Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 68 (2004) 1061–1069
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

New anticancer strategies targeting HIF-1

Eun-Jin Yeo[a], Yang-Sook Chun[b], Jong-Wan Park[a,*]

[a]Department of Pharmacology, College of Medicine, Seoul National University, 28 Yongon-dong, Chongno-gu, Seoul 110-799, Republic of Korea
[b]Human Genome Research and Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea

Received 11 January 2004; accepted 18 February 2004

Abstract

Hypoxia-inducible factor-1 (HIF-1), which is present at high levels in human tumors, plays crucial roles in tumor promotion by up-regulating its target genes, which are involved in anaerobic energy metabolism, angiogenesis, cell survival, cell invasion, and drug resistance. Therefore, it is apparent that the inhibition of HIF-1 activity may be a strategy for treating cancer. Recently, many efforts to develop new HIF-1-targeting agents have been made by both academic and pharmaceutical industry laboratories. The future success of these efforts will be a new class of HIF-1-targeting anticancer agents, which would improve the prognoses of many cancer patients. This review focuses on the potential of HIF-1 as a target molecule for anticancer therapy, and on possible strategies to inhibit HIF-1 activity. In addition, we introduce YC-1 as a new anti-HIF-1, anticancer agent. Although YC-1 was originally developed as a potential therapeutic agent for thrombosis and hypertension, recent studies demonstrated that YC-1 suppressed HIF-1 activity and vascular endothelial growth factor expression in cancer cells. Moreover, it halted tumor growth in immunodeficient mice without serious toxicity during the treatment period. Thus, we propose that YC-1 is a good lead compound for the development of new anti-HIF-1, anticancer agents.

Keywords: HIF; Cancer; Hypoxia; Angiogenesis; Anticancer chemotherapy; YC-1

1. Introduction

Although many anticancer regimens have been introduced to date, their survival benefits are negligible, which is the reason that a more innovative treatment is required. Basically, the identification of the specific molecular features of tumor promotion has allowed for rational drug discovery in cancer treatment, and drugs have been screened based upon the modulation of specific molecular targets in tumor cells [1]. Target-based drugs should satisfy the following two conditions. First, they must act by a described mechanism. Second, they must reduce tumor growth in vivo, associated with this mechanism. Many key factors have been found to be involved in the multiple steps of cell growth signal-transduction pathways. Targeting these factors offers a strategy for preventing tumor growth; for example, competitors or antibodies blocking ligand–receptor interaction, and receptor tyrosine kinase inhibitors, downstream pathway inhibitors (i.e., RAS farnesyl transferase inhibitors, mitogen-activated protein kinase and mTOR inhibitors), and cell-cycle arresters (i.e., cyclin-dependent kinase inhibitors) could all be used to inhibit tumor growth [2]. In addition to the intracellular events, tumor environmental factors should be considered to treat solid tumors. Of these, hypoxia is an important cancer-aggravating factor because it contributes to the progression of a more malignant phenotype, and to the acquisition of resistance to radiotherapy and chemotherapy [3]. Thus, transcription factors that regulate these hypoxic events are good targets for anticancer therapy and in particular HIF-1 is one of most compelling targets [4,5]. In this paper, we introduce the roles of HIF-1 in tumor promotion and provide a summary of new anticancer strategies designed to inhibit HIF-1 activity.

2. Role of HIF-1 in adaptation to hypoxia

Oxygen is essentially required by the aerobic metabolisms of most eukaryotic organisms. It functions as a

Abbreviations: HIF, hypoxia-inducible factor; ARNT, aryl hydrocarbon nuclear receptor translocator; HRE, hypoxia response element; ODDD, oxygen-dependent degradation domain; pVHL, von Hippel-Lindau protein; bHLH, basic-helix–loop–helix; PAS, Per-Arnt-Sim

* Corresponding author. Tel.: +82 2 7408289; fax: +82 2 7457996.
E-mail address: parkjw@snu.ac.kr (J.-W. Park).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.02.040

scavenger to remove harmful electron and hydrogen ions, both of which are waste by-products that remain after mitochondrial oxidative phosphorylation. However, when oxygen delivery is disrupted in cardiovascular, pulmonary, and hematological diseases, this energy metabolism is seriously impaired. Thus, organisms have developed numerous adaptive mechanisms to enable cells to survive in oxygen-depleted conditions. Hypoxic adaptation includes reflex hyperventilation, the increased production of red blood cells, and new vessel formation, which in combination lead to increased oxygen delivery from the atmosphere to tissues [6]. At the cellular level, adaptation involves a switch of energy metabolism from oxidative phosphorylation to anaerobic glycolysis, which increases glucose uptake, and expressions of stress proteins related to cell survival or death [7]. The regulations of most proteins required for hypoxic adaptation occurs at the gene level, which involves transcriptional induction via the binding of a transcription factor HIF-1, to the HRE on the regulated genes [8]. To date, more than 60 putative hypoxia-inducible genes have been found to be directly regulated by HIF-1. Wang and Semenza [9] affinity-purified HIF-1 by using oligonucleotide containing the HRE sequence, 5′-RCGTG-3′. HIF-1 is a heterodimer composed of two bHLH proteins of the PAS family, HIF-1 and ARNT (or HIF-1β). Of these, HIF-1α is the key protein, as it determines the presence of HIF-1 and transactivates the hypoxia-inducible genes.

