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REVIEW

Modulation of hypoxia-inducible factors (HIF) from an integrative pharmacological perspective

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Abstract Oxygen homeostasis determines the activity and expression of a multitude of cellular proteins and the interplay of pathways that affect crucial cellular processes for development, physiology, and pathophysiology. Hypoxia-inducible factors (HIFs) are transcription factors that respond to changes in available oxygen in the cellular environment and drives cellular adaptation to such conditions. Selective gene expression under hypoxic conditions is the result of an exquisite regulation of HIF, from the pre-transcriptional stage of the HIF gene to the final transcriptional activity of HIF protein. We provide a dissected analysis of HIF modulation with special focus on hypoxic conditions and HIF pharmacological interventions that can guide the application of any future HIF-mediated therapy.

Keywords HIF · Modulation · Pharmacology

Introduction

The hypoxia-inducible transcription factors (HIFs) play a central role in the regulation of oxygen homeostasis and are the master regulators of the events that occur under hypoxic conditions. The majority of publications so far are concentrated on the effect of oxygen deprivation on HIF expression and most of the chemical compounds that modulate HIF are reported using cancer cells in such conditions. A lower number of publications about non-hypoxic stimuli that modulate HIF have been published, even though hypoxia is only one factor among several HIF-inducing stimuli [1, 2]. As reviewed elsewhere, different non-hypoxic stimuli have been proven to enhance HIF levels through the activation of regulatory mechanisms distinct from protein stabilization [2] or regardless of the oxygen concentration [3, 4]. Therefore, HIF activation does not exclusively occur in a hypoxic environment but also in normoxic conditions mediated by various stimuli such as transition metals, nitric oxide, reactive oxygen species, growth factors, mechanical stress or oncogene activation [4–6]. HIF is a heterodimeric transcription factor composed of an inducible α (1, 2, or 3) subunit that confers the sensitivity to oxygen changes and a β-subunit, which is constitutively expressed (also termed aryl receptor nuclear translocator, ARNT). Their target genes have important roles in many physiological and pathological events such as angiogenesis, vascular remodeling, erythropoiesis, glucose utilization, iron transport, cell proliferation, cell survival, apoptosis, and tumor progression [7, 8]. Most of the studies have concentrated on HIF-1α, which is by far the better understood isoform, and information regarding HIF-2α is mainly mentioned in this review when studied alongside HIF-1α. The abundance of HIF-1α is controlled through transcriptional, post-transcriptional, and post-translational mechanisms. The main mode of HIF-α regulation occurs at the level of protein stability and has been extensively reported. Under normoxic conditions, the proline residues of the oxygen-dependent degradation domain (ODD), which is positioned within the N-terminal transactivation domain (N-TAD) are targeted by O_2-dependent prolyl hydroxylases (PHDs). Once the PHDs cause the O_2-dependent hydroxylation of prolyl residues in HIF, the von Hippel–Lindau tumor suppressor protein (pVHL)

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recognizes them and initiates the ubiquitination and degradation process of HIF via the proteasomal system. It has also been reported that the ubiquitin ligase hypoxia-associated factor (HAF) ubiquitinates HIF-1α for proteasomal degradation. HAF turns off the HIF-1α response in acute hypoxia but turns on the HIF-2α response in prolonged hypoxia [9]. Therefore, post-translational protein regulation of HIF is the major process for its modulation and traditionally has received higher priority and attention in the scientific literature. However, in the last 5 years the volume of manuscripts and reviews concerning the pre-transcriptional and transcriptional regulation of HIF has increased exponentially. In fact, HIF-1α can also be regulated at the mRNA level, not only under hypoxic but also non-hypoxic conditions as, has been reviewed elsewhere [10]. Under hypoxic conditions, HIF-α proteins can translocate to the nucleus and form a heterodimer with the constitutively expressed HIF-1β or ARNT and bind to the hypoxia response elements (HREs) to initiate the transcriptional activation of the target genes [11]. The ability of each isoform to control independent cellular processes seems to be important, either by targeting preferential genes or by itself regulatory feedback of HIF-α isoforms. HIF-2α is a key regulator for iron homeostasis and erythropoiesis [12]. Moreover, genes associated with pluripotency, such as Oct4 and Sox2, are also recognized as HIF-2α-specific target genes [13, 14]. The recent work of Heikkila et al. shows that the interaction of HIF-3α variants with HIF-1α or 2α was exclusively associated with the negative regulation of these isoforms by inhibiting their nuclear translocation. Long variants of HIF-3α were capable of efficient induction of an HRE-reporter in the hamster ovary cell line ChoK1 but inhibited the transcriptional activation of the reporter by HIF-1α and HIF-2α under conditions where ARNT is likely to be a limiting factor [15]. HIF-3α is subject to alternative splicing in human tissues and cancer cells and is regulated by HIF-1α but not HIF-2α [16]. Although relatively little has been reported about the pharmacological regulation of HIF-3α, it is likely that drugs that affect the other α isoforms may also have an effect on HIF-3α. For this review, we have disclosed the available knowledge of HIF by an integrative analysis of its regulation through the pharmacological arsenal described to date, from a previous stage of the HIF transcriptional level to the regulation of the activity of HIF as a transcription factor.

Pre-transcriptional regulation of HIF

Epigenetics is the study of heritable changes in gene function that occur without alterations in the DNA sequence, such as DNA methylation, histone modifications, histone variants, and nucleosome positioning, amongst others [17]. The epigenetic changes that occur under hypoxia control the cellular response to such stressful conditions. Conformational changes of chromatin and the methylation status of DNA during hypoxia and the association of HIF with epigenetic regulators such as histone deacetylases (HDACs) to form a complex for further transcriptional activation are important for gene regulation [18]. However, little is known about the epigenetic regulation driving the transcriptional activation of HIF genes. Epigenetic events can influence not only the transcriptional regulation of HIF [19] but also the occupancy of HIF at the binding motifs (HRE) of its target genes [20]. The most well understood epigenetic process is DNA methylation by covalent modification of cytosine at CpG dinucleotides associated with gene silencing. Active promoters are associated with unmethylated CpG islands and an open chromatin conformation, whereas inactive promoters are characterized by a repressive chromatin structure and hypermethylated CpG islands. Abnormal DNA methylation of certain promoters is a primary mechanism for the inactivation of the transcription of genes and is of importance for the understanding of pathologies such as cancer. It has been recently demonstrated that tumor-associated CpG demethylation favors positive auto-regulation of HIF-1α and its target genes [19]. The drug 5-AZA-dC increased HIF-1α, but not HIF-2α, protein levels in the human colon cancer cell line HCT116 grown under hypoxia. 5-AZA-dC can modify the transcription of genes by changing the status of DNA methylation and appears to be a potent tool for dissecting the DNA methylated transcriptional control of HIF and is likely to be used in further therapeutic interventions [19]. In fact, this drug is currently in Phase I trials for the treatment of lymphoma and intestinal neoplasms (http://www.clinicaltrials.gov). Recent evidence demonstrates that DNA methylation is an important process for the regulation of proteins that leads to negative regulation of HIF, such as PHD3. Although regulation of HIF is finely controlled by PHDs, a loss of PHD3 expression by aberrant promoter CpG methylation does not correlate with an increase in HIF-1α protein levels or an increase in the transcriptional activity of HIF in cancer cell lines [21]. Currently, there is no evidence for DNA methylation-mediated regulation of other genes that regulate HIF accumulation such as von Hippel–Lindau ubiquitin ligase complex genes [21]. The gene silencing mediated by CpG island hypermethylation is associated in many cases with the deacetylation of histones within the regulatory region of genes in certain pathologies. Camptothecin, a selective inhibitor of DNA Top I, increases the modifications of histones that indicate active chromatin (euchromatin) in the exonic regions of HIF-1α gene in HCT116 cells [22]. Topoisomerase I (Top I) inhibition by Camptothecin also affects histone modifications at the HIF-1α gene locus, but at later time points, promoting a more accessible chromatin structure in a manner dependent on

cyclin-dependent kinase activity [22]. HIF-1α gene transcription is dependent on a core promoter sequence, the HREs that mediate activation and inhibition by binding motifs located upstream from the transcription initiation site. Several putative HREs have been identified in the HIF-1α promoter in the human endothelial cell line HMEC-1, suggesting a positive auto-regulatory feed back with upregulation of HIF-1α mRNA [23]. Hypoxia also induces HIF-1α mRNA expression via activation of other transcription factors such as early growth response 1 (EGR1) in the prostate cancer cell line DU145 [24], SP1 [23] and nuclear factor kappaB (NF-κB) in pulmonary artery smooth muscle cells [25]. Statins like simvastatin, atorvastatin, and lovastatin are known to decrease the DNA binding of SP1, NF-κB as well as HIF-1α in human endothelial and vascular smooth muscle cells [26]. Although it remains unclear, these results suggest that the statin might produce a synergetic effect on HIF transcriptional activation by decreasing the trans-activation mediated by SP1, NF-κB and the positive auto-regulation of HIF.

Transcriptional regulation of HIF

Pharmaceutical agents that modulate trans-activation mediated by HIF might also affect HIF auto-transcriptional activation, however, for most agents, this assumption remains to be clarified. Camptothecin can interfere with specific transcriptional regulatory steps of HIF. The activation of antisense transcription is likely due to a more open chromatin conformation, most likely provoked by interference of Camptothecin with transcription regulation [27]. Camptothecin is also able to alter the alternative splicing of HIF-1α mRNA co-transcriptionally in HCT116 cells [22]. Some compounds affect mRNA stability. The semi-synthetic compound derived from a plant toxin podophyllotoxin GL331 down-regulates HIF-1α expression without decreasing the stability of HIF-1α mRNA, probably through transcriptional repression. GL331 decreases the binding of the nuclear components derived from human lung adenocarcinoma CL1-5 to the promoter of HIF-1α gene [28]. Flavopiridol is a flavonoid derived from an indigenous plant from India that inhibits HIF-1α in the human glioma cell lines U87MG and T98G by a proteasome-independent pathway, mainly at the level of gene transcription [29].

Post-transcriptional regulation of HIF

RNA-binding proteins (RBPs) associate with mRNAs specifically or non-specifically and regulate non-coding RNAs, including microRNAs (miRNAs) and antisense RNAs [30]. These powerful regulators can cause complex effects on HIF. For example, the HIF antisense (aHIF) transcript destabilizes HIF-1α mRNA in lung epithelial cells, while the RBP human antigen R (HuR) stabilizes HIF transcripts [31]. The pre-translation events that determine HIF levels seem to operate under exquisite regulation and depend on hypoxia [31]. For instance, increased aHIF expression after hypoxia decreases HIF-1α transcripts which in turn, can also contribute to its own negative feedback by increasing the HRE-mediated up-regulation of aHIF [31]. At the post-transcriptional level, Camptothecin can also act by impairing the balance of cellular antisense and sense transcripts at the HIF-1α gene locus in HCT116 cells [22]. In fact, Baranello et al. describe an increment in the level of the antisense transcripts by increased antisense transcription and decreased HIF-1α mRNA levels under normoxic and hypoxic conditions. For instance, the increase of HIF-1α after Camptothecin treatment occurs for the antisense sequence located at the 5′ end and the 3′ ends of the HIF gene.

