Hypoxia-Inducible Factors in Cancer

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Low oxygen concentrations (hypoxia) are detrimental to most species on Earth; thus, cells have evolved with adaptations allowing them to withstand transient hypoxia. As with other survival pathways, cancer cells have co-opted these mechanisms to keep up with the metabolic demands of rapid growth and proliferation in harsh tumor microenvironments. The most well-studied oxygen response pathway involves hypoxia-inducible factors (HIF) and their regulation by the von Hippel–Lindau protein (pVHL) and the prolyl hydroxylases (PHD1-3). This study from Zhong and colleagues, published in *Cancer Research* in 1999, was the first to show increased HIF1α expression in several cancer types and in metastases, suggesting a role for HIFs in disease progression. Since publication, significant progress has been made in the understanding of tumor hypoxia responses and efforts to target this pathway as a therapeutic strategy for patients with cancer are underway.

See related article by Zhong and colleagues, Cancer Res 1999;59:5830–5

Hypoxia-inducible factors (HIF) are now widely recognized as critical contributors to many features of solid tumors including angiogenesis, metabolic reprogramming, and metastasis, thanks to a series of discoveries from the 1990s through the early 2000s defining the hypoxia signaling pathway and its connection to cancer biology. One of these seminal studies comes from Zhong and colleagues who observed increased HIF1α expression in primary and metastatic tumor samples compared with normal tissue in several cancer types (1).

HIFs are the major transcription factors involved in the hypoxic response (2), acting as heterodimers and binding to hypoxia response elements (HRE) in the regulatory regions of target genes, many of which are involved in protumorigenic processes. The HIFα (HIF1α or HIF2α) subunit is tightly regulated and only expressed under low oxygen conditions while the HIF1β subunit is constitutively expressed. In normoxia, the von Hippel Lindau (VHL) complex (consisting of pVHL, elongin BC, and CUL2) polyubiquitylates HIFα subunits, tagging them for proteasomal degradation. Polyubiquitylation by the VHL complex requires hydroxylation of two proline residues within the oxygen-dependent degradation domain of HIFα, which is catalyzed by the prolyl hydroxylases (PHD1-3). This PHD hydroxylation reaction requires oxygen, iron, and α-ketoglutarate, and produces carbon dioxide and succinate. Hypoxia prevents hydroxylation of the HIFα subunits, blocking subsequent ubiquitylation by VHL and preventing proteasomal degradation. HIFα can thereby dimerize with HIF1β, bind to HREs, and promote transcription of target genes. Examples of HIF target genes known to be involved in tumor progression include angiogenic factors *VEGF* and *PDGF* and metabolic genes *GLUT1*, *HK2*, and *LDHA*.

In their article, Zhong and colleagues provide some of the first *in vivo* evidence that HIF1α could play a major role in tumor progression through both physiologic (induced by hypoxia) and nonphysiologic mechanisms (1). Using IHC analysis, Zhong and colleagues interrogated 174 normal tissue samples, 131 primary tumors, 36 metastatic tumors, and 12 benign tumors across many tissue types for HIF1α expression. They found that while most of the normal tissues did not express HIF1α, both primary and metastatic tumor samples had much higher HIF1α accumulation. Furthermore, the authors correlated HIF1α expression with p53 accumulation, indicating oncogenic alterations could also affect HIF1α expression.

We now have a much better understanding of the reasons for elevated HIFα expression in cancer. As solid tumors grow beyond the diffusion limits of oxygen, they initiate the hypoxia program, stabilizing HIF and promoting transcription of proangiogenic factors like *VEGF* (**Fig. 1**). However, blood vessels formed through tumor angiogenesis are often tortuous and leaky, with poor tissue perfusion. As such, most solid tumors remain hypoxic and cancer cells must adapt to oxygen starvation to overcome an intrinsically stressful tumor microenvironment, often co-opting the hypoxia-induced signaling pathways required for normal cell survival in times of transient low oxygen. This is best characterized in clear cell renal cell carcinoma (ccRCC), where approximately 90% of tumors inactivate pVHL, leading to constitutive stabilization of HIFα. Increased HIF1α expression in metastases presented in this study is also consistent with this idea, as cells that can survive prolonged hypoxia are selected for and can go on to metastasize (**Fig. 1**; ref. 3).

Significant advances have been made in the understanding of HIF biology in cancer, particularly in deciphering the distinct roles of family members HIF1α and HIF2α. These studies have also revealed that surprisingly, HIF1α expression is not always oncogenic. While the findings in the highlighted study suggest a correlation of HIF1α with worse prognosis in many cancer types, studies in neuroblastoma and ccRCC have shown that HIF1α expression is correlated with a favorable prognosis (3). Furthermore, in some cancer types, HIF2α is correlated with prognosis while HIF1α is not, suggesting that the roles of each HIFα family member are unique, tissue dependent, and should be considered when targeted for cancer therapy.

