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Hypoxia-Inducible Factors in Cancer Stem Cells and Inflammation

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Abstract

Hypoxia-inducible factors (HIF) mediate metabolic switch 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 and activated immune effector cells exhibit high HIF activity in normoxic environments and that HIF activity is critical in maintenance of cancer stem cells as well as differentiation and function of inflammatory cells. Since inflammation and cancer stem cells are two major barriers to effective cancer therapy, targeting HIF may provide a new approach for the ultimate challenges.

Keywords

Leukemia-initiating cells; tumor microenvironment; myeloid-derived suppressor cells; dendritic cells; tumor-associated macrophages; cancer immunotherapy

Challenges in targeting 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 cancer stem cells 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 cancer stem cells, many cancer stem cells are enriched by conventional cancer therapy [5, 6]. The ineffective elimination of cancer stem cells is considered as a major cause for cancer relapses following conventional therapy, and thus presents as a major barrier to effective cancer treatment [7].

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In addition to heterogeneity among transformed cancer cells, cancer tissues also consist of non-cancerous 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 suggest that these host cells may also be targeted for cancer therapy. Again, since conventional cancer therapy was developed without emphasizing tumor microenvironment, a major new focus for cancer therapy is to limit cancer development by targeting the tumor microenvironment.

Based on these considerations, it would be of interest to identify druggable targets critical for cancer stem cells and tumor-promoting microenvironments. In this context, we and others have revealed selective activation of the HIF pathway and metabolic switch of cancer stem cells [8–10]. Remarkably, recent studies have demonstrated that HIF may play a major role in inflammation, including the adaptive and innate inflammatory response [11] [12, 13]. The shared requirement for HIF in cancer stem cells and inflammatory cells raised the interesting prospect that cancer stem cells 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 focus on potential cross-fertilization of HIF research on cancer cells and host inflammatory cells, and 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-1 α , an oxygen-sensitive transcriptional activator [16]. Recent studies suggest that by directly regulating expression and activity of pyruvate kinase muscle isozyme 2 (PKM2), HIF-1 α can serve as a master switch for oxygen-regulation in cellular metabolism [17].

As illustrated in Figure 1, HIF is a heterodimer consisting of 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, called HIF1 α , HIF2 α and HIF3 α . All three HIF α subunits heterodimerize with a HIF-1 β subunit and are subject to posttranslational regulations that are dictated by oxygen concentration in the environment. Although HIF-3 α lacks a transactivation domain and is generally considered to be a negative regulator for HIF-1 α and HIF-2 α function, a notable exception was reported recently [18]. Despite the general similarity in regulation and function between HIF-1 α and HIF-2 α , they differ in their sensitivity to oxygen-deprivation, target gene binding and tissue distribution [14].

The critical role of HIF in cancer biology was established when a major renal tumor suppressor gene VHL was identified as the E3 ligase responsible ubiquitinylation of HIF-1 α

and HIF-2 α [19–23]. VHL recognizes hydroxylated residues at Pro402 and/or Pro562 in HIF-1 α and Pro 405 and Pro 531 in HIF-2 α , by means of the prolyl hydroxylase domain protein (PHD) [24]. VHL is inactivated in most renal cancer samples, leading to increased expression of HIF-1 α and HIF-2 α protein [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.cbiportal.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 ~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-1 α accumulation [25, 26], while a more recent study suggests otherwise [32].

Over-expression of either HIF-1 α and/or HIF-2 α is a marker for poor prognosis in the majority of the 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 shRNA silencing of *Hif1a* and over-expression of VHL strongly reduced leukemia-initiating activity [9].

Despite the overwhelming association between HIF levels in cancer tissue and poor prognosis of cancer patients, conflicting reports have emerged in renal cancer, where over-expression of HIF-1 α has been associated with either poor or favorable prognosis, depending on the methods used to evaluate HIF-1 α levels [34, 35]. In the 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 that in the cells that give rise to leukemia, promote, rather than suppress 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 that of its function as a tumor suppressor can be explained by both the transcriptional and non-transcriptional activity of HIF.

