Involvement of Hypoxia-Inducible Factor 1 in Human Cancer

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Abstract

Hypoxia-inducible factor 1 (HIF-1) mediates transcriptional responses to hypoxia. HIF-1 is composed of an O2- and growth factor-regulated HIF-1α subunit and a constitutively-expressed HIF-1β subunit. Four lines of evidence indicate that HIF-1 contributes to tumor progression. First, HIF-1 controls the expression of gene products that stimulate angiogenesis, such as vascular endothelial growth factor, and promote metabolic adaptation to hypoxia, such as glucose transporters and glycolytic enzymes, thus providing a molecular basis for involvement of HIF-1 in tumor growth and angiogenesis. Second, in mouse xenograft models, tumor growth and angiogenesis are inhibited by loss of HIF-1 activity and stimulated by HIF-1α overexpression. Third, immunohistochemical analyses of human tumor biopsies indicate that HIF-1α is overexpressed in common cancers and that the level of expression is correlated with tumor grade, angiogenesis, and mortality. Fourth, in addition to intratumoral hypoxia, genetic alterations in tumor suppressor genes and oncogenes induce HIF-1 activity.

Key words: angiogenesis, glycolysis, immunohistochemistry, oxygen

Introduction

The initial discoveries of molecular oncology focused on genes that when mutated resulted in increased cellular proliferation because this was a phenotype that could be easily assayed in tissue culture. The discovery of the crucial role of angiogenesis in tumor progression (1) has provided an example of a critical aspect of cancer biology that could not be well studied ex vivo. It has also focused attention on the metabolic requirements of tumor cells, as angiogenesis occurs as a response to tissue hypoxia (reviewed in ref. 2). In addition, many commonly-occurring mutations in oncogenes and tumor suppressor genes that result in increased cell proliferation have been found to also stimulate angiogenesis (2). Intratumoral microvascular density has been shown to be of prognostic significance in many common human cancers (3–5). Remarkably when cervical cancers and soft tissue sarcomas that are accessible to Eppendorf microelectrode measurements of intratumoral PO2 have been studied, a significant association has been observed between intratumoral hypoxia (PO2 <10 mm Hg or 1.5% O2) and invasion, metastasis, treatment failure, and patient death (6, 7). Thus, tumor progression is associated both with angiogenesis and intratumoral hypoxia. The basis for this apparent paradox is that tumor vasculature is structurally and functionally abnormal resulting in perfusion that is spatially and temporally heterogeneous (8). An important conclusion drawn from these findings is that tumor cell survival is dependent upon adaptation to O2 concentrations far below those present in normal tissue (reviewed in ref. 2).

Regulation of Gene Expression by HIF-1

When cells are exposed to hypoxia or growth factors, HIF-1α accumulates within the nucleus, dimerizes with HIF-1β, binds to target genes in a sequence-specific manner, and activates their transcription. Over 40 different HIF-1 target genes have been identified and the majority of these are known to play important roles in tumor progression such as angiogenesis, cell proliferation or survival, remodeling of the extracellular milieu, glucose metabolism, and iron homeostasis (Table 1; see ref. 9 for citations). Proteins encoded by these genes, such as vascular endothelial growth factor (VEGF) mediate increased O2 delivery via new blood vessel formation. Other protein products of HIF-1-regulated genes, such as glucose transporters and glycolytic enzymes allow the cell to adapt their metabolism to an O2-deprived environment. Finally, expression of a third group of gene products may influence the balance between apoptotic and anti-apoptotic signals that determines cell survival. In addition to the role of HIF-1 as a DNA binding protein, HIF-1α has also been shown to exert biological effects via protein-protein interactions. Under hypoxic conditions HIF-1α has been shown to interact with the tumor suppressor protein p53, which is itself a DNA-binding transcription factor. This interaction appears to increase the half-life of p53 (10) and decrease the half-life of HIF-1α as a result of increased ubiquitination.
Table 1. HIF-1-regulated Gene Products that Contribute to Tumor Progression

<table>
<thead>
<tr>
<th>Function</th>
<th>Gene Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiogenesis</td>
<td>Plasminogen activator inhibitor 1, vascular endothelial growth factor (VEGF), VEGF receptor FLT-1</td>
</tr>
<tr>
<td>Cell proliferation and survival</td>
<td>Adrenomedullin, cyclin G2, endothelin 1, erythropoietin, heme oxygenase 1, insulin-like growth factor (IGF) 2, IGF binding protein-1, -2, -3, nitric oxide synthase 2, NIP3, p21, transforming growth factor β</td>
</tr>
<tr>
<td>Extracellular microenvironment</td>
<td>Carbonic anhydrase 9, collagen type V (α1), prolyl-4-hydroxylase α (I)</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td>Glucose transporters GLUT1, GLUT3</td>
</tr>
<tr>
<td></td>
<td>Glycolytic enzymes aldolase A, aldolase C, glyceraldehyde-3-phosphate dehydrogenase, enolase 1, hexokinase 1, hexokinase 2, lactate dehydrogenase A, phosphofructokinase L, phosphoglycerate kinase 1, pyruvate kinase M, triosephosphate isomerase</td>
</tr>
<tr>
<td>Iron homeostasis</td>
<td>Ceruloplasmin, transferrin, transferrin receptor</td>
</tr>
<tr>
<td>Other transcription factors</td>
<td>DEC1, p35sri, peroxisomal proliferator-activated receptor α</td>
</tr>
</tbody>
</table>

by MDM2, a ubiquitin protein ligase that binds to p53 (11). The HIF-1α-mediated stabilization of p53 may contribute to hypoxia-mediated apoptosis, which may in turn represent a critical selective force for p53 loss of function (12).

