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HIFs, Angiogenesis, and Cancer

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ABSTRACT

Tumor hypoxia was first described in the 1950s by radiation oncologists as a frequent cause of failure to radiotherapy in solid tumors. Today, it is evident that tumor hypoxia is a common feature of many cancers and the master regulator of hypoxia, hypoxia-inducible factor-1 (HIF-1), regulates multiple aspects of tumorigenesis, including angiogenesis, proliferation, metabolism, metastasis, differentiation, and response to radiation therapy. Although the tumor hypoxia response mechanism leads to a multitude of downstream effects, it is angiogenesis that is most crucial and also most susceptible to molecular manipulation. The delineation of molecular mechanisms of angiogenesis has revealed a critical role for HIF-1 in the regulation of angiogenic growth factors. In this article, we review what has been described about HIF-1: its structure, its regulation, and its implication for cancer therapy and we focus on its role in angiogenesis and cancer. J. Cell. Biochem. 114: 967–974, 2013.

KEY WORDS: HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1); ANGIOGENESIS; CANCER

Severely hypoxic regions in tumors result from a combination of rapid cell division and aberrant angiogenesis. Tumor hypoxia is a common feature of many cancers and essentially occurs when the growth of the tumor outstrips the accompanying angiogenesis. All cells must be within 1–2 mm^3 of a blood supply for survival [Folkman, 1990]. It is therefore not surprising that many parts of a developing tumor are hypoxic. The transcriptional factor hypoxia-inducible factor-1 (HIF-1) plays an essential role in the adaptive response of cells to reduced oxygen tension. It functions as a master regulator of oxygen and undergoes conformational changes in response to varying oxygen concentrations.

Angiogenesis represents an essential step in tumor proliferation, expansion, and metastasis. Tumor cells may express both proangiogenic and/or antiangiogenic factors. Under normal circumstances, angiogenesis is controlled through the equilibrium of these factors. This balance is disrupted in malignancy, a shift in the equilibrium to a proangiogenic state occurs at an early to mid-stage in tumor development. This leads to activation of an ''angiogenic switch'' and, consequently, the formation of new vasculature [Hanahan and Folkman, 1996]. HIF can directly activate the expression of a number of pro-angiogenic factors, including VEGF, VEGF receptors FLT-1 and FLK-1, plasminogen activator inhibitor-1 (PAI-1), angiopoietins (ANG-1 and -2), platelet-derived growth factor-B (PDGF-B), the TIE-2 receptor, and matrix metalloproteinases MMP-2 and -9[Hickey and Simon, 2006]. Of all the proangiogenic factors induced by HIF, VEGF appears to have a central role in the angiogenic process: it is the target of many proangiogenic factors, but it also regulates molecules that are implicated in endothelial proliferation. It has been suggested that VEGF may be a proximate angiogenic factor through which others act. The degree of angiogenesis and the expression of angiogenic factors have been associated with prognosis in several human

Abbreviations used: HIF-1, hypoxia-inducible factor-1; HER2, human epidermal growth factor receptor 2; IGFR, insulin-like growth factor receptor; EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; AKT, protein kinase B; FRAP, FKBP-rapamycin-associated protein; FKBP, FK-506-binding protein; FIH-1, factor inhibiting HIF-1; VEGF, vascular endothelial growth factor; IGF-2, insulin-like growth factor 2; VHL, von Hippel–Lindau tumor suppressor protein.

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neoplasms. Improved understanding of the molecular mechanisms involved in angiogenesis and tumor hypoxia-response will allow for future targeted therapies to be developed that may, either alone or in combination with established modalities like chemotherapy, surgery, and radiotherapy, provide improved cure and control rates over current therapeutic strategies.