PKM2 and metabolic switch in cancer cells

PKM2 is the final enzyme in glycolysis and consists of two isoforms that arise from alternative splicing. Most tissues express the PKM1 isoform with products directed into oxidative phosphorylation to efficiently produce ATP. PKM2, by contrast, may exist either in tetrameric or dimeric forms to direct oxidative phosphorylation or glycolysis, respectively. Because 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-1 α can stimulate expression of PKM2. Second, PKM2 and HIF-1 α form heterodimers and migrate into the nuclei, where they act as a master switch to over-express genes crucial for a robust glycolysis that produces both energy and metabolic intermediates for biosynthesis. Therefore, HIF-1 α plays a critical role for metabolic switch in cancer cells.

In addition to PKM2, HIF-1 α has been shown to antagonize cMyc activity by inducing expression of MX11, 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-1 α as a major regulator of VEGF expression [40, 41]. HIF-1 α -p300/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].

MDR1

The link between HIF and MDR1 was first reported in non-transformed endothelial and epithelial cell lines [43]. The authors showed that *HIF1 α* is responsible for hypoxia-induced *MDR1* expression through stimulation of the *MDR1* promoter sequence. Subsequently, it was shown in head and neck cancer cell lines that inhibition of HIF-1 α increased cancer cell sensitivity to paclitaxel [44]. The contribution of HIF to multidrug resistance is now substantiated in many types of cancer, including gastric cancer, colon cancer, multiple myeloma, AML, laryngeal cancer, and sacral chordoma [45–51]. These observations explained the poor prognosis of patients with cancer over-expressing HIF and suggest an important role for HIF inhibitors in combinatorial cancer therapy.

Tumor growth inhibition by HIF-1 α

Despite compelling evidence for oncogenic effect of the HIF family members, over activation of HIF may cause growth arrest and/or apoptosis. At least three mechanisms have been proposed to explain tumor suppressor activity of HIF-1 α . First, HIF-1 α has been shown to stabilize p53, perhaps by binding to MDM2 [52]. Second, among HIF-1 α targets are genes capable of inducing apoptosis, including *RPT801* [53], *NIP3* [54, 55] and *NIX* [55]. Third, HIF-1 α has been shown to antagonize *Myc* by preventing its repression of p21 [56]. In combination, these mechanisms may reconcile the inconsistencies in the mouse genetic data which suggest that the *Hif1a* gene may either promote or suppress tumor growth depending on the tumor types investigated [33] [37].

HIF and cancer stem cells

An important advance in cancer biology was made with the demonstration that somatically transformed cancer cells are heterogeneous in establishing cancer in a new host. In many solid and hematological cancers, a small subset of cancer-initiating cells can be prospectively isolated based on either cell-surface phenotypes or their ability to exclude fluorescent dyes (side population). Although it has been suggested that therapeutic elimination of cancer stem cells may hold the key to reduce cancer relapse and drug resistance, few druggable targets have been identified that provide a means for selective

elimination of cancer stem cells [57–59] [60]. As reviewed below, accumulating data suggest that HIF may emerge as a long-sought target.

Glioma stem cells and HIF

The involvement of HIF pathway in glioma stem cells was first reported by Li *et al.* [8]. Using xenograft glioma initiating and *in vitro* tumorsphere formation assays and CD133 as the cancer stem cell markers, the authors observed significant enhancement of stem cell activity when the stem cells were cultured under a hypoxic environment. Interestingly, the stem cell activity under both normoxic and hypoxic environments are reduced when either *HIF1a* or *HIF2a* are silenced by shRNA. Since *HIF2a* mRNA levels correlate with glioma activity and glioma progression and prognosis, the authors emphasized that HIF2a is critical for the glioma stem cell activity. However, it should be noted that small hairpin RNA (shRNA) silencing showed an equally important role for *HIF1a* in cancer stem cell activity. The lack of correlation between *HIF1a* mRNA levels and stem cell activity may simply reflect the fact that HIF-1 α protein levels are regulated by post-transcriptional mechanisms. The HIF targets responsible for CSC activity remain to be identified.