Effect of Altering HIF-1 Activity in Mouse Xenograft Models

In order to determine whether HIF-1 activity is important for tumorigenesis, cultured tumor cell lines which have been genetically altered to increase or decrease HIF-1 activity have been analyzed in mouse xenograft assays. Loss of HIF-1 activity is associated with decreased angiogenesis and tumor growth (13–18). Forced overexpression of HIF-1α in HCT116 human colon carcinoma cells results in increased growth, vascular density, and vascular permeability of tumor xenografts (11). In addition to stimulation of angiogenesis, tumor xenograft growth is also stimulated by HIF-1 via its activation of glycolytic metabolism as a means of maintaining ATP levels under conditions of reduced O2 availability (19). These data indicate that the increased HIF-1α expression observed in human tumor biopsies (see below) is not an epiphenomenon but is an important factor contributing to tumor progression and clinical outcome.

Expression of HIF-1α in Human Cancer and its Clinical Significance

In order to analyze the expression of HIF-1α in human tumor biopsies, monoclonal antibodies were generated and immunohistochemical assays were developed (20, 21). Analysis of an increasing number of common human cancers has resulted in several important findings (Table 2). HIF-1α is overexpressed in most common human cancers, including breast, brain, cervical, colon, lung, ovarian, and prostate cancer. Significant tumor-specific associations have been found between HIF-1α overexpression and a variety of biomarkers including Ki67 (proliferation), VEGF or MVD (angiogenesis), and mutant p53 (resistance against apoptosis) (21–25). The most interesting and important results, however, have addressed the relationship of HIF-1α overexpression to overall survival, as described below.

In early-stage cervical carcinoma, HIF-1α overexpression is correlated with patient mortality (26). In early-stage esophageal cancer, HIF-1α overexpression is correlated with failure to achieve a complete response to photodynamic therapy (27). In oropharyngeal squamous cell carcinoma, the degree of HIF-1α overexpression is correlated with both radiation resistance and patient mortality (28). In contrast, HIF-1α overexpression is correlated with patient survival and increased apoptosis in non-small cell lung carcinoma (29). A recent analysis of ovarian cancers (22) provides important data that help interpret the lung cancer data. In ovarian cancer, HIF-1α overexpression was correlated with apoptotic rate as in the lung cancer study. However, in ovarian cancers expressing mutant p53, HIF-1α overexpression was not associated with apoptosis. Furthermore, the combination of mutant p53 and HIF-1α overexpression was associated with a highly significant (p<0.0001) seven-fold increased relative risk of patient mortality (22). In the study of early-stage esophageal cancer discussed above, the combination of HIF-1α overexpression and expression of the anti-apoptotic protein Bcl2 was significantly associated with treatment failure (27).

An important but not surprising conclusion is that the con...
Table 2. HIF-1α Immunohistochemistry and Human Cancer

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survey</td>
<td>Ki67, mutant p53</td>
<td>20, 21</td>
</tr>
<tr>
<td>Brain</td>
<td>grade, MVD⁴</td>
<td>25</td>
</tr>
<tr>
<td>Breast</td>
<td>grade, Ki67, VEGF, ER⁵, MVD (in DCIS⁶)</td>
<td>23</td>
</tr>
<tr>
<td>Cervix</td>
<td>mortality</td>
<td>26</td>
</tr>
<tr>
<td>Esophagus</td>
<td>response to photodynamic therapy</td>
<td>27</td>
</tr>
<tr>
<td>Wilms</td>
<td>VEGF²</td>
<td>24</td>
</tr>
<tr>
<td>Lung-NSCC³</td>
<td>apoptosis, survival</td>
<td>29</td>
</tr>
<tr>
<td>Oropharynx-SCC⁶</td>
<td>radiation resistance, mortality</td>
<td>28</td>
</tr>
<tr>
<td>Ovarian</td>
<td>MVD, mortality (with mutant p53)</td>
<td>22</td>
</tr>
</tbody>
</table>


quences of HIF-1α overexpression in a tumor are dependent upon the cancer type, tumor grade/stage, and/or the presence of other genetic alterations, especially mutations that inactivate pro-apoptotic tumor suppressors or activate anti-apoptotic oncoproteins. Most importantly, the finding of particular subpopulations of cancer patients in which HIF-1α overexpression results in a greatly increased risk of mortality may provide a means of identifying, at the time of initial diagnosis, patients who require more aggressive therapy in order to survive their disease.

