflammation. Another report utilized a somewhat complementary strategy involving synthetic peptides centered on Pro⁵⁶⁴ in the ODD [67]. Delivery of these peptides into cells and tissues resulted in normoxic activation of HIF-1, as reflected by induction of several HIF target genes and by stimulation of angiogenesis. Apparently, either the Pro⁵⁶⁴-based peptide competed with HIF-1 subunits for binding to the EGLN proteins, or it was first hydroxylated by an EGLN and then it competed with HIF-1 for binding to pVHL.

A number of small molecule prolyl hydroxylase inhibitors (N-oxalylglycine; pyridine 2,4-dicarboxylate;

ciclopirox olamine; FG0041) can stabilize HIF-1 and induce HIF target gene expression [50,68-73]. Most of these compounds act by chelating iron, competitively inhibiting 2OG, or both. A potential limitation of these classes of compounds is that they may exhibit little selectivity among the various Fe(II)/2OG dioxygenases. Thus, investigators should be careful not to assume that any biological effects that are observed are mediated through inhibition of HIF hydroxylation, at least not without corroborating evidence. Even if compounds with increased specificity for the EGLN prolyl hydroxylases can be identified or created, it may be necessary to target specific EGLN family members to minimize possible untoward side effects. Nonetheless, a few of these compounds have recently been shown to have protective effects in animal models of ischemia [74,75].

Other potentially promising approaches for activating HIF are being evaluated in a number of laboratories. Possible strategies for increasing HIF activity include inhibiting pVHL-dependent ubiquitination of HIF-subunits, blocking the binding of HIF- to pVHL, creating novel types of hypoxia mimetics, designing gene therapy approaches to target regulators of HIF such as the EGLN proteins, and utilizing endogenous glycolytic metabolites to selectively increase the metabolic function of hypoxic cells (for example, see [76]).

The most obvious applications for therapies aimed at downregulating HIF activity are in the treatment of cancer. Selective inhibitors of HIF could be useful for reducing tumor vascularization and growth, reducing tumor cell survival, and decreasing the resistance of tumors to existing radiation treatments and chemotherapies [18]. A complex mix of signal transduction pathways, tumor suppressor proteins, and oxygen-sensitive mechanisms are believed to contribute to HIF up-regulation during tumorigenesis making it difficult to predict the effects of therapies targeted at any one mechanism [77]. Nevertheless, decreased tumor growth was demonstrated in a study that used peptides derived from the C-TAD of HIF-1 to compete with endogenous HIF-1 for binding to the transcription co-activators CBP/p300 [78].

Recently, the approximately 2000 representative compounds in the NCI "Diversity Set" was screened with the goal of identifying compounds capable of inhibiting the activation of HIF in intact cells, as measured using an HRE-driven luciferase reporter gene [79]. Two of the small handful of compounds identified are approved chemotherapeutic agents that inhibit topoisomerase-I. How these drugs inhibit HIF activity is not known. Several other potential anti-cancer drugs were found to inhibit HIF activation using more traditional approaches. These include the guanylate-cyclase activator YC-1 [80], the HSP90 inhibitor 17-allyl-aminogeldanancin (17-AAG) [81,82], two thioredoxin inhibitors [83], and a disrupter of microtubule polymerization 2-methoxyestradiol (2ME2) [84]. For each of these drugs, the mechanism of HIF inactivation is not yet known.

CONCLUSION

Several lines of evidence suggest that HIF may be an important therapeutic target for CNS disorders characterized by hypoxia. In this review, we discussed the major oxygen

sensitive pathways that regulate HIF stability and activity. We also discussed the potential for targeting the enzymes that post-translationally hydroxylate HIF as a way for manipulating HIF activity in pathological conditions.