MOLECULAR BIOLOGY OF HIF

HIF-1 was identified and purified as a nuclear factor that was induced in hypoxic cells and bound to the *cis*-acting hypoxia response element (HRE) located in the 3′-flanking region of the human *EPO* gene, which encodes erythropoietin, the primary humoral regulator of red blood cell production that undergoes hypoxia-induced transcription. HIF-1 is a heterodimeric transcription factor composed of a HIF-1α subunit and a HIF-1β subunit which exist as a series of isoforms encoded by distinct genetic loci. Both HIF-1 subunits are members of the basic helix-loop-helix (HLH)-containing PER-ARNT-SIM (PAS)-domain family of transcription factor. Interactions between HLH-PAS domains from the two subunits mediate their dimerization, and individual basic regions of the two subunits then make contact with their corresponding DNA sequences, namely HRE [Michel et al., 2000]. Based on the available data, HIF-1α the highly regulated subunit, and HIF-1β the constitutive subunit also called aryl hydrocarbon receptor nuclear translocator (ARNT). ARNT2 and ARNT3 could also be implicated in the formation of different putative dimers with HIF-1α increasing adaptability to reduced oxygen availability. The N-terminal half of HIF-1α contains bHLH and PAS domains that are required for dimerization and DNA binding. The C-terminal half contains domains required for degradation and transactivation: the oxygen-dependent degradation domain (ODDD) which confers oxygen-dependent instability, two independent transactivation domains (N-TAD and C-TAD) and in between, an inhibitory domain (ID) that negatively regulates the transactivation domains [Jiang et al., 1997] (Fig. 1).

In addition to HIF-1α and -β, two other proteins have been identified. These are additional α isoforms termed HIF-2α and HIF-3α. HIF-2α is closely related to HIF-1α and both are able to interact with HREs to upregulate transcriptional activity. HIF-2α shares 48% amino acid sequence identity with HIF-1α and accordingly shares a number of structural and biochemical similarities with HIF-1α. In contrast to ubiquitously expressed HIF-1α, though, HIF-2α is primarily expressed in the lung, carotid body, and endothelium. HIF-2α has several unique transcriptional targets such as Oct4 and TGFα. These targets are outside the canonical pro-angiogenic hypoxic response, which suggests an important and specific role for HIF-2α in regulating other cellular processes such as pluripotency [Semenza, 2003]. A study by Li et al. has demonstrated differential protein and mRNA expression of HIF-1α and HIF-2α between the non-stem and cancer stem cell. Further, this study elucidated that HIF-2α was only significantly present in the cancer stem cell population [Li et al., 2009]. Little is known about the third HIFa isoform. Several splice variants of HIF-3α have been shown to be a dominant-negative regulator of the other two alpha isoforms and has a limited expression pattern in the eye and the cerebellum, dimerizes with HIF-1β, and binds to HREs. Some HIF-3α isoforms are also thought to be direct transcriptional targets of HIF-1α activity under hypoxia. Current studies are still unclear as to the primary function and regulatory mechanism through which HIF-3α and its variants function [Maynard et al., 2003].

The expression of over 40 genes is known to be activated at the transcriptional level by HIF-1 as determined by the most stringent criteria, including the induction of gene expression in response to hypoxia, the presence of a functionally essential HIF-1-binding site in the gene, and an effect of HIF-1 gain-of-function or loss-of-function on expression of the gene. However, a study of global gene expression using DNA microarrays indicates that more than 2% of all human genes are directly or indirectly regulated by HIF-1 in arterial endothelial cells (ECs) [Manalo et al., 2005].

REGULATION OF HIF-1

During hypoxia, HIF-1α becomes stabilized and translocates from the cytoplasm to the nucleus, where it dimerizes with HIF-1β and bind to HREs located within regulatory elements of HIF target genes. Cell culture studies have shown that HIF stabilization and DNA-binding activity is induced at oxygen concentrations below 6%

