

The role of hypoxia-inducible factors in leukemias

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Abstract

Hypoxia, understood as low partial oxygen pressure, has become one of the most explored fields in recent years. Cellular response to hypoxia is mediated by hypoxia-inducible factors (HIFs) – potent transcription regulators, and their downstream pathways. In general, HIFs modify energy metabolism, inflammation and immune response, enhance cancer invasion, metastasis, resistance to treatment, and relapse. The influence of HIFs on the progression of leukemia is still under investigation in various studies, but in mice and some human models HIFs have been recognized as leukemia immortalizers by promoting leukemic stem cell quiescence and inhibiting their cell cycle. This makes leukemic stem cells resistant to most known treatment approaches. The role of HIFs in solid tumors and leukemia makes them almost ideal targets for an anticancer treatment. Although the first attempts with new molecules are encouraging, there is a need to investigate the ambiguous role of HIFs to develop a modern antileukemic treatment.

Key words: leukemia, hypoxia, hypoxia-inducible factor-1

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Leukemias remain an issue for contemporary medicine. The huge success in chronic myeloid leukemia (CML) treatment has not yet been matched by treatments for acute leukemias. Even tyrosine kinase inhibitors (TKI) – the main players in CML treatment – are not able to eradicate leukemic stem cells (LSCs). LSCs are a small subset of quiescent, self-renewable cells capable of re-establishing the whole tumor after chemotherapy. LSCs share marrow niches with hematopoietic stem cells (HSCs), but the niches are also inhabited by mesenchymal stem cells, bone cells, immune cells, and other cell types. The marrow microenvironment contributes considerably to the protection and development of leukemic cells. The bone marrow hematopoietic niche is characterized by low partial oxygen pressure, and this is essential to retaining HSCs, LSCs and other progenitor cells for long periods. Hypoxia is a very strong stimulus for adaptive cell changes mediated by the hypoxia-inducible factor (HIF) family. It has been shown that through HIFs, hypoxia optimizes solid tumor growth via metabolic modifications, stimulation of angiogenesis, cancer stem cell (CSC) maintenance and differentiation, and modification of the function of inflammatory cells. However, in leukemias, the role of hypoxia remains unclear, and the influence of HIFs on leukemogenesis is still controversial. On the other hand, some research has established the influence of HIFs on leukemic cell proliferation and resistance to chemotherapy. This paper attempts to summarize current knowledge about the role of hypoxia and HIFs in leukemias.

Hypoxia

Hypoxia is defined as a state of reduced or inadequate oxygen availability. Although different tissues and cells have varying degrees of susceptibility to hypoxia, at the cellular level, hypoxia and hypoxic responses generally occur at $PO_2 \leq \sim 1$ kPa ($\leq \sim 7$ – 10 mm Hg or $\sim 1\%$ O_2).¹ There are multiple physiologic and pathological contexts in which cells experience conditions of insufficient oxygen availability.²

Hypoxia has been reported to contribute in a variety of pathological states, including solid tumors with incomplete neoplastic vascularization; ischemic injury, such as myocardial infarction or transplants; and obesity with impaired adipose tissue metabolism.² Other conditions that can be added to this list include vascular diseases (e.g., sickle cell disease), obstructive sleep apnea and chronic infection (e.g., granulomas).

Tissue oxygen concentration is much lower than in arterial blood enriched with oxygen from the air in the lungs. Tumor cells are not only prepared to survive total anoxia, but are skilled at taking advantage of the state to ensure uninhibited and uncontrolled growth through completely altered (but impaired) metabolism.³ Local oxygen concentration plays a leading role in tumor progression due to impaired and incomplete vessel networks, resulting

in reduced cell nutrition in hypoxic zones. Low oxygen levels cause the activation of mTOR kinase 1 (mTORC1) and modify tumor metabolism through the HIF signaling pathway, leading to tumor growth.⁴

The role of hypoxia-inducible factors

As Semenza wrote: “[HIF] transcription factors are master regulators of the cellular response to hypoxia and coordinate a transcriptional program that ensures optimal functional, metabolic, and vascular adaptation to O_2 shortages”.⁵ HIF-1 α is also known to be responsible for cancer angiogenesis, growth and survival, glucose metabolism, invasion and metastasis, and immune regulation via the control of Th17/Treg balance.^{6–10} HIF-2 α is also expressed in a variety of cells, including endothelial cells and immune cells such as tumor-associated macrophages, and is reported to play an opposite role to HIF-1 α in the regulation of angiogenesis, but mainly in iron metabolism, erythrocytosis control, and somatic stem cell self-renewal.^{11–16}