Whereas previous strategies were directed at one or two HIF-response genes (for example, EPO and VEGF), targeting HIF has the potential advantage of simultaneously regulating the expression of a large number of genes involved in virtually all aspects of the response to hypoxia. However, it may not always be advantageous to regulate all HIF response genes at once. Moreover, therapies directed at HIF will most likely need to discriminate between the different HIF isoforms. Of course, the same concern applies to drugs designed to target the HIF prolyl hydroxylases. Finally, it is quite possible that additional targets of the prolyl and asparaginyl hydroxylases exist that are critical for the cellular adaptation to hypoxia.

ACKNOWLEDGEMENTS

Research in the author's laboratory is funded by National Institutes of Health grants NS34400 and NS42224. M. C. Barone acknowledges postdoctoral support from the Accademia dei Lincei Endowment Fund at the University of Rochester.

ABBREVIATIONS

ARNT	= Aryl hydrocarbon receptor nuclear translocator
CBP	= CREB-binding protein
C-TAD	= C-terminal transactivating domain
EGLN	= Egg-laying nine
EPO	= Erythropoietin
FIH1	= Factor inhibiting HIF-1
GFP	= Green fluorescent protein
HIF	= Hypoxia-inducible factor
HRE	= Hypoxia response element
2OG	= 2-oxoglutarate
OGD	= Oxygen and glucose deprivation
ODD	= Oxygen-dependent degradation domain
VEGF	= Vascular endothelial growth factor
VHL	= Von Hippel-Lindau disease

REFERENCES

- [1] Erecinska, M.; Silver, I. A. *Respir. Physiol.*, **2001**, 128(3), 263-276.
- [2] Acker, T.; Acker, H. *J. Exp. Biol.*, **2004**, 207(Pt. 18), 3171-3188.
- [3] Sharp, F. R.; Bernaudin, M. *Nat. Rev. Neurosci.*, **2004**, 5(6), 437-448.
- [4] Semenza, G. L. *Trends Mol. Med.*, **2001**, 7(8), 345-350.
- [5] Kietzmann, T.; Knabe, W.; Schmidt-Kastner, R. *Eur. Arch. Psychiatry Clin. Neurosci.*, **2001**, 251(4), 170-178.
- [6] Piret, J. P.; Mottet, D.; Raes, M.; Michiels, C. *Biochem. Pharmacol.*, **2002**, 64(5-6), 889-892.
- [7] Semenza, G. L. *Annu. Rev. Cell Dev. Biol.*, **1999**, 15 551-578.
- [8] Wenger, R. H. *J. Exp. Biol.*, **2000**, 203 Pt. 8 1253-1263.