HIF-1α is composed of 826 amino acids [9]. Its N-terminal contains the bHLH and the PAS domains, which are essential for dimerization and DNA binding [10], and its C-terminal contains two transactivation domains and a nuclear localization signal [11]. The central portion of HIF-1α (aa. 401-603) contains ODDD, which determines the stability of HIF-1α protein [12]. In aerobic conditions, HIF-1-prolyl hydroxylases modify two proline residues in the amino acid motif LXXLAP located at either end of ODDD [13–17]. Then, pVHL, a part of the E3 ubiquitin ligase protein complex, binds to the modified HIF-1α, which results in the ubiquitination and proteasomal degradation of HIF-1α [18,19]. Since the enzymatic reaction of prolyl hydroxylation requires oxygen as a substrate, hypoxia limits this hydroxylation, thereby precluding the binding of pVHL and thus leading to the stabilizing HIF-1α [20]. In addition to prolyl hydroxylation, the acetylation of a lysine residue (Lys532) within the ODDD also mechanistically regulates HIF-1α stability. Lysine acetylation also enhances the interaction between HIF-1α and pVHL, since the expression of ARD1, an HIF-1α acetyltransferase, decreases under hypoxic conditions, HIF-1α escapes from acetylation and becomes stable [21]. On the other hand, homology searches in the gene bank and cloning experiments found other members of this family, such as HIF-2α (also known as endothelial PAS protein-1 or MOP2) [22,23] and HIF-3α [24]. HIF-2α is highly similar to HIF-1α in protein structure, but exhibits restricted tissue-specific expression. HIF-2α is also tightly regulated by oxygen tension and its complex with HIF-1β appears to be directly involved in hypoxic gene regulation, as is HIF-1α [25]. However, although HIF-3α is homologous to HIF-1α, the physiological role of HIF-3α is uncertain. It could function as a negative regulator of hypoxia-inducible gene expression [26].

3. Role of HIF-1 in tumor promotion

Within solid tumors, hypoxia commonly develops because the rate of tumor cell proliferation outpaces the rate of vessel formation, thus the blood supply is compromised due to aberrant vasculature formation. Tumor hypoxia is presumed to occur early during tumor growth, when a tumor diameter reaches only a few millimeters [27]. Since HIF-1α is induced by a hypoxic stimulus, it is no wonder that HIF-1α protein is highly expressed in solid tumors containing hypoxic regions. Furthermore, the expression of HIF-1α may be enhanced by genetic alterations in oncogenes or tumor suppressor genes, regardless of oxygen tension. For instance, gain-of-function mutations in the oncogenes Ras, Src, Her2, or mTOR, and loss-of-function mutations in the tumor suppressor genes VHL, PTEN, or p53, are associated with a higher HIF-1α expression in human cancers [28]. In some cancer cell-lines, tumor growth factors also induce HIF-1α expression. The bindings of these factors to their receptors activate respective receptor tyrosine kinases, which in turn activate the PI3K/AKT/mTOR pathway. Finally, mTOR stimulates the expression of HIF-1α even under normoxic conditions [29–31]. In addition, reactive oxygen species (ROS), which are well-known tumor promoters, can stabilize HIF-1α and stimulate the transcriptional activity of HIF-1. Park et al. [32] demonstrated that HIF-1α is expressed in normoxic gastric cancer cells, and that its expression is closely related to ROS activity. They also showed that infection with *H. pylori*, which in gastric epithelial cells contributes to the initiation and progression of gastric cancer, stimulates ROS production and stabilizes HIF-1α even under normoxia. The mechanisms of HIF-1α expression in tumors are illustrated in Fig. 1. Based on the functions of proteins up-regulated by HIF-1, it may be expected that H1F-1 contributes to tumor progression and metastasis. The possible roles of HIF-1-targeted gene products in tumor promotion are summarized in Fig. 2. Indeed, immunohistochemical analyses in biopsy specimens have shown that HIF-1α is overexpressed in human tumors [33], and that its levels are associated with vascular density in various solid tumors [34]. In cancer patients, HIF-1α levels in tumors have been positively related to tumor aggressiveness and a poor prognosis [35–37]. Moreover, animal studies using xenografted human tumors have shown that the over-expression of HIF-1α enhances tumor growth and angiogenesis [38,39]. Taken together, HIF-1 is likely to play a central role in tumor growth and progression, and