HIF is a heterodimeric complex consisting of 2 subunits: an oxygen-sensitive HIF- α and an unchangeable oxygen-stable HIF- β (aryl hydrocarbon receptor nuclear transporter – ARNT).¹⁷ Both subunits are members of the basic helix-loop-helix (bHLH) PAS family of transcription factors.¹⁷ Three HIF- α homologs have been discovered: HIF-1 α , HIF-2 α and less known HIF-3 α .^{18–20} HIF-1 α and HIF-2 α heterodimerize to HIF-1 β and translocate to the nucleus, where the complex is bound to hypoxia response elements (HREs) in the promoters of target genes. When oxygen is available, prolyl-hydroxylases (PHDs) are active and HIF1- α is degraded by the PHD-mediated oxygen-hydroxylation of proline (Pro-403 or Pro-564).²¹ The hydroxylated HIF1- α is recognized by the von Hippel-Lindau protein (pVHL), which is the recognition component of an E3 ubiquitin-protein ligase. This leads to ubiquitination and consequent degradation by proteasome.²¹ In hypoxic conditions, PHDs are inactivated, which leads to HIF- α accumulation. However, cells are doubly protected against HIF activation effects, and the shield is factor-inhibiting HIF (FIH) hydroxylating asparagine residues in HIF1- α and HIF-2 α , which prevents HIFs from building active transcriptional complexes with cofactors.²² Both PHDs and FIH require α -ketoglutarate (2-oxoglutarate) as a co-substrate. On the other hand, hypoxia diminishes PHD- and FIH-dependent HIF- α hydroxylation, resulting in full activation of the HIF- α pathway.²³

HIF1- α expression is also regulated by growth factors, cytokines and other signaling pathways, such as the phosphatidylinositol 3-kinase (PI3K) pathway and mitogen-activated protein kinase (MAPK) pathway. HIF1- α plays a role in cancer progression by activating the transcriptional programs to maintain the ability to self-renew and the multipotency of cancer stem cells in a hypoxic

environment.²⁴ This has been described for renal cell cancer, hepatocellular cancer, colorectal cancer and other cancers.^{25–27}

In the O₂-independent mechanism of HIF stabilization, bacterial products are recognized by toll-like receptors (TLRs) expressed on myeloid cells, signaling through the nuclear factor-light-chain-enhancer of activated B cells (NF-κB) to increase HIF1-α transcription.²⁸ Similarly, T cell receptor (TCR) ligation upon antigen presentation on T lymphocytes results in increased HIF1-α transcription and HIF-1α protein accumulation, even in the presence of oxygen. The mechanism of HIF-1α mRNA expression has not yet been identified, but activation of PI3K and mTOR seems to be involved in TCR-related activation of HIF-1α.²⁹ On the other hand, HIF-1α controls the Th17/Treg balance by promoting transcription of RORγt – a key regulator of Th17 differentiation – and proteosomal degradation of forkhead box P3 (FOXP3) – a key regulator of Treg differentiation. This leads to a sustained proinflammatory and auto-aggressive reaction.¹⁰

HIF transcriptional targets also include glycolysis and angiogenesis regulating genes, e.g., glucose transporter 1 (*GLUT1*), phosphoglycerate kinase 1 (*PGK1*) and vascular endothelial growth factor (*VEGF*).^{30–32} As McNamee et al. wrote: “While some HIF-1α targets are conserved across multiple cell types, HIF-1α is also clearly capable of mediating cell type-specific transcriptional responses. HIF-1α is regulated at multiple stages, including transcriptional, translational, and post-translational levels.”²

Hematopoietic stem cells (HSCs) are able to enter quiescence and are characterized by self-renewal capability, and HIF-1α protein is reported to enhance these features.³³ Conditional deletion of the HIF-1α gene causes HSC proliferation and reduces self-renewal potential in serial transplantations.³⁴ On the other hand, HSCs cultured in hypoxic conditions have increased quiescence *in vitro* and *in vivo* when the HIF-1 pathway is activated.³⁵

HIF-2α is not necessary for the function of adult HSCs *in vivo*.^{35,36} Human bone marrow CD34+ cells hardly express HIF-2α, but HIF-2α itself has been identified as a STAT5 target gene in HSCs. STAT5 plays crucial roles in self-renewal in mouse and human HSCs, and its persistent activation leads to leukemic transformation.³⁷ As Forristal et al. wrote: “It is clear that normal adult mouse and human [bone marrow] hematopoietic cells in steady state have low levels of HIF-2α”.³⁵ HIF-2α is also expressed by some hematological neoplastic cells, such as acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) cells.^{35,38–40}

HIF-2α is also an important transcriptional regulator of the cellular hypoxia response, including hypoxic regulation of erythropoietin (EPO) synthesis and macrophage function.^{12,41} The interaction between HIF-1α and HIF-2α in hypoxic gene regulation remains unclear. However, these transcriptional regulators can have competitive or even antagonistic functions.^{2,42}

In summary, the HIF complex can cause transactivation of the target genes, leading to cell adaptation to hypoxic conditions, and plays a crucial role in many processes including enhanced cell proliferation (renal and colorectal cancer), cancer vascularization via VEGF, metastases, glycolysis regulation multidrug resistance (MDR), HSC quiescence and self-renewal, and many other processes. All these pathways have been widely described for solid tumors, but the roles of HIFs in leukemia seem to be inconsistent and remain unclear.