Acute lymphocytic leukemia (ALL)

Early work on cancer stem cells was criticized for using xenograft transplantation as the CSC activity (tumor-initiating activity) assayed in the xenogeneic host may be artificially affected by rejection of human cells by the innate immunity of the recipient mice [61]. Wang and colleagues used a syngeneic transplantation model to demonstrate that the c-Kit⁺Sca-1⁺ population in their mouse model of ALL are the leukemia initiating cells [9]. Since the c-Kit⁺Sca-1⁺ cells are the necessary and sufficient population for an *in vitro* colony-forming unit (CFU), they used this model to identify inhibitors that may target the ALL stem cells. They found that echinomycin, a natural product that binds to HRE and thus inhibits HIF activity efficiently eliminated the CFU with an IC₅₀ of 30–100 pM. *In vivo*, low dose administration of echinomycin (10 μ g/kg or 30 μ g/m²) cured 100% of syngeneic mice that received lethal doses of leukemia cells. The involvement of HIF-1 α is substantiated by three lines of evidence. First, *Hif1a*, but not *Hif2a* gene, is expressed at substantially higher levels in the leukemia stem cell population than the bulk of leukemia blasts. Second, shRNA silencing of *Hif1a* reduced CFU and leukemia-initiating activity. Third, Hif-1 α was found to enhance Notch signaling by preventing negative feedback regulation of the *Hes1* gene, a key Notch target known to be involved in stem cell self-renewal. However, it remains to be established whether up-regulation of *Hes1* is responsible for HIF-mediated maintenance of LSC.

Using a HIF reporter, Wang et al. showed that under normoxic conditions, HIF is active exclusively within the c-Kit⁺Sca-1⁺ leukemia stem cell (LSC) subset [9]. This high HIF activity was due to selective loss of VHL-expression in the stem cell subset, which is critical for LSC function as ectopic expression of *Vhl* eliminates the LSC [9].

AML

Historically, prospective identification of AML stem cells by John Dick and colleagues in 1994 marked the revival of the cancer stem cell concept [4]. Several groups have shown that the abundance of AML stem cells is a biomarker for poor prognosis [62–64]. Gene expression signatures derived from comparison of AML stem cells and AML blasts have proven valuable in predicting the outcome of newly diagnosed patients [65]. An important prediction of the cancer stem cell concept is that therapeutics that selectively eliminate stem cells should prevent drug resistance and cancer relapse, the two most pressing issues in cancer therapy. Since echinomycin can selectively eliminate AML stem cells [9], the planned clinical trials using echinomycin [66] may provide an opportunity to test this concept

Using the AML stem cell markers identified by Dick's group, Wang and colleagues [9] showed that *HIF1a* mRNA and protein is over-expressed in the human AML stem cells in comparison to the bulk of AML blasts. In both naïve and treated AML samples, echinomycin efficiently inhibits CFU activity, with an IC₅₀ of about 100 pM. More importantly, echinomycin is 100–1000 fold more efficient in inducing apoptosis of the CD34⁺CD8[−] AML stem cells in comparison to the bulk of AML blasts. To test the therapeutic potential, the authors established human AML in the NOD.SCID mice according to the methods described by the Dick laboratory [4] and treated them with a low dose of echinomycin [9]. Short-term treatment with echinomycin not only reduced leukemia blast burden, but also preferentially reduced the frequency of AML stem cells within the leukemia cells, marking a major difference with the conventional therapeutics. As evidence of functional inactivating AML stem cells, the remaining AML blast(s) could no longer initiate AML in the new host.

