

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

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 HIF1 α 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 HIF α (HIF1 α or HIF2 α) subunit is tightly regulated and only expressed under low oxygen conditions while the HIF1 β subunit is constitutively expressed. In normoxia, the von Hippel Lindau (VHL) complex (consisting of pVHL, elongin BC, and CUL2) polyubiquitylates HIF α subunits, tagging them for proteasomal degradation. Polyubiquitylation by the VHL complex requires hydroxylation of two proline residues within the oxygen-dependent degradation domain of HIF α , 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 HIF α subunits, blocking subsequent ubiquitylation by VHL and preventing proteasomal degradation. HIF α can thereby dimerize with HIF1 β , 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 HIF1 α 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 HIF1 α expression. They found that while most of the normal tissues did not express HIF1 α , both primary and metastatic tumor samples had much higher HIF1 α accumulation. Furthermore, the authors correlated HIF1 α expression with p53 accumulation, indicating oncogenic alterations could also affect HIF1 α expression.

We now have a much better understanding of the reasons for elevated HIF α 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 micro-environment, 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 HIF α . Increased HIF1 α 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 HIF1 α and HIF2 α . These studies have also revealed that surprisingly, HIF1 α expression is not always oncogenic. While the findings in the highlighted study suggest a correlation of HIF1 α with worse prognosis in many cancer types, studies in neuroblastoma and ccRCC have shown that HIF1 α expression is correlated with a favorable prognosis (3). Furthermore, in some cancer types, HIF2 α is correlated with prognosis while HIF1 α is not, suggesting that the roles of each HIF α 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 HIF1 α degradation by pVHL in ccRCC demonstrated that HIF1 α stabilization alone was not sufficient to induce xenograft growth, suggesting HIF1 α may not be the critical substrate of VHL (4). In addition, ectopic expression of HIF1 α in *VHL*^{-/-} ccRCC cell lines inhibited cell proliferation and tumor growth (5). Conversely, expression of pVHL degradation-resistant HIF2 α overrides the effects of pVHL reexpression, leading to tumor growth in xenograft models of ccRCC (6). Together, this

study from Zhong and colleagues, published in *Cancer Research* in 1999, was the first to show increased HIF1 α 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

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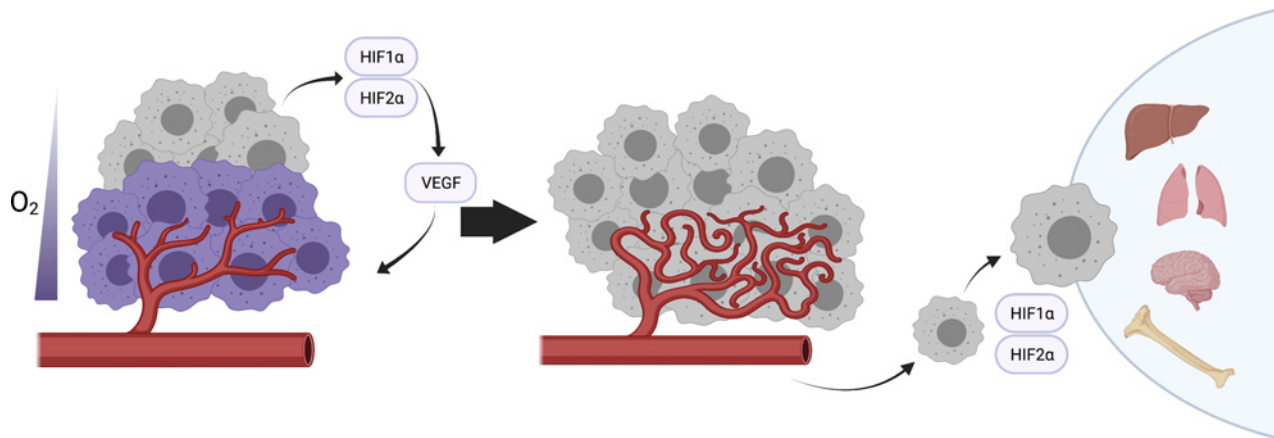


Figure 1.

As tumors grow beyond the diffusion limits of O_2 , cancer cells stabilize HIF α , 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, HIF1 α is a tumor suppressor while HIF2 α is an oncogene. It should be noted that in other cancer types, including in gliomas and KRAS-mutant lung cancer, HIF2 α acts as a tumor-suppressor (7, 8). Analysis of HIF target genes shows that HIF1 α and HIF2 α 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 HIF2 α has led to the identification of small-molecule inhibitors that allosterically disrupt its heterodimerization with HIF1 β and can safely be used in humans (10). Positive results from clinical trials have led to FDA approval of the first HIF2 α 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 HIF1 α inhibitor.

In the years since the discovery of overexpressed HIF1 α 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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References

- Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, et al. Overexpression of hypoxia-inducible factor 1 α in common human cancers and their metastases. *Cancer Res* 1999;59:5830–5.
- Lee P, Chandel NS, Simon MC. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nat Rev Mol Cell Biol* 2020;21:268–83.
- Keith B, Johnson RS, Simon MC. HIF1 α and HIF2 α : sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer* 2012;12:9–22.
- Maranchie JK, Vasselli JR, Riss J, Bonifacino JS, Linehan WM, Klausner RD. The contribution of VHL substrate binding and HIF1- α to the phenotype of VHL loss in renal cell carcinoma. *Cancer Cell* 2002;1:247–55.
- Shen C, Beroukhi R, Schumacher SE, Zhou J, Chang M, Signoretti S, et al. Genetic and functional studies implicate HIF1 α as a 14q kidney cancer suppressor gene. *Cancer Discov* 2011;1:222–35.
- Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin WG. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. *Cancer Cell* 2002;1:237–46.
- Acker T, Diez-Juan A, Aragonés J, Tjwa M, Brusselmans K, Moons L, et al. Genetic evidence for a tumor suppressor role of HIF-2 α . *Cancer Cell* 2005;8:131–41.
- Mazumdar J, Hickey MM, Pant DK, Durham AC, Sweet-Cordero A, Vachani A, et al. HIF-2 α deletion promotes Kras-driven lung tumor development. *Proc Natl Acad Sci U S A* 2010;107:14182–7.
- Hu C-J, Wang L-Y, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1 α (HIF-1 α) and HIF-2 α in hypoxic gene regulation. *Mol Cell Biol* 2003;23:9361–74.
- Choueiri TK, Kaelin WG. Targeting the HIF2-VEGF axis in renal cell carcinoma. *Nat Med* 2020;26:1519–30.