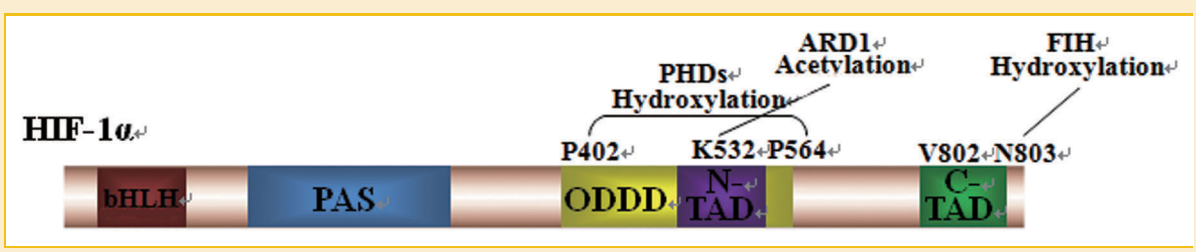

Fig. 1. Domain structure of HIF-1α and target residues involved in its regulation. Structural analysis of the HIF-1α protein revealed that HIF-1α contains four distinct domains including a bHLH domain for DNA binding and dimerization, a PAS domain for dimerization and target gene specificity, an oxygen-dependent degradation domain (ODDD) required for degradation by the ubiquitin–proteasome pathway, and two transactivation domains located in the C-terminal portion of the protein. Hydroxylation of the proline residues 402 and 564, which are located at the oxygen-dependent degradation domain (ODDD) of HIF-1, mediates interactions with the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex that targets HIF-1 for proteasomal degradation. This hydroxylation is catalyzed by three prolyl hydroxylases namely prolyl-4-hydroxylase-domain proteins (PHD1, PHD2, PHD3).

oxygen and is maximal at 0.5% oxygen tensions. Once stabilized, the HIF-α/HIF-1β heterodimer activates transcription by recruiting the transcriptional activators p300 and CBP. The oxygen sensors in the HIF-1α pathway are two kinds of oxygen-dependent hydroxylases. One is prolyl hydroxylase which could hydroxylize the proline residues 402 and 564, which are part of a conserved consensus sequence LXXLAP, at the ODDD of HIF-1 [Höpfl et al., 2004]. Hydroxylation of these two prolyl residues mediates interactions with the von Hippel–Lindau (VHL) E3 ubiquitin ligase complex that targets HIF-1 for proteasomal degradation [Kaelin, 2005]. Three prolyl hydroxylases namely prolyl-4-hydroxylase-domain proteins (PHD1, PHD2, PHD3) have been characterized [Masson et al., 2001]. PHD1 is specifically localized in the nucleus, PHD2 is mainly localized to the cytoplasm and PHD3 seems to have no preference [Metzen et al., 2003]. These three homologs were originally designated EGLN2,1,3, respectively, on the basis of protein sequence homology to EGL-9, the HIF-1 prolyl hydroxylase of *Caenorhabditis elegans*. All three PHDs have the potential to hydroxylate HIF-1a in vitro with their relative activities as PHD2 ≫ PHD3 > PHD1, and PHD2 was shown to be the key limiting enzyme that controls the HIF-1α turnover in vivo. These iron-dependent enzymes convert proline into hydroxyproline, a reaction that requires oxygen, 2-oxoglutarate, and ascorbate. The activity of these enzymes is governed by the O_2 concentration within the cell, which defines these proteins as oxygen sensors. The requirement for iron as a cofactor explains the observed hypoxia-mimetic effects of iron chelators such as desferrioxamine (DFO) and iron antagonists such as cobalt chloride ($CoCl_2$). Besides controlling the activity of the PHDs, the O_2 concentration also controls the expression of PHD2 and PHD3 mRNAs. Thus, in a manner similar to p53, HIF-1a governs its own stability by controlling the expression of PHD2 [Berra et al., 2003]. The other is hydroxylation of Asn803 at the C-terminal TAD domain. Hydroxylation of the asparagyl-residue, however, does not lead to HIF-1a degradation and is therefore not directly involved in the oxygen-sensing mechanism. The hydroxylation of the Asn803 residue leads to a steric inhibition of the interaction between HIF-1α and its co-activator CBP/p300, interfering with its recruitment. This recruitment is critical for HIF-1a activation. This asparagyl hydroxylase was first described as factor-inhibiting HIF-1 (FIH-1) [Hewitson et al., 2002]. FIH-1 is mainly located in the cytoplasm, but some fraction is likely to reside in the nucleus as well. Linke et al. [Metzen et al., 2003] have demonstrated the crucial role of the adjacent residue valine802 in targeting asparagine803 hydroxylation. This Val–Asn motif seems to be primordial in maintaining the interaction between p300/CBP and the HIF-1a-C-TAD. FIH-1 does not influence HIF-1α stability but like the PHDs, is a member of the 2-oxoglutarate and iron-dependent dioxygenase family. Like PHD1, FIH-1 transcription is totally independent of the oxygen concentration and seems to be constitutively expressed in all cell types so far studied [Metzen et al., 2003]. Interestingly, Safran and Kaelin [2003] reported the in vitro interaction of FIH-1 and pVHL with histone deacetylases. This recruitment could help FIH-1 modulate HIF-1α transactivation under normoxic conditions.