The use of engineered RNA molecules is becoming widely applied for intervention in specific gene regulation, such as the ablation of the HIF gene during hypoxia [32]. The modification of HIF gene expression using small RNA molecules has been considered as part of a combination cancer therapy with other anti-tumor agents in established tumors [33]. Cellular translation of HIF mRNA has been shown to be enhanced or reduced by several regulatory factors as reviewed by Galban and Gorospe [34]. For instance, the cytoplasmic polyadenylation-element-binding protein (CPEB) and polypyrimidine tract-binding protein (PTB) increase HIF-1α translation while the following several miRNAs: miR-17-92, miR-199a, miR-107, and miR-22, repress HIF-1α in different cellular models [35–39]. The induction of HIF-1α translation in the mouse embryonic fibroblast cell line NIH3T3 is enhanced by the RNA sequences for ribosome assembly (IRES) located within the 5′-untranslated region (5′-UTR) of HIF [40]. The modulation of gene expression at the post-transcriptional level is also exerted by interaction of RBPs with the HIF-1α 3′-UTR, like that produced by the occupancy of HuR, PTB, as well as the CPEB 1 and 2. Tristetraprolin is a protein that can function as a tumor suppressor and is also able to bind directly to the 3′-UTR of HIF-1α mRNA to down-regulate HIF mRNA stability in HCT116 cells [41]. In addition, androgenic hormones can modulate the binding of some of these proteins like HuR, to the AU-rich 3′ UTRs of HIF-1α in Jurkat cell lysates [42]. Since HuR promotes the translation of HIF [35], it has been suggested that low-molecular-weight inhibitors of HuR, like dehydromutactin, MS-444, and okicenone might be able to modulate HIF expression [34]. On the other hand, the inhibition of

HIF-1α translation is also known to be caused by the RBP iron regulatory protein (IRP) and microRNAs, as reviewed by Galban and Gorospe [34]. Therefore, proteins and non-coding RNAs that interact with the HIF-1α mRNA are also interesting targets for HIF regulation.

Inhibition of HIF-α translation

Although global repression of protein translation occurs under hypoxic conditions, some selected proteins are induced in order to respond to such stress, most of which are targeted by HIF-mediated transactivation. Some drugs can cause a reduction in the synthesis of HIF proteins under hypoxia, and in some cases this effect is produced by inhibition of those pathways that increase HIF protein production during hypoxia such as the growth-signaling pathway that includes PI3K/AKT/mTOR/p70S6K kinases. The treatment of cells with specific inhibitors of PI3K and mTOR indicates their requirement to increase HIF-1α expression [43]. mTOR is positioned as an upstream activator of HIF and the mTOR inhibitor rapamycin inhibits HIF-1α-dependent transcription induced by hypoxia [44], HIF-1α protein accumulation by inhibition of HIF-1α protein synthesis [45], as well as promotion of HIF-1α protein degradation [44]. Rapamycin functions as an anticancer agent by regulating HIF activity in the liver [46] and in kidney cancer cell lines and mouse models [47]. The recently discovered HIF inhibitor FM19G11 can impair HRE-mediated trans-activation mediated by HIF as well as down-regulating HIFα proteins under hypoxic conditions. FM19G11 represses the target genes of two of the α-subunits of HIF affecting the differentiation status of ependymal stem cells under low oxygen concentration [13]. It is plausible that FM19G11 reduces HIF synthesis affecting the level of HIFα isoforms and also FM19G11 could favor degradation of the already formed HIFα proteins by proteasome-independent degradation. On the other hand, FM19G11 mediates the molecular effects by activation of the AKT/mTOR pathway in HCT116 cells under normoxia causing an increase in HIFα proteins [48]. This dual effect dependent on the oxygen concentration has also been shown for other drugs such as the plant-derived flavonoid quercetin, indicating a fine regulation depending on oxygen homeostasis. In fact, quercetin inhibits HIF-1α protein synthesis under hypoxia in the human prostate cancer cell line LNCaP, colon cancer cell line CX-1, and breast cancer cell line SkBr3 [49]. However, under normoxia increases HIF-1α in the human colon epithelial cell lines HCT116 and SW620 [50], as well as HIF-1α/HIF-2α by impairing its degradation by PHDs in the human prostate adenocarcinoma cell lines LNCaP, DU-145 and PC-3 or HCT116 cells [50, 51]. Genistein, another flavonoid and inhibitor of tyrosine kinases, completely blocks the synthesis of both HIF-1 subunits in human retinal pigment epithelium cells [52] as well as HIF-1 DNA-binding activity [53]. Silibinin, an additional flavonoid isolated from milk thistle (*Silybum marianum*) inhibits expression of HIF-1α through suppression of protein translation in PC-3 and LNCaP cells [54]. Wondonin reduces the stability of HIF-1α protein and activity in the human keratinocyte cells HaCaT [55]. Some inhibitors of the enzymes that wind or unwind DNA for the synthesis of proteins inhibit HIF-1α protein accumulation. For instance, the Top I inhibitor topotecan (NSC-609699) in the human glioma cell line U251 [56, 57], as well as the inhibitors of Top II, like NSC-644221 in a cell type-dependent manner [58], inhibit HIF-1α by decreasing HIF-1α protein translation. These Top I and Top II inhibitors are also able to decrease the transcriptional activation. EZN-2208, a compound derived from the topoisomerase inhibitor SN38, (the active part of Camptothecin-11) down-regulates HIF-1α/HIF-2α protein in preclinical neuroblastoma models [59] as wells as the expression of proteins controlled by HIF-1α in a U251-HRE glioblastoma xenograft model [60]. In addition to Camptothecin, other DNA damage inducing agents like Mitomycin C and NSC-652287 also inhibit HIF-1α protein synthesis in the human embryonic kidney cell line HEK293 [61]. Drugs that target microtubules can also influence HIF-1α level by microtubule-dependent regulation. Among these drugs are taxotere, epothilone B, discodermolide, vincristine, 2-methoxyestradiol (2ME2), and colchicine. These drugs affect HIF-1α protein by de novo-inhibition of HIF-1α protein synthesis, but can also inhibit HIF-1α transcriptional activity in the human ovarian cancer cell line 1A9. However, no changes in mRNA levels were observed [62]. There is some controversy regarding the microtubule-dependent regulation of HIF-1α by 2ME2, which can also regulate HIF-2α in the human breast cancer cell line MDA-MB-231 [63], as according to Hagen et al. [64] its diminution in HEK293 cells is due to an effect on protein degradation rather than affecting the protein synthesis. These apparently differing results observed for 2ME2 might be due to the particular cell type used in each study. The anti-epidermal growth factor receptor monoclonal antibody cetuximab (Erbitux) reduces HIF-1α at the level of protein synthesis in the epidermoid carcinoma cell line A431 under both normoxic and hypoxic conditions through the RAS [65], a molecule that contributes to stabilizing HIF-1α. 103D5R is another small molecule that strongly reduces HIF-1α protein synthesis under normoxia and hypoxia in cells derived from different cancer types, including glioma, prostate, and breast cancers, whereas HIF-1α mRNA levels and HIF-1α degradation are not affected [66].

Modulation of HIF post-translation

HIF proteins can undergo different post-translational modifications that determine its stability. To find out whether the repression of HIF occurs before or after the translation of the protein, pharmacological tools can be used to inhibit protein synthesis (Cycloheximide) or proteasomal degradation (MG132) [49]. One of the mentioned modifications is the well-documented ubiquitination of HIF which leads to degradation by the proteasome. Also, S-nitrosylation up-regulates as well as stabilizes HIF-1α in a murine mammary carcinoma cell line 4T1 and murine melanoma cell line B16F10 [67]. In contrast, the acetylation of HIF-1α enhances its degradation by the VHL pathway in HEK293 cells and the fibrosarcoma cell line HT1080 [68] and phosphorylation destabilizes HIF-1α in murine embryonic fibroblasts (MEFs) [69]. However, it still remains unclear whether full-length HIF-1α is acetylated in vivo and its putative role in HIF-1α stability [70]. In any case, this proposed model cannot explain the HDAC-inhibitor-mediated, VHL and ubiquitination-independent degradation of HIF-1α [70, 71]. Intermittent hypoxia produces changes in the abundance of HIF-1α phosphorylated/non-phosphorylated forms with enhanced HIF-1α stabilization during each period of hypoxia in the human endothelial cell lines EAhy926 and HMEC-1 [72]. The different phosphorylation status of HIF-1α and HIF-2α determines expression of genes involved in the DNA repair process in HCT116 cell line [73]. Compound C, an inhibitor of the key intracellular energy sensor AMP-activated protein kinase (AMPK) that enhances phosphorylation, prevents HIF-1α stabilization in the human osteosarcoma cell line 143B [74]. However, this stabilization might be mediated by a process independent of AMPK in MEFs [75]. HIF protein can also be SUMOylated by ubiquitin E3 ligases that control its degradation by the ubiquitin–proteasome pathway in the human cervical cancer cell line HeLa [76]. Noscapine, another microtubule modulator agent, promotes the degradation of HIF-1α protein via the proteasome in the human glioma cell lines U87MG and T98G [77]. Noscapine acts in a similar way to the heat shock protein 90 (Hsp90) inhibitor, geldanamycin [78] by blocking the accumulation of HIF-1α protein in the nucleus [77]. An alternative means of protein homeostasis is the VHL-independent mechanism mediated by Hsp90, which protects proteins degradation through its ATPase activity [79]. Several studies have demonstrated that Hsp90 is associated with HIF-1α and this association may be required for the hypoxic activation of HIF-1α by stabilizing the protein or by enhancing its DNA binding [80]. Inhibitors of Hsp90 dissociate Hsp90 from HIF-1α and induce O_2/PHD/VHL-independent degradation of HIF-1α. In contrast, Hur et al. [81] reported that the Hsp90 inhibitor Radicicol does not affect the stability of HIF-1α, but significantly reduces DNA binding of HIF-1α in the human hepatoma cell line Hep3B under hypoxic conditions. The receptor of activated protein C kinase (RACK1) promotes the O_2/PHD/VHL-independent degradation of HIF-1α by competition with Hsp90 for binding to HIF-1α and recruitment of the ubiquitin ligase complex for further HIF-ubiquitination and degradation [82]. Some proteins such as the stress kinase c-Jun NH2-terminal kinase 1 (JNK1) mediate degradation of HIF-1α by a VHL-independent mechanism that involves both chaperones Hsp90 and Hsp70. JNK1 deficiency impairs HIF-1α protein stabilization as well as its transcriptional activity [83]. Another example of Hsp90 inhibition that induces HIF-1α degradation is 17-allylaminogeldanamycin (17AAG), an analogue of the flavonoid geldanamycin, which requires the contribution of RACK1 for such effect in HEK293T cells [84]. The regulation of HIF by flavonoids seems to be complex. The flavonoid apigenin possesses anti-tumor properties and is able to reduce HIF levels. Apigenin decreases HIF-1α protein stability and expression by inhibition of PI3K/AKT/p70S6K but increases p53 pathways in the human ovarian cancer cell lines OVCAR-3 and A2780/CP70 [85]. This mode of action contrasts with that of FM19G11 which increases HIF-1α proteins and impairs tumor cell growth under normoxia by activation of both AKT and p53 pathways in HCT116 cells [48], suggesting that the regulation of HIF is complex. In addition, Flavopiridol inhibits HIF-1α by a proteasome-independent pathway but mainly acts at the level of gene transcription [77]. Geldanamycin and the thioredoxin redox inhibitors facilitate HIF protein degradation by the proteasome pathway in a similar way to the mentioned Noscapine [77, 86]. Thioredoxin (Trx-1) inhibitors exert heterogeneous effects on protein regulation, since it was reported that novel drugs like AJM290 and AW464 (quinols) might increase HIF-1α but decrease functional transcriptional activity, DNA binding, and degradation in the human breast cancer cell line MDA-MB-468 [87]. Interestingly, the anti-tumor agent PX-12, an inhibitor of Trx-1, causes degradation of HIF-1α in a Trx-1 independent manner in a range of cancer cell lines [88]. PX-478 inhibits HIF-1α protein synthesis, the accumulation of HIF-1α by increased polyubiquitination and further degradation, as well as HIF-1α activity in a wide range of cancer cell types [89, 90].