- [9] Wang, G. L.; Jiang, B. H.; Rue, E. A.; Semenza, G. L. *Proc. Nat. Acad. Sci. USA*, **1995**, 92(12), 5510-5514.
- [10] Wang, G. L.; Semenza, G. L. *J. Biol. Chem.*, **1995**, 270(3), 1230-1237.
- [11] Kallio, P. J.; Okamoto, K.; O'Brien, S.; Carrero, P.; Makino, Y.; Tanaka, H.; Poellinger, L. *EMBO J.*, **1998**, 17(22), 6573-6586.
- [12] Ryan, H. E.; Lo, J.; Johnson, R. S. *EMBO J.*, **1998**, 17(11), 3005-3015.
- [13] 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.; Keshert, E.; Keshet, E. *Nature*, **1998**, 394(6692), 485-490.
- [14] 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.; Semenza, G. L. *Genes Dev.*, **1998**, 12(2), 149-162.
- [15] Viores, S. A.; Youssri, A. I.; Luna, J. D.; Chen, Y. S.; Bhargava, S.; Viores, M. A.; Schoenfeld, C. L.; Peng, B.; Chan, C. C.; LaRoche, W.; Green, W. R.; Campochiaro, P. A. *Histol. Histopathol.*, **1997**, 12(1), 99-109.
- [16] Ang, S. O.; Chen, H.; Hirota, K.; Gordeuk, V. R.; Jelinek, J.; Guan, Y.; Liu, E.; Sergueeva, A. I.; Miasnikova, G. Y.; Mole, D.; Maxwell, P. H.; Stockton, D. W.; Semenza, G. L.; Prchal, J. T. *Nat. Genet.*, **2002**, 32(4), 614-621.
- [17] Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J. *Adv. Exper. Med. Biol.*, **2001**, 502, 365-376.
- [18] Semenza, G. L. *Nat. Rev. Cancer*, **2003**, 3(10), 721-732.
- [19] Compernelle, 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.; Carmeliet, P. *Nat. Med.*, **2002**, 8(7), 702-710.
- [20] Tian, H.; Hammer, R. E.; Matsumoto, A. M.; Russell, D. W.; McKnight, S. L. *Genes Dev.*, **1998**, 12(21), 3320-3324.
- [21] Talks, K. L.; Turley, H.; Gatter, K. C.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J.; Harris, A. L. *Am. J. Pathol.*, **2000**, 157(2), 411-421.
- [22] Hu, C. J.; Wang, L. Y.; Chodosh, L. A.; Keith, B.; Simon, M. C. *Mol. Cell Biol.*, **2003**, 23(24), 9361-9374.
- [23] Tomita, S.; Ueno, M.; Sakamoto, M.; Kitahama, Y.; Ueki, M.; Maekawa, N.; Sakamoto, H.; Gassmann, M.; Kageyama, R.; Ueda, N.; Gonzalez, F. J.; Takahama, Y. *Mol. Cell Biol.*, **2003**, 23(19), 6739-6749.
- [24] Kaelin, W. G. Jr. *Genes Dev.*, **2002**, 16(12), 1441-1445.
- [25] 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.; Ratcliffe, P. J. *Nature*, **1999**, 399(6733), 271-275.
- [26] Cockman, M. E.; Masson, N.; Mole, D. R.; Jaakkola, P.; Chang, G. W.; Clifford, S. C.; Maher, E. R.; Pugh, C. W.; Ratcliffe, P. J.; Maxwell, P. H. *J. Biol. Chem.*, **2000**, 275(33), 25733-25741.
- [27] Ivan, M.; Kondo, K.; Yang, H.; Kim, W.; Valiando, J.; Ohh, M.; Salic, A.; Asara, J. M.; Lane, W. S.; Kaelin, W. G. Jr. *Science*, **2001**, 292(5516), 464-468.
- [28] 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.; Ratcliffe, P. J. *Science*, **2001**, 292(5516), 468-472.