To test whether echinomycin can be used to treat relapsed AML, Wang and coworkers[67] used the relapsed AML from mice with heterozygous knock-ins of two genes that are frequently mutated in human AML: *FLT3^{ITD}* and *MLL^{PTD}* (*Mll^{PTD/WT}:Flt3^{ITD/WT}*). The *Mll^{PTD/WT}:Flt3^{ITD/WT}* mice developed spontaneous AML and responded to treatment with a DNA hypomethylating agent and/or a histone deacetylase (HDAC) inhibitor; however, the AML invariably relapsed [68]. The authors[67] transplanted the relapsed AML from CD45.2 mice into CD45.1 mice and followed the expansion and therapeutic response using CD45.2 as a leukemia marker [64]. They observed that short-term treatment with echinomycin treatment cured 40–60% of mice with a high burden of relapsed AML at the time of treatment. Again the bone marrow from the cured mice did not harbor dormant AML stem cells as was evidenced by its inability to initiate AML in the new hosts. Surprisingly, echinomycin-treated bone marrow cells are fully competent in hematopoiesis in transplantation. Therefore, therapeutic elimination of cancer stem cells can be achieved in relapsed AML without significant adverse effect on tissue stem cells. Thus, although cancer stem cells and tissue stem cells may share self-renewal programs, therapeutic elimination of cancer stem cells can be achieved without harming tissue homeostasis.

Chronic myeloid leukemia

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder that results from an acquired genetic change in a single hemopoietic stem cell. The hallmark of CML is generation of BCR-ABL chimera protein, a constitutively active tyrosine kinase, as a result of gene translocation [69]. Induction of BCR-ABL induces expression of HIF-1 α and its target gene *VEGF* [70]. Kinase inhibitors (such as imatinib) failed to eradicate CML LSCs. Instead, the targeted therapy selected for imatinib-resistant cells with high levels of BCR-ABL and active HIF-1 α , resulting in anaerobic glycolysis and increased survival *in vitro* [71]. Using a mouse model of CML, Zhang *et al.* showed that *Hif1a*-deficient HSPCs expressing BCR-ABL failed to generate CML in secondary recipients. Deletion of *Hif1a* impairs the propagation of CML by impairing cell-cycle progression and inducing apoptosis of LSCs [72]. In addition, recent studies also showed that HIF1- α supports maintenance of CML LSCs despite effective BCR-ABL1 inhibition in hypoxic environments [73]. These observations indicated that Hif-1 α is crucial for maintenance of CML LSCs.

Taken together, the above examples illustrate that HIF plays a critical role in the stem cells for both solid tumors, and hematological malignancies, serving as a druggable target for therapeutic elimination of cancer stem cells. The fact that HIF is active in cancer stem cells regardless of hypoxia suggests that these cells have acquired mechanisms to protect HIF under normoxic conditions.

Breast cancer stem cells (BCSCs)

Breast cancer is the first solid tumor in which cancer stem cells were prospectively isolated [3]. Using xenograft model of human breast cancer cell lines, Conley *et al.* observed that anti-angiogenic agents increased the proportion of BCSCs by inducing hypoxia. The impact of hypoxia is mediated by HIF-1 α , but not HIF-2 α , and correlates with Wnt signaling in BCSC [74]. Using the mouse mammary tumor virus polyoma virus middle T (MMTV-PyMT) oncogene-induced breast cancer model, Schwab *et al.* [75] demonstrated that conditional deletion of the *Hif1a* gene in the mammary epithelial cells cause reduced tumor growth and metastasis. Interestingly, this depressed tumor growth and metastasis corresponds to a reduction in the number and function of BCSC [75].

A key issue is how HIF-1 α induces BCSC. One key regulator of BCSC activity is a *Hippo* pathway effector molecule called TAZ [76]. By comparing gene expression profile among 1600 cases of human breast cancer samples, Xiang *et al.* [77] showed a significant correlation between known HIF target genes and TAZ, raising the intriguing possibility that TAZ may be a direct target of HIF-1 α . This hypothesis is validated by shRNA silencing, chromatin-immunoprecipitation, promoter activity, TAZ target gene co-expression and hypoxia responses. In addition to controlling TAZ expression, HIF-1 α also activates transcription of the *SIAH1* gene, which encodes a ubiquitin ligase that is required for proteasome-dependent degradation of LATS2. Degradation of LATS2 allowed nuclear localization of TAZ. Apart from expression and function of TAZ, hypoxia also regulates the interaction between BCSC and mesenchymal stem cells (MSC) and tumor-associated macrophages (TAM) through two feed-forward mechanisms [78]. First, HIF-1 α induces expression of CXCL16 to recruit MSC. Second, the MSC produces CCL5 to recruit TAM.