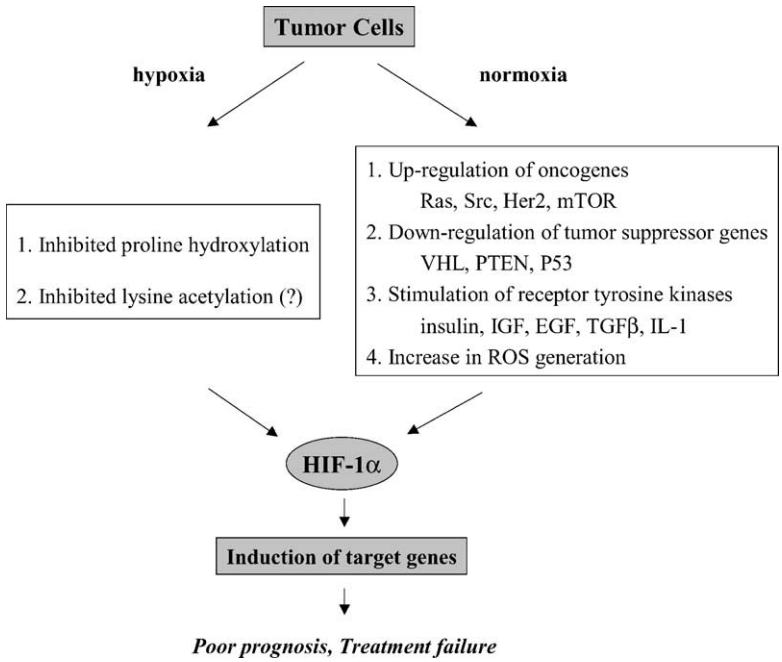
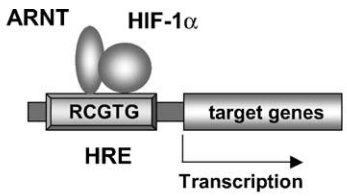


Fig. 1. Mechanisms of HIF-1α expression in tumor cells and the roles of HIF-1α in tumor promotion. HIF-1α is overexpressed in solid tumors by either hypoxic or non-hypoxic stimuli and up-regulates the expression of various genes that favor tumor growth and metastasis, and which confer resistance to anticancer therapies. (?): not well defined.



Blood Supply	Energy Production	Growth/Survival	Invasion/Metastasis	Resistance
α1B-AR	AK-3	Adrenomedullin	AMF	P-gp
Adrenomedullin	Aldolase- A & C	CA-9	Cathepsin D	
ANP	Enolase-1	Ceruloplasmin	Collagen V	
Endothelin 1	GAPDH	Cyclin G2	Fibronectin 1	
ENG	GLUT- 1 & 3	DEC1 & 2	Keratin 14, 18, 19	
ETS-1	HK- 1 & 2,	EPO	MMP-2	
HO-1	LDH-A	HO-1	PAI-1	
leptin	PGK-1	IGF-2	uPAR	
LRP1	PK-M	IGFBP-1, 2, 3	Vimentin	
NOS-2	TPI	NOS-2		
PAI-1		TGF-α		
TGF-β3		TGF-β3		
VEGF		Transferrin		
VEGF-R		Transferrin-R		
		VEGF		
		VEGF-R		

Fig. 2. HIF-1-targeted genes related with tumor promotion. Abbreviations: ADM, adrenomedullin; AK-3, adenylate kinase 3; AMF, autocrine motility factor; ANP, atrial natriuretic peptide; α_{1B}-AR, α_{1B}-adrenergic receptor; CA-9, carbonic anhydrase 9; DEC1 and 2, differentiated embryo-chondrocyte expressed gene 1 and 2; ENG, endoglin; EPO, erythropoietin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT-1 and -3, glucose transporter 1 and 3; HK-1 and 2, hexokinase 1 and 2; HO-1, heme oxygenase 1; IGF-2, insulin-like growth factor 2; IGFBP-1, -2 and -3, IGF-binding protein 1, 2 and 3; LDH-A, lactate dehydrogenase A; LRP1, LDL-receptor-related protein 1; MMP-2, matrix metalloproteinase 2; NOS-2, nitric oxide synthase 2; P-gp, P-glycoprotein multidrug resistance transporter; PAI-1, plasminogen activator inhibitor 1; PGK-1, phosphoglycerate kinase 1; PK-M, pyruvate kinase M; TGF-, transforming growth factor; TPI, triosphosphate isomerase; uPAR, urokinase plasminogen activator receptor; VEGF-R, VEGF receptor; VEGF, vascular endothelial growth factor.

strategies to inhibit HIF-1 may be the basis of appropriate anticancer therapies.

Since HIF-2α is expressed in a number of cancer cell lines and involved in hypoxic gene regulation, HIF-2α is also suggested to be associated with tumor promotion, but may not contribute to the growth of most tumors. In breast cancer cell lines that express both HIF-1α and HIF-2α, HIF-1α rather than HIF-2α appears to predominantly contribute to the transcriptional response to hypoxia [40]. However, HIF-2α may take over the role of HIF-1α in tumors that express only HIF-2α. Indeed, in VHL-defective 786-O renal cell carcinoma cells, the transcriptional response to hypoxia depended on expression levels of HIF-2α [40]. Moreover, the ectopic expression of HIF-2α led to enhanced growth of 786-O tumors grafted in nude mice [41,42]. Therefore, HIF-2α could be a target for cancer treatment.

4. Genetic approaches for HIF-1-targeting anticancer therapy

Many approaches have been made to inhibit the expression or the transcriptional activity of HIF-1. Antisense HIF-1α plasmid has been used to reduce HIF-1α levels at the genetic level. Sun et al. [43] demonstrated that HIF-1α expression in tumors is suppressed by the intratumoral gene transfer of an antisense HIF-1α plasmid, and that this leads to the down-regulation of VEGF and reduced tumor vascular density. Moreover, this gene therapy resulted in the complete and permanent rejection of small EL-4 tumors. Another genetic approach to inhibit HIF-1 activity made use of the dominant-negative isoforms of HIF-1α mRNA. After HIF-1α has been stabilized, the next essential steps - required prior to HIF-1 binding to the HRE consensus sequence and the transactivation of hypoxia-inducible genes are its nuclear translocation and dimerization with ARNT. Thus, these translocation and dimerization processes may be good targets for the inhibition of HIF-1 activity.

