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Hypoxia-inducible factors in cancer stem cells and inflammation

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Hypoxia-inducible factors (HIF) mediate metabolic switches in cells in hypoxic environments, including those in both normal and malignant tissues with limited supplies of oxygen. Paradoxically, recent studies have shown that cancer stem cells (CSCs) and activated immune effector cells exhibit high HIF activity in normoxic environments and that HIF activity is critical in the maintenance of CSCs as well as the differentiation and function of inflammatory cells. Given that inflammation and CSCs are two major barriers to effective cancer therapy, targeting HIF may provide a new approach to developing such treatments.

Challenges in targeting the tumor microenvironment

An important paradigm shift in cancer research has been the realization that cancer tissue is not a homogenous population of clonally expanded cancer cells [1–3]. It has been established in multiple cancer types that cancer cells are hierarchical: while a small subset of CSCs have a high capacity for self-renewal and are responsible for initiating cancer, the bulk of cancer cells lack self-renewal and cancer-initiating capacity [3,4]. Perhaps because conventional therapeutic approaches were not developed to target CSCs, many such cells are enriched by conventional cancer therapy [5,6]. The ineffective elimination of CSCs is considered a major cause of cancer relapse following conventional therapy and, thus, is a major barrier to effective cancer treatment [7].

In addition to heterogeneity among transformed cancer cells, cancer tissues also comprise noncancerous host cells [1,2]. Together, cancer cells and host cells form a tumor microenvironment that enables tumor initiation and progression. The dependence of cancer on the host cells suggests that these host cells could also be targeted for cancer therapy. Again, because conventional cancer therapy was developed without emphasizing the tumor microenvironment, a major new focus for cancer therapy is to limit cancer development by targeting this microenvironment.

Based on these considerations, it would be of interest to identify druggable targets that are critical for CSCs and tumor-promoting microenvironments. In this context, studies have highlighted the selective activation of the HIF pathway and metabolic switch of CSCs [8–10]. Remarkably, recent studies demonstrated that HIF may have a major role in inflammation, including the adaptive and innate inflammatory responses [11–13]. The shared requirement for HIF in CSCs and inflammatory cells raised the interesting prospect that CSCs and inflammation, two important challenges in cancer therapy, may be addressed by targeting HIF. Here, we review the critical role for HIF in immunology and cancer biology, with a focus on the potential cross-fertilization of HIF research on cancer cells and host inflammatory cells. We also explore the translational potential of this new concept. Readers are referred to outstanding recent reviews for the general concept and involvement of HIF in cancer biology and immunology [11,14,15].

HIF and cancer: an overview

The modes of energy production in a cell are usually dictated by the oxygen levels in the environment: oxidative phosphorylation occurs in a well-oxygenated environment (normoxia), while glycolysis is switched on when oxygen levels drop below 1% (hypoxia) [15]. In a search for the fundamental mechanism of the oxygen-mediated metabolic switch, Gregg Semenza and colleagues identified HIF-1α an oxygen-sensitive transcriptional activator [16]. Recent studies suggest that, by directly regulating the expression and activity of pyruvate kinase muscle isozyme 2 (PKM2), HIF-1α can serve as a master switch for oxygen regulation in cellular metabolism [17].

As illustrated in Figure 1, HIF is a heterodimer comprising α and β subunits. The heterodimers translocate into the nucleus, where they interact with specific DNA sequences called HIF-responsive elements (HREs). By binding to the HRE, HIF may either activate or repress gene expression. At least three different genes have been identified that encode a subunit of HIF, namely HIF1α, HIF2α and HIF3α. All three HIFα subunits heterodimerize with a HIF-1β subunit and are subject to post-translational regulations that are dictated by the oxygen concentration in the environment. Although HIF-3α lacks a transactivation domain and is generally considered to be a negative regulator for HIF-1α and HIF-2α function, a notable exception was reported recently [18]. Despite the
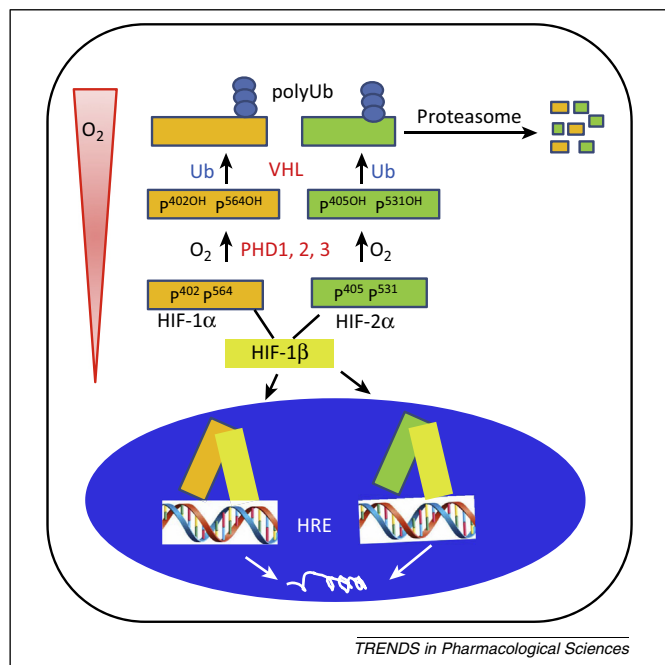
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Figure 1. Regulation of hypoxia-inducible factors (HIF) activity in response to oxygen levels. Under hypoxic conditions, stabilized HIF-1α and HIF-2α dimerize with HIF-1β. The heterodimers translocate into the nucleus to regulate gene transcription. In the presence of oxygen, HIF-1α and HIF-2α proteins are hydroxylated by prolyl hydroxylase domain proteins (PHD) 1–3 at the proline residues indicated. Hydroxylated HIF is recognized by von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase (VHL), which causes polyubiquitinylation (polyUb) and proteasome-mediated degradation of HIF. Abbreviation: HRE, HIF-responsive element.