Therefore, BCSC-intrinsic signaling not only maintains the number and function of the BCSC, but also drives formation of the tumor microenvironment.

HIF in adaptive and innate inflammation

Hypoxia-resistant HIF activity is not limited to cancer cells. Accumulating evidence shows that HIFs play a role in regulation of innate and adaptive immune cells that normally reside in normoxic environments. Activated T cells express two isoforms of HIF-1 α : short form called 1.1 and a longer form called 1.2 [79]. The shorter 1.1 form appears to suppress production of inflammatory cytokines by T cells [80]. Accumulating data demonstrates that HIF is a key regulator for Treg/TH17 development, differentiation and function of cytotoxic T lymphocytes, (CTL), and innate immune effectors such as dendritic cells, neutrophils and myeloid-derived suppressor cells (MDSCs).

T helper 17 (TH17) /Treg cell differentiation

Although Th17 and Treg cells share important pathways in their differentiation from naïve T cells, they eventually bifurcate into distinct phenotypes with opposite activities, with TH17 cells being pro-inflammatory and Tregs being anti-inflammatory. Recent studies suggest that the functional switch between these cell types may be mediated by HIF-1 α .

HIF-1 α activates *ROR γ t* transcription and forms a tertiary complex with ROR γ t and p300 to activate the IL-17A gene. By contrast, Hif-1 α binds to Foxp3 and causes its degradation, resulting in a blockade of Treg differentiation. As a result of altered Th17/Treg differentiation, mice with HIF-1 α -deficient T are resistant to experimental autoimmune encephalomyelitis [81]. When naïve T cells were cultured in the presence of TGF- β and IL6, they showed upregulation of the glycolytic activity and induction of glycolytic enzymes. A critical role for glycolysis in Th17 development is suggested as blocking glycolysis with 2-dexoglucose inhibits TH17 cell development. Consistent with a critical role for HIF in Th17 differentiation, HIF-1 α was selectively expressed in TH17 cells, while *Hif1a* deficiency inhibits TH17 differentiation [82]. Function of HIF-1 α in TH17 was not restricted to regulation of the metabolic switch. HIF-1 α controlled expression of anti-apoptotic Bcl-2 family genes in collaboration with Notch signaling and thus supports TH17 survival in human samples [83]. Taken together, these data highlight HIF-1 α -dependent pathways in regulating the balance between differentiation of TH17 and Treg cells.

Despite the *in vitro* effect of HIF-1 α on Treg differentiation, the inhibitory function of HIF on Treg function in the tumor microenvironment remains to be substantiated. Surprisingly, HIF promotes differentiation of CD4⁺/CD25⁺Treg cells in the case of hypoxia caused by rapid tumor progression [84]. Tregs may be enriched in cancer tissue by HIF-1 α -dependent production of chemokines, such as CCL-28 [85]. Additional studies are needed to determine if the apparent contradiction was attributable to tumor microenvironment, and if so, how tumor microenvironment alters the role for HIF-1 α in Treg differentiation.

Cytotoxic T lymphocyte differentiation and function

CTLs are the key adaptive effectors in elimination of intracellular pathogens and tumor cells. HIF-1 α determines CTL fate by regulating transcription of genes encoding glucose

transporters, rate-limiting glycolytic enzymes, cytolytic effector molecules, and essential chemokine and adhesion receptors that regulate T cell trafficking [86]. Activated T cells express high levels of HIF-1 α proteins even under normoxia, although hypoxia further elevates the HIF-1 α [87] [86]. The induction of HIF-1 α is controlled by mTORC1 [86]. Genetic studies in mice demonstrated that deletion of *Vhl* in CTLs inhibits persistent viral infection and neoplastic growth, while that of *Hif1a* attenuates CTL effector function during viral infection [87]. Surprisingly, deletion of *Hif1a* in activated T cells also enhanced production of inflammatory cytokines. These data argue for a negative regulatory role for HIF-1 α in T cell-mediated inflammatory responses [80, 88]. Further studies are needed to shed light on T cell-intrinsic HIF-1 α function in the tumor microenvironment.