In this respect, two HIF-1α variant cDNAs that we recently found may be useful. One HIF-1-inhibitory cDNA was designated HIF-1αZ because it is induced by zinc [44]. This variant is derived from an alternatively spliced mRNA that lacks exon 12 of the *HIF1a* locus. The direct joining of exons 11 and 13 generates an immediate termination codon and a new short frame in the 13th exon. This alternative mRNA-splicing produces a truncated HIF-1α protein of 557 amino acids, which conserves both the bHLH and PAS domains of HIF-1α. However, HIF-1αZ loses the C-terminal end of ODDD, TAD, and NLS motifs, which results in impaired regulation by oxygen tension and a lack of transactivation activity. The expression of HIF-1αZ inhibits the hypoxic activation of HIF-1 and reduces the mRNA expressions of HIF-1-targeted genes. This inhibitory action of HIF-1αZ is due to its competing with HIF-1α for dimerization with ARNT.

The second HIF-1-inhibitory cDNA recently found is called HIF-$1\alpha^{516}$ [45]. This variant was derived from an alternatively spliced mRNA lacking exons 11 and 12 of the *HIF1a* locus. In this variant, exons 10 and 13 are directly joined, which generates an immediate termination codon. Compared to HIF-1αZ, HIF-$1\alpha^{516}$ is constitutively expressed in various human cell-lines and translates into a 516-amino acid polypeptide. HIF-$1\alpha^{516}$ conserves both the bHLH and PAS domains, but like HIF-1αZ lacks the C-terminal half of ODDD, and all TADs. Expressed HIF-$1\alpha^{516}$ was also found to inhibit HIF-1 activity in hypoxic cells and to reduce the mRNA expressions of HIF-1-targeted genes. HIF-$1\alpha^{516}$ also inhibits the HIF-1 dimerization process by sequestering ARNT.

In addition, although it is not an HIF-1α variant, inhibitory PAS domain protein (IPAS) may also usefully inhibit HIF-1 dimerization [46,47]. This protein is translated by an alternatively spliced variant of mouse HIF-3α mRNA, and contains exons 2–6 of the *HIF3α* locus; however, its 1 and 7 exons differ from those of wild-type HIF-3α mRNA. However, since exons 1 and 7 are also located in the *HIF3a* locus, this variant is considered a spliced mRNA transcribed by the *Hif3a* gene. The protein structure of IPAS is similar to those of the human HIF-1α variants, HIF-1αZ and HIF-$1\alpha^{516}$. It contains a bHLH domain and a PAS domain, but has no transactivation function due to the lack of TAD. It functions as a dominant-negative regulator of HIF-1-mediated gene induction. IPAS is expressed in hepatoma cells and selectively impairs the induction of hypoxia-inducible genes regulated by HIF-1, which result in retarded tumor growth and low vascular density in vivo. In mice, IPAS was found to be selectively expressed in Purkinje cells of the cerebellum and in the corneal epithelium of the eye. Moreover, IPAS expression in the cornea is associated with low levels of VEGF expression under hypoxic conditions.

The inhibitory process of HIF-1 dimerization by truncated HIF-1α or HIF-3α isoforms is illustrated in Fig. 3. However, although these anti-HIF-1 approaches using antisense plasmid and dominant-negative isoforms may be experimentally relevant in cell culture, it has proven to be almost impossible to achieve to any high therapeutic effect in patients. If techniques for in vivo gene transfer become more understood in the future, these genetic materials could provide new anticancer strategies.

5. Pharmacological approaches for an HIF-1-targeting anticancer therapy

Several approaches have been examined experimentally to chemically target cellular processes that regulate HIF-1α expression. For example, HSP90 is a molecular chaperone involved in the folding of HIF-1α protein [48,49]. A previous study of the HSP90 inhibitor geldanamycin showed that HIF-1α can be destabilized when HSP90

