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Minireview

Molecules and Cells

HIF-1 and HIF-2 Transcription Factors - Similar but Not Identical

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Hypoxia inducible factor (HIF)-1 and HIF-2 are heterodimeric transcription factors mediating the cellular response to hypoxia. Recent data indicate that not only ubiquitous HIF-1α, but also more cell-specific HIF-2α, is an important regulator of the hypoxia response. Although both α subunits are highly conservative at protein level, share similar domain structure, heterodimerize with HIF-1β, and bind to the same DNA sequence called hypoxia responsive element (HRE), their effect on the expression of some genes may vary. In this review we stressed the differences between the isoforms, their structure and expression pattern. Moreover, we described diversity of coactivators and proteins which interact with HIFs, and which are responsible for the specificity of their action. Finally, recent data showing link between HIFs and specific microRNA have been presented.

INTRODUCTION

Hypoxia is defined as a reduction of oxygen amount available to a cell, tissue or organism. The decline of O_2 level can cause alteration in gene transcription or may result in posttranslational modifications of proteins, leading to changes of cell metabolism. Hypoxia inducible factors (HIFs), are the master regulators of oxygen homeostasis and play a role in development, postnatal physiology as well as disease pathogenesis. From multiple HIF isoforms, HIF-1α and HIF-2α are the best understood and described. HIF-1α is recognized to control more than 100 genes and it is suggested that in endothelial cells more than 2% of all human genes may be both directly or indirectly regulated by this factor (Manalo et al., 2005). Recently, more data found selective HIF-2α responsive genes (Covello et al., 2006; Raval et al., 2005), and indicate the importance of this isoform in hypoxic gene regulation. Here, we summarize the function of HIF-1α and HIF-2α and stress the differences between the isoforms, which influence some biological action, both during development as well as during pathologic conditions, such as tumors and vascular disease.

Hypoxia inducible factor - isoforms with similar stuctures

Hypoxia inducible factor (HIF) is a dimer composed of 120 kDa oxygen-regulated α-subunit and the constitutively expressed 91-94 kDa β-subunit (also known as the aryl hydrocarbon receptor nuclear translocator, ARNT). There are three isoforms of the HIF-α subunit (HIF-1α, HIF-2α, and HIF-3α), and three paralogues of HIF-1β (Arnt1, Arnt2 and Arnt3) (reviewed in: Zagorska and Dulak, 2004). HIF-1α and HIF-2α are particularly critical for the hypoxia response, and are able to form complex with HIF-1β. The role of HIF-3α is less well understood, but it has been suggested that the alternative splice form of HIF-3α binds to and inhibits transcriptional activity of HIF-1α (Makino et al., 2001). HIF-2α has a similar structure to HIF-1α, but the pattern of their expression varies, as HIF-2α in contrast to widely present HIF-1α, is only expressed in certain tissues (Wiesener et al., 2003). Both α and β subunits are members of the family of the basic helix-loop-helix (bHLH) and PER-ARNT-SIM (PAS) domain-containing transcription factors (Wang et al., 1995). Apart from those domains, which are important for DNA binding and dimerization, a central oxygen-dependent degradation domain (ODD) as well as two transactivation domains: the N-terminal activation domain (NAD) and C-terminal activation domain (CAD) (located in the TAD domain) are present (Fig. 1). The N-terminal transactivation domains of HIF-1α and HIF-2α are essential for targeting gene specificity (Dayan et al., 2006; Hu et al., 2007), while the CAD contributes to the regulation of most, but not all, HIF target genes.

Oxygen-dependent HIF-α degradation

HIF-1α is constitutively transcribed and translated in cells, but under normoxia it has an extremely short half-life of less than 5 minutes (Huang et al., 1996). HIF-1α protein levels is tightly regulated by several mechanisms, but the most notable of these is the degradation pathway (Fig. 2). Under aerobic conditions, HIF-1α undergoes proteasomal degradation via the ubiquitin-dependent pathway. This involves posttranslational hydroxylation of specific proline residues (Pro402, Pro564) within ODD domain by prolyl hydroxylases (PHDs), non-heme, oxygen-, Fe(II)- and 2-oxoglutarate-dependent dioxygenases. Three PHDs proteins have been identified so far: PHD1, 2, and 3 (Epstein et al., 2001). Although *in vitro* all three hydroxylate HIF-1α (Bruick and McKnight, 2001), *in vivo* PHD2 isoform plays a major role in normoxic HIF-1α regulation (Appelhoff et al., 2004; Berra et al., 2003). Hydroxylated HIF-1α is bound by