Leukemias, hypoxia and HIFs

Leukemia is the uncontrolled and uninhibited proliferation of hematopoietic cells. Acute leukemias are defined by proliferation of immature hematopoietic cells that fail to differentiate and accumulate in bone marrow and other organs, inhibiting the growth of normal hematopoietic cells. Acute myeloid leukemia (AML) has a high risk of relapse, despite therapeutic advances. Most known treatments target cycling cells, so the concept of relapse deriving from a quiescent surviving population of cells has arisen. LSCs derive from HSCs homing to the most hypoxic bone marrow areas.^{43–45} As mentioned above, tissue oxygen concentration is much lower than in arterial blood enriched in O₂ from the lungs. In bone marrow, the oxygen concentration is even lower than in other tissues, ranging from < 6% oxygen near blood vessels to anoxia in not vascularized regions. The components of the hematopoietic niche vary in oxygen level.^{45,46} The boosting role of hypoxia in rapid growth, proliferation, metabolism, metastases and mortality of solid tumors has been described.⁴⁷ The role of hypoxia in leukemias is not as well established, but the hypoxic BM niche increases poor response to treatment.⁴⁵

It is accepted that gradients of O₂ from below 1% in the hypoxic niche to 6% in the sinusoidal cavity exist in human bone marrow. Hypoxia is essential for long-term HSC survival and function.⁴⁸ Molecular regulation of the influence of hypoxia on HSCs has not been established yet, but some studies have shed new light on the crucial role of HIF1 in mediating the effect of hypoxia on HSCs.³⁴

Resistance to standard treatment modalities leading to leukemia relapse may be related to increased HIF expression.⁴⁹ Poor outcomes of antileukemic treatment have been linked with overexpression of HIF-1α in some studies, including an impact on survival.^{50–52} In other studies, overexpression of HIF-1α has simply been reported – in AML, acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML).^{34,35} Similarly, HIF-2α overexpression has been described in both AML and ALL, but has not yet been correlated with outcomes.³⁵

There is a variety of mechanisms and molecular pathways that allow both HIF-1α and HIF-2α to contribute to leukemia survival, including metabolism, promoting cells in quiescence and immune dysregulation. HIF-1α

also plays the opposite role, inhibiting the expression of tumor expression genes.^{34,53,54} Most of the widely used antileukemic drugs target cycling cells, so AML cells quiesced by HIFs become resistant to standard cytosine treatment. Another HIF-1 α -mediated mechanism promoting leukemia resistance is LSC support, as in CML, T cell acute lymphoblastic leukemia (T-ALL) and AML.⁴⁴ This is a vicious circle: on one hand, LSCs sheltered in naturally hypoxic bone marrow niches stabilize HIF-1 α , on the other hand, that same HIF-1 α keeps them in quiescence and lets them survive any treatment, as in CML.⁵⁵ Moreover, in AML cells, HIF-1 α is stabilized under normoxic conditions as well.⁵⁶ HIF-1 α (in cooperation with the Notch pathway) is able to arm LSCs with a powerful tool: self-renewal, which lets them survive all known treatment modalities.⁵⁷

Perspectives and conclusions

Taking into account the data cited above, it has been assumed lately that HIF inhibitors or PHD stimulators/enhancers could be a potent weapon in the antileukemic war. Some new agents have been tested; one of the first was echinomycin, which was known to inhibit HIF-1 α DNA binding activity. This antibiotic targets AML cells through apoptosis. Echinomycin has no impact on self-renewal and differentiation of HSCs, which makes it a perfect drug to eradicate leukemia.⁵⁸ Another well-known drug, L-ascorbic acid in high concentrations, has also been shown to inhibit the expression of HIF-1 α in CML cells. It is particularly important in CML treatment to find a molecule capable of impacting LSCs that are completely resistant to TKI-based modalities. EZN-2208 (pegylated SN38) has also been shown to inhibit the expression and transcriptional activity of HIF-1 α in APL.⁵⁹ None of these molecules target HSCs. Another molecule, TH-302, is a hypoxia-activated prodrug that has been reported to preferentially decrease proliferation, reduce HIF-1 α expression and induce cell-cycle arrest in AML cells.⁶⁰ This is an example of an alternative strategy “using” hypoxia to activate prodrugs in the bone marrow niche and target LSCs in their homeland.

On the other hand, some research has shown evidence that PHD inhibition can inhibit tumor growth and invasiveness.⁵⁷ This data derives from solid tumor investigation; evidence is still needed in relation to leukemia, but those trials definitely demonstrated the complex and ambiguous role of HIFs in cancer.

This data corroborates the view that hypoxia and HIF-mediated signaling play a crucial role in leukemia. As noted above, there are some confusing and even contrary results, but most mouse trials have unequivocally confirmed the proleukemic role of HIFs. Therefore, it can be assumed that HIFs inhibitors may potentially be successful in treating human leukemia.

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