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Roles of the hypoxia response system in hematopoietic and leukemic stem cells

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Abstract Stem cells exhibit a number of characteristic features, including the capacity for self-renewal and differentiation into multiple cell types, stress resistance, and drug efflux activity. These specific biological characteristics are supported by signals from the surrounding niche and the stemcell-specific transcription factor set, including hypoxia and the machinery that senses low oxygen levels. These properties are essential for normal stem cells, and when defective may induce cellular senescence and tumorigenesis. In contrast, cancer stem cells in tumor tissue utilize these biological characters driven by stemcell-specific molecular mechanisms and acquire indefinite self-renewal capacity, drug resistance, and metastatic ability. A fuller understanding of the differences between normal and malignant stem cells in the biological and molecular context is, therefore, necessary to the development of therapies against cancer stem cells. In this review, we discuss the effect of hypoxic microenvironment on normal and malignant stem cells and describe their molecular machinery with an emphasis on hematopoietic stem cells and their malignant counterparts, leukemic stem cells.

Keywords Hematopoietic stem cell · Leukemic stem cell · Stem cell niche · Hypoxia

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Introduction

Molecular oxygen