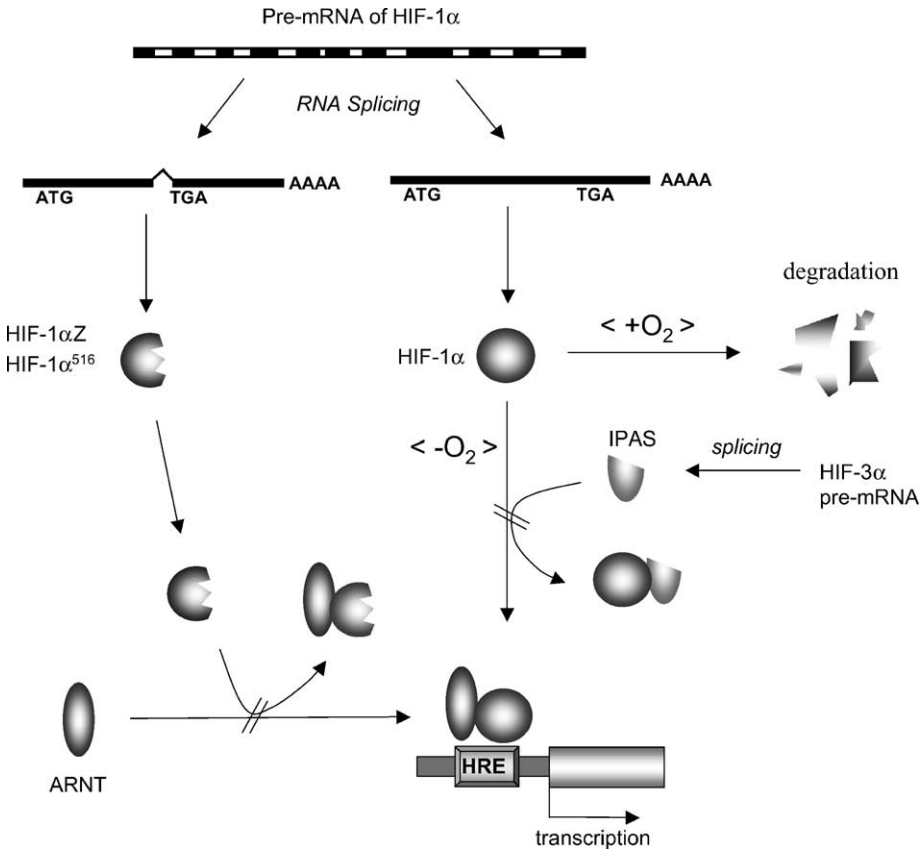


Fig. 3. Inhibition of HIF-1 dimerization by HIF-1α and HIF-3α isoforms. Truncated PAS-containing proteins are translated from the alternative splicing of HIF-1α or HIF-3α mRNA, and bind to the endogenous wild-types of HIF-1α or ARNT, thus competing with HIF-1α/ARNT dimerization. Abbreviations: $+O_2$, normoxic conditions; $-O_2$, hypoxic conditions.

binding is inhibited [50]. The oncogenes Ras [51] and Src [52] are also responsible for stabilizing HIF-1α. Pharmacological agents that inhibit the activity of the Ras oncogene, such as the farnesyl transferase inhibitors, could potentially exert anticancer effects by inhibiting HIF-1; moreover, the inhibition of Src kinase could be targeted for HIF-1 inhibitor development. On the other hand, tumor suppressor genes, such as VHL, PTEN, and p53, have been reported to inhibit HIF-1α expression. VHL protein binds to HIF-1α prolyl-hydroxylated by molecular oxygen, and in so doing targets HIF-1α for ubiquitin-mediated degradation [53]. PTEN protein also inhibits the cellular process of HIF-1α stabilization by antagonizing the phosphotidyl-3-inositol kinase (PI3K) pathway. In some cancer cells, such as prostate and breast cancer cells, HIF-1α is expressed even under aerobic conditions by PI3K pathway activation [54]. Another mechanism implicated in the negative regulation of HIF-1α is the binding of the p53 tumor suppressor gene to HIF-1 [55]. However, the role of p53 in the regulation of HIF-1α remains controversial. Therefore, it is possible that pharmacologic approaches taken to activate these tumor suppressor genes may also be applicable to the inhibition of HIF-1.

Signal transduction pathways can be targeted to inhibit HIF-1. A growing body of evidence indicates that the PI3K pathway modulates both the protein expression and the transcriptional activity of HIF-1α [29–31]. The growth factor-mediated activation of PI3K contributes to the expression of HIF-1α in tumors. This mechanism was first demonstrated in prostate cancer cells, in which HIF-1α is constitutively expressed even under aerobic conditions [29]. Growth factors reported to show this effect, include epidermal growth factor [29], insulin-like growth factor [56], insulin [31,57], and interleukin-1 [57]. These growth factors are known to bind to their receptors and to activate receptor tyrosine kinases, which in turn activate the PI3K/AKT/mTOR pathway. Finally, mTOR stimulates the translation of HIF-1α [29,30]. However, this signal transduction system appears to contribute to HIF-1α expression under aerobic rather than under hypoxic conditions. Therefore, the clinical usage of pharmacological agents inhibiting these pathways may be limited to the treatment of some unique tumors that overexpress HIF-1α under aerobic conditions. Of the anticancer agents that have been developed, mTOR inhibitors, such as rapamycin and CCI-779, and receptor tyrosine kinase inhibitors, such as trastuzumab, ZD-1839, and imatinib, are potentially re-evaluable as HIF-1 inhibitors.

Recently, two compound types—microtubule-targeting agents and topoisomerase-I inhibitors were reported to inhibit HIF-1α stability and transactivation by unidentified mechanisms. Microtubule polymerization and subsequent

depolymerization are features of mitosis, and agents that interfere with these processes lead to mitotic arrest and death in cancer cells. Moreover, many types of anticancer agents currently used may be categorized as microtubule-targeting agents. Recently, Mabjeesh et al. [58] demonstrated that these microtubule-targeting agents inhibit the expression of HIF-1α in normoxic and hypoxic cancer cells. Two microtubule destabilizing agents (2-methoxyestradiol and vincristine) and a microtubule stabilizing agent (taxol) were found to inhibit HIF-1α expression. And, the expressions of genes targeted by HIF-1, such as VEGF, glucose transporter 1, and endothelin 1, and HRE reporter activity were also found to be inhibited by these agents. It was also demonstrated that 2-methoxyestradiol has anti-angiogenic and anticancer effects on grafted human breast tumors in immune-deficient mice.