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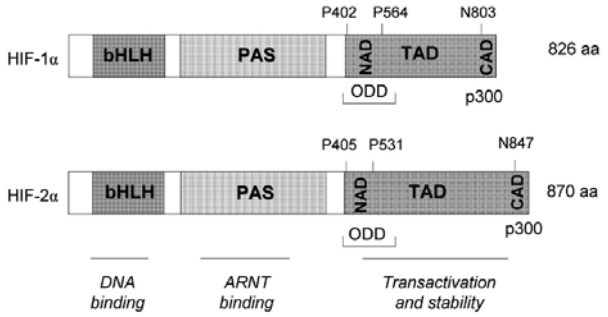

Fig. 1. Structure of hypoxia inducible factor-1α and 2α. The main domains are: basic helix-loop-helix domain (bHLH), Per-AHR-ARNT-Sim homology domains (PAS), oxygen-dependent degradation domain (ODD), and transactivation domains (TAD, with C-terminal CAD and N-terminal NAD). The homology in the DNA and ARNT (HIF-β) binding domains is high, in contrast to the carboxy-terminal transcription domains which exhibit only 42% of homology (the highest homology is observed in the ODD and p300/CBP binding motif).

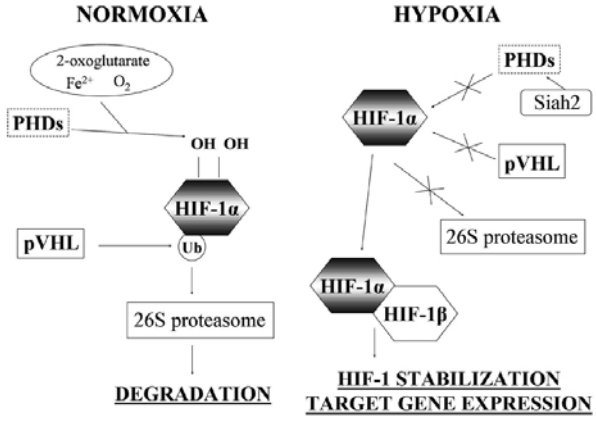

Fig. 2. Schematic representation of hypoxic control of HIF-1α regulation and downstream HIF-1α activity. Under normoxic conditions, HIF-1α undergoes proteasomal degradation by a mechanism that involves hydroxylation of proline residues on HIF-1α by prolyl hydroxylases (PHDs) and subsequent ubiquitination by the pVHL protein E3 ubiquitin ligase system. Under hypoxic conditions, Siah2 ligase degrades PHDs, HIF-1α is stabilized and heterodimerizes with HIF-1β to regulate transcription of downstream genes.

the von Hippel-Lindau protein (pVHL), which recruits the elongin-C/ elongin-B/cullin-2 E3-ubiquitin-ligase complex, thus targeting HIF-1α for degradation by the 26S proteasome (Cockman et al., 2000; Maxwell et al., 1999). During hypoxia, or in case of the lack of cofactors - Fe(II) or 2-oxoglutarate, PHDs are inactive, what prevents binding of pVHL. Therefore, HIF-1α/HIF-2α escape ubiquitination and proteasomal degradation, and can be transported to the nucleus where, after dimerization with HIF-1β (Chilov et al., 1999) and recruitment of numerous coactivators they both bind to the same hypoxia responsive element (HRE) at the target gene loci. HRE has a core five-nucleotide sequence RCGTG (R: A/G), which is well conserved among numerous hypoxia responsive genes (Semenza et al., 1996).

The inactivation of PHDs in the hypoxia, and concomitant HIF-1α stabilization, is also caused by PHDs degradation, which is mediated by the E3 ubiquitin ligases Siah1a and Siah2 (Nakayama et al., 2004). Interestingly, although PHD2 was found to associate with Siah2, its stability was not affected by the ligase. Nakayama et al. reported that Siah2 regulates PHD1 and 3 under oxygen concentration of 2% to 5%, thereby allowing accumulation of HIF-1α (Nakayama et al., 2004). The mechanism of the PHDs degradation by Siah2 is dependent on p38 and Akt kinases activity. It was shown, that Siah2 is subjected to phosphorylation by p38, what increases its activity under hypoxia conditions. The phosphorylation of Siah2 was inhibited when cells were treated with p38 inhibitor SB203580 (Khurana et al., 2006). Moreover, also the role of Akt pathway was suggested, as Siah2 mRNA induction by hypoxia is blocked by treatment with the PI3K inhibitor LY294002. Concomitantly, Siah2 is upregulated when an active form of Akt is introduced into cells (Nakayama et al., 2007).