Although O_2-sensitive prolyl and asparaginyl hydroxylation events are two principal mechanisms regulating the HIF isoforms, several additional regulatory pathways have also been uncovered. Hydroxylation is not the only post-translational modification that induces HIF-1α destabilization. The arrest-defective-1 protein (ARD1) is a member of a large and diverse super-family of acetyltransferases that includes histone acetyltransferases. Acetylation by ARD1 of lysine532 located in the ODDD domain, close to hydroxylproline564, appears to favor recruitment of pVHL and thus degradation by the proteasomal system [Carrozza et al., 2003]. Thus, ARD1 like PHDs will act as a negative regulator of HIF-1α by making it less stable. Since *N*-acetyltransferase activity is not known to be dependent on oxygen it could be expected that the modification occurs under both hypoxic and normoxic conditions (Fig. 2).

Many oncogenes also have effects on HIF-1α. Tumor suppressor genes p53 influence the levels and functions of HIF-1. The wild-type (wt) form of p53 protein was involved in inhibiting HIF-1 activity [Jeong et al., 2002], and in inducing angiogenesis inhibitors such as thrombospondin-1, while loss of wt p53 (by gene deletion or mutation) could enhance HIF-1α accumulation in hypoxia, and augment HIF-1-dependent expression of VEGF in tumor cells. In addition, inactivation of other tumor suppressors such as PTEN which is an antagonist of PI-3K signaling could function by removing phosphate at the third position of phosphatidylinositol biphosphate and triphosphate. Loss of the tumor suppressor function of PTEN would augment HIF-1-mediated gene expression and restoration of PTEN could inhibit the expression of HIF-1α [Zundel et al., 2000]. Besides, a battery of growth factors and cytokines from stromal and parenchymal cells have effects on HIF-1α too, such as epidermal growth factor (EGF) [Jiang et al., 2001], transforming growth factor-α (TGF-α) [Zhong et al., 2000], insulin-like growth factor-1 (IGF-1) and IGF-2 [Krishnamachary et al., 2003], heregulin [Treins et al., 2002], and interleukin-1β [Laughner et al., 2001] via autocrine and paracrine pathways. They not only induce the expression of HIF-1α protein, HIF-1 DNA-binding activity and transactivity, but also make HIF-1 target gene expression under normoxia or hypoxia.

HIF-1 AND ANGIOGENESIS

INTRODUCTION OF ANGIOGENESIS

Angiogenesis represents an essential step in tumor proliferation, expansion, and metastasis, which is particularly relevant to the pathology of virtually all human tumors. It is generally accepted that neovascularization is critical for tumor progression since the supply of oxygen and nutrients becomes limited in tumor cells that are located more than 100 μm away from a blood vessel. Given this, there are two stages of tumor progression regarding its vasculature. During the initial avascular stage of tumor growth (tumor mass <0.5 mm), nutrition and oxygen supplementation can be achieved by diffusion. When tumor mass grows larger than 0.5 mm, nutrition through diffusion is no longer sufficient and formation of new vasculature is necessary for further growth (vascular stage). The tumor remains in a dormant state until it can stimulate blood vessel growth from nearby pre-existing capillaries, a process known as angiogenesis [Hanahan and Weinberg, 2000]. So it is apparent that the angiogenic process in solid tumors is crucial for advanced tumors' growth and progression to a metastatic state. It has been proposed that microvessel density (MVD) is an indicator of biological aggressiveness and metastatic

