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Signal transduction to hypoxia-inducible factor 1

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Abstract

Hypoxia-inducible factor 1 (HIF-1) is a transcriptional activator that functions as a master regulator of O_2 homeostasis. HIF-1 target genes encode proteins that increase O_2 delivery and mediate adaptive responses to O_2 deprivation. HIF-1 activity is regulated by the cellular O_2 concentration and by the major growth factor-stimulated signal transduction pathways. In human cancer cells, both intratumoral hypoxia and genetic alterations affecting signal transduction pathways lead to increased HIF-1 activity, which promotes angiogenesis, metabolic adaptation, and other critical aspects of tumor progression.

Keywords: Growth factor; MAP kinase; Phosphatidylinositol 3-kinase; Prolyl hydroxylase; Receptor tyrosine kinase

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that functions as a master regulator of oxygen homeostasis. Over 40 HIF-1 target genes have been identified thus far which encode proteins that play key roles in critical developmental and physiological processes including angiogenesis/vascular remodeling, erythropoiesis, glucose transport, glycolysis, iron transport, and cell proliferation/survival (Table 1). HIF-1 is a heterodimer composed of an inducibly-expressed HIF-1α subunit and a constitutively-expressed HIF-1β subunit [1]. The unique feature of HIF-1 is the regulation of HIF-1α expression and activity based upon the cellular O_2 concentration. However, because HIF-1 is critical for a variety of essential biological processes, HIF-1α expression and activity are also regulated by major signal transduction pathways including those involving phosphatidylinositol 3-kinase (PI3K) and ERK/MAPK. In this review I will describe mechanisms by which hypoxia and growth factor signaling regulate HIF-1 and the implications of these findings for cancer biology and therapy.

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Abbreviations: HIF-1, hypoxia-inducible factor 1; PI3K, phosphatidylinositol 3-kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; VHL, von Hippel–Lindau tumor suppressor protein; VEGF, vascular endothelial growth factor; FIH-1, factor inhibiting HIF-1; FRAP, FKBP12-rapamycin associated protein.

1. Hypoxia signal transduction

HIF-1α is subject to rapid ubiquitination and proteasomal degradation under non-hypoxic conditions [2–4] and this process is inhibited under hypoxic conditions [5], resulting in an exponential increase in HIF-1α levels as the cellular O_2 concentration is decreased (Fig. 1), both in cultured cells [6] and *in vivo* [7]. The molecular basis for this regulation is the O_2-dependent hydroxylation of proline residues 402 and 564 in HIF-1α by any one of three enzymes in mammals that have been designated prolyl hydroxylase-domain proteins [8] or HIF-1α prolyl hydroxylases [9]. Prolyl hydroxylation of HIF-1α is required for the binding of the von Hippel–Lindau tumor suppressor protein (VHL) which is the recognition component of an E3 ubiquitin-protein ligase that targets HIF-1α for proteasomal degradation [10,11]. VHL loss-of-function in clear cell renal carcinomas results in constitutive overexpression of HIF-1α and downstream target genes such as *VEGF* which encodes vascular endothelial growth factor and accounts for the highly vascular nature of these tumors [12]. In addition to promoting ubiquitination and degradation of HIF-1α, VHL forms a ternary complex with HIF-1α and the co-repressor FIH-1 (factor inhibiting HIF-1), and both VHL and FIH-1 recruit histone deacetylases that may contribute to the loss of HIF-1α transcriptional activity under non-hypoxic conditions [13]. These findings

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Table 1
HIF-1 regulated genes

Gene product	References
Aminopeptidase A	[37]
Adenylate kinase 3	[38]
α1B-adrenergic receptor	[39]
Adrenomedullin	[40]
Aldolase A (ALDA)	[41,42]
Aldolase C	[41]
Carbonic anhydrase 9	[43]
Ceruloplasmin	[44]
Collagen type V, α1	[37]
DEC1	[37]
Endocrine gland-derived VEGF	[45]
Endothelin-1	[46]
Enolase 1	[41]
Erythropoietin	[47]
ETS-1	[48]
Glucose transporter 1 (GLUT1)	[38,41,42]
Glucose transporter 3	[41]
Glyceraldehyde-3-phosphate dehydrogenase	[41,42]
Heme oxygenase-1	[49]
Hexokinase 1	[41]
Hexokinase 2	[41]
Insulin-like growth factor 2 (IGF-2)	[14]
IGF binding protein 1	[50]
IGF factor binding protein 2	[14]
IGF factor binding protein 3	[14]
Intestinal trefoil factor	[51]
Lactate dehydrogenase A (LDHA)	[41,42]
LDL receptor-related protein 1	[37]
Nitric oxide synthase 2	[52,53]
NIP3	[54,55]
NIX	[55]
p21	[56]
p35srj	[57]
6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3	[58]
Phosphofructokinase L (PFKL)	[41]
Phosphoglycerate kinase 1	[41,42,56]
Plasminogen activator inhibitor 1	[59]
Prolyl-4-hydroxylase α(I)	[60]
Pyruvate kinase M (PKM)	[41]
Transferrin	[61]
Transferrin receptor	[62,63]
Transforming growth factor β_3	[64]
Transglutaminase 2	[37]
Triosephosphate isomerase	[41]
Vascular endothelial growth factor (VEGF)	[41,42,56]
VEGF receptor FLT-1	[65]

indicate that HIF-1 is regulated by a unique hypoxia signal transduction pathway that is based upon a unique oxygen-dependent posttranslational modification. They also demonstrate that tumor suppressor gene loss-of-function can lead to HIF-1α overexpression in human cancer cells.