The regulation of HIF-2α protein stability is similar to that of HIF-1α isoform and relies on O_2-dependent degradation through the PHDs/pVHL/elongin pathway in well-oxygenated cells. The difference was found in the specific proline residues of HIF-2α ODD, as Pro405 and Pro531 are affected by PHDs (Fig. 1). HIF-2α is stabilized during hypoxia and its protein levels increases with prolonged hypoxia (Holmquist-Mengelbier et al., 2006).

Other mechanisms of HIF-1α regulation

Oxygen-dependent regulation of HIF-1α degradation involves pVHL, however there has been increasing evidence of pVHL-independent pathways of HIF-1α degradation. Several different O_2/PHDs/pVHL-independent mechanisms have been identified so far (Koh et al., 2008; Liu et al., 2007a; 2007b; Luo et al., 2009).

Hypoxia-associated factor (HAF), which has been detected in both normal and tumor-derived cell lines and in proliferating tissues but is undetectable in normal, non-proliferating tissue, has been shown to regulate HIF-1α protein stability (Koh et al., 2008). Koh et al. demonstrated that HAF promotes HIF-1α degradation independently of cellular oxygen tension. HAF overexpression diminishes HIF-1α, whereas HAF knockdown increased HIF-1α levels independently of pVHL or oxygen. Interestingly, the HAF action is selective for HIF-1α, as neither HAF knockdown nor overexpression caused any change in the levels of HIF-2α. This suggests that HAF is a novel HIF-1α isoform specific E3 ligase (Koh et al., 2008).

Extracellular heat shock protein 90 (Hsp90) is a molecular chaperone among whose client proteins is also HIF-1α. Accordingly, HIF-1α stability can be regulated by the receptor of activated protein kinase C (RACK1), which competes with Hsp90 for binding to HIF-1α and promotes the ubiquitination and degradation of HIF-1α in cells exposed to the Hsp90 inhibitor 17-allylaminogeldanamycin (17-AAG) (Liu et al., 2007a) or the calcineurin inhibitor cyclosporine A (Liu et al., 2007b). Recently, also Hsp70 and carboxyl terminus of Hsc70-interacting protein (CHIP) were identified as HIF-1α-interacting proteins that selectively regulate ubiquitination and degradation of HIF-1α, but not HIF-2α (Luo et al., 2009).

HIF-1α may also undergo other posttranslational modifications. The oxygen-dependent degradation of HIF-1α by pVHL can be mediated by hypoxia induced HIF-1α small ubiquitin-like modifier (SUMO)ylation. Interestingly, SUMOylation can either positively (Bae et al., 2004; Carbia-Nagashima et al., 2007) or negatively (Berta et al., 2007; Cheng et al., 2007) regulate HIF-1α stability. It was also shown that SUMOylation promotes the binding of pVHL to HIF-1α even when the latter is not hydroxylated on Pro402 or Pro564 (Cheng et al., 2007). Recent data shows that also HIF-2α is a target protein of SUMO modifier

(van Hagen et al., 2009).

Phosphorylation is another mechanism of HIF-α stabilization. HIF-1α undergoes phosphorylation by ERK-MAPK, resulting in increased transcriptional activity (Richard et al., 1999). It has been shown that p42/44 and p38 kinase phosphorylated HIF-1α/HIF-2α *in vitro* (Richard et al., 1999; Sodhi et al., 2000), whereas inhibitors of these pathways blocked HIF-1α-mediated reporter gene expression (Hur et al., 2001).

Transcriptional coactivators of HIF-1 and HIF-2

HIF-mediated transcription is dependent on the activity of several coactivators, which are recruited to form, together with HIF-α and β, an active HIF complex. This process is mediated by transactivation domains, the centrally located NAD, and the CAD, situated at the C-terminus end of the HIF-α proteins (Fig. 1).