- [29] Yu, F.; White, S. B.; Zhao, Q.; Lee, F. S. *Proc. Nat. Acad. Sci. USA*, **2001**, 98(17), 9630-9635.
- [30] Sorond, F. A.; Ratan, R. R. *Antioxid. Redox. Signal.*, **2000**, 2(3), 421-436.
- [31] Epstein, A. C.; Gleadle, J. M.; McNeill, L. A.; Hewitson, K. S.; O'Rourke, J.; Mole, D. R.; Mukherji, M.; Metzzen, 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.; Ratcliffe, P. J. *Cell*, **2001**, 107(1), 43-54.
- [32] Bruick, R. K.; McKnight, S. L. *Science*, **2001**, 294(5545), 1337-1340.
- [33] Bruick, R. K.; McKnight, S. L. *Science*, **2002**, 295(5556), 807-808.
- [34] Lando, D.; Peet, D. J.; Whelan, D. A.; Gorman, J. J.; Whitelaw, M. L. *Science*, **2002**, 295(5556), 858-861.
- [35] Jeong, J. W.; Bae, M. K.; Ahn, M. Y.; Kim, S. H.; Sohn, T. K.; Bae, M. H.; Yoo, M. A.; Song, E. J.; Lee, K. J.; Kim, K. W. *Cell*, **2002**, 111(5), 709-720.
- [36] Ravi, R.; Mookerjee, B.; Bhujwalla, Z. M.; Sutter, C. H.; Artemov, D.; Zeng, Q.; Dillehay, L. E.; Madan, A.; Semenza, G. L.; Bedi, A. *Genes Dev.*, **2000**, 14(1), 34-44.
- [37] Makino, Y.; Kanopka, A.; Wilson, W. J.; Tanaka, H.; Poellinger, L. *J. Biol. Chem.*, **2002**, 277(36), 32405-32408.
- [38] Makino, Y.; Cao, R.; Svensson, K.; Bertilsson, G.; Asman, M.; Tanaka, H.; Cao, Y.; Berkenstam, A.; Poellinger, L. *Nature*, **2001**, 414(6863), 550-554.
- [39] Bardos, J. I.; Ashcroft, M. *Bioessays*, **2004**, 26(3), 262-269.
- [40] Aravind, L.; Koonin, E. V. *Genome Biol.*, **2001**, 2(3), 7.1-7.8.
- [41] Schofield, C. J.; Ratcliffe, P. J. *Nat. Rev. Mol. Cell Biol.*, **2004**, 5(5), 343-354.
- [42] Taylor, M. S. *Gene*, **2001**, 275(1), 125-132.
- [43] Darby, C.; Cosma, C. L.; Thomas, J. H.; Manoil, C. *Proc. Nat. Acad. Sci. USA*, **1999**, 96(26), 15202-15207.
- [44] Trent, C.; Tsuing, N.; Horvitz, H. R. *Genetics*, **1983**, 104(4), 619-647.
- [45] Metzzen, E.; Berchner-Pfannschmidt, U.; Stengel, P.; Marxsen, J. H.; Stolze, L.; Klinger, M.; Huang, W. Q.; Wotzlaw, C.; Hellwig-Burgel, T.; Jelkmann, W.; Acker, H.; Fandrey, J. *J. Cell Sci.*, **2003**, 116(Pt 7), 1319-1326.
- [46] Freeman, R. S.; Hasbani, D. M.; Lipscomb, E. A.; Straub, J. A.; Xie, L. *Mol. Cells*, **2003**, 16(1), 1-12.
- [47] Lipscomb, E. A.; Sarmiere, P. D.; Freeman, R. S. *J. Biol. Chem.*, **2001**, 276(7), 5085-5092.
- [48] Huang, J.; Zhao, Q.; Mooney, S. M.; Lee, F. S. *J. Biol. Chem.*, **2002**, 277(42), 39792-39800.
- [49] Berra, E.; Benizri, E.; Ginouves, A.; Volmat, V.; Roux, D.; Pouyssegur, J. *EMBO J.*, **2003**, 22(16), 4082-4090.
- [50] Hirsilä, M.; Koivunen, P.; Günzler, V.; Kivirikko, K. I.; Myllyharju, J. *J. Biol. Chem.*, **2003**, 278 30772-30780.
- [51] Lieb, M. E.; Menzies, K.; Moschella, M. C.; Ni, R.; Taubman, M. B. *Biochem. Cell Biol.*, **2002**, 80(4), 421-426.