The oncogene Ras stabilizes HIF-1α. Ras can interact with intracellular membranes via the farnesyl group. Without farnesylation, Ras does not interact with other regulatory molecules and the MAPK pathway is not activated. Therefore, disruption of Ras by farnesyltransferase inhibitors, such as tipifarnib (R115777) [91] and lonafarnib (SCH66336) [92] destabilizes HIF-1α and decreases HIF transcriptional activity [93, 94]. Another agent developed

for circulatory disorders, YC-1 inhibits HIF-1α protein synthesis under normoxia and affects protein stability in the human hepatocellular liver carcinoma cell line HepG2 grown under hypoxia [95].

Some commonly used non-steroidal anti-inflammatory drugs like Ibuprofen reduce the protein levels of HIF-1α and HIF-2α in prostate cancer cells grown under normoxic and hypoxic conditions [96]. A possible explanation for inhibition of the accumulation of HIF-1α is the increment of expression of the VHL tumor suppressor protein and further degradation of the protein under hypoxic conditions [97]. It has recently been reported that Honokiol obtained from *Magnolia grandifloris* and used for thousands of years in traditional Asian medicine modulates HIF at different levels. Honokiol regulates the HIF-1α protein accumulation as well as its transcriptional activity in subcutaneous murine colon carcinoma [98].

Modulation of HIF transcriptional activity

Epigenetic regulation of HIF function

In the last decade, it has become increasingly evident that epigenetic events that occur under hypoxia can drive the molecular response to hypoxia and also control the expression of HIF-1α. To induce target genes, HIF-1α requires a conductive chromatin environment to allow binding of specific factors to specific sequences. Transcriptionally repressed chromatin, heterochromatin, is compact and restricts access of transcription factors to DNA, while transcriptionally active chromatin, euchromatin, allows access of transcription factors to DNA. Histones are proteins that package and order the DNA-forming nucleosomes and their modification play a role in gene regulation. Hypoxia induces chromatin modifications by modulation of enzymes that modify the histones, which causes global repression of transcription [99]. Therefore, intervention with pharmacological agents may help to elucidate the mechanisms that control the molecular response to hypoxia prior to the activation of transcription. There are several ways of modulating chromatin structure, including histone tail modification and nucleosome remodeling that results from the action of ATP-dependent remodeling complexes. Drug discovery related to modulation of gene expression cannot be done at the DNA level alone. One important family of mammalian chromatin remodeling complexes by nucleosome remodeling is mediated by a family of ATP-dependent enzymes named SWI/SNF (switch/sniff) that modulate the expression. SWI/SNF interacts with only certain classes of transcription factors and this property enables SWI/SNF to be selectively recruited to particular promoters. SWI/SNF complexes can be targeted to chromatin by p300, a co-activator of HIF, while histone acetylation by CBP/p300 facilitates the recruitment of SWI/SNF in *Xenopus* oocytes [100]. SWI/SNF is required for several of the cellular responses induced by hypoxia. Moreover, HIF-1α is a direct target of the SWI/SNF chromatin-remodeling complex [102] and SWI/SNF components are found associated with the HIF-1α promoter and modulation of SWI/SNF levels results in pronounced changes in HIF-1α expression and its ability to trans-activate target genes in the human osteosarcoma cell line U2OS [102]. In addition to acetylation, chromatin can also be modified through methylation, ADP-ribosylation, ubiquitination, SUMOylation and phosphorylation, amongst others. The acetylation status of histones that are located within the regulatory regions determine the accessibility of crucial transcription factors such as HIF to their binding sites and the formation of multiprotein co-regulatory complexes that define gene-specific transcriptional responses under hypoxic conditions [32, 102–104]. In general, histone acetylation leads to increased access of transcription factors to the DNA. Acetylation status is reversibly regulated by a dynamic balance between histone acetyl transferases (HATs) and HDACs. HDACs include a large family of enzymes that remove the acetyl groups of histones, transcription factors, coactivators, and other proteins. FM19G11 which inhibits HIF-α proteins and transcriptional activity, also showed a predominant involvement in epigenetic-associated events [13]. FM19G11 causes a reduction of overall histone acetylation with significant repression of p300, a histone acetyltransferase required as a co-factor for HIF-transcriptional activation, in rat ependymal stem cells. Type I/II HDAC-inhibitors impair HIF function by either reducing functional HIF-1α levels or repressing HIF-α transactivation activity [105]. Low doses of HDAC-inhibitors that promote acetylation of HIF are not sufficient to cause HIF-1α degradation but are sufficient to repress HIF-1α transactivation potential under both normoxic and hypoxic conditions [20]. The repression of HIF function by the induction of hyperacetylation of histones by HDAC inhibitors might explain their effects in the repression of tumor growth and may be promising anti-cancer drugs for clinical goals [70, 106, 107]. The different therapeutic treatments that include HDAC inhibitors in combination with other drugs have been reviewed elsewhere [108]. Although HDAC inhibitors are generally well tolerated, the accumulation of acetylated histones and non-specific targets may induce undesired side-effects depending on the dose, route, and drug [109]. The stimulatory transcriptional effects of the HDAC inhibitor trichostatin A (TSA) could be possibly caused by hyperacetylation of histones or other transcription regulators, since the drug did not increase endogenous HIF-1α levels [20]. However, the authors

proposed that HDAC inhibitors-mediated repression, like that produced by TSA requires both HIF-α protein and HRE binding sites and is independent of the direct acetylation of HIF-α. It has also been reported that the HDAC inhibitor FK228 is able to decrease the HIF-1α binding activity in the human fibrosarcoma cell lines LLC and HT1080 [110]. The transactivation potential of HIF-1α needs certain deacetylation activity that is also impaired by HDAC inhibitors [20]. Histone methylation or demethylation is another histone modification due to environmental influence that occurs in dynamic regulation driven by methyltransferases and demethylases. Hypoxia can increase histone methylation, the repressive marks of histones that impair transcription, which are also observed at promoters of hypoxia-regulated genes [99]. The methylation of lysine residues in histones by the specific histone methyltransferase (HMTs) is also implicated in alterations of chromatin structure and the regulation of crucial genes under hypoxic conditions [111]. However, little is known about how these epigenetic events can influence the transcriptional activation mediated by HIF but this transcription factor exhibits a preferential binding for transcriptionally active loci [112]. The regulation of Jumonji-domain-containing histone demethylases (JHDMs) that belong to a family of dioxygenases and share similarities with PHDs are regulated by HIF by binding within the promoters of histone demethylases (JARID1B, JMJD1A, JMJD2B, and JMJD2C) [113–117]. Therefore, HIF also contributes to control global levels of histone methylation under hypoxic conditions that modulate gene expression and determines cell type-specific responses to hypoxia. A combination of inhibitors of DNA methyltransferase (DNMT) and HDACs have been used alone or in conjunction with other pharmacological tools [108]. Histone demethylase enzymes in the Jumonji gene family are in some cases induced by hypoxia in a HIFα-dependent manner [118] and contribute to control the cell response by epigenetic regulation under hypoxic conditions [119]. Therefore, besides HIF-mediated gene transcription, modulation of histone methylation is another way of cell adaptation to hypoxic stress. In addition, some of these proteins like JMJD1A could also potentially regulate gene transcription in hypoxia independent of its histone demethylase activity [118]. It is remarkable that the concurrence of DNA methylation and histone deacetylation can regulate gene expression including HIF in pathological processes and in fact, synergy of both processes can silence HIF-2α transcription in MYCN-amplified neuroblastoma cells [120]. Phosphorylation of the N-terminal tail of histone H3 by MAP-kinase signaling or de-phosphorylation by phosphatases are crucial processes that might be involved in the regulation of the transcriptional activation and inactivation processes [121]. Histone phosphorylation is caused by signaling kinases like AMPK that activate transcription promoted by stress [122], such as hypoxia conditions. However, little is known about the influence of the phosphorylation status of histones in the regulatory region of HIF target genes and the activity of HIF as a transcription factor. It has been reported that AMPK is rapidly activated in vitro by both physiologically and pathophysiologically low-oxygen conditions, but this event is independent of HIF-1α activity [123]. It is noteworthy that the first ubiquitinated protein to be identified was histone H2A [124]. Despite the early discovery of ubiquitinated histones, it has only been in the last five or so years that we have begun to understand how histone ubiquitination is regulated [125]. However, the role that histone ubiquitination plays in chromatin folding and/or function that control cells grown under hypoxic conditions remains to be clarified.

HIF DNA binding

Effective transcriptional activation first requires a proper heterodimerization of HIF-1α with HIF-1β in the absence of DNA [126]. Jiang et al. reported a functional analysis of HIF-1α and the identification of protein domains required for HIF-1α heterodimerization, DNA binding, and the final transcriptional activation [126, 127], and therefore there are some drugs that can act at the mentioned stages. At the heterodimerization stage, it has been reported that Acriflavine inhibits heterodimerization of HIF-1α with HIF-1β in HEK293 cells [128, 129]. The search of chemical products that affect the protein–DNA interactions offer another possibility to intervene on the control of endogenous gene regulation. Polyamides are a class of DNA-binding molecules used to disrupt protein–DNA interactions in a sequence-specific manner [130]. Echinomycin (NSC-13502) is a DNA-binding product that inhibits binding of HIF-1α and HIF-1β proteins to HRE sequences in U251 cells [131]. Echinomycin acts in a similar way to polyamide but has less sequence preference than polyamide in U251 cells [132]. The anthracycline chemotherapeutic agents doxorubicin and daunorubicin are potent inhibitors of HIF-1α-mediated gene transcription by blocking its binding to DNA in Hep3B cells and dimerization of HIF-1α and HIF-1β in HEK293 cells [133]. Inhibitors of Hsp90 activity such as geldanamycin in HMEC-1 cells and the monkey kidney fibroblast cell line COS-7 [134] and Radicicol also affect HRE binding by the HIF-1α and HIF-1β heterodimer [81]. In addition, the iron-binding porphyrin hemin inhibits HIF-1α-dependent gene transactivation through its binding with Hsp90 in HCT116 cells [135]. The metabolite actinomycin D, an inhibitor of transcription was shown to abolish hypoxia-induced HIF-1α binding activity in Hep3B cells [11]. The novel thioredoxin inhibitors AJM290 and

AW464 significantly inhibited HIF-1α transactivation activity by blocking HIF-1α HRE-DNA binding [87, 136]. The same authors also identified DJ12, a compound that also blocks HIF-1α HRE-DNA binding and transcriptional activation [136]. In addition to external pharmacological tools, cell-signaling molecules like IL-1β and TNF-α can cause a moderate activation of HIF-1α DNA binding under normoxic conditions. The treatment of HepG2 cells grown under hypoxia with these cytokines strongly increases HIF-1α activity compared to the effect of hypoxia alone [137].