The topoisomerase-I inhibitors are the second type of compounds reported to inhibit HIF-1α stability. To identify HIF-1 inhibitors by mass screening, Rapisarda et al. [59] prepared a stable cell-line transfected with the luciferase reporter gene, which was under the control of three copies of a canonical hypoxia-responsive element (U251-HRE). After screening approximately 2000 compounds, they identified four that inhibited the HIF-1-dependent induction of luciferase. Of these four, three compounds were identified as analogues of CPT, which inhibits topoisomerase-I activity. CPT compounds belong to a class of chemotherapeutic agents that stabilize the complex formed between topoisomerase-I and DNA, which results in cell-cycle arrest and cell death. Although the mechanism of HIF-1 inhibition has not been determined, these compounds could be developed as anti-HIF agents because they are effective HIF-1 inhibitors.

6. Anti-HIF-1, anticancer effects of YC-1

YC-1 [3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole] was first described by Ko et al. [60]. In isolated platelets, YC-1 inhibits platelet aggregation, ATP release, phosphoinositide breakdown, and the elevation of intracellular free calcium. These pharmacological actions of YC-1 are derived from the activation of platelet soluble gualylate cyclase (sCG) and the elevation of cyclic guanosine monophosphate (cGMP). In mice, YC-1 was found to prevent intravascular thrombus formation by inhibiting platelet aggregation [60]. Similarly, YC-1 was found to activate sCG in vascular and corpus cavernosal smooth muscle cells, promoting vasodilation and penile erection [61–64]. Biochemical studies on expressed sGC showed that the stimulatory effect of YC-1 on sGC is enhanced by the physiologic sGC activators nitric oxide (NO) and carbon monoxide (CO) [65,66]. Indeed, combined treatment with YC-1 and natural sGC activators was found to synergistically inhibit platelet aggregation and to relax arterial smooth muscle. Thus, YC-1 is viewed as a research tool for investigating sGC- and cGMP-mediated cellular processes, and as a potential drug for treating platelet-rich thrombosis, vasospasm, or male erectile dysfunction.

However, we recently found that YC-1 has novel effects on HIF-1α expression and tumor promotion [67]. Initially, YC-1 was used as a NO mimic to examine the effects of NO signaling on hypoxic response in Hep3B hepatoma cells. It was found to diminish the hypoxic induction of both EPO and VEGF mRNA, and to suppress HRE-binding by enhancing HIF-1 and HIF-1α protein expression. It was also found to inhibit non-hypoxic HIF-1α induction by cobalt or desferrioxamine. However, sGC inhibitors failed to block these effects of YC-1 on HIF-1α, and further treatment with 8-bromo-cGMP also failed to inhibit the hypoxic induction of HIF-1α. These results indicate that the HIF-1-inhibitory effect of YC-1 is unlikely to be mediated by sGC/cGMP signal transduction, rather that the YC-1 effect is probably achieved by a novel cellular process linked with the oxygen-sensing pathway [67]. Since HIF-1 plays a crucial role in tumor promotion and angiogenesis, YC-1, as a novel HIF-1 inhibitor, could be further developed as a novel anticancer agent targeting HIF-1 and tumor angiogenesis. Indeed, YC-1 effectively halted tumor growth in immunodeficient mice grafted with five types of human tumor cells [68]. These YC-1-treated tumors showed reduced HIF-1α expression and poor

Table 1
Potential anticancer agents targeting HIF-1

Class	Inhibitor	Mechanism	Reference
sGC activator	YC-1	sGC/cGMP-independent inhibition of HIF-1α protein expression via uncertain target	[67,68]
HSP90 inhibitor	Geldanamycin radicicol	Destabilization of HIF-1α protein or inhibition of DNA binding of HIF-1	[48–50]
PI3K inhibitor	Wortmannin LY294002	Inhibition of HIF-1α protein expression by blocking the translational initiation of HIF-1α mRNA	[29–31,56,57]
mTOR inhibitor	Rapamycin CCI-779		
Microtubule modifier	2-Methoxyestradiol vincristine taxol	Inhibition of HIF-1α protein expression via uncertain target	[58]
Topoisomerase I inhibitor	Topotecan	Inhibition of HIF-1α protein expression via uncertain target	[59]
Inhibitor		Via uncertain target	

sCG, soluble guanylate cyclase; HSP70, heat-shock protein 90; PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin.

vascularization, and YC-1 suppressed the expressions of the HIF-1-regulated genes, i.e., VEGF and glycolytic enzymes, in these grafted tumor tissues. In addition, the suppression of HIF-1α by YC-1 was associated with blocked angiogenesis, and tumor growth inhibition in these YC-1-treated tumors. Moreover, YC-1 treatment did not cause serious toxicity or impair innate immunity during its treatment period [68]. Thus, we believe that YC-1 should be regarded as a good lead compound for the development of novel anti-angiogenic, anticancer agents [69,70]. YC-1 and other small molecules found to have anti-HIF or anticancer properties are summarized in Table 1.