The data obtained from many studies indicate that the p300/Creb-binding protein (CBP) is a central integrating coactivator (Arany et al., 1996) which, after binding with the HIF-1α or HIF-2α CAD helps, to recruit the accessory coactivators, like steroid receptor coactivator (SRC-1), transcription intermediary factor-2 (TIF-2), or the redox factor Ref-1. The coactivators play two main roles: they stabilize the transcription initiation complex containing RNA polymerase II and also posses histone acetyltransferase activity that is required for the polymerase to access DNA within chromatin and transcribe it into RNA.

SRC-1 and TIF-2 are the members of the SRC-1/p160 family of transcriptional coactivators harboring histone acetyltransferase activity, and are able to interact with HIF-1α and enhance its transactivation potential in a hypoxia-dependent manner (Carrero et al., 2000). Ruas et al. (2005) have found that SRC-1 does not interact directly with HIF-1α but is recruited to the complex by CBP in a hypoxia-dependent manner. By the use of different biochemical assays they showed that depletion of CBP from cell extracts abrogated interaction between SRC-1 and HIF-1α (Ruas et al., 2005).

Ref-1, a nuclear protein possessing both redox and apurinic endonuclease DNA repair activities (Xanthoudakis and Curran, 1992), has also been shown to be involved in the formation of active HIF complex. Overexpression of Ref-1 enhances the transcriptional activity of HIF-1α (Huang et al., 1996) and the mechanism of this action relies on the redox-dependent interactions between HIF-1 and transcriptional coactivators, including p300 and CBP (Carrero et al., 2000). Ref-1 takes an active part in the formation of hypoxia-inducible transcriptional complex regulating the main target gene of HIF-1, the vascular endothelial growth factor (VEGF) expression in rat pulmonary artery endothelial cells (Ziel et al., 2004).

Interestingly, the specific coactivators which bound only HIF-2α have been found. For example NF-κB essential modulator (NEMO) is unique for HIF-2α, and enhances HIF-2α-mediated transcription activity at normoxia (Bracken et al., 2005). Similarly, the transcription factor Ets1 has been demonstrated to interact exclusively with HIF-2α for the transcription of VEGF receptor 2 (Elvert et al., 2003). Another Ets family transcription factor, Elk-1, cooperates with HIF-2α to activate the target genes CITED-2, erythropoietin (EPO), insulin-like growth factor-binding protein-3 (IGFBP3), and plasminogen activator inhibitor-1 (PAI-1) (Aprelikova et al., 2006; Hu et al., 2007). Another group of accessory molecules contains the factors which are required for transcription of specific HIF target genes. For example, hepatocyte nuclear factor 4 (HNF-4), acting together with HIF-1, is a liver- and kidney-specific transcription factor reported to be obligatory for EPO transcription (Zhang et al., 1999). The deletion mutant of HNF-4, lacking the carboxyl terminal transactivation domain, which is essential for protein-protein interactions with HIF-1α, has been shown to prevent hypoxic induction of EPO (Tsuchiya et al., 2002).

The regulation of the transcriptional coactivators binding is controlled by asparaginyl hydroxylase, also termed factor inhibiting HIF-1 (FIH-1), which was identified as a 2-oxoglutarate- and Fe(II)-dependent oxygenase, similarly as PHDs which modify proline residues in ODD domain (Lando et al., 2002). FIH-1 modifies the asparagine 803 of human HIF-1α, located in the CAD (Fig. 1), what leads to blockage of p300/CBP coactivators recruitment to HIF-1α. Similarly, FIH-1 is capable of modifying the key asparagine residue (N851) within the HIF-2α CAD, thereby suppressing its activity (Lando et al., 2002).

HIF-1α and HIF-2α - similar isoforms with distinct functions

The existence of different isoforms of HIFα subunits raises the question about the specificity of their action as well as the spectrum of target genes regulated by both subunits. The data obtained from studies of knockout animals showed that both HIF proteins are non-redundant and play important role in the development. The knockout of both subunits is lethal, however, embryos die of different causes: HIF-1α$^{-/-}$ embryos die by E11 due to cardiac and vascular defects, whereas HIF-2α$^{-/-}$ embryos do not survive beyond E16.5 because of bradycardia, vascular defects, and incomplete lung maturation (reviewed in: Huang and Bunn, 2003).