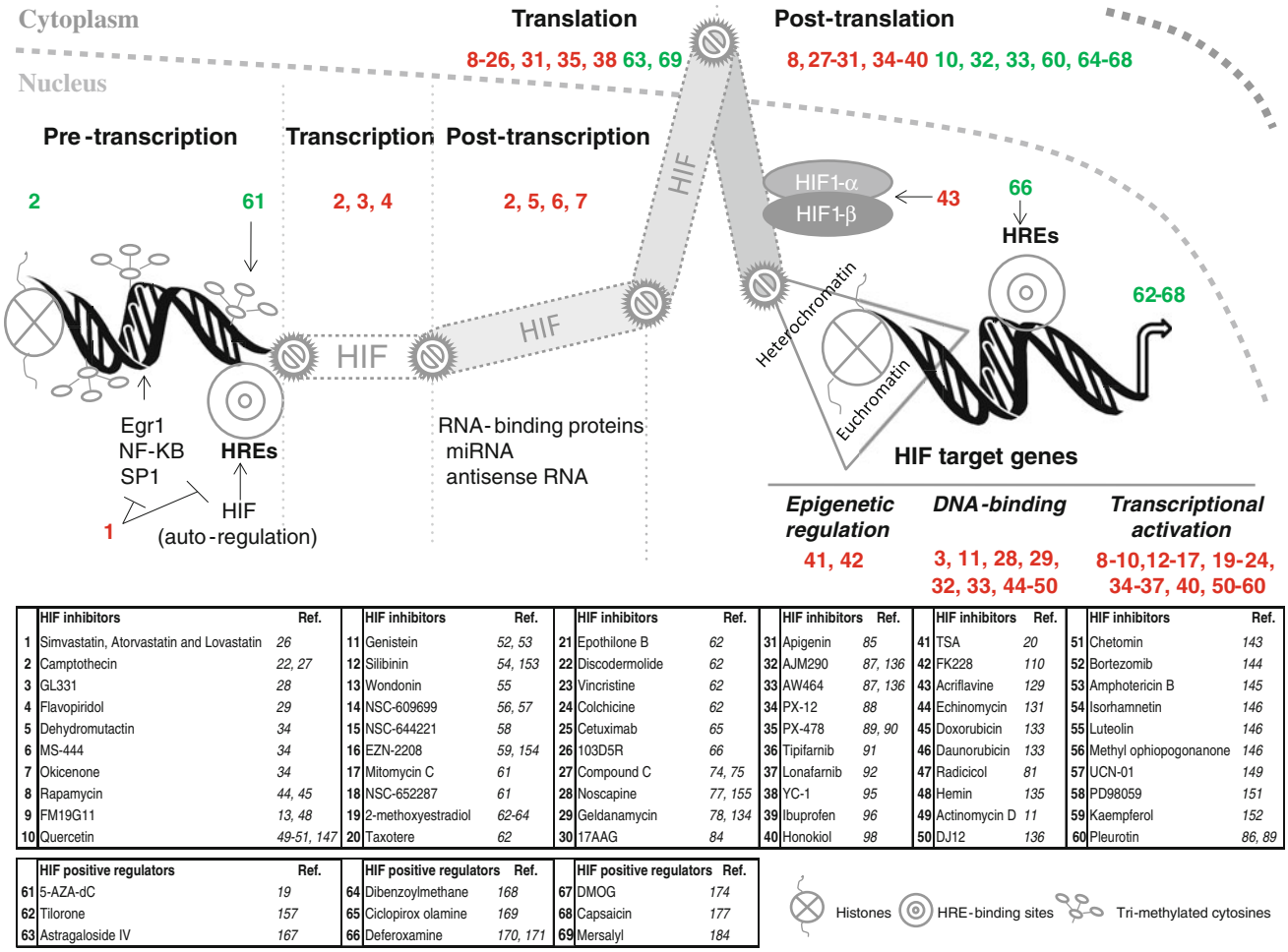
HIF transactivation

The association between the histone acetyl transferase CBP/p300 with HIF-1α is crucial for the activation of gene transcription and can be disrupted by the hydroxylation of HIF by FIH (factor inhibiting HIF) in HEK293 cells [138] that blocks association with co-activators in HEK293T cells [139]. FIH-1 binds to VHL that also functions as a transcriptional co-repressor that inhibits HIF-1α transactivation by recruiting HDACs [138]. It is accepted that the HAT activity enhances the hypoxia inducible activity of HIF-1α [140]. However, hyperacetylation of p300 mediated by HDAC-inhibitors represses the HIF-1α-p300 complex in vivo indicating that deacetylase activity is indispensable for the transactivation potential of HIF. In fact, HDACs are known to be interacting partners for HIF-1α and some of them (HDAC4, 5 and 7) interfere with FIH-1 binding to the inhibitory domain causing increased transcriptional activity [18]. It has also been recently reported that the transcription factor NF-κB suppresses the HIF-1α transcriptional response by competing for p300 binding in the human osteosarcoma cell line MG63, the human osteoblast cell lines hFOB 1.19 and MC3T3-E1, and the murine mesenchymal cell line C3H10T(1/2) [141]. The CBP/p300-interacting transactivator 2 (Cited2) is an interacting transcriptional modulator and a proposed negative regulator of HIF-1α by competition for the binding sites of CBP/p300 [142]. The impairment of formation of the basal transcription complex that is necessary for HIF-mediated transcriptional activation is also an object of intervention at the pharmacological level. In addition to cellular proteins, there are also external pharmacological tools that regulate this interaction. The compound chetomin was identified through a target-based high-throughput screen as a disrupter of HIF binding to p300 in Hep3B and HepG2 cells, which affects HIF-1α/HIF-2α-mediated transactivation [143]. The proteasome inhibitor bortezomib (PS-341) also represses HIF-1α transcriptional activity by stimulation of the interaction between HIF and FIH under hypoxic conditions, impairing p300 recruitment in multiple myeloma cells [144]. In Hep3B and HEK293 cells, the antifungal agent amphotericin B enhances FIH binding to HIF and blocks p300 recruitment with a resultant repression of HIF-1α transcriptional activity [145]. For other drugs, their mechanisms of action require further studies because they are still elusive or unclear. Some flavonoids, like isorhamnetin, luteolin, methyl ophiopogonanone (MOB) [146] and quercetin [147] can also regulate the HIF-1α-mediated transcriptional activation. The phosphorylation status of HIF is critical for HIF-1α transcriptional activity and the expression of its target gene under hypoxic conditions [148]. The protein kinase C inhibitor UCN-01 also modulates the HRE transcriptional activation mediated by HIF in human endothelial cells [149]. Flavonoids also inhibit HIF-1α activity by impairing the MAPK-dependent phosphorylation of HIF-1α, thereby decreasing its nuclear accumulation in HeLa cells [150]. The MAPK inhibitor PD98059 blocks the trans-activation but not the stabilization or DNA binding ability of HIF-1α in Hep3B cells [151]. The flavonoid kaempferol effectively inhibits HIF-1α activity in the human hepatocarcinoma cell line HuH-7 under hypoxic conditions by its relocalization into the cytoplasm by inhibition of HIF-1α phosphorylation rather than suppression of protein levels [152]. Silibinin inhibits hypoxia-induced HIF-1α accumulation and transcriptional activity that is correlated with inhibition of the mTOR/p70S6K/4E-BP1 signaling pathway in HeLa and Hep3B cells [153]. FM19G11 decreases the transcriptional activity mediated by HRE in hypoxia, but increased mTOR and HIF-1α accumulation in HCT116 cells in normoxia. The topoisomerase inhibitors NSC607097 [57] and the compound EZN-2208 derived from the SN38 (10-hydroxy-7-ethyl-camptothecin) down-regulate HIF-1α-mediated trans-activation of downstream targets in the cell line U251-HRE [154]. Noscapine appears to interfere with microtubule function and sensitizes chemoresistant ovarian cancer cells to cisplatin through inhibition of HIF-1α transcriptional activity in the human ovarian cancer cell line C13K [155]. The thioredoxin redox inhibitors, PX-12, pleurotin and PX-478 [86, 89] as well as Wondonin [55], a novel compound derived from sponges inhibit the HIF-1α-mediated transactivation.

HIF-positive regulators

HIF-positive regulators increase protein expression or decrease degradation, thereby stabilizing HIF and increasing HIF-mediated transactivation. The central nervous system requires a constant supply of oxygen and glucose, and neurons have developed specific mechanisms to adapt to hypoxia/ischemia. HIFs have emerged as master regulators of neuroprotection and survival, especially for the application of neuronal degenerative diseases and aging

[156]. Tilorone, a novel potent activator of HIF-1α and its downstream target genes, provides prophylaxis against brain stroke and traumatic spinal cord injury and helps ameliorate these conditions [157]. In neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis, the associated accumulation of toxins, lead to neuroinflammation and degeneration, HIF-1α functions as a neuroprotector. The neuroprotection is associated with VEGF-mediated expression through activation of HIF-1α [158]. In addition, some pre-conditioning strategies that induce HIF are applied for ischemic rescue in the brain and for myocardial infarction [159, 160]. HIF is important for development [161], angiogenesis, and normal and pathological functioning of the heart [162, 163]. Results obtained in the last decade and reviewed elsewhere indicate that HIF-1α exerts a critical role in mediating cardioprotection [164]. Induction of RTEF-1, a positive regulator of HIF-1α transcription that acts by binding the MCAT-like elements in the HIF-1α promoter region in endothelial cells, accelerates recovery from ischemia [165]. Calcineurin is activated by calcium and calmodulin and plays a key role in physiological responses such as cardiac hypertrophy. Calcineurin dephosphorylates RACK-1, which impairs its dimerization and consequently inhibits HIF-1α ubiquitination and proteasomal degradation in HEK293T cells under both hypoxic and non-hypoxic conditions [166]. Astragaloside IV, a constituent of *Astragalus membranaceus*, is an example of an external chemical compound that increases HIF and effects heart function. Recent findings demonstrate that Astragaloside IV can stimulate HIF-1α accumulation through the PI3K/Akt pathway of human umbilical vein endothelial cells (HUVEC) cultured under hypoxic conditions [167]. The authors proposed that Astragaloside IV promotes angiogenesis and protects against cardiac hypoxia during myocardial ischemia. It has been proposed that dibenzoylmethane, a natural dietary compound, induces HIF-1α by inhibition of protein degradation and promotes activation of HIF-1 as measured by reporter gene assay in LNCaP, PC-3, and HEK293 cell lines, with possible applications in ischemic diseases [168]. The antimycotic ciclopirox olamine induces HIF-1α stability, HIF-1α transcriptional activity and modulates angiogenesis [169]. Deferoxamine can induce HIF-1α by inhibiting its degradation [170] as well as potentiating HIF DNA binding [171] and has been proposed in the reduction of brainstem blood [172] or prevention of cardiac hypertrophy [173]. Deferoxamine and the prolylhydroxylase inhibitor dimethyloxalylglycine (DMOG) can also stimulate HIF-2α in human lung endothelial and epithelial cells [174]. DMOG induces HIF-1α activation with a possible positive effect in ischemia–reperfusion injury [175]. Capsaicin-sensitive afferent neurons are related to the transmission of cardiac nociception in acute myocardial infarction [176]. Capsaicin induces HIF-1α expression and binding activity under normoxic conditions, most likely by inhibiting NF-κB activation, which may trigger stress-signaling pathways [177]. The role of HIF-1α preconditioning has also been shown to have excellent preventive and therapeutic effects in various experimental models of kidney disease [178]. However, HK-2 cells exhibit a non-lethal but dysfunctional phenotype under hypoxic conditions, which reflects the epithelial pathology of ischemic acute renal failure [179]. In human kidney HK-2 cells, all trans-retinoic acid (ATRA) treatment induces HIF-1α under normoxic conditions and hypoxia. ATRA induces stabilization of HIF-1α mRNA but not of HIF-1α protein [180]. Other transcription factors such as Stat3, are associated with HIF-1α in hypoxia by direct interaction that causes its stabilization in human renal carcinoma cells [181]. Human cytomegalovirus (HCMV) may cause significant alterations to cellular physiology, not only related to the innate immune responses but also to altered cellular processes affected by an increment in HIF expression [182]. Mersalyl [o-[(3-hydroxymercuri-2-methoxypropyl) carbamoyl]phenoxyacetic acid] is an organic mercurial diuretic with antiviral activity [183] that induces expression of HIF-1α by a mechanism involving the IGF-1/MAPK pathways in Hep3B cells [184]. Some pathways including the MAPK and PI3K/AKT cascades are involved in HIF activation regardless of the oxygen tension [4, 5]. Some attempts have tried to elucidate whether the regulation of HIF in normoxia or hypoxia is similar or different. For instance, an overexpression of HIF induced by doxycycline in HEK293 cells served to study a HIF-1α-dependent gene regulation under normoxia or hypoxia [185]. This strategy allowed the authors to differentiate the hypoxia-dependent from hypoxia-independent effects on HIF expression and some of its known target genes (i.e., VEGF or EPO) as well as the consequences in a crucial process such as apoptosis. HIF-1α and the mentioned target genes increase in a time-dependent manner after treatment with doxycycline but at a lower level in comparison to the induction caused by hypoxia. The authors tried to explain the effect by the requirement of cofactors for HIF-1α-induced action on target genes and the absence of post-translational modifications under normoxia suggesting a cofactor-dependent process in HIF-1α and hypoxia-related apoptotic process. For the non-hypoxic stimuli of lipopolysaccharides (LPS), the upregulation of HIF-1α protein takes more time compared to hypoxic induction but are elevated for a longer period of time [2]. In addition, the non-hypoxic induction of functional HIF-1α is not only cell type-specific but also depends on the differentiation status of the cells as in the innate immune response [186]. Therefore, it seems that the regulation of expression