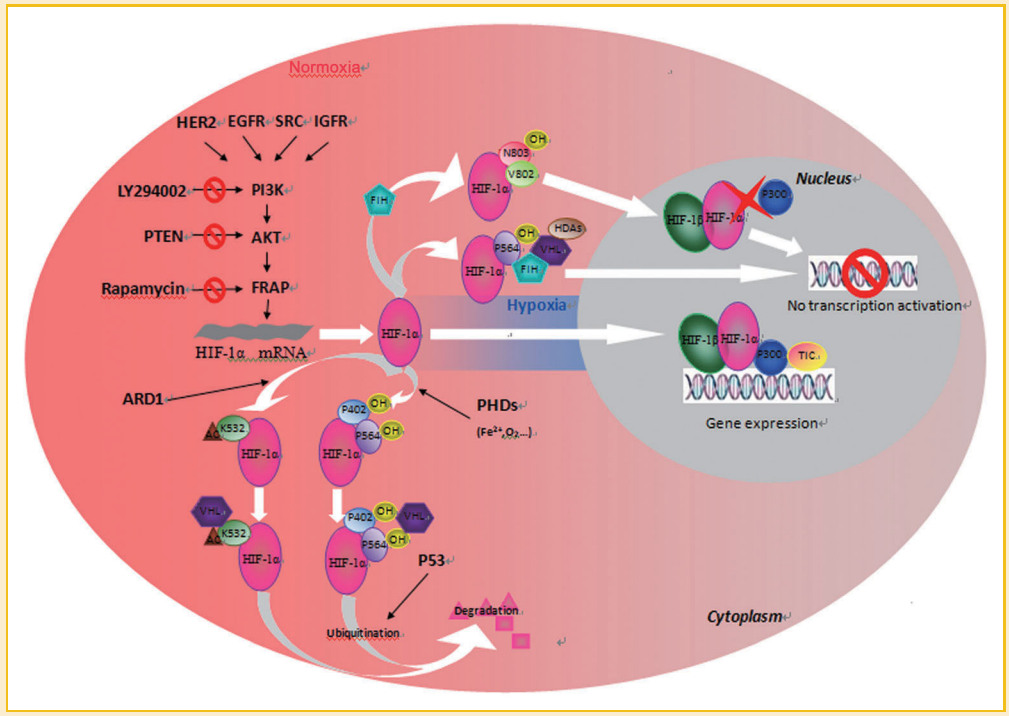

Fig. 2. Regulation of the stability and translation of HIF-1α. Steady-state levels of HIF-1α protein are regulated at the level of synthesis and stability. Activation of tyrosine kinases, such as SRC and the HER2neu, IGF and EGF receptors, stimulates the PI3K–AKT–FRAP signal transduction pathway, which leads to increased translation of HIF-1α mRNA into protein. Binding of p53 to HIF-1α under hypoxic conditions recruits MDM2, which also targets HIF-1α for ubiquitination and degradation. Under hypoxic or low oxygen conditions, the HIF-1α subunit enters the nucleus, dimerizes with HIF-1α, and binds to a core DNA sequence (TACGTG) within the hypoxia response element adjacent to the HIF-1 target gene (transcription initiation complex; TIC).

potential in many primary tumors and has association with prognosis in several human neoplasms.

INVOLVEMENT OF HIFS IN ANGIOGENESIS

Angiogenesis result from the expression of angiogenic factors by tumor cells as a response to certain stimuli. Tumor hypoxia, oncogenes (such as *ras*, VHL, and *bcl-2*), cytokines, proangiogenic cell growth factors (such as bFGF and EGF), and hormones are important stimuli that cause the increased production of tumor VEGF. Among them, the most well-studied external stimulus is hypoxia, which actually is a key signal for the induction of angiogenesis [Levy et al., 1995].

The growth of new capillaries from existing blood vessels is a complex multi-step process involving extracellular matrix components. It begins with an enlargement of the parent vessel, which then sprouts or divides by intussusception or bridging, and subsequently splits into individual capillaries. This process can be divided into four stages (1) proteolytic degradation of the basement membrane and surrounding extracellular matrix, (2) EC proliferation, (3) EC migration, and (4) tube formation and structural reorganization [Carmeliet, 2000].

HIF-1 regulates genes encoding angiogenic growth factors. HIF-1 activates transcription of genes encoding angiogenic growth factors, which are secreted by hypoxic cells and stimulate ECs, leading to angiogenesis. Angiogenesis is a complex process, involving multiple gene products expressed by different cell types, all contributing to an integrated sequence of events. Consistent with a major role for hypoxia in the overall process, many genes involved in different steps of angiogenesis are independently responsive to hypoxia in tissue culture. Examples include VEGF, angiopoietin1 (ANGPT1) and ANGPT2, placental growth factor (PLGF), and PDGF-B and their various receptors, and genes involved in matrix metabolism, including matrix metalloproteinase, plasminogen activator receptors and inhibitors, and procollagen prolyl hydroxylase. Among angiogenic molecules, VEGF appears to have a central role in the angiogenic process: it is the target of many proangiogenic factors, but it also regulates molecules that are implicated in endothelial proliferation. HIF-1 directly activates VEGF and VEGF receptor (VEGFR-1) transcription by binding to HRE, and plays an important role during normal growth and tumor formation [Koop et al., 2003].