Although HIF-1α and HIF-2α are closely related and activate HRE-dependent gene expression (Wenger, 2002), both subunits differ in their transactivation domains, implying they may have unique target genes and require distinct transcriptional cofactors. Interestingly, the NAD has recently been shown to contribute to HIF-1 and HIF-2 target gene specificity (Hu et al., 2007). Using a number of deletion mutants of HIF-1α and HIF-2α, as well as HIF-1α/HIF-2α hybrid proteins, Hu et al. proved that the NAD confers target gene specificities of HIF-1α and HIF-2α, whereas the CAD promotes the expression of HIF-1α/HIF-2α common target genes. These data clearly implicate the importance of the NAD and not the CAD in regulating HIF target gene specificity.

Moreover, despite the fact that all α subunits are highly conservative at protein level, share similar domain structure and heterodimerize with HIF-1β, they can have distinct, tissue-specific expression pattern (Holmquist-Mengelbier et al., 2006; Rosenberger et al., 2002). HIF-1α is expressed ubiquitously, whereas HIF-2α expression is limited to endothelium, kidney, lung, heart, and small intestine (Gordan et al., 2007). Additionally, cell-type specific pattern of expression have been noticed. For example, in kidney, in tubular cells only HIF-1α have been found, whereas HIF-2α was expressed in endothelial cells and fibroblasts (Rosenberger et al., 2002). In neuroblastomas, HIF-2α but not HIF-1α is strongly expressed in well-vascularized areas and expression of HIF-2α promotes growth and metastasis in later stages and is associated with more aggressive disease (Holmquist-Mengelbier et al., 2006).

Results by Sowter et al. indicate that in the breast carcinoma and endothelial cell lines, the major HIF isoform required for induction of a set of well-characterized hypoxic genes is HIF-1α, however, for hypoxia-induced cell migration both subunits are necessary (Sowter et al., 2003). Similarly, Blancher et al. have found that HIF-1α is responsible for regulation of a broad spectrum of hypoxia-inducible genes, but HIF-2α inhibited growth of breast cells (Blancher et al., 2000). This correlates with the pattern of expression of both subunits: in six human breast cancer cell lines HIF-1α was expressed at various levels, whereas HIF-2α was low or absent from the more aggressive cell lines (Blancher et al., 2000). On the other hand, HIF-2α

overexpression is especially important in the development of renal carcinoma in patients with the von Hippel-Lindau syndrome; and in this setting, HIF-2α may act as a renal cancer oncogene (Kondo et al., 2003).

HIF-2α is highly expressed in embryonic vascular endothelial cells and activates the expression of target genes whose products modulate vascular function and angiogenesis. Recently, Skuli et al. have introduced a model of mice with HIF-2α-deficient endothelial cells and found that although developed normally, such animals are characterized by increased vessel permeability, aberrant endothelial cell ultrastructure, and pulmonary hypertension. Moreover, these animals exhibited defective tumor angiogenesis associated with increased hypoxic stress and tumor cell apoptosis (Skuli et al., 2009).

Although HIF-1α and HIF-2α share several common targets such as VEGF, both isoforms may regulate distinct transcriptional targets. HIF-1α, but not HIF-2α is responsible for the regulation of transcription of genes encoding enzymes involved in the glycolytic pathway (Hu et al., 2003). In liver, erythropoietin production is preferentially regulated by HIF-2α (Rankin et al., 2007), whereas the glycolytic enzyme - phosphoglycerate kinase (Pgk) and the proapoptotic gene BCL2/adenovirus E1B–interacting protein 1, (NIP3/Bnip3) are characterized as preferential HIF-1α targets (Hu et al., 2003; Raval et al., 2005). Oct4, a crucial transcription factor regulating stem cell self-renewal is a specific, direct target of HIF-2α but not of HIF-1α (Covello et al., 2006). In addition, predominantly HIF-2α mediates transcription of cyclin D1 and transforming growth factor α (TGFα) (Covello et al., 2006; Raval et al., 2005). Difference between both subunits have been demonstrated also in maintenance of iron homeostasis (Mastrogiannaki et al., 2009). By using conditional knockout mice lacking HIF-1α or HIF-2α in the intestinal epithelium Mastrogiannaki et al. have shown that only HIF-2α regulates the transcription of the gene encoding divalent metal transporter 1 (DMT1), the principal intestinal iron transporter and via this pathway maintains iron balance (Mastrogiannaki et al., 2009).