	HIF inhibitors	Ref.
1	Simvastatin, Atorvastatin and Lovastatin	26
2	Camptothecin	22, 27
3	GL331	28
4	Flavopiridol	29
5	Dehydromutactin	34
6	MS-444	34
7	Okicenone	34
8	Rapamycin	44, 45
9	FM19G11	13, 48
10	Quercetin	49-51, 147
11	Genistein	52, 53
12	Silibinin	54, 153
13	Wondonin	55
14	NSC-609699	56, 57
15	NSC-644221	58
16	EZN-2208	59, 154
17	Mitomycin C	61
18	NSC-652287	61
19	2-methoxyestradiol	62-64
20	Taxotere	62
21	Epothilone B	62
22	Discodermolide	62
23	Vincristine	62
24	Colchicine	62
25	Cetuximab	65
26	103D5R	66
27	Compound C	74, 75
28	Noscapine	77, 155
29	Geldanamycin	78, 134
30	17AAG	84
31	Apigenin	85
32	AJM290	87, 136
33	AW464	87, 136
34	PX-12	88
35	PX-478	89, 90
36	Tipifarnib	91
37	Lonafarnib	92
38	YC-1	95
39	Ibuprofen	96
40	Honokiol	98
41	TSA	20
42	FK228	110
43	Acriflavine	129
44	Echinomycin	131
45	Doxorubicin	133
46	Daunorubicin	133
47	Radicicol	81
48	Hemin	135
49	Actinomycin D	11
50	DJ12	136
51	Chetomin	143
52	Bortezomib	144
53	Amphotericin B	145
54	Isorhamnetin	146
55	Luteolin	146
56	Methyl ophiopogonanone	146
57	UCN-01	149
58	PD98059	151
59	Kaempferol	152
60	Pleurotin	86, 89

	HIF positive regulators	Ref.
61	5-AZA-dC	19
62	Tilorone	157
63	Astragaloside IV	167
64	Dibenzoylmethane	168
65	Ciclopirox olamine	169
66	Deferoxamine	170, 171
67	DMOG	174
68	Capsaicin	177
69	Mersalyl	184

Fig. 1 Pharmacological intervention of HIF regulation. The mode of action of both negative HIF regulators (*red numbers*) and the positive HIF regulators (*green numbers*) are indicated at the corresponding regulatory stage

modulated by different non-hypoxic stimuli is distinct from the hypoxia-induced response. It is also noteworthy that some of the HIF regulators mentioned here are not selective inhibitors of HIF and can affect the general machinery in the cells. For instance, the microtubule-stabilizing and microtubule-destabilizing drugs that disrupt microtubule function affecting the cell cytoskeleton or the HDAC-inhibitors that influence the chromatin remodeling and transcriptional activation of many genes. HIF regulation by pharmacological compounds is the result of complex molecular events occurring under different oxygen concentrations that requires further studies for better comprehension (Fig. 1).

Concluding remarks

The HIF-α subunit is precisely regulated on multiple levels depending on the oxygen availability. Pre-transcription, transcription, translation, post-translation and the capability of HIF-mediated transactivation to initiate the transcription of target genes are important sites of regulation. Transcriptional activity is regulated by epigenetic events and by the capability to form heterodimers by HIF protein–protein interactions. Controlling HIF modulation by molecular or pharmacological strategies seems to be crucial for treatment of significant malignancies directly influenced by angiogenesis such as cancer or cardiac diseases. The multifaceted aspects of HIF regulation provide different possibilities for therapeutic intervention to up- or down-regulate HIF according to the desired therapeutic effect, making HIF an attractive target. Although there are many efforts to identify new HIF modulators, there is still room for the discovery of new drugs and molecular strategies that modulate HIF at different levels. These findings contribute to clarify the cellular effects caused by HIF modulation for further and secure interventions in clinical trials.

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References

1. Lopez-Lazaro M (2009) Role of oxygen in cancer: looking beyond hypoxia. Anticancer Agents Med Chem 9:517–525
2. Kuschel A, Simon P, Tug S (2011) Functional regulation of HIF-1alpha under normoxia—is there more than posttranslational regulation? J Cell Physiol
3. Hellwig-Burgel T, Stiehl DP, Wagner AE, Metzen E, Jelkmann W (2005) Review: hypoxia-inducible factor-1 (HIF-1): a novel transcription factor in immune reactions. J Interferon Cytokine Res 25:297–310
4. Chun YS, Kim MS, Park JW (2002) Oxygen-dependent and -independent regulation of HIF-1alpha. J Korean Med Sci 17:581–588
5. Semenza G (2002) Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol 64:993–998
6. Bardos JI, Ashcroft M (2005) Negative and positive regulation of HIF-1: a complex network. Biochim Biophys Acta 1755: 107–120
7. Semenza GL (2000) HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 88:1474–1480
8. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732
9. Koh MY, Lemos R Jr, Liu XP, Powis G (2011) The hypoxia associated factor (HAF) switches cells from HIF-1{alpha} to HIF-2{alpha} dependent signaling promoting stem cell characteristics, aggressive tumor growth and invasion. Cancer Res 71:4015–4027
10. Gorlach A (2009) Regulation of HIF-1alpha at the transcriptional level. Curr Pharm Des 15:3844–3852
11. Wang GL, Semenza GL (1993) Characterization of hypoxia-inducible factor 1 and regulation of DNA binding activity by hypoxia. J Biol Chem 268:21513–21518
12. Anderson ER, Xue X, Shah YM (2011) Intestinal hypoxia-inducible factor (HIF)-2{alpha} is critical for efficient erythropoiesis. J Biol Chem 286:19533–19540
13. Moreno-Manzano V, Rodriguez-Jimenez FJ, Acena-Bonilla JL, Fustero-Lardies S, Erceg S, Dopazo J, Montaner D, Stojkovic M, Sanchez-Puelles JM (2010) FM19G11, a new hypoxia-inducible factor (HIF) modulator, affects stem cell differentiation status. J Biol Chem 285:1333–1342
14. Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, 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
15. Heikkila M, Pasanen A, Kivirikko KI, Myllyharju J (2011) Roles of the human hypoxia-inducible factor (HIF)-3alpha variants in the hypoxia response. Cell Mol Life Sci
16. Pasanen A, Heikkila M, Rautavuoma K, Hirsila M, Kivirikko KI, Myllyharju J (2010) Hypoxia-inducible factor (HIF)-3alpha is subject to extensive alternative splicing in human tissues and cancer cells and is regulated by HIF-1 but not HIF-2. Int J Biochem Cell Biol 42:1189–1200
17. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A (2009) An operational definition of epigenetics. Genes Dev 23:781–783
18. Kato H, Tamamizu-Kato S, Shibasaki F (2004) Histone deacetylase 7 associates with hypoxia-inducible factor 1alpha and increases transcriptional activity. J Biol Chem 279:41966–41974
19. Koslowski M, Luxemburger U, Tureci O, Sahin U (2011) Tumor-associated CpG demethylation augments hypoxia-induced effects by positive autoregulation of HIF-1alpha. Oncogene 30:876–882
20. Fath DM, Kong X, Liang D, Lin Z, Chou A, Jiang Y, Fang J, Caro J, Sang N (2006) Histone deacetylase inhibitors repress the transactivation potential of hypoxia-inducible factors independently of direct acetylation of HIF-alpha. J Biol Chem 281:13612–13619
21. Place TL, Fitzgerald MP, Venkataraman S, Vorrink SU, Case AJ, Teoh ML, Domann FE (2011) Aberrant promoter CpG methylation is a mechanism for impaired PHD3 expression in a diverse set of malignant cells. PLoS ONE 6:e14617
22. Baranello L, Bertozzi D, Fogli MV, Pommier Y, Capranico G (2010) DNA topoisomerase I inhibition by camptothecin induces escape of RNA polymerase II from promoter-proximal pause site, antisense transcription and histone acetylation at the human HIF-1alpha gene locus. Nucleic Acids Res 38:159–171
23. Minet E, Ernest I, Michel G, Roland I, Remacle J, Raes M, Michiels C (1999) HIF1A gene transcription is dependent on a core promoter sequence encompassing activating and inhibiting sequences located upstream from the transcription initiation site and cis elements located within the 5′UTR. Biochem Biophys Res Commun 261:534–540
24. Sperandio S, Fortin J, Sasik R, Robitaille L, Corbeil J, de Belle I (2009) The transcription factor Egr1 regulates the HIF-1alpha gene during hypoxia. Mol Carcinog 48:38–44
25. Belaiba RS, Bonello S, Zahringer C, Schmidt S, Hess J, Kietzmann T, Gorlach A (2007) Hypoxia up-regulates hypoxia-inducible factor-1alpha transcription by involving phosphatidylinositol 3-kinase and nuclear factor kappaB in pulmonary artery smooth muscle cells. Mol Biol Cell 18:4691–4697
26. Dichtl W, Dulak J, Frick M, Alber HF, Schwarzacher SP, Ares MP, Nilsson J, Pachinger O, Weidinger F (2003) HMG-CoA reductase inhibitors regulate inflammatory transcription factors in human endothelial and vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 23:58–63
27. Capranico G, Marinello J, Baranello L (2010) Dissecting the transcriptional functions of human DNA topoisomerase I by selective inhibitors: implications for physiological and therapeutic modulation of enzyme activity. Biochim Biophys Acta 1806:240–250
28. Chang H, Shyu KG, Lee CC, Tsai SC, Wang BW, Hsien Lee Y, Lin S (2003) GL331 inhibits HIF-1alpha expression in a lung cancer model. Biochem Biophys Res Commun 302:95–100
29. Newcomb EW, Ali MA, Schnee T, Lan L, Lukyanov Y, Fowkes M, Miller DC, Zagzag D (2005) Flavopiridol downregulates hypoxia-mediated hypoxia-inducible factor-1alpha expression in human glioma cells by a proteasome-independent pathway: implications for in vivo therapy. Neuro Oncol 7:225–235
30. Mattick JS, Makunin IV (2006) Non-coding RNA. Hum Mol Genet 15(Spec No 1):R17–R29
31. Uchida T, Rossignol F, Matthay MA, Mounier R, Couette S, Clottes E, Clerici C (2004) Prolonged hypoxia differentially regulates hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression in lung epithelial cells: implication of natural antisense HIF-1alpha. J Biol Chem 279:14871–14878
32. Rodriguez-Jimenez FJ, Moreno-Manzano V, Lucas-Dominguez R, Sanchez-Puelles JM (2008) Hypoxia causes downregulation of mismatch repair system and genomic instability in stem cells. Stem Cells 26:2052–2062
33. Li L, Lin X, Staver M, Shoemaker A, Semizarov D, Fesik SW, Shen Y (2005) Evaluating hypoxia-inducible factor-1alpha as a cancer therapeutic target via inducible RNA interference in vivo. Cancer Res 65:7249–7258
34. Galban S, Gorospe M (2009) Factors interacting with HIF-1alpha mRNA: novel therapeutic targets. Curr Pharm Des 15:3853–3860
35. Galban S, Kuwano Y, Pullmann R Jr, Martindale JL, Kim HH, Lal A, Abdelmohsen K, Yang X, Dang Y, Liu JO, Lewis SM, Holcik M, Gorospe M (2008) RNA-binding proteins HuR and