Dendritic cells (DCs) activation

As the primary antigen-presenting cells, DCs play crucial role in linking the innate and the adaptive immune systems. LPS and hypoxia lead to increased glycolytic activity and increased DC maturation. The role for HIF-1 α is confirmed as knocking down of HIF-1 α in DCs significantly reduced glucose utility and inhibited DC maturation as evidenced by reduced stimulation of allogeneic T cells [89]. While the role for HIF-1 α in tumor-infiltrating DC has not been investigated, the induction of B7H1/PD-L1 by HIF-1 α [90, 91] and the immune suppression by B7H1-expressing myeloid DC in human cancer [92] suggest that HIF-1 α expressed in the tumor-infiltrating DC may contribute to the immune-suppressive tumor microenvironment.

Tumor-associated myeloid suppressor cells

Tumor growth alters myeloid development in the host, with significant phenotypic and functional changes in the myeloid compartment [93]. A major change in the tumor-bearing host and the tumor microenvironment is the expansion of myeloid suppressor cells, including tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC) and myeloid dendritic cells (mDC) [93]. Accumulating data demonstrate that HIF-1 α contributes to expansion, differentiation and effector function of the myeloid suppressor cells within the tumor microenvironment.

As discussed above, HIF-1 α may contribute to mDC-mediated immune suppression by regulating expression of B7H1/PD-L1. Both TAM and MDSC suppress tumor immunity by producing soluble factors such as nitric oxide (NO) and reactive oxygen species (ROS) [94] [93], and through expression of cell-surface proteins such as B7H1/PD-L1. HIF-1 α , but not HIF-2 α , upregulates the expression of B7H1/PD-L1 by binding to the HRE of the PD-L1 proximal promoter to enhance expression of PD-L1 [90]. In addition, HIF-1 α is necessary for optimal expression of arginase and iNOs in both TAM and MDSC and is thus responsible for NO production [95]. Interestingly, MDSC have been shown to differentiate into TAM in the tumor microenvironment, and this process is requires expression of HIF-1 α MDSC [95].

The contribution of HIF to tumor microenvironment has been demonstrated mostly using transplantable tumor models. As a notable exception, myeloid-specific HIF-1 α -deletion a transgenic model of breast cancer reduces tumor growth and progression [96]. This is

achieved without alternation of VEGF-A levels and vascularization. To test whether this deletion affects macrophage functions, the authors compared WT and *Hif1a*^{-/-} bone marrow-derived macrophages under normoxic and hypoxic culture conditions. The authors observed that hypoxia exacerbated inhibition of T cell proliferation and that such exacerbation is *Hif1a*-dependent. Since TAM are known to inhibit T cell function [93], it would be of interest to determine whether the delay in tumor growth was attributable to *Hif1a* deletion in TAM.

Epigenetic and genetic mechanisms for HIF activities under normoxia: cross-fertilization between cancer biology and immunology

The critical role for HIF in cancer cell metabolism, cancer stem cell function, and immunity raised a fundamental but largely unanswered question: how is HIF activity maintained in circulating leukemia stem cells, cancer cells and hematopoietic cells in a normoxic environment? Accumulating data indicate that HIF activation is stimulated by increased transcription and translation of the *HIF1a* gene and inactivation of oxygen-mediated HIF degradation, through both genetic and epigenetic mechanisms.