Recently, new molecular target of HIF-1α has been discovered (Loboda et al., 2009b). It is well known, that in human cells low oxygen tension attenuates the expression of the stress inducible protein, heme oxygenase-1 (HO-1), which degrades heme to carbon monoxide, iron and biliverdin and posses antioxidant, anti-inflammatory and anti-apoptotic functions (Otterbein et al., 2003). HO-1 has also pro-angiogenic activity, similarly to IL-8, chemokine acting as an angiogenesis mediator (Yoshida et al., 1998). Interestingly, both IL-8 (Zhang et al., 2005) and HO-1 (Gong et al., 2002) expression is regulated by NF-E2-related factor 2 (Nrf2), a member of the basic leucine zipper (bZIP) transcription factors family. We showed that hypoxia, prolyl hydroxylase inhibitor dimethyloxaloylglycine (DMOG) or overexpression of stable form of HIF-1α down-regulates the expression of HO-1 and IL-8 in human microvascular endothelial cells. Importantly, we observed both the diminishment of Nrf2 expression and activity after HIF-1 induction. Interestingly, although both HO-1 and IL-8 are upregulated by Nrf2, and both are decreased by HIF-1 activation via its influence on Nrf2, the regulations of HO-1 and IL-8 were mutually independent. Importantly, as Nrf2 controls set of antioxidant and detoxifying genes, its down-regulation by HIF-1α may cause the alteration of the expression of many genes. Further studies should also elucidate the role and extent of influence of HIF-2 on Nrf2 regulation. Interestingly, recent paper by Fang et al. (2009), suggested the importance of additional transcription factors for HIF-1 and HIF-2 dependent regulation of gene expression. They found that IL-8 is up-regulated in primary human macrophages after hypoxia and interestingly, after both siRNA against HIF-1α and HIF-2α the reduction of hypoxia-induced IL-8 mRNA has been observed, however, only the effect of HIF-2α siRNA reached statistical significance. What is more interesting, the authors checked if the NF-κB plays a role in hypoxic signal transduction, however, they conclude that other than NF-κB transcription factors have to be involved in this regulation.

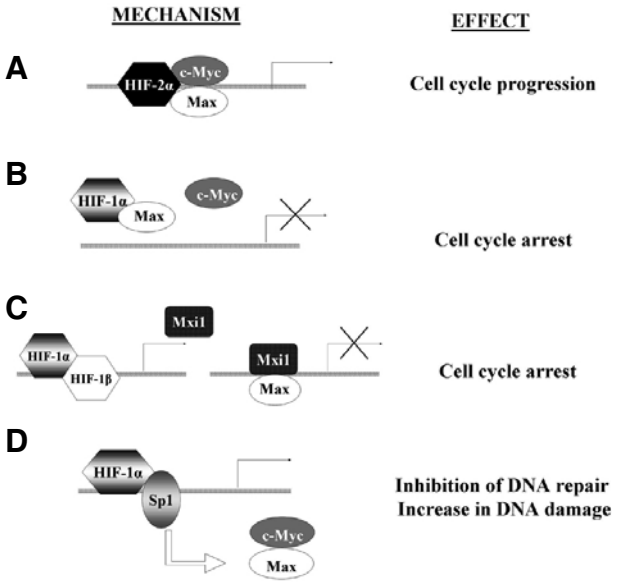

Fig. 3. HIF-1α and HIF-2α effect on c-Myc activity. HIF-2α enhances c-Myc activity (A). c-Myc:Max heterodimer-mediated transcriptional activation is atenuated by HIF-1α through the binding of Max and rendering c-Myc inactive (B). Furthermore, HIF-1 can activate the expression of Mxi1, which binds Max and represses a subset of c-Myc target genes (C). Additionally, displacement of c-Myc with Sp-1 transcription factor HIF-1α results in the inhibition of DNA repair genes expression (D).

HIF-1α and HIF-2α and protein interaction

Both HIF-1α and HIF-2α bind to the same DNA sequence, and the broad panel of genes is similarly regulated by both subunits. However, opposite effects of HIF-1 and HIF-2 observed in the case of some genes may result from different protein partners which interact with these transcription factors.