When tumors express VEGF to generate their own blood supply, the resulting blood vessels are usually immature compared with normal blood vessels and lack surrounding pericytes. VEGF is necessary to maintain the viability of these immature blood vessels. In addition, VEGF plays a key role in maintaining the viability of existing tumor blood vessels. Deletion of HIF-1α in ECs disrupted an autocrine loop necessary for hypoxic induction of both VEGFR-1 and VEGFR-2 by VEGF signaling [Olsson et al., 2006]. Though the expression of both VEGFR-1 and VEGFR-2 is upregulated by hypoxia, VEGFR-1 is directly upregulated by hypoxia via an HIF-binding enhancer element located in the VEGFR-1 promoter, while

the upregulation of VEGFR-2 is through a post-transcriptional regulation [Tang et al., 2004]. Disruption of VEGFR-1/VEGF/VEGFR-2 autocrine signaling resulted in decreased EC proliferation and tube formation in vitro and severely impaired tumor angiogenesis and reduced tumor growth in vivo. On the other hand, activation of VEGF receptors initiates multiple intracellular downstream signaling pathways that lead to tumor angiogenesis.

Cell-autonomous effects of HIFs in vascular endothelial cells. In addition to controlling the production of angiogenic factors in hypoxic or ischemic tissue, HIF-1 also controls cell-autonomous responses to hypoxia within ECs. HIF-1 controlled VEGF production leads to autocrine signal transduction that is critical for angiogenesis. Many of the biological processes in angiogenesis, extracellular matrix invasion, and tube formation by ECs are stimulated under hypoxic conditions through HIF-1, which activates the transcription of scores of genes whose protein products play critical roles in these processes [Tang et al., 2004]. Manalo et al. [2005] compared gene expression profiles in arterial ECs cultured under non-hypoxic versus hypoxic conditions and in non-hypoxic cells infected with adenovirus encoding β-galactosidase versus a constitutively active form of HIF-1α (AdCA5). They found many genes up-regulated by both hypoxia and AdCA5 encoded cytokines/growth factors, receptors, and other signaling proteins. Transcription factors accounted for the largest group of HIF-1-regulated genes, indicating that HIF-1 controls a network of transcriptional responses to hypoxia in ECs. Lately, infection of ECs with AdCA5 under non-hypoxic conditions was sufficient to induce increased basement membrane invasion and tube formation similar to the responses induced by hypoxia, indicating that HIF-1 mediates cell-autonomous activation of ECs.

HIF-2α is highly expressed in embryonic vascular ECs and activates the expression of target genes whose products modulate vascular function and angiogenesis. Skuli et al. [2009] describe a genetic model designed to test the physiologic consequences of deleting HIF-2α in murine ECs. They found mice with HIF-2α-deficient ECs developed normally but displayed a variety of phenotypes. Moreover, these animals exhibited defective tumor angiogenesis associated with increased hypoxic stress and tumor cell apoptosis. Immortalized HIF-2α-deficient ECs displayed decreased adhesion to extracellular matrix proteins and expressed reduced levels of transcripts encoding fibronectin, integrins, endothelin-β receptor, angiopoietin 2, and delta-like ligand 4 (Dll4). Together, these data identify unique cell-autonomous functions for HIF-2α in vascular ECs.

HIFs influence the biological behavior of vascular endothelial cells. Due to the distinctive coexpression of HIF-1α and HIF-2α subunits in ECs, Han et al. examined the genetic elimination of HIF transcriptional activity in response to physiological hypoxic conditions by using a genetic model in which the required HIF-β subunit, also called ARNT, to HIF transcriptional responses was depleted. They demonstrate that ARNT promotes ECs migration and vessel outgrowth and is indispensible for the proliferation and preservation of ECs in response to hypoxia. Thus, these results demonstrate that ARNT plays a critical intrinsic role in ECs and support an important collaboration between HIF-1 and HIF-2 transcriptional activity in these cells [Han et al., 2012].