2. Signaling of the PI3K-AKT-FRAP pathway to HIF-1α

Stimulation of cells with a variety of growth factors and cytokines, including epidermal growth factor (EGF), fibroblast growth factor 2, heregulin, insulin, insulin-like growth factor 1 and 2, and interleukin-1β induce the expression of HIF-1α protein, HIF-1 DNA-binding activity, and HIF-1 target gene expression under non-hypoxic conditions [14–17]. Binding of these ligands to their cognate receptor tyrosine kinases activates a variety of signal transduction pathways, including PI3K and the serine-threonine protein kinases AKT (protein kinase B) and FRAP (FKBP12-rapamycin associated protein; also known as mammalian target of rapamycin). Constitutive activity of the $HER2^{neu}$ receptor tyrosine kinase in NIH-3T3 cells or stimulation of $HER2^{neu}$ activity by heregulin in MCF-7 breast cancer cells leads to induction of HIF-1α protein expression that can be blocked by wortmannin or LY294002, inhibitors of PI3K, or by rapamycin, an inhibitor of FRAP [16]. $HER2^{neu}$ overexpression is a poor prognostic sign in human breast cancer, is associated with increased angiogenesis, and defines a subset of patients who are candidates for treatment with the monoclonal antibody Herceptin which binds to the receptor. Remarkably, the induction of HIF-1α protein expression in heregulin-treated MCF-7 cells is not due to decreased degradation but rather to increased protein synthesis and is mediated by nucleotide sequences in the 5′-untranslated region of the *HIF1A* gene [16]. These results are consistent with the fact that the only known targets of FRAP are p70 s6 kinase and eIF-4E binding protein, both of which function as regulators of translation. In addition to $HER2^{neu}$ overexpressing breast cancer cells, prostate cancer cells have been shown to overexpress HIF-1α and VEGF in a PI3K- and FRAP-dependent manner as a result of EGF receptor signaling [18]. *PTEN* is a tumor suppressor gene that encodes a phosphatase that dephosphorylates the products of the PI3K reaction and thus negatively regulates the PI3K-AKT-FRAP pathway. PTEN loss of function is associated with angiogenesis and tumor progression in gliomas and prostate cancers. Forced overexpression of recombinant PTEN in glioma cells results in a dramatic reduction in HIF-1α expression [19]. The rapamycin ester CCI-779 is presently in clinical trials as a cancer chemotherapeutic [20] and the data presented above suggest that its efficacy may be due in part to the inhibition of HIF-1 and downstream target genes.

3. Signaling of the MAP kinase pathway to HIF-1α

Receptor tyrosine kinase activity also leads to signaling *via* the ERK (p42 and p44) and p38 MAP kinase pathways. HIF-1α is phosphorylated by p42, p44, p38α, and p38γ *in vitro* [21,22], although the precise residues have not been defined. In CCL39 cells, RAF-1 overexpression is associated with phosphorylation of p42/p44 and a mobility shift of HIF-1α that is blocked by treatment with PD098059, an inhibitor of the MAP kinase kinase MEK-1. RAF-1 activity is associated with increased HIF-1-dependent reporter gene expression but no increase in the steady-state levels