7. Conclusion

It is clear that the modulation of HIF-1 activity can be good strategy for the treatment of a wide range of hypoxia- or ischemia-related pathologies. Moreover, HIF-1 activity upregulation may promote cell survival during hypoxia or ischemia, and increase angiogenesis at oxygen-deficient tissues. In contrast, the inhibition of HIF-1 activity could prevent angiogenic activity and the survival of pathological tissues with hypoxic or inflamed regions. Thus, HIF-1 inhibitors may be widely useful as therapeutic agents for the treatment of various diseases associated the over-activation of HIF-1, such as tumor cardiovascular remodeling, preeclamsia, and other angiogenesis-related diseases. However, since the experimental and clinical relevance of the association between HIF-1 and tumor aggressiveness has become more solid, the most compelling use of HIF-1 inhibitors is as anticancer agents. Much effort is being expended to develop new HIF-1-targeting agents by academia and the pharmaceutical industry. The success of these efforts will result in a new chemotherapeutic drug class, namely, HIF-1-targeting anticancer drugs, which hopefully will improve the prognoses of many cancer patients.

Acknowledgments

This work was supported by a Korean Research Foundation Grant, KRF-03-015-E00090.

References

[1] Saijo N, Tamura T, Nishio K. Strategy for the development of novel anticancer drugs. Cancer Chemother Pharmacol 2003;52:S97–101.
[2] Elsayed YA, Sausville EA. Selected novel anticancer treatments targeting cell signaling proteins. Oncologist 2001;6:517–37.
[3] Harris AL. Hypoxia—a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38–47.
[4] Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med 2002;8:S62–67.
[5] Blancher C, Harris AL. The molecular basis of the hypoxia response pathway: tumour hypoxia as a therapy target. Cancer Metastasis Rev 1998;17:187–94.
[6] Czyzyk-Krzeska MF. Molecular aspects of oxygen sensing in physiological adaptation to hypoxia. Respir Physiol 1997;110: 99–111.
[7] Bunn HF, Poyton RO. Oxygen sensing and molecular adaptation to hypoxia. Physiol Rev 1996;76:839–85.
[8] Semenza GL, Agani F, Booth G, Forsythe J, Iyer N, Jiang BH, et al. Structural and functional analysis of hypoxia-inducible factor 1. Kidney Int 1997;51:553–5.
[9] Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 1995;270:1230–7.
[10] Jiang BH, Rue E, Wang GL, Roe R, Semenza GL. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem 1996;271:17771–8.
[11] Jiang BH, Zheng JZ, Leung SW, Roe R, Semenza GL. Transactivation and inhibitory domains of hypoxia-inducible factor 1 alpha. Modulation of transcriptional activity by oxygen tension. J Biol Chem 1997;272:19253–60.
[12] Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1 alpha is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 1998;95:7987–92.
[13] Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science 2001;294:1337–40.
[14] Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, et al. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001;107:43–54.
[15] Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 2001;292:464–8.
[16] Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 2001;292:468–72.
[17] Masson N, Willam C, Maxwell PH, Pugh CW, Ratcliffe PJ. Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J 2001;20:5197–206.
[18] Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, Conaway JW. Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. Proc Natl Acad Sci USA 2000;97:10430–5.
[19] Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999;399:271–5.
[20] Masson N, Ratcliffe PJ. HIF prolyl and asparaginyl hydroxylases in the biological response to intracellular O(2) levels. J Cell Sci 2003;116:3041–9.
[21] Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, et al. Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell 2002;111:709–20.
[22] Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev 1997;11:72–82.
[23] Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii-Kuriyama Y. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development. Proc Natl Acad Sci USA 1997;94:4273–8.
[24] Gu YZ, Moran SM, Hogenesch JB, Wartman L, Bradfield CA. Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit. HIF3alpha. Gene Exp 1998;7:205–13.
[25] Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, et al. Induction of endothelial PAS domain protein-1 by hypoxia:

characterization and comparison with hypoxia-inducible factor-1alpha. Blood 1998;92:2260–8.
[26] Hara S, Hamada J, Kobayashi C, Kondo Y, Imura N. Expression and characterization of hypoxia-inducible factor (HIF)-3alpha in human kidney: suppression of HIF-mediated gene expression by HIF-3alpha. Biochem Biophys Res Commun 2001;287:808–13.
[27] Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992;3:65–71.
[28] Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med 2002;8:S62–67.
[29] Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, et al. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000;60:1541–5.
[30] Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. 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 2001;21:3995–4004.
[31] Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. Biol Chem 2002;277:27975–81.
[32] Park JH, Kim TY, Jong HS, Kim TY, Chun YS, Park JW, et al. Gastric epithelial reactive oxygen species prevent normoxic degradation of hypoxia-inducible factor-1alpha in gastric cancer cells. Clin Cancer Res 2003;9:433–40.
[33] Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, et al. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res 1999;59:5830–5.
[34] Zagzag D, Zhong H, Scalzitti JM, Laughner E, Simons JW, Semenza GL. Expression of hypoxia-inducible factor 1alpha in brain tumors: association with angiogenesis, invasion, and progression. Cancer 2000;88:2606–18.
[35] Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000;60:4693–6.
[36] Bos R, Zhong H, Hanrahan CF, Mommers EC, Semenza GL, Pinedo HM, et al. Levels of hypoxia-inducible factor-1 alpha during breast carcinogenesis. J Natl Cancer Inst 2001;93:309–14.
[37] Aebersold DM, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH, et al. Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res 2001;61:2911–6.
[38] Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, Stratford IJ, et al. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. Proc Natl Acad Sci USA 1997;94:8104–9.
[39] Ryan HE, Poloni M, McNulty W, Elson D, Gassmann M, Arbeit JM, et al. Hypoxia-inducible factor-1alpha is a positive factor in solid tumor growth. Cancer Res 2000;60:4010–5.
[40] Sowter HM, Raval RR, Moore JW, Ratcliffe PJ, Harris AL. Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. Cancer Res 2003;63:6130–4.
[41] Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin Jr WG. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. Cancer Cell 2002;1:237–46.
[42] Kondo K, Kim WY, Lechpammer M, Kaelin Jr WG. Inhibition of HIF2alpha Is Sufficient to Suppress pVHL-defective tumor growth. PLoS Biol 2003;1:439–44.
[43] Sun X, Kanwar JR, Leung E, Lehnert K, Wang D, Krissansen GW. Gene transfer of antisense hypoxia inducible factor-1 alpha enhances the therapeutic efficacy of cancer immunotherapy. Gene Ther 2001;8:638–45.
[44] Chun YS, Choi E, Yeo EJ, Lee JH, Kim MS, Park JW. A new HIF-1 alpha variant induced by zinc ion suppresses HIF-1-mediated hypoxic responses. J Cell Sci 2001;114:4051–61.
[45] Chun YS, Choi E, Kim TY, Kim MS, Park JW. A dominant-negative isoform lacking exons 11 and 12 of the human hypoxia-inducible factor-1alpha gene. Biochem J 2002;362:71–9.
[46] Makino Y, Cao R, Svensson K, Bertilsson G, Asman M, Tanaka H, et al. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. Nature 2001;414:550–4.
[47] Makino Y, Kanopka A, Wilson WJ, Tanaka H, Poellinger L. Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3alpha locus. J Biol Chem 2002;277:32405–8.
[48] Minet E, Mottet D, Michel G, Roland I, Raes M, Remacle J, et al. Hypoxia-induced activation of HIF-1: role of HIF-1alpha-Hsp90 interaction. FEBS Lett 1999;460:251–6.
[49] Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. J Biol Chem 2002;277:29936–44.
[50] Mabjeesh NJ, Post DE, Willard MT, Kaur B, Van Meir EG, Simons JW, et al. Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteosome pathway in prostate cancer cells. Cancer Res 2002;62:2478–82.
[51] Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. J Biol Chem 2001;276:9519–25.
[52] Jiang BH, Agani F, Passaniti A, Semenza GL. V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: involvement of HIF-1 in tumor progression. Cancer Res 1997;57:5328–35.
[53] Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999;399:271–5.
[54] Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, et al. Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev 2003;14:391–6.
[55] Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q, et al. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev 2000;14:34–44.
[56] Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 2002;277:38205–11.
[57] Stiehl DP, Jelkmann W, Wenger RH, Hellwig-Burgel T. Normoxic induction of the hypoxia-inducible factor 1alpha by insulin and interleukin-1beta involves the phosphatidylinositol 3-kinase pathway. FEBS Lett 2002;512:157–62.
[58] Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, et al. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell 2003;3:363–75.
[59] Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH, et al. Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. Cancer Res 2002;62:4316–24.
[60] Ko FN, Wu CC, Kuo SC, Lee FY, Teng CM. YC-1, a novel activator of platelet guanylate cyclase. Blood 1994;84:4226–33.
[61] Mulsch A, Bauersachs J, Schafer A, Stasch JP, Kast R, Busse R. Effect of YC-1, an NO-independent, superoxide-sensitive stimulator of soluble guanylyl cyclase, on smooth muscle responsiveness to nitrovasodilators. Br J Pharmacol 1997;120:681–9.

[62] Wegener JW, Nawrath H. Differential effects of isoliquiritigenin and YC-1 in rat aortic smooth muscle. Eur J Pharmacol 1997;323: 89–91.
[63] Mizusawa H, Hedlund P, Brioni JD, Sullivan JP, Andersson KE. Nitric oxide independent activation of guanylate cyclase by YC-1 causes erectile responses in the rat. J Urol 2002;167:2276–81.
[64] Hsieh GC, O'Neill AB, Moreland RB, Sullivan JP, Brioni JD. YC-1 potentiates the nitric oxide/cyclic GMP pathway in corpus cavernosum and facilitates penile erection in rats. Eur J Pharmacol 2003;458:183–9.
[65] Lee YC, Martin E, Murad F. Human recombinant soluble guanylyl cyclase: expression, purification, and regulation. Proc Natl Acad Sci USA 2000;97:10763–8.
[66] Friebe A, Koesling D. Mechanism of YC-1-induced activation of soluble guanylyl cyclase. Mol Pharmacol 1998;53:123–7.
[67] Chun YS, Yeo EJ, Choi E, Teng CM, Bae JM, Kim MS, et al. Inhibitory effect of YC-1 on the hypoxic induction of erythropoietin and vascular endothelial growth factor in Hep3B cells. Biochem Pharmacol 2001;61:947–54.
[68] Yeo EJ, Chun YS, Cho YS, Kim J, Lee JC, Kim MS, et al. YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. J Natl Cancer Inst 2003;95:516–25.
[69] Giaccia A, Siim BG, Johnson RS. HIF-1 as a target for drug development. Nat Rev Drug Discov 2003;2:803–11.
[70] Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721–32.