THERAPEUTIC IMPLICATIONS

After demonstrating the central role of hypoxia and HIF-1 in the activation of numerous pathways responsible for tumor progression, it is not surprising that hypoxic cells targeting has become amajor therapeutic goal in cancer therapy. Therapeutic approaches using HIF-1 inhibitors include those that target HIF-1 directly or indirectly. HIF-1 direct inhibitors identified to date interfere with the following general mechanisms: (i) control of HIF-1a synthesis, (ii) folding, stabilization and nuclear translocation, and (iii) HIF-1a transactivation of target genes [Makino et al., 2001] (Table I). For example, EZN-2968 is a RNA antagonist, which is composed of a third-generation oligonucleotide, a technology that specifically binds and inhibits the expression of HIF-1a mRNA. EZN-2968 induced a potent, selective, and durable antagonism of HIF-1 mRNA and protein expression (IC50, 1–5 nM) under both normoxia and hypoxia associated with cell growth inhibition in human prostate and glioblastoma cells [Greenberger et al., 2008]. Another bright example of direct targeting of HIF-1 is the use of PX-478 and YC-1 which have shown impressive activity in tumor xenograft models [Macpherson and Figg, 2004;

TABLE I. Recent Inhibitors That Target the HIF-1 Pathway

Mechanism of HIF-1 inhibition	Agent	Refs.
HIF-1α degradation	GA and analogs	Porter et al. [2009]
	LW6	Lee et al. [2010]
	AC1-004	Won et al. [2009]
	Radical and analogs	Hur et al. [2002]
	SCH66336	Han et al. [2005]
	Apigenin	Fang et al. [2005]
	LAQ824	Qian et al. [2006]
	FK228	Lee et al. [2003]
	Trichostain A	Kong et al. [2006]
	YC-1 and analogs	Li et al. [2008]
	PX-478	Belozerov and Van [2005]
	PX-12	Welsh et al. [2003b]
	Pleurotin	Welsh et al. [2003b]
	AJM 290	Jones and Harris [2006]
	AW464	Jones et al. [2006]
HIF-1α protein accumulation	GN26361	Shimizu et al. [2010]
	P2630	Yewalkar et al. [2010]
	(R)-(−)-Moracin O	Xia et al. [2011]
	(R)-(−)-Moracin P	Xia et al. [2011]
	Manassantin A	Kasper et al. [2009]
	(−)-Cryptopleurine	Cai et al. [2006]
	Sibiriquinone A	Dat et al. [2007]
	Caulerpin	Liu et al. [2009]
	10-Hydroxyglaucanetin	Coothankandaswamy et al. [2010]
HIF-1 dimerization	Chaetocin	Isham et al. [2007]
ARNT degradation	Curcumin	Choi et al. [2006]
	Resveratrol	Zhang et al. [2005]
HIF-1α translation	Wortmannin	Xia et al. [2011]
	LY294002	Xia et al. [2011]
	CCI-779	Wan et al. [2006]
	Camptothecins	Verma and Hansch [2009]
	2ME2 and analogs	LaVallee et al. [2008]
Transcriptional activity	YC-1 and analogs	Li et al. [2008]
	PX-478	Belozerov and Van [2005]
	PX-12	Welsh et al. [2003b]
	Pleurotin	Welsh et al. [2003b]
	Ajm290	Jones and Harris [2006]
	Aw464	Jones et al. [2006]
	Chetomin	Kung et al. [2004a]
	Bortezomib	Kaluz et al. [2006]
	Amphotericin B	Yeo et al. [2006]
DNA binding	AJM290	Jones and Harris [2006]
	AW464	Jones et al. [2006]
	Echinomycin	Kong et al. [2005]