PTB promote the translation of hypoxia-inducible factor 1alpha. Mol Cell Biol 28:93–107
36. Taguchi A, Yanagisawa K, Tanaka M, Cao K, Matsuyama Y, Goto H, Takahashi T (2008) Identification of hypoxia-inducible factor-1 alpha as a novel target for miR-17-92 microRNA cluster. Cancer Res 68:5540–5545
37. Rane S, He M, Sayed D, Vashistha H, Malhotra A, Sadoshima J, Vatner DE, Vatner SF, Abdellatif M (2009) Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. Circ Res 104:879–886
38. Yamakuchi M, Lotterman CD, Bao C, Hruban RH, Karim B, Mendell JT, Huso D, Lowenstein CJ (2010) P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. Proc Natl Acad Sci USA 107:6334–6339
39. Yamakuchi M, Yagi S, Ito T, Lowenstein CJ (2011) MicroRNA-22 regulates hypoxia signaling in colon cancer cells. PLoS ONE 6:e20291
40. Lang KJ, Kappel A, Goodall GJ (2002) Hypoxia-inducible factor-1alpha mRNA contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia. Mol Biol Cell 13:1792–1801
41. Kim TW, Yim S, Choi BJ, Jang Y, Lee JJ, Sohn BH, Yoo HS, Yeom YI, Park KC (2010) Tristetraprolin regulates the stability of HIF-1alpha mRNA during prolonged hypoxia. Biochem Biophys Res Commun 391:963–968
42. Sheflin LG, Zou AP, Spaulding SW (2004) Androgens regulate the binding of endogenous HuR to the AU-rich 3′UTRs of HIF-1alpha and EGF mRNA. Biochem Biophys Res Commun 322:644–651
43. Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E (2002) Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. J Biol Chem 277:27975–27981
44. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT (2002) Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol 22:7004–7014
45. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL (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
46. Wang W, Jia WD, Xu GL, Wang ZH, Li JS, Ma JL, Ge YS, Xie SX, Yu JH (2009) Antitumoral activity of rapamycin mediated through inhibition of HIF-1alpha and VEGF in hepatocellular carcinoma. Dig Dis Sci 54:2128–2136
47. Thomas GV, Tran C, Mellinghoff IK, Welsbie DS, Chan E, Fueger B, Czernin J, Sawyers CL (2006) Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Nat Med 12:122–127
48. Rodriguez-Jimenez FJ, Moreno-Manzano V, Mateos-Gregorio P, Royo I, Erceg S, Murguia JR, Sanchez-Puelles JM (2010) FM19G11: a new modulator of HIF that links mTOR activation with the DNA damage checkpoint pathways. Cell Cycle 9:2803–2813
49. Lee DH, Lee YJ (2008) Quercetin suppresses hypoxia-induced accumulation of hypoxia-inducible factor-1alpha (HIF-1alpha) through inhibiting protein synthesis. J Cell Biochem 105:546–553
50. Jeon H, Kim H, Choi D, Kim D, Park SY, Kim YJ, Kim YM, Jung Y (2007) Quercetin activates an angiogenic pathway, hypoxia inducible factor (HIF)-1-vascular endothelial growth factor, by inhibiting HIF-prolyl hydroxylase: a structural analysis of quercetin for inhibiting HIF-prolyl hydroxylase. Mol Pharmacol 71:1676–1684
51. Park SS, Bae I, Lee YJ (2008) Flavonoids-induced accumulation of hypoxia-inducible factor (HIF)-1alpha/2alpha is mediated through chelation of iron. J Cell Biochem 103:1989–1998
52. Wang B, Li H, Yan H, Xiao JG (2005) Genistein inhibited hypoxia-inducible factor-1alpha expression induced by hypoxia and cobalt chloride in human retinal pigment epithelium cells. Methods Find Exp Clin Pharmacol 27:179–184
53. Wang GL, Jiang BH, Semenza GL (1995) Effect of protein kinase and phosphatase inhibitors on expression of hypoxia-inducible factor 1. Biochem Biophys Res Commun 216:669–675
54. Jung HJ, Park JW, Lee JS, Lee SR, Jang BC, Suh SI, Suh MH, Baek WK (2009) Silibinin inhibits expression of HIF-1alpha through suppression of protein translation in prostate cancer cells. Biochem Biophys Res Commun 390:71–76
55. Jun HO, Kim Y, Kwon YW, Hong SS, Kim KW, Shin J, Kim TY (2007) Wondonin, a novel compound, inhibits hypoxia-induced angiogenesis through hypoxia-inducible factor 1 alpha. FEBS Lett 581:4977–4982
56. Rapisarda A, Zalek J, Hollingshead M, Braunschweig T, Uranchimeg B, Bonomi CA, Borgel SD, Carter JP, Hewitt SM, Shoemaker RH, Melillo G (2004) Schedule-dependent inhibition of hypoxia-inducible factor-1alpha protein accumulation, angiogenesis, and tumor growth by topotecan in U251-HRE glioblastoma xenografts. Cancer Res 64:6845–6848
57. Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH, Melillo G (2002) Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. Cancer Res 62:4316–4324
58. Creighton-Gutteridge M, Cardellina JH 2nd, Stephen AG, Rapisarda A, Uranchimeg B, Hite K, Denny WA, Shoemaker RH, Melillo G (2007) Cell type-specific, topoisomerase II-dependent inhibition of hypoxia-inducible factor-1alpha protein accumulation by NSC 644221. Clin Cancer Res 13:1010–1018
59. Pastorino F, Loi M, Sapra P, Becherini P, Cilli M, Emionite L, Ribatti D, Greenberger LM, Horak ID, Ponzoni M (2010) Tumor regression and curability of preclinical neuroblastoma models by PEGylated SN38 (EZN-2208), a novel topoisomerase I inhibitor. Clin Cancer Res 16:4809–4821
60. Sapra P, Zhao H, Mehlig M, Malaby J, Kraft P, Longley C, Greenberger LM, Horak ID (2008) Novel delivery of SN38 markedly inhibits tumor growth in xenografts, including a camptothecin-11-refractory model. Clin Cancer Res 14:1888–1896
61. Lou JJ, Chua YL, Chew EH, Gao J, Bushell M, Hagen T (2010) Inhibition of hypoxia-inducible factor-1alpha (HIF-1alpha) protein synthesis by DNA damage inducing agents. PLoS ONE 5:e10522
62. Escuin D, Kline ER, Giannakakou P (2005) Both microtubule-stabilizing and microtubule-destabilizing drugs inhibit hypoxia-inducible factor-1alpha accumulation and activity by disrupting microtubule function. Cancer Res 65:9021–9028
63. Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P (2003) 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell 3:363–375
64. Hagen T, D'Amico G, Quintero M, Palacios-Callender M, Hollis V, Lam F, Moncada S (2004) Inhibition of mitochondrial respiration by the anticancer agent 2-methoxyestradiol. Biochem Biophys Res Commun 322:923–929
65. Luwor RB, Lu Y, Li X, Mendelsohn J, Fan Z (2005) The antiepidermal growth factor receptor monoclonal antibody cetuximab/C225 reduces hypoxia-inducible factor-1 alpha, leading to transcriptional inhibition of vascular endothelial growth factor expression. Oncogene 24:4433–4441
66. Tan C, de Noronha RG, Roecker AJ, Pyrzynska B, Khwaja F, Zhang Z, Zhang H, Teng Q, Nicholson AC, Giannakakou P,

Zhou W, Olson JJ, Pereira MM, Nicolaou KC, Van Meir EG (2005) Identification of a novel small-molecule inhibitor of the hypoxia-inducible factor 1 pathway. Cancer Res 65:605–612
67. Li F, Sonveaux P, Rabbani ZN, Liu S, Yan B, Huang Q, Vujaskovic Z, Dewhirst MW, Li CY (2007) Regulation of HIF-1alpha stability through S-nitrosylation. Mol Cell 26:63–74
68. Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, Kim KW (2002) Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell 111:709–720
69. Xu D, Yao Y, Lu L, Costa M, Dai W (2010) Plk3 functions as an essential component of the hypoxia regulatory pathway by direct phosphorylation of HIF-1alpha. J Biol Chem 285:38944–38950
70. Liang D, Kong X, Sang N (2006) Effects of histone deacetylase inhibitors on HIF-1. Cell Cycle 5:2430–2435
71. Kong X, Lin Z, Liang D, Fath D, Sang N, Caro J (2006) Histone deacetylase inhibitors induce VHL and ubiquitin-independent proteasomal degradation of hypoxia-inducible factor 1alpha. Mol Cell Biol 26:2019–2028
72. Toffoli S, Feron O, Raes M, Michiels C (2007) Intermittent hypoxia changes HIF-1alpha phosphorylation pattern in endothelial cells: unravelling of a new PKA-dependent regulation of HIF-1alpha. Biochim Biophys Acta 1773:1558–1571
73. To KK, Sedelnikova OA, Samons M, Bonner WM, Huang LE (2006) The phosphorylation status of PAS-B distinguishes HIF-1alpha from HIF-2alpha in NBS1 repression. EMBO J 25:4784–4794
74. Chua YL, Hagen T (2011) Compound C prevents Hypoxia-Inducible Factor-1alpha protein stabilization by regulating the cellular oxygen availability via interaction with Mitochondrial Complex I. BMC Res Notes 4:117
75. Emerling BM, Viollet B, Tormos KV, Chandel NS (2007) Compound C inhibits hypoxic activation of HIF-1 independent of AMPK. FEBS Lett 581:5727–5731
76. van Hagen M, Overmeer RM, Abolvardi SS, Vertegaal AC (2010) RNF4 and VHL regulate the proteasomal degradation of SUMO-conjugated Hypoxia-Inducible Factor-2alpha. Nucleic Acids Res 38:1922–1931
77. Newcomb EW, Lukyanov Y, Schnee T, Ali MA, Lan L, Zagzag D (2006) Noscapine inhibits hypoxia-mediated HIF-1alpha expression and angiogenesis in vitro: a novel function for an old drug. Int J Oncol 28:1121–1130
78. Mabjeesh NJ, Post DE, Willard MT, Kaur B, Van Meir EG, Simons JW, Zhong H (2002) Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteasome pathway in prostate cancer cells. Cancer Res 62:2478–2482
79. Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. Nat Rev Cancer 5:761–772
80. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM (2002) Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. J Biol Chem 277:29936–29944
81. Hur E, Kim HH, Choi SM, Kim JH, Yim S, Kwon HJ, Choi Y, Kim DK, Lee MO, 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
82. Liu YV, Semenza GL (2007) RACK1 vs. HSP90: competition for HIF-1 alpha degradation vs. stabilization. Cell Cycle 6:656–659
83. Zhang D, Li J, Costa M, Gao J, Huang C (2010) JNK1 mediates degradation HIF-1alpha by a VHL-independent mechanism that involves the chaperones Hsp90/Hsp70. Cancer Res 70:813–823
84. Liu YV, Baek JH, Zhang H, Diez R, Cole RN, Semenza GL (2007) RACK1 competes with HSP90 for binding to HIF-1alpha and is required for O(2)-independent and HSP90 inhibitor-induced degradation of HIF-1alpha. Mol Cell 25:207–217
85. Fang J, Xia C, Cao Z, Zheng JZ, Reed E, Jiang BH (2005) Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. FASEB J 19:342–353
86. Welsh SJ, Williams RR, Birmingham A, Newman DJ, Kirkpatrick DL, Powis G (2003) The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1alpha and vascular endothelial growth factor formation. Mol Cancer Ther 2:235–243
87. Jones DT, Pugh CW, Wigfield S, Stevens MF, Harris AL (2006) Novel thioredoxin inhibitors paradoxically increase hypoxia-inducible factor-alpha expression but decrease functional transcriptional activity, DNA binding, and degradation. Clin Cancer Res 12:5384–5394
88. Kim YH, Coon A, Baker AF, Powis G (2010) Antitumor agent PX-12 inhibits HIF-1alpha protein levels through an Nrf2/PMF-1-mediated increase in spermidine/spermine acetyl transferase. Cancer Chemother Pharmacol
89. Welsh S, Williams R, Kirkpatrick L, Paine-Murrieta G, Powis G (2004) Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1alpha. Mol Cancer Ther 3:233–244
90. Koh MY, Spivak-Kroizman T, Venturini S, Welsh S, Williams RR, Kirkpatrick DL, Powis G (2008) Molecular mechanisms for the activity of PX-478, an antitumor inhibitor of the hypoxia-inducible factor-1alpha. Mol Cancer Ther 7:90–100
91. Mesa RA (2006) Tipifarnib: farnesyl transferase inhibition at a crossroads. Expert Rev Anticancer Ther 6:313–319
92. Liu M, Bryant MS, Chen J, Lee S, Yaremko B, Lipari P, Malkowski M, Ferrari E, Nielsen L, Prioli N, Dell J, Sinha D, Syed J, Korfmacher WA, Nomeir AA, Lin CC, Wang L, Taveras AG, Doll RJ, Njoroge FG, Mallams AK, Remiszewski S, Catino JJ, Girijavallabhan VM, Bishop WR et al (1998) Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice. Cancer Res 58:4947–4956
93. Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A (2001) Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. J Biol Chem 276:9519–9525
94. Blancher C, Moore JW, Robertson N, Harris AL (2001) Effects of ras and von Hippel–Lindau (VHL) gene mutations on hypoxia-inducible factor (HIF)-1alpha, HIF-2alpha, and vascular endothelial growth factor expression and their regulation by the phosphatidylinositol 3′-kinase/Akt signaling pathway. Cancer Res 61:7349–7355
95. Lau CK, Yang ZF, Lam CT, Tam KH, Poon RT, Fan ST (2006) Suppression of hypoxia inducible factor-1alpha (HIF-1alpha) by YC-1 is dependent on murine double minute 2 (Mdm2). Biochem Biophys Res Commun 348:1443–1448
96. Palayoor ST, Tofilon PJ, Coleman CN (2003) Ibuprofen-mediated reduction of hypoxia-inducible factors HIF-1alpha and HIF-2alpha in prostate cancer cells. Clin Cancer Res 9:3150–3157
97. Jones MK, Szabo IL, Kawanaka H, Husain SS, Tarnawski AS (2002) von Hippel Lindau tumor suppressor and HIF-1alpha: new targets of NSAIDs inhibition of hypoxia-induced angiogenesis. FASEB J 16:264–266
98. Lan KL, Lan KH, Sheu ML, Chen MY, Shih YS, Hsu FC, Wang HM, Liu RS, Yen SH (2011) Honokiol inhibits hypoxia-inducible factor-1 pathway. Int J Radiat Biol 87:579–590
99. Johnson AB, Denko N, Barton MC (2008) Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. Mutat Res 640:174–179
100. Huang ZQ, Li J, Sachs LM, Cole PA, Wong J (2003) A role for cofactor-cofactor and cofactor-histone interactions in targeting