- [52] Cioffi, C. L.; Liu, X. Q.; Kosinski, P. A.; Garay, M.; Bowen, B. R. *Biochem. Biophys. Res. Comm.*, **2003**, 303(3), 947-953.
- [53] Metzzen, E.; Berchner-Pfannschmidt, U.; Stengel, P.; Marxsen, J. H.; Stolze, L.; Klinger, M.; Huang, W. Q.; Wotzlaw, C.; Hellwig-Burgel, T.; Jelkmann, W.; Acker, H.; Fandrey, J. *J. Cell Sci.*, **2002**, 116 1319-1326.
- [54] Appelhoff, R. J.; Tian, Y. M.; Raval, R. R.; Turley, H.; Harris, A. L.; Pugh, C. W.; Ratcliffe, P. J.; Gleadle, J. M. *J. Biol. Chem.*, **2004**, 279(37), 38458-38465.
- [55] Hewitson, K. S.; McNeill, L. A.; Riordan, M. V.; Tian, Y. M.; Bullock, A. N.; Welford, R. W.; Elkins, J. M.; Oldham, N. J.; Bhattacharya, S.; Gleadle, J. M.; Ratcliffe, P. J.; Pugh, C. W.; Schofield, C. J. *J. Biol. Chem.*, **2002**, 277(29), 26351-26355.
- [56] Mahon, P. C.; Hirota, K.; Semenza, G. L. *Genes Dev.*, **2001**, 15(20), 2675-2686.
- [57] Dann, C. E.; Bruick, R. K.; Deisenhofer, J. *Proc. Nat. Acad. Sci. USA*, **2002**, 99(24), 15351-15356.
- [58] Lee, C.; Kim, S. J.; Jeong, D. G.; Lee, S. M.; Ryu, S. E. *J. Biol. Chem.*, **2003**, 278(9), 7558-7563.
- [59] Elkins, J. M.; Hewitson, K. S.; McNeill, L. A.; Seibel, J. F.; Schlemminger, I.; Pugh, C. W.; Ratcliffe, P. J.; Schofield, C. J. *J. Biol. Chem.*, **2003**, 278(3), 1802-1806.
- [60] Hewitson, K. S.; Schofield, C. J. *Drug Discov. Today*, **2004**, 9(16), 704-711.
- [61] Zhang, G. J.; Safran, M.; Wei, W.; Sorensen, E.; Lassota, P.; Zhelev, N.; Neuberger, D. S.; Shapiro, G.; Kaelin, W. G. Jr. *Nat. Med.*, **2004**, 10(6), 643-648.
- [62] Grimm, C.; Wenzel, A.; Groszer, M.; Mayser, H.; Seeliger, M.; Samardzija, M.; Bauer, C.; Gassmann, M.; Reme, C. E. *Nat. Med.*, **2002**, 8(7), 718-724.
- [63] Sakanaka, M.; Wen, T. C.; Matsuda, S.; Masuda, S.; Morishita, E.; Nagao, M.; Sasaki, R. *Proc. Nat. Acad. Sci. USA*, **1998**, 95(8), 4635-4640.
- [64] Morita, M.; Ohneda, O.; Yamashita, T.; Takahashi, S.; Suzuki, N.; Nakajima, O.; Kawauchi, S.; Ema, M.; Shibahara, S.; Udono, T.; Tomita, K.; Tamai, M.; Sogawa, K.; Yamamoto, M.; Fujii-Kuriyama, Y. *EMBO J.*, **2003**, 22(5), 1134-1146.
- [65] Vincent, K. A.; Shyu, K. G.; Luo, Y.; Magner, M.; Tio, R. A.; Jiang, C.; Goldberg, M. A.; Akita, G. Y.; Gregory, R. J.; Isner, J. M. *Circulation*, **2000**, 102(18), 2255-2261.
- [66] Elson, D. A.; Thurston, G.; Huang, L. E.; Ginzinger, D. G.; McDonald, D. M.; Johnson, R. S.; Arbeit, J. M. *Genes Dev.*, **2001**, 15(19), 2520-2532.
- [67] Willam, C.; Masson, N.; Tian, Y. M.; Mahmood, S. A.; Wilson, M. I.; Bicknell, R.; Eckardt, K. U.; Maxwell, P. H.; Ratcliffe, P. J.; Pugh, C. W. *Proc. Nat. Acad. Sci. USA*, **2002**, 99(16), 10423-10428.