Belozerov and Van, 2005]. Other HIF-1 inhibitors do not target HIF-1 directly, but rather target the different pathways activated by HIF-1 (indirect HIF-1 inhibitors); these include use of inhibitors that target PI3K (such as wortmannin and LY294002 [Yan et al., 2012]), Hsp-90 (such as GA and analogs [Porter et al., 2009]), co-activator CBP/p300 (such as Chetomin [Kung et al., 2004b]), thioredoxin (one of the major cellular thiol/disulfide antioxidant systems involved in opposing the oxidant stress), topoisomerases (such as camptothecins [Verma and Hansch, 2009]), microtubule complexes, histone deacetylases, kinases (such as PI-3 kinases and MEK1), and guanyl cyclase [Rapisarda et al., 2004]. Other indirect HIF-1 inhibitors have been in clinical practice for some time; for instance, Bevacizumab targets VEGF, Sunitinib and sorafinib target VEGFR and PDGFR while other relatively new drugs like temsirolimus target the mTOR pathway, all of which lead to inhibition of the action of HIF [Welsh et al., 2003a]. Besides that, it is well documented that alpha-ketoglutarate (AKG) treatment of Hep3B hepatoma cells in hypoxia-induced HIF-alpha (hypoxia-inducible factor) degradation and reduced vascular endothelial growth factor (VEGF) synthesis. Recently, the antiproliferative activity of AKG on colon adenocarcinoma Caco-2, HT-29, and LS-180 cells in normoxic conditions was revealed. Taking into consideration an anticancer activity both in hypoxic and normoxic conditions, AKG may be considered as a new potent chemopreventive agent [Rzeski et al., 2012].

Angiogenesis plays a central role in both local tumor growth and distant metastasis. The importance of angiogenesis for tumor growth, but also the prognostic significance of angiogenesis and angiogenic factors, provides the theoretical background for using antiangiogenic strategies as a form of anticancer treatment. Based on the low mutation rate of the genetically stable ECs, antiangiogenic therapy was initially thought to be a tumor-specific treatment. Initial xenograft studies supported these theoretical predictions: widespread activity, limited toxicity, and no resistance. The angiogenesis antagonists can be classified into five distinct types: protease inhibitors that impact extracellular matrix remodeling, inhibitors of activated EC proliferation, inhibitors of survival signaling via vascular adhesion molecules, and agents that interfere with the generation of angiogenic molecules or receptor activity. The PI3K/Akt signaling pathway plays an important role in tumor angiogenesis. Akt is a major downstream target of PI3K in the angiogenic pathway. In animal studies, the siRNA-mediated inhibition of Akt effectively reduced ovarian tumor growth and angiogenesis [Jiang and Liu, 2008]. Recently, Seung et al. reported programmed cell death 6 (PDCD6) inhibited VEGF-induced phosphorylation of Akt signaling pathway, including mammalian target of rapamycin (mTOR), tuberous sclerosis complex 2 (TSC-2), p70 ribosomal protein S6 kinase (p70S6K), and glycogen synthase kinase-3β (GSK-3β) and effectively decreased the expression of cyclin D1 through direct interactions with VEGFR-2. These findings strongly suggest that PDCD6 can inhibit tumor growth via suppression of tumor angiogenesis in the cellular physiological condition through targeting PI3K/mTOR/p70S6K kinase signaling pathway [Seung et al., 2012]. Antiangiogenic cancer treatment is still largely experimental and its clinical potential will not be realized for several years to come. In order to get more details please see the review by Bamias and Dimopoulos [2003].

CONCLUSION

Tumor cell hypoxia, via HIF-1, appears to be a crucial stimulant for the angiogenic process to allow tumor growth beyond a few millimetres as well as the development of metastasis. Although the tumor hypoxia response mechanism leads to a multitude of downstream effects, it is angiogenesis that is most crucial and also most susceptible to molecular manipulation. Hence, there is not surprising that specific targeting of HIF-1 will be of crucial importance as complete inhibition of its expression will certainly have repercussions on progression of tumor angiogenesis. However, a number of questions that have yet to be answered, hinder the current development of novel HIF or angiogenesis inhibitors. As in the case of every other protein implicated in cancer pathogenesis, HIF-1 overexpression does not occur in every cancer and when it does occur it is not always associated with increased mortality [Seung et al., 2012]. This evidence suggests that HIF-1 will not be a therapeutic target in all cancers. On the other hand, agents that suppress angiogenesis may be beneficial in the patient who is harboring an occult carcinoma but may cause undesirable side effects if the patient is harboring an cardiovascular disease. Thus, much work remains to be done. At the same time, there are some limitations in the application of antiangiogenic therapy in cancer, such as EC heterogeneity, tumor cell heterogeneity, impact of the tumor microenvironment [Kaelin, 2002]. Thus, much work remains to be done to discover optimal therapeutic agents, to identify appropriate patient populations in which their administration will result in an improved clinical outcome, and to reduce the potential for development of drug resistance.

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