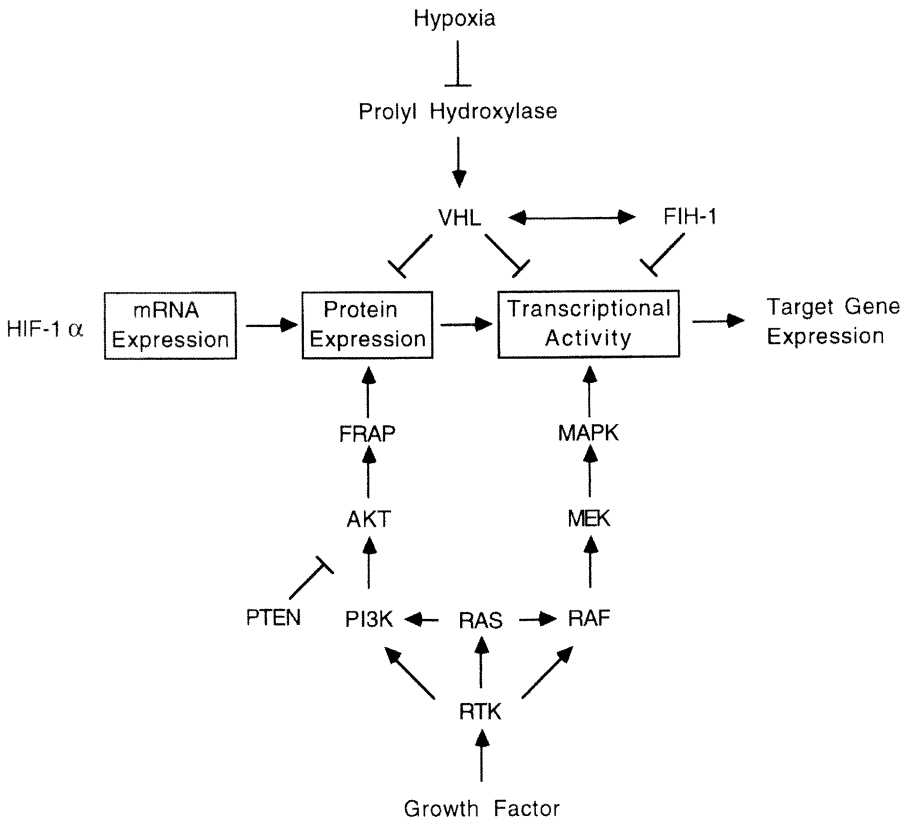

Fig. 1. Oxygen- and growth factor-regulated signal transduction pathways determine HIF-1α protein expression and transcriptional activity. HIF-1α is induced by hypoxia (*top*) in all cell types. In contrast, activation of the PI3K or MAPK pathway (*bottom*) has cell- and stimulus-specific effects on HIF-1α. See text for details and abbreviations.

of HIF-1α protein [21], suggesting an effect on HIF-1α transactivation domain function. A constitutively-active G protein-coupled receptor encoded by the Kaposi sarcoma-associated herpes virus also stimulates *VEGF* gene transcription in a HIF-1-dependent manner by increasing HIF-1α transactivation domain function, an effect that can be blocked by inhibitors of MEK1 (PD098059) and p38 (SKF86002) [22]. HIF-1α transactivation domain function in hypoxic Hep3B cells is also inhibited by PD098059 and, to a lesser extent, by SKF86002 [23]. Remarkably, activation of the PI3K-AKT-FRAP pathway by heregulin stimulation of MCF-7 cells results in increased HIF-1α protein synthesis but no stimulation of HIF-1α transactivation domain function [16], suggesting that in these cells MAP kinase activity does not stimulate transactivation domain function. Thus, the ability of MAP kinase to regulate HIF-1α may be stimulus- and/or cell-type-specific. It should be noted that H-RAS has been shown to induce VEGF expression *via* either the PI3K or MAP kinase pathway depending upon the cell type analyzed [24]. Increased *VEGF* gene transcription in H-RAS-transformed cells is dependent upon the presence of an intact HIF-1 binding site in the *VEGF* promoter [25]. Whereas H-RAS can stimulate HIF-1-dependent gene transcription, the small G protein RAC1 has been shown to be required for hypoxia-induced HIF-1α protein expression and transactivation domain function [23]. Thus, many essential signal transduction pathways modulate HIF-1 activity.

4. Implications for cancer biology and therapy

Intratumoral hypoxia and genetic alterations that dysregulate signal transduction pathways result in the dramatic overexpression of HIF-1α in the majority of human cancers analyzed by immunohistochemistry [26,27]. As described above, these tumor-specific physiologic and genetic alterations stimulate HIF-1α protein synthesis or stability as well as transactivation domain function (Fig. 1), although the latter cannot be assayed by immunohistochemistry. HIF-1α overexpression is associated with tumor VEGF expression and vascularization [28–30]. In breast cancer, this association is observed in pre-invasive ductal carcinoma *in situ*, indicating that HIF-1 is playing a critical role in the initiation of tumor angiogenesis [28]. Furthermore, HIF-1α overexpression is associated with treatment failure and/or patient mortality in oropharyngeal squamous cell cancer [31], early-stage cervical cancer [32], p53-mutant ovarian carcinoma [33], oligodendroglioma [34], and BCL2-positive esophageal cancer [35]. Genetic manipulations in human tumor xenograft models have established causal relationships between the level of HIF-1 activity and tumor

growth and angiogenesis (reviewed in [36]). Two hypotheses that have important therapeutic implications follow from these results. First, patients with high levels of HIF-1α overexpression demonstrated by immunohistochemical analysis of diagnostic tumor biopsies may require more aggressive therapy in order to survive their disease. This hypothesis will require clinical testing and of course can only be tested in those conditions where alternative therapies exist. Second, inhibition of HIF-1 activity may represent a novel approach to the treatment of cancers with high levels of HIF-1α overexpression. For example, patients with glioblastoma multiforme have a mean survival of less than 1 year regardless of therapy and these tumors uniformly express HIF-1α at high levels. A therapeutic window for HIF-1 inhibitors may exist because of the tremendous overexpression of HIF-1α in these tumors relative to surrounding normal tissue. This window may be opened further by combination therapy with angiogenesis inhibitors that would further increase intratumoral hypoxia and dependence upon HIF-1-mediated adaptive mechanisms.

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