Genetic mechanisms taught by cancer: the AML example

The accumulation of HIF1 α protein and its transcriptional activity are strictly regulated. As shown in Figure 2, many of these mechanisms are targeted in AML, and a number of genomic alterations in AML have either been shown or have the potential to enhance HIF activity. First, AML-associated mutations seem to target the canonical HIF degradation pathway. Under normoxia, HIF-1 α is degraded by VHL, which recognizes HIF-1 α when it is hydroxylated at Pro402 and/or Pro562 by the prolyl hydroxylase domain protein (PHD) [24]. Mutations of gene encoding PHD have observed in AML only at low frequency. However, the function of PHD is enhanced IDH1 and/or IDH2 [25, 26], which are mutated at a combined frequency of ~15% in AML patients [27–30]. One study showed that IDH mutations increase HIF1 α accumulation [25, 26]; while a later study suggested an opposite effect [32]. The differences between the two remain to be reconciled. Second, *FLT3*-ITD (internal tandem repeats) and TKD (tyrosine kinase domain) mutations activate FLT3 [97, 98]. Since FLT3 signaling activates the PI3K-mTOR pathway [99], and since mTOR activation increases HIF1 α protein accumulation [100–103], it would be intriguing to test whether *FLT3* mutations cause HIF activation in AML. Third, loss of *TP53* function in tumor cells has been shown to cause defective MDM2-mediated degradation of HIF-1 α [104]. Since p53 mutations are observed in approximately 10% of AML samples, and since this mutation is associated with devastating prognosis [105], it would be of great interest to determine whether this mutation contributes to increased HIF-1 α accumulation in AML. Given the fact that MDM2 is an E3 ligase for HIF-1 α [106, 107], p53 mutations may increase HIF-1 α levels by decreasing MDM2. Fourth, *NPM1* is mutated in 30–40% of AML samples that have a normal karyotype [108, 109]. Since NPM1 sequesters and protects p14ARF against degradation [110], NPM mutations may stimulate HIF activities by inactivating the p14ARF-mediated aberrant delocalization of HIF1 α [111].

Epigenetic mechanism of HIF1 α activation: unanswered questions from cancer stem cells and immune effector cells

As the offspring of cancer stem cells, cancer cells should have inherited genetic alterations that enable oxygen-resistant HIF activity. Therefore, for cancers that show selective activation of HIF within the cancer stem cell compartment, including AML, ALL, CLL and glioma, genetic alterations in HIF regulators do not offer full explanation. There are at least two mechanisms for selective HIF-1 α activation in mouse leukemia stem cells [9]: increased *Hif1a* mRNA accumulation and lack of expression of *Vhl*. Both *Hif1a* upregulation and *Vhl* downregulation are necessary for the maintenance of LSC. Since LSCs are a self-renewing population that also “differentiates” into cancer cells, it is of great interest to determine the epigenetic mechanism that ensures the heritable gene expression pattern of *Hif1a* and *Vhl* and how this pattern is lost when the LSCs “differentiate” into cancer cells. Elucidation of this mechanism will also help to formally demonstrate the existence of epigenetic programs that maintain the LSC activity by selective activation of HIF-1 α in this compartment.

Similarly, how oxygen-resistant HIF-1 α activity is induced in the immune effector cells remains largely unexplained. Accumulating data suggest that activation of either T-cell receptor [81, 82, 112] or Toll-like receptors (TLR) [113] induce accumulation of *Hif1a* mRNA, perhaps through suppression of miR-200 [112]. However, additional mechanisms are needed to explain oxygen-resistant HIF-1 α activity. AKT-PI3K pathway induces accumulation of HIF-1 α through activation of mTOR to increase translation of HIF-1 α [114]. In addition, mTOR activation prevents oxygen-induced HIF-1 α degradation [102]. In the latter case, the mTOR-induced HIF protection against degradation requires functional interaction between mTOR and the HIF-1 α domain that is also involved in hydroxylation of HIF-1 α . Therefore, mTOR may inhibit HIF degradation through a yet undefined mechanism.

Recent studies demonstrated that, much like adaptive immunity, innate effector cells may be also capable of immune memory, i.e., they can be trained to mount a more robust or much reduced recall response [12, 13]. For instance, stimulation of monocytes with glucan trained the monocytes to mount a more robust innate immune response to secondary stimulation in vitro and increased host resistance to infections by *Candida albicans* and *S. aureus* [12]. This training is associated with extensive epigenetic alterations in the monocytes [13]. Surprisingly, the training is associated with a metabolic switch from oxidative phosphorylation to glycolysis [12]. Given the critical role for HIF-1 α in the metabolic switch, researchers used a genetic model to determine the involvement of HIF-1 α in the recall response of innate effectors [12]. Indeed, targeted mutation of *Hif1a* in myeloid cells is sufficient to ablate trained immunity. The critical role for HIF-1 α in trained immunity and the extensive epigenetic reprogramming during the process raised an interesting issue as to whether epigenetic mechanisms may be directly responsible for oxygen-resistant HIF activation in monocytes.