Genetic approaches have helped to clarify contexts in which HIF can promote or inhibit tumor growth. Blockade of HIF1α degradation by pVHL in ccRCC demonstrated that HIF1α stabilization alone was not sufficient to induce xenograft growth, suggesting HIF1α may not be the critical substrate of VHL (4). In addition, ectopic expression of HIF1α in $VHL^{-/-}$ ccRCC cell lines inhibited cell proliferation and tumor growth (5). Conversely, expression of pVHL degradation-resistant HIF2α overrides the effects of pVHL reexpression, leading to tumor growth in xenograft models of ccRCC (6). Together, this

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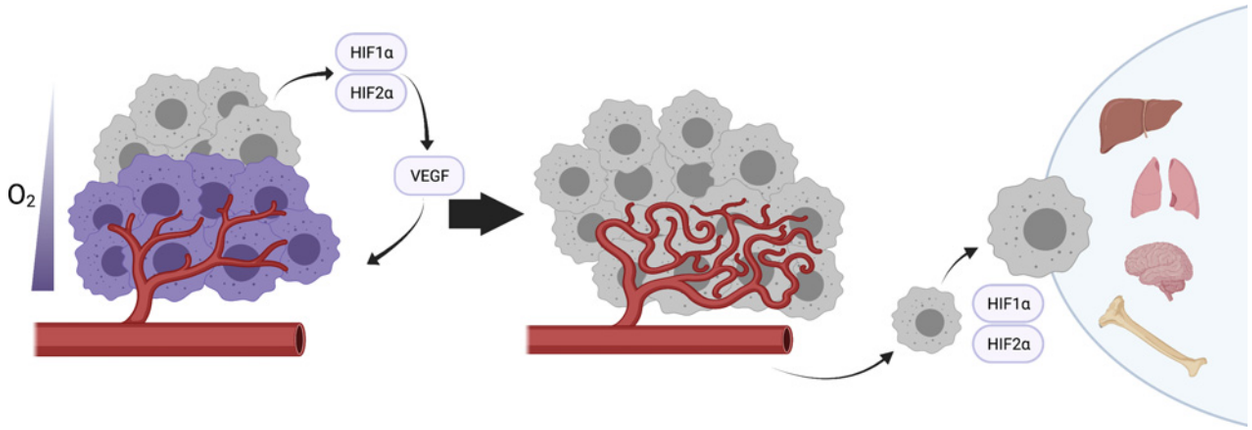

Figure 1.
As tumors grow beyond the diffusion limits of O_2, cancer cells stabilize HIFα, upregulating expression of HIF target genes, which include angiogenic factors like VEGF. Blood vessels formed during tumor angiogenesis are often tortuous and leaky, leading to poor tissue oxygen perfusion. Cancer cells must constitutively express HIF to survive in this perpetually hypoxic environment. These cells are primed for metastasis, as they are preselected to survive in harsh environments. In addition, several genes known to be involved in invasion and metastasis are regulated by HIF.

suggests that in ccRCC, HIF1α is a tumor suppressor while HIF2α is an oncogene. It should be noted that in other cancer types, including in gliomas and KRAS-mutant lung cancer, HIF2α acts as a tumor-suppressor (7, 8). Analysis of HIF target genes shows that HIF1α and HIF2α regulate expression of many overlapping but also unique targets, likely mediating these differential effects (9).

Targeting HIF has long been an attractive goal for cancer therapy, but because of the difficulties involved with targeting transcription factors, HIF has historically been considered undruggable. However, discovery of a druggable cavity in HIF2α has led to the identification of small-molecule inhibitors that allosterically disrupt its heterodimerization with HIF1β and can safely be used in humans (10). Positive results from clinical trials have led to FDA approval of the first HIF2α inhibitor (Belzutifan, from Merck) for treatment of *VHL*-associated RCC. Investigations are ongoing to maximize the therapeutic efficacy of this inhibitor (such as through combinations with other therapies and research into resistance mechanisms) as well as continued efforts to develop a HIF1α inhibitor.

In the years since the discovery of overexpressed HIF1α in cancer, the importance of the hypoxia response pathway in human biology has been widely recognized—including through the awarding of the 2019 Nobel Prize in Physiology or Medicine to William G. Kaelin Jr, Sir Peter J. Ratcliffe, and Gregg L. Semenza for their pioneering work on oxygen sensing and adaptation to hypoxia. These fundamental studies helped to shape our current understanding of the complex and dynamic nature of local tumor ecosystems. We anticipate many more exciting discoveries in the field of HIF biology, particularly through investigations into noncanonical mechanisms of HIF regulation and oxygen-dependent interactions between multiple cell types in local microenvironments. As cancer research and therapy increasingly shifts to considering the entire tumor microenvironment, we will continue to see their impact on future patient care.

Authors' Disclosures

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