Using a yeast two-hybrid system, several proteins which interact with HIF-1α have been identified. Stabilization of HIF-1α is enhanced by Jab1 (Jun activation domain-binding protein-1) (Bae et al., 2002), whereas F29 peptide facilitates the interaction of the HIF-1α/β heterodimer with its target DNA sequence (Choi and Park, 2009). Moreover, negative regulator of HIF-1α, MgcRacGAP (male germ cell Rac GTPase Activating Protein), lowering its transcriptional activity have been identified in human cervical carcinoma cells (HeLa) or human embryonic kidney 293 cells (293T) (Lyberopoulou et al., 2007). Unfortunately, little is known about HIF-2α partners.

Currently, our understanding of the complex regulation of gene expression by both subunits is much deeper and boarder, because the interaction between HIF-1α/HIF-2α and c-Myc/Max complexes are well known. c-Myc transcription factor regulates the expression of genes involved in cell proliferation, glucose and energy metabolism, apoptosis and differentiation and has been found to be overexpressed in many tumor types. This basic helix-loop-helix leucine zipper (B-HLH-LZ) factor het-

erodimerizes with Max protein, and binds to consensus E-box elements in c-Myc regulated genes (Grandori et al., 2000). Transcriptional repressors belonging to Mad family (Mad1, Mad2/Mxi1, Mad3, Mad4) are known to be inversely regulated than c-Myc protein (Chin et al., 1995).

HIF-1α and HIF-2α exert opposite effects on c-Myc oncoprotein activity. HIF-2α enhances c-Myc activity by binding and stabilizing c-Myc/Max complexes, what promotes cell cycle progression (Fig. 3A). On the other hand, HIF-1α inhibits c-Myc function and causes cell cycle arrest at G1/S phase. The effect of HIF-1α is complex and several mechanisms have been proposed. Firstly, HIF-1α binds Max protein and thereby competes with c-Myc (Fig. 3B). Secondly, HIF-1α induces Mxi1, the c-Myc antagonist (Fig. 3C). Lofsted et al. have shown that hypoxia up-regulates Mxi1 mRNA and protein in neuroblastoma and breast cancer cells and have confirmed that Mxi1 is a direct HIF-1α target gene (Lofstedt et al., 2009). In murine hepatoma cell line Mxi1 induction by hypoxia was also found to be HIF-1 dependent (Corn et al., 2005). Additionally, concomitantly with Mxi1 up-regulation, diminishment in c-Myc target genes expression, like cyclin dependent kinase 4 (CDK4) or ornithine decarboxylase (ODC) was observed (Corn et al., 2005). Concomitantly, shift from Myc:Max to Mxi-1:Max heterocomplexes under hypoxic conditions was noted (Corn et al., 2005). Inhibition of c-Myc protein stability by HIF-1α is also caused by favoring proteasome-dependent degradation of c-Myc (Zhang et al., 2007). Recently, link between HIF-1α, c-Myc and specific miRNA has been found. Zhang et al. have identified c-Myc antagonist, MNT protein, as a target of miR-210, which is a master miRNA responsible for HIF-1α regulation (see also below) (Zhang et al., 2009).

Interestingly, recent data suggest, that HIF-1 may not only counter the activity of c-Myc, but paradoxically, it may cooperate with dysregulated c-Myc in tumor cells. Kim et al. found the increased production of pro-angiogenic VEGF as well as induction of hexokinase 2 and pyruvate dehydrogenase kinase 1 leading to promotion of glycolysis resulted from HIF-1 and dysregulated c-Myc cooperation (Kim et al., 2007).

HIF-1 and c-Myc axis can be additionally dysregulated by the involvement of other transcription factors. Recently, the mechanism of HIF-1α competition between c-Myc and Sp-1 transcription factor have been proposed. Data show that such competition caused by Sp-1 binding by HIF-1α results in the inhibition of DNA repair gene expression and the induction of DNA damage. Particularly, the DNA repair genes MSH2, MSH6 and NBS1 were downregulated by activation of the HIF-1-c-Myc axis (reviewed in: Yoo et al., 2009). Our results showed that expression of Ref-1, another protein with endonuclease DNA repair activity (Xanthoudakis and Curran, 1992) is diminished after hypoxia in human microvascular endothelial cells (Loboda et al., 2009a). Moreover, when we studied the effect of specific HIF isoforms, we observed down-regulation of Ref-1 after adenoviral HIF-1α overexpression and inhibition of HIF-1α by specific siRNA reversed the inhibitory effect of hypoxia on Ref-1 expression (Loboda et al., 2009a). If this effect involves the HIF-1-c-Myc axis and needs the displacement of the Max/c-Myc heterocomplexes by Sp-1 transcription factor is not known yet. However, the similar effect of HIF-2α on Ref-1 expression observed by our group (Fig. 4) indicates that it might be a general mechanism. The requirement of HIF-1α for the hypoxic downregulation of DNA repair genes suggests a critical role for HIF-1α in hypoxia-induced genetic alterations.