general similarity in regulation and function between HIF-1α and HIF-2α, they differ in their sensitivity to oxygen deprivation, and target gene binding and tissue distribution [14].

The critical role of HIF in cancer biology was established when a major renal tumor suppressor gene von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase (*VHL*) was identified as the E3 ligase responsible ubiquitinylation of HIF-1α and HIF-2α [19–23]. VHL recognizes hydroxylated residues at Pro402 and/or Pro562 in HIF-1α and Pro 405 and Pro 531 in HIF-2α, by means of the prolyl hydroxylase domain protein (PHD) [24]. VHL is inactivated in most renal cancer samples, leading to increased expression of the HIF-1α and HIF-2α proteins [19–23]. Mutations of PHD protein-encoding genes, EGLN1 (PHD2), EGLN2 (PHD1), and EGLN3 (PHD3) have been observed in various cancers at low frequency (http://www.cbioportal.org/). The function of PHD is enhanced by isocitrate dehydrogenase 1 (IDH1) and/or IDH2 [25,26], which are mutated at a combined frequency of approximately 15% in patients with acute myeloid leukemia (AML) [27–30] and low-grade glioma or glioblastoma [31]. Two reports suggested that IDH mutations lead to increase in HIF-1α accumulation [25,26], while a more recent study suggests otherwise [32].

Overexpression of HIF-1α and/or HIF-2α is a marker for poor prognosis in most cancer types tested, including common cancers, such as breast, prostate, colon, hepatocellular, pancreatic, brain, and ovarian cancers, as well as a host of less common cancers [14]. In transgenic mouse

cancer models, heterozygous deletion of *Hif1a* reduced the growth of thymic lymphoma [33], while short hairpin (sh)RNA silencing of *Hif1a* and overexpression of VHL strongly reduced leukemia-initiating activity [9].

Despite the overwhelming association between HIF levels in cancer tissue and poor prognosis of patients with cancer, conflicting reports have emerged in renal cancer, where overexpression of HIF-1α has been associated with either a poor or favorable prognosis, depending on the methods used to evaluate HIF-1α levels [34,35]. In mouse cancer models, recent studies suggest that, while local deletion of *Hif1a* in lung tissue had no impact on KRas-driven lung cancer, deletion of *Hif2a* paradoxically accelerated lung cancer development [36]. More recently, broad deletion of *Hif1a* in adult mice, including in the cells that give rise to leukemia, promoted, rather than suppressed Mll-AF9a-induced leukemia [37]. These contradicting observations may be explained by the broad spectrum of HIF target genes: the cancer-promoting effect is exemplified by HIF-mediated induction and function of PKM2, vascular endothelial cell growth factor (VEGF), and multidrug resistance gene (MDR1), while its function as a tumor suppressor can be explained by both its transcriptional and nontranscriptional activity.

PKM2 and metabolic switches in cancer cells

PKM2 is the final enzyme in glycolysis and comprises two isoforms that arise from alternative splicing. Most tissues express the PKM1 isoform with products directed into oxidative phosphorylation to efficiently produce ATP. By contrast, PKM2 may exist either in tetrameric or dimeric forms to direct oxidative phosphorylation or glycolysis, respectively. Given that PKM2 exists predominantly in its dimeric form in cancer cells, it contributes to a high rate of aerobic glycolysis in cancer cells regardless of hypoxia, a phenomenon known as the Warburg effect. HIF regulates PKM2 through two mechanisms [17]: first, HIF-1α can stimulate expression of PKM2. Second, PKM2 and HIF-1α form heterodimers and migrate into the nuclei, where they act as a master switch to overexpress genes crucial for a robust glycolysis that produces both energy and metabolic intermediates for biosynthesis. Therefore, HIF-1α has a critical role in metabolic switches in cancer cells. In addition to PKM2, HIF-1α has been shown to antagonize cMyc activity by inducing expression of MXl1, thus reducing mitochondrial biogenesis [38].

VEGF and cancer neoangiogenesis

The increase in tumor volume demands a corresponding increase in angiogenesis. By regulating VEGF expression, both cellular and viral oncogenes not only regulate the growth of cancer cells, but also allow cancer progression in the host. Optimal VEGF expression was found to depend on both hypoxia and oncogenes [39]. These observations led to the identification of HIF-1α as a major regulator of VEGF expression [40,41]. The HIF-1α–p300/CREB-binding protein (CBP) complex binds to a HIF-responsive element in the 5′ promoter region of the gene encoding VEGF [40,41]. This HRE sequence was specifically targeted by echinomycin [9,42].