- [68] Mole, D. R.; Schlemminger, I.; McNeill, L. A.; Hewitson, K. S.; Pugh, C. W.; Ratcliffe, P. J.; Schofield, C. J. *Bioorg. Med. Chem. Lett.*, **2003**, *13*(16), 2677-2680.
- [69] Mabweesh, N. J.; Willard, M. T.; Harris, W. B.; Sun, H. Y.; Wang, R.; Zhong, H.; Umbreit, J. N.; Simons, J. W. *Biochem. Biophys. Res. Comm.*, **2003**, *303*(1), 279-286.
- [70] Schlemminger, I.; Mole, D. R.; McNeill, L. A.; Dhanda, A.; Hewitson, K. S.; Tian, Y. M.; Ratcliffe, P. J.; Pugh, C. W.; Schofield, C. J. *Bioorg. Med. Chem. Lett.*, **2003**, *13*(8), 1451-1454.
- [71] Warnecke, C.; Griethe, W.; Weidemann, A.; Jurgensen, J. S.; Willam, C.; Bachmann, S.; Ivashchenko, Y.; Wagner, I.; Frei, U.; Wiesener, M.; Eckardt, K. U. *FASEB J.* **2003**, *17*(9), 1186-1188.
- [72] Wright, G.; Higgin, J. J.; Raines, R. T.; Steenbergen, C.; Murphy, E. *J Biol. Chem.*, **2003**, *278*(22), 20235-20239.
- [73] Ivan, M.; Haberberger, T.; Gervasi, D. C.; Michelson, K. S.; Gunzler, V.; Kondo, K.; Yang, H.; Sorokina, I.; Conaway, R. C.; Conaway, J. W.; Kaelin, W. G. Jr. *Proc. Nat. Acad. Sci. USA*, **2002**, *99*(21), 13459-13464.
- [74] Nwogu, J. I.; Geenen, D.; Bean, M.; Brenner, M. C.; Huang, X.; Buttrick, P. M. *Circulation*, **2001**, *104*(18), 2216-2221.
- [75] Linden, T.; Katschinski, D. M.; Eckhardt, K.; Scheid, A.; Pagel, H.; Wenger, R. H. *FASEB J.*, **2003**, *17*(6), 761-763.
- [76] Dalgard, C. L.; Lu, H.; Mohyeldin, A.; Verma, A. *Biochem. J.*, **2004**, *380*(Pt. 2), 419-424.
- [77] Giaccia, A.; Siim, B. G.; Johnson, R. S. *Nat. Rev. Drug Discov.*, **2003**, *2*(10), 803-811.
- [78] Kung, A. L.; Wang, S.; Klco, J. M.; Kaelin, W. G.; Livingston, D. M. *Nat. Med.*, **2000**, *6*(12), 1335-1340.
- [79] Rapisarda, A.; Uranchimeg, B.; Scudiero, D. A.; Selby, M.; Sausville, E. A.; Shoemaker, R. H.; Melillo, G. *Cancer Res.*, **2002**, *62*(15), 4316-4324.
- [80] Yeo, E. J.; Chun, Y. S.; Cho, Y. S.; Kim, J.; Lee, J. C.; Kim, M. S.; Park, J. W. *J. Natl. Cancer Inst.*, **2003**, *95*(7), 516-525.
- [81] Isaacs, J. S.; Jung, Y. J.; Neckers, L. *J. Biol. Chem.*, **2004**, *279*(16), 16128-16135.
- [82] Mabweesh, N. J.; Post, D. E.; Willard, M. T.; Kaur, B.; Van Meir, E. G.; Simons, J. W.; Zhong, H. *Cancer Res.*, **2002**, *62*(9), 2478-2482.
- [83] Welsh, S. J.; Williams, R. R.; Birmingham, A.; Newman, D. J.; Kirkpatrick, D. L.; Powis, G. *Mol. Cancer Ther.*, **2003**, *2*(3), 235-243.
- [84] Mabweesh, N. J.; Escuin, D.; LaVallee, T. M.; Pribluda, V. S.; Swartz, G. M.; Johnson, M. S.; Willard, M. T.; Zhong, H.; Simons, J. W.; Giannakakou, P. *Cancer Cell*, **2003**, *3*(4), 363-375.