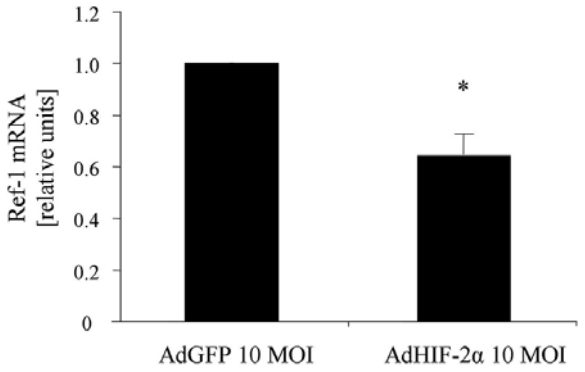

Fig. 4. Adenoviral overexpression of stabilized form of HIF-2α diminishes Ref-1 mRNA level in human microvascular endothelial cells assessed by real-time RT-PCR. Control cells were transduced with control vector harboring green fluorescent protein (AdGFP) at the same MOI.

Regulation of HIF-1α by miRNAs

Recently, a link between hypoxia and specific microRNAs (miRNAs), short non-coding transcripts involved in a wide variety of cellular processes have been established. One member of this large family, miR-210 was identified to be a master miRNA involved in the regulation of HIF-1α in broad spectrum of cell lines, and was found to be overexpressed in most cancer types. Camps et al. (2008) studied expression of miR-210 in 219 human early breast cancer samples as well as in the human breast adenocarcinoma cell line MCF7, hepatoblastoma cell line Hep3B, uterine cervix adenocarcinoma cell line HeLa, and renal cancer cell line RCC4 stably transfected with either an empty vector or pVHL. Using microarrays technique and real-time PCR they found that miR-210 was induced significantly by hypoxia in all models tested. Moreover, to show the mechanism of this induction the authors used siRNA against HIF-1α or HIF-2α, and siRNA-mediated suppression of HIF-1α only caused abrogation of the hypoxic response of miR-210 (Camps et al., 2008). Very recent data suggest, that miR-210 may be used as a marker for tumor hypoxia (Huang et al., 2009). Interestingly, Kushibiki proposed miR-210 as a marker of photodynamic therapy efficacy (Kushibiki, 2009). As a consequence of this therapy, hypoxia is induced and in turn miR-210 generation is up-regulated, followed by an increase of VEGF expression as well as another miRNA, miR-296 expression (Kushibiki, 2009). As miR-210 can regulate numerous targets (Fasanaro et al., 2009) including downregulation of MNT protein, a known c-Myc antagonist (Zhang et al., 2009), the various interactions of HIF-1 and HIF-2 in regulation of this microRNA have to be investigated.

HIF-1α was also reported as a target of miR-20b in different tumor cell lines, including liver, breast, prostate and melanoma cancer cell lines (Lei et al., 2009), whereas in lung cancer cell miR-17-92 cluster has been identified to regulate HIF-1α (Taguchi et al., 2008). As predicted, inverse correlation between HIF-1α and miRNA has been detected, moreover, also expression of target gene of HIF-1α, VEGF, was dependent on miR-20b (Lei et al., 2009). Interestingly, expression of VEGF has been shown to be regulated by a group of regulatory microRNAs, including miR-16, miR-20a, miR-20b, let-7b, miR-17-5p, miR-27a, miR-106a, miR-106b, miR-107, miR-193a, miR-210, miR-320 and miR-361 (Hua et al., 2006) and most of these miRNA have been identified as responsive to hypoxia. Little is known about specific miRNA regulated by HIF-2α but it seems highly possible that such miRNA will be found in the near future.

SUMMARY

Although HIF-1α is the best known and widely described isoform, many data suggest that HIF-2α is, at least, the same important as HIF-1α. The understanding of the biology of both isoforms will be crucial for better diagnosis of pathological conditions in which hypoxia plays a predominant role. Moreover, it will be crucial for the development of new drugs acting either as agonists or antagonists of the hypoxia response pathway.

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