p300, SWI/SNF and Mediator for transcription. EMBO J 22:2146–2155
101. Varga-Weisz PD, Becker PB (2006) Regulation of higher-order chromatin structures by nucleosome-remodelling factors. Curr Opin Genet Dev 16:151–156
102. Kenneth NS, Mudie S, van Uden P, Rocha S (2009) SWI/SNF regulates the cellular response to hypoxia. J Biol Chem 284:4123–4131
103. Safronova O, Morita I (2010) Transcriptome remodeling in hypoxic inflammation. J Dent Res 89:430–444
104. Watson JA, Watson CJ, McCann A, Baugh J (2010) Epigenetics, the epicenter of the hypoxic response. Epigenetics 5:293–296
105. Chen S, Sang N (2011) Histone deacetylase inhibitors: the epigenetic therapeutics that repress hypoxia-inducible factors. J Biomed Biotechnol 2011:197946
106. Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW, Kim KW (2001) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med 7:437–443
107. Monneret C (2007) Histone deacetylase inhibitors for epigenetic therapy of cancer. Anticancer Drugs 18:363–370
108. Tomasi TB, Magner WJ, Khan AN (2006) Epigenetic regulation of immune escape genes in cancer. Cancer Immunol Immunother 55:1159–1184
109. Acharya MR, Sparreboom A, Venitz J, Figg WD (2005) Rational development of histone deacetylase inhibitors as anticancer agents: a review. Mol Pharmacol 68:917–932
110. Mie Lee Y, Kim SH, Kim HS, Jin Son M, Nakajima H, Jeong Kwon H, Kim KW (2003) Inhibition of hypoxia-induced angiogenesis by FK228, a specific histone deacetylase inhibitor, via suppression of HIF-1alpha activity. Biochem Biophys Res Commun 300:241–246
111. Lee SH, Kim J, Kim WH, Lee YM (2009) Hypoxic silencing of tumor suppressor RUNX3 by histone modification in gastric cancer cells. Oncogene 28:184–194
112. Xia X, Kung AL (2009) Preferential binding of HIF-1 to transcriptionally active loci determines cell-type specific response to hypoxia. Genome Biol 10:R113
113. Wellmann S, Bettkober M, Zelmer A, Seeger K, Faigle M, Eltzschig HK, Buhrer C (2008) Hypoxia upregulates the histone demethylase JMJD1A via HIF-1. Biochem Biophys Res Commun 372:892–897
114. Beyer S, Kristensen MM, Jensen KS, Johansen JV, Staller P (2008) The histone demethylases JMJD1A and JMJD2B are transcriptional targets of hypoxia-inducible factor HIF. J Biol Chem 283:36542–36552
115. Krieg AJ, Rankin EB, Chan D, Razorenova O, Fernandez S, Giaccia AJ (2010) Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. Mol Cell Biol 30:344–353
116. Xia X, Lemieux ME, Li W, Carroll JS, Brown M, Liu XS, Kung AL (2009) Integrative analysis of HIF binding and transactivation reveals its role in maintaining histone methylation homeostasis. Proc Natl Acad Sci USA 106:4260–4265
117. Pollard PJ, Loenarz C, Mole DR, McDonough MA, Gleadle JM, Schofield CJ, Ratcliffe PJ (2008) Regulation of Jumonji-domain-containing histone demethylases by hypoxia-inducible factor (HIF)-1alpha. Biochem J 416:387–394
118. Yang J, Ledaki I, Turley H, Gatter KC, Montero JC, Li JL, Harris AL (2009) Role of hypoxia-inducible factors in epigenetic regulation via histone demethylases. Ann N Y Acad Sci 1177:185–197
119. Tausendschon M, Dehne N, Brune B (2011) Hypoxia causes epigenetic gene regulation in macrophages by attenuating Jumonji histone demethylase activity. Cytokine 53:256–262
120. Qing G, Skuli N, Mayes PA, Pawel B, Martinez D, Maris JM, Simon MC (2010) Combinatorial regulation of neuroblastoma tumor progression by N-Myc and hypoxia inducible factor HIF-1alpha. Cancer Res 70:10351–10361
121. Nowak SJ, Corces VG (2004) Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. Trends Genet 20:214–220
122. Bungard D, Fuerth BJ, Zeng PY, Faubert B, Maas NL, Viollet B, Carling D, Thompson CB, Jones RG, Berger SL (2010) Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation. Science 329:1201–1205
123. Laderoute KR, Amin K, Calaoagan JM, Knapp M, Le T, Orduna J, Foretz M, Viollet B (2006) 5′-AMP-activated protein kinase (AMPK) is induced by low-oxygen and glucose deprivation conditions found in solid-tumor microenvironments. Mol Cell Biol 26:5336–5347
124. Goldknopf IL, Busch H (1975) Remarkable similarities of peptide fingerprints of histone 2A and nonhistone chromosomal protein A24. Biochem Biophys Res Commun 65:951–960
125. Zhang Y (2003) Transcriptional regulation by histone ubiquitination and deubiquitination. Genes Dev 17:2733–2740
126. Jiang BH, Rue E, Wang GL, Roe R, Semenza GL (1996) Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem 271:17771–17778
127. Jiang BH, Zheng JZ, Leung SW, Roe R, Semenza GL (1997) Transactivation and inhibitory domains of hypoxia-inducible factor 1alpha. Modulation of transcriptional activity by oxygen tension. J Biol Chem 272:19253–19260
128. Berra E, Richard DE, Gothie E, Pouyssegur J (2001) HIF-1-dependent transcriptional activity is required for oxygen-mediated HIF-1alpha degradation. FEBS Lett 491:85–90
129. Lee K, Zhang H, Qian DZ, Rey S, Liu JO, Semenza GL (2009) Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. Proc Natl Acad Sci USA 106:17910–17915
130. Nickols NG, Dervan PB (2007) Suppression of androgen receptor-mediated gene expression by a sequence-specific DNA-binding polyamide. Proc Natl Acad Sci USA 104:10418–10423
131. Kong D, Park EJ, Stephen AG, Calvani M, Cardellina JH, Monks A, Fisher RJ, Shoemaker RH, Melillo G (2005) Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res 65:9047–9055
132. Nickols NG, Jacobs CS, Farkas ME, Dervan PB (2007) Modulating hypoxia-inducible transcription by disrupting the HIF-1-DNA interface. ACS Chem Biol 2:561–571
133. Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL (2009) Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. Proc Natl Acad Sci USA 106:2353–2358
134. Minet E, Mottet D, Michel G, Roland I, Raes M, Remacle J, Michiels C (1999) Hypoxia-induced activation of HIF-1: role of HIF-1alpha–Hsp90 interaction. FEBS Lett 460:251–256
135. Lee JM, Lee WH, Kay HY, Kim ES, Moon A, Kim SG (2011) Hemin, an iron-binding porphyrin, inhibits HIF-1alpha induction through its binding with heat shock protein 90. Int J Cancer
136. Jones DT, Harris AL (2006) Identification of novel small-molecule inhibitors of hypoxia-inducible factor-1 transactivation and DNA binding. Mol Cancer Ther 5:2193–2202
137. Hellwig-Burgel T, Rutkowski K, Metzen E, Fandrey J, Jelkmann W (1999) Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. Blood 94:1561–1567
138. Mahon PC, Hirota K, Semenza GL (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

139. Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK (2002) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 16:1466–1471
140. Carrero P, Okamoto K, Coumailleau P, O'Brien S, Tanaka H, Poellinger L (2000) Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha. Mol Cell Biol 20:402–415
141. Mendonca DB, Mendonca G, Aragao FJ, Cooper LF (2011) NF-kappaB suppresses HIF-1alpha response by competing for P300 binding. Biochem Biophys Res Commun 404:997–1003
142. Yin Z, Haynie J, Yang X, Han B, Kiatchoosakun S, Restivo J, Yuan S, Prabhakar NR, Herrup K, Conlon RA, Hoit BD, Watanabe M, Yang YC (2002) The essential role of Cited2, a negative regulator for HIF-1alpha, in heart development and neurulation. Proc Natl Acad Sci USA 99:10488–10493
143. Kung AL, Zabludoff SD, France DS, Freedman SJ, Tanner EA, Vieira A, Cornell-Kennon S, Lee J, Wang B, Wang J, Memmert K, Naegeli HU, Petersen F, Eck MJ, Bair KW, Wood AW, Livingston DM (2004) Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell 6:33–43
144. Shin DH, Chun YS, Lee DS, Huang LE, Park JW (2008) Bortezomib inhibits tumor adaptation to hypoxia by stimulating the FIH-mediated repression of hypoxia-inducible factor-1. Blood 111:3131–3136
145. Yeo EJ, Ryu JH, Cho YS, Chun YS, Huang LE, Kim MS, Park JW (2006) Amphotericin B blunts erythropoietin response to hypoxia by reinforcing FIH-mediated repression of HIF-1. Blood 107:916–923
146. Hasebe Y, Egawa K, Yamazaki Y, Kunimoto S, Hirai Y, Ida Y, Nose K (2003) Specific inhibition of hypoxia-inducible factor (HIF)-1 alpha activation and of vascular endothelial growth factor (VEGF) production by flavonoids. Biol Pharm Bull 26:1379–1383
147. Triantafyllou A, Liakos P, Tsakalof A, Chachami G, Paraskeva E, Molyvdas PA, Georgatsou E, Simos G, Bonanou S (2007) The flavonoid quercetin induces hypoxia-inducible factor-1alpha (HIF-1alpha) and inhibits cell proliferation by depleting intracellular iron. Free Radic Res 41:342–356
148. Lee M, Hwang JT, Lee HJ, Jung SN, Kang I, Chi SG, Kim SS, Ha J (2003) AMP-activated protein kinase activity is critical for hypoxia-inducible factor-1 transcriptional activity and its target gene expression under hypoxic conditions in DU145 cells. J Biol Chem 278:39653–39661
149. Kruger EA, Blagosklonny MV, Dixon SC, Figg WD (1998) UCN-01, a protein kinase C inhibitor, inhibits endothelial cell proliferation and angiogenic hypoxic response. Invasion Metastasis 18:209–218
150. Triantafyllou A, Mylonis I, Simos G, Bonanou S, Tsakalof A (2008) Flavonoids induce HIF-1alpha but impair its nuclear accumulation and activity. Free Radic Biol Med 44:657–670
151. Hur E, Chang KY, Lee E, Lee SK, Park H (2001) Mitogen-activated protein kinase kinase inhibitor PD98059 blocks the trans-activation but not the stabilization or DNA binding ability of hypoxia-inducible factor-1alpha. Mol Pharmacol 59:1216–1224
152. Mylonis I, Lakka A, Tsakalof A, Simos G (2010) The dietary flavonoid kaempferol effectively inhibits HIF-1 activity and hepatoma cancer cell viability under hypoxic conditions. Biochem Biophys Res Commun 398:74–78
153. Garcia-Maceira P, Mateo J (2009) Silibinin inhibits hypoxia-inducible factor-1alpha and mTOR/p70S6K/4E-BP1 signalling pathway in human cervical and hepatoma cancer cells: implications for anticancer therapy. Oncogene 28:313–324
154. Sapra P, Kraft P, Pastorino F, Ribatti D, Dumble M, Mehlig M, Wang M, Ponzoni M, Greenberger LM, Horak ID (2011) Potent and sustained inhibition of HIF-1alpha and downstream genes by a polyethyleneglycol-SN38 conjugate, EZN-2208, results in anti-angiogenic effects. Angiogenesis
155. Su W, Huang L, Ao Q, Zhang Q, Tian X, Fang Y, Lu Y (2011) Noscapine sensitizes chemoresistant ovarian cancer cells to cisplatin through inhibition of HIF-1alpha. Cancer Lett 305:94–99
156. Correia SC, Moreira PI (2010) Hypoxia-inducible factor 1: a new hope to counteract neurodegeneration? J Neurochem 112:1–12
157. Ratan RR, Siddiq A, Aminova L, Langley B, McConoughey S, Karpisheva K, Lee HH, Carmichael T, Kornblum H, Coppola G, Geschwind DH, Hoke A, Smirnova N, Rink C, Roy S, Sen C, Beattie MS, Hart RP, Grumet M, Sun D, Freeman RS, Semenza GL, Gazaryan I (2008) Small molecule activation of adaptive gene expression: tilorone or its analogs are novel potent activators of hypoxia inducible factor-1 that provide prophylaxis against stroke and spinal cord injury. Ann N Y Acad Sci 1147:383–394
158. Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, Marklund SL, Wyns S, Thijs V, Andersson J, van Marion I, Al-Chalabi A, Bornes S, Musson R, Hansen V, Beckman L, Adolfsson R, Pall HS, Prats H, Vermeire S, Rutgeerts P, Katayama S, Awata T, Leigh N, Lang-Lazdunski L, Dewerchin M, Shaw C, Moons L, Vlietinck R, Morrison KE, Robberecht W, Van Broeckhoven C, Collen D, Andersen PM, Carmeliet P (2003) VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. Nat Genet 34:383–394
159. Theus MH, Wei L, Cui L, Francis K, Hu X, Keogh C, Yu SP (2008) In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. Exp Neurol 210:656–670
160. Akita T, Murohara T, Ikeda H, Sasaki K, Shimada T, Egami K, Imaizumi T (2003) Hypoxic preconditioning augments efficacy of human endothelial progenitor cells for therapeutic neovascularization. Lab Invest 83:65–73
161. Semenza GL, Agani F, Iyer N, Kotch L, Laughner E, Leung S, Yu A (1999) Regulation of cardiovascular development and physiology by hypoxia-inducible factor 1. Ann N Y Acad Sci 874:262–268
162. Semenza GL (2003) Angiogenesis in ischemic and neoplastic disorders. Annu Rev Med 54:17–28
163. Semenza GL (2007) Vasculogenesis, angiogenesis, and arteriogenesis: mechanisms of blood vessel formation and remodeling. J Cell Biochem 102:840–847
164. Tekin D, Dursun AD, Xi L (2010) Hypoxia inducible factor 1 (HIF-1) and cardioprotection. Acta Pharmacol Sin 31:1085–1094
165. Jin Y, Wu J, Song X, Song Q, Cully BL, Messmer-Blust A, Xu M, Foo SY, Rosenzweig A, Li J (2011) RTEF-1, an upstream gene of HIF-1{alpha}, accelerates recovery from Ischemia. J Biol Chem 286:22699–22705
166. Liu YV, Hubbi ME, Pan F, McDonald KR, Mansharamani M, Cole RN, Liu JO, Semenza GL (2007) Calcineurin promotes hypoxia-inducible factor 1alpha expression by dephosphorylating RACK1 and blocking RACK1 dimerization. J Biol Chem 282:37064–37073
167. Zhang L, Liu Q, Lu L, Zhao X, Gao X, Wang Y (2011) Astragaloside IV stimulates angiogenesis and increases HIF-1{alpha} accumulation via PI3K/Akt pathway. J Pharmacol Exp Ther 338:485–491

168. Mabjeesh NJ, Willard MT, Harris WB, Sun HY, Wang R, Zhong H, Umbreit JN, Simons JW (2003) Dibenzoylmethane, a natural dietary compound, induces HIF-1 alpha and increases expression of VEGF. Biochem Biophys Res Commun 303:279–286
169. Linden T, Katschinski DM, Eckhardt K, Scheid A, Pagel H, Wenger RH (2003) The antimycotic ciclopirox olamine induces HIF-1alpha stability, VEGF expression, and angiogenesis. FASEB J 17:761–763
170. Wang GL, Semenza GL (1993) Desferrioxamine induces erythropoietin gene expression and hypoxia-inducible factor 1 DNA-binding activity: implications for models of hypoxia signal transduction. Blood 82:3610–3615
171. Prass K, Ruscher K, Karsch M, Isaev N, Megow D, Priller J, Scharff A, Dirnagl U, Meisel A (2002) Desferrioxamine induces delayed tolerance against cerebral ischemia in vivo and in vitro. J Cereb Blood Flow Metab 22:520–525
172. Hishikawa T, Ono S, Ogawa T, Tokunaga K, Sugiu K, Date I (2008) Effects of deferoxamine-activated hypoxia-inducible factor-1 on the brainstem after subarachnoid hemorrhage in rats. Neurosurgery 62:232–240 (discussion 240-231)
173. Yang T, Brittenham GM, Dong WQ, Levy MN, Obejero-Paz CA, Kuryshev YA, Brown AM (2003) Deferoxamine prevents cardiac hypertrophy and failure in the gerbil model of iron-induced cardiomyopathy. J Lab Clin Med 142:332–340
174. Asikainen TM, Ahmad A, Schneider BK, Ho WB, Arend M, Brenner M, Gunzler V, White CW (2005) Stimulation of HIF-1alpha, HIF-2alpha, and VEGF by prolyl 4-hydroxylase inhibition in human lung endothelial and epithelial cells. Free Radic Biol Med 38:1002–1013
175. Ockaili R, Natarajan R, Salloum F, Fisher BJ, Jones D, Fowler AA 3rd, Kukreja RC (2005) HIF-1 activation attenuates post-ischemic myocardial injury: role for heme oxygenase-1 in modulating microvascular chemokine generation. Am J Physiol Heart Circ Physiol 289:H542–H548
176. Zhang RL, Guo Z, Wang LL, Wu J (2011) Degeneration of capsaicin sensitive sensory nerves enhances myocardial injury in acute myocardial infarction in rats. Int J Cardiol
177. Patel PS, Yang S, Li A, Varney ML, Singh RK (2002) Capsaicin regulates vascular endothelial cell growth factor expression by modulation of hypoxia inducing factor-1alpha in human malignant melanoma cells. J Cancer Res Clin Oncol 128:461–468
178. Nangaku M, Inagi R, Miyata T, Fujita T (2008) Hypoxia and hypoxia-inducible factor in renal disease. Nephron Exp Nephrol 110:e1–e7
179. Leonard MO, Cottell DC, Godson C, Brady HR, Taylor CT (2003) The role of HIF-1 alpha in transcriptional regulation of the proximal tubular epithelial cell response to hypoxia. J Biol Chem 278:40296–40304
180. Fernandez-Martinez AB, Jimenez MI, Hernandez IS, Garcia-Bermejo ML, Manzano VM, Fraile EA, de Lucio-Cazana FJ (2011) Mutual regulation of hypoxic and retinoic acid related signalling in tubular proximal cells. Int J Biochem Cell Biol
181. Jung JE, Kim HS, Lee CS, Shin YJ, Kim YN, Kang GH, Kim TY, Juhnn YS, Kim SJ, Park JW, Ye SK, Chung MH (2008) STAT3 inhibits the degradation of HIF-1alpha by pVHL-mediated ubiquitination. Exp Mol Med 40:479–485
182. McFarlane S, Nicholl MJ, Sutherland JS, Preston CM (2011) Interaction of the human cytomegalovirus particle with the host cell induces hypoxia-inducible factor 1 alpha. Virology 414:83–90
183. Kramer MJ, Cleeland R, Grunberg E (1975) Mersalyl: a diuretic with antiviral properties. Antimicrob Agents Chemother 8:295–299
184. Agani F, Semenza GL (1998) Mersalyl is a novel inducer of vascular endothelial growth factor gene expression and hypoxia-inducible factor 1 activity. Mol Pharmacol 54:749–754
185. Meissner U, Allabauer I, Repp R, Rascher W, Dotsch J (2003) Inducible expression of hypoxia-inducible factor 1alpha (HIF-1alpha) as a tool for studying HIF-1alpha-dependent gene regulation during normoxia in vitro. Pharmacology 69:74–78
186. Frede S, Stockmann C, Freitag P, Fandrey J (2006) Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-kappaB. Biochem J 396:517–527