Molecular Basis of HIF-1α Overexpression in Human Cancer

As described above, intratumoral hypoxia represents a major selective force during tumor progression. The adaptation to hypoxia occurs via two mechanisms. First, hypoxia directly increases the half-life of HIF-1α by decreasing the rate of ubiquitination and proteasomal degradation of the protein (30–33). Recent studies have demonstrated that the tumor suppressor protein VHL interacts with HIF-1α and targets the protein for ubiquitination (34–36) and that VHL loss of function results in the constitutive high-level expression of HIF-1α (37). The binding of VHL requires hydroxylation of HIF-1α on proline 564 by an enzyme that requires O₂, iron, and oxoglutarate as substrates, which is characteristic of prolyl-4-hydroxylases (38, 39).

In addition to the effects of intratumoral hypoxia on the half-life of HIF-1α, growth factor stimulation also induces HIF-1α expression (40). In the case of signaling via the HER2 receptor, activation of a signal transduction pathway involving phosphatidylinositol-3-kinase (PI3K) and the serine-threonine kinases AKT (protein kinase B) and FKBP-rapamycin-associated protein (mammalian target of rapamycin) has been shown to result in a dramatic increase in HIF-1α protein synthesis (41). Thus, gain-of-function mutations in oncoproteins which activate growth factor-PI3K signaling pathways lead to increased HIF-1α expression via this mechanism, as do loss-of-function mutations in the tumor suppressor gene encoding PTEN, a phosphatase that negatively regulates the PI3K pathway (42, 43). Activation of SRC, which lies downstream of receptor tyrosine kinases and upstream of the PI3K pathway, also induces HIF-1α expression (14). Most recently, the tumor suppressor p14ARF has been shown to sequester HIF-1α in the nucleolus such that p14ARF loss-of-function increases HIF-1 DNA-binding and transcriptional activity in cancer cells (44). Thus, the high level expression of HIF-1α in human cancers demonstrates both as a response to the physiological stimulus of hypoxia and as a result of tumor-specific genetic alterations (Table 3). The induction of HIF-1 activity provides a basis for the observation that mutations in oncogenes and tumor suppressor genes result in stimulation of VEGF expression and angiogenesis as well as the induction of aerobic glycolysis, also known as the Warburg effect (2).

Translating Basic Research into Clinical Applications

Do the basic science discoveries summarized above have potential clinical applications? First, HIF-1α immunohistochemistry on standard formalin-fixed and paraffin-embedded tumor biopsies obtained at the time of initial diagnosis may be useful in identifying individuals at high risk of failing standard therapy. The studies performed to date suggest that this approach may be useful for entire cancer types (e.g. oropharyngeal squamous cell cancer) or for certain subpopulations of patients (e.g. early-stage invasive cervical cancer) or may be useful in combination with other biomarkers (e.g. Bcl2 or mutant p53). Thus, for each cancer type the relationship of HIF-1α expression to disease outcome must be established. Subsequently, clinical trials will be necessary to determine whether more intensive clinical regimens may prolong survival of patients with HIF-1α-overexpressing cancers.

Second, a small molecule inhibitor of HIF-1 activity may be of therapeutic utility. Here, the issue of a therapeutic window is of great importance since complete systemic inhibition
Table 3. Molecular Mechanisms Underlying Increased HIF-1 Activity

<table>
<thead>
<tr>
<th>Alteration in tumor</th>
<th>Mechanism of HIF-1α induction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>decreased ubiquitination</td>
<td>29–32</td>
</tr>
<tr>
<td>VHL loss-of-function</td>
<td>decreased ubiquitination</td>
<td>33–38</td>
</tr>
<tr>
<td>p53 loss-of-function</td>
<td>decreased ubiquitination</td>
<td>1</td>
</tr>
<tr>
<td>PTEN loss-of-function</td>
<td>increased synthesis</td>
<td>40–42</td>
</tr>
<tr>
<td>PI3K/AKT/FRAP signaling</td>
<td>increased synthesis</td>
<td>40–42</td>
</tr>
<tr>
<td>SRC gain-of-function</td>
<td>increased synthesis</td>
<td>14, 40</td>
</tr>
<tr>
<td>p14ARF loss-of-function</td>
<td>decreased nuclear sequestration</td>
<td>43</td>
</tr>
</tbody>
</table>

of HIF-1 activity would not be tolerated. However, because tumor cells are more hypoxic and express higher levels of HIF-1α than normal cells, a therapeutic window may exist. Particularly appealing is the potential effect of combination therapy utilizing an angiogenesis inhibitor, which would cut off the tumor’s supply of oxygen, and a HIF-1 inhibitor, which would prevent hypoxic adaptation. High-throughput screening for inhibitors of HIF-1-mediated gene transcription, presently being performed by the Developmental Therapeutics Program of the National Cancer Institute (45), may identify small molecules for use in proof-of-principle studies in animal models and as lead compounds for the development of novel chemotherapeutic agents suitable for clinical testing.

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References

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