Taken together, in both immune effectors and cancer stem cells, a steady state of oxygen-resistant HIF-1 α accumulation has been achieved. A major challenge remains in defining how this crucial state is achieved through non-genetic, perhaps epigenetic, mechanisms.

Concluding remarks

HIF was discovered in the process of investigating how living organisms adjust to varying oxygen environment [15]. More recent studies have demonstrated that this pathway is operative even under normoxic environment and that such oxygen-resistant function is critical in cancer biology and immunology. HIF activation is responsible for a metabolic switch that allows more sustained proliferation with less DNA damage. These features are important for the maintenance of cancer stem cells. In the immune system, activation and/or function re-programming of immune effectors is often imprinted through oxygen-resistant HIF accumulation. The converging feature suggests cross-fertilization between cancer biologists and immunologists may bring new insights on the mechanism of HIF regulation and new approaches for cancer therapy.

As summarized in Figure 3, cancer cells and immune effectors are the major components that constitute the tumor microenvironment. In this environment, the immune system not only fails to defend the host against cancer but often actively aid carcinogenesis, with functions ranging from promoting cancer stem cell activity [115, 116], angiogenesis [117], to facilitating malignant transformation [118] and metastasis [119]. Therefore, effective control of the tumor microenvironment will likely complement the traditional approach of cancer therapy, aiming at eliminating cancer cells. Since most contributors to the tumor-promoting microenvironment rely on HIF, it is likely that targeting HIF will not only aid in the therapeutic elimination of cancer stem cells, but also result in depriving cancer cells of a habitable microenvironment.

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Highlights

Hypoxia-inducible factors maintain cancer stem cells

Hypoxia-inducible factors set the cancer microenvironment

Genetic and epigenetic mechanisms confers oxygen-resistance of hypoxia-inducible factors

Hypoxia-inducible factors are targets for therapeutic elimination of cancer stem cells and reprogramming of the tumor microenvironment.

Fig. 1. Regulation of hypoxia-inducible factors (HIF) activity in response to oxygen levels. Under hypoxic conditions, stabilized HIF-1 α and HIF-2 α dimerize with HIF-1 β . The heterodimers translocate into the nucleus to regulate gene transcription. In the presence of oxygen, HIF-1 α and HIF-2 α proteins are hydroxylated by the prolyl hydroxylase domain protein (PHD)1-3 at the indicated proline residues. Hydroxylated HIF is recognized by VHL, which causes polyubiquitinylation and proteasome-mediated degradation of HIF.

Fig. 2. Prevalent mutations in acute myeloid leukemia (AML) potentially affect HIF levels in cancer cells. Mutations of genes encoding isocitrate dehydrogenase (IDH) 1 and 2, which are mutated in 10–15% of AML, inactivates PHD to suppress HIF hydroxylation. FLT3 mutations (found in 25–35% AML cases) stimulates PI3K/AKT pathway to enhance HIF translation. NPM mutation, which was found in 30% of AML with normal karyotype (NC), reduces p14ARF, and HIF inhibitors. *TP53* mutation reduces expression of MDM2, an alternative E3 ubiquitinylation ligase E3 for HIF. This in turn may reduce HIF ubiquitinylation.

Fig. 3.

A central role for HIF in tumor microenvironment. In addition to its critical role for maintenance and proliferation of cancer stem cells (CSC) and cancer cells (CaC), HIF promotes differentiation and/or proliferation of host cells within the tumor microenvironment, including endothelial cells (EC), dendritic cells (DC), tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC). Apart from regulating cells that affect T cell function, HIF also directly regulates activation and differentiation of various functional subsets of T cells, including cytolytic T lymphocytes (CTL), regulatory T cells (Treg), and IL-17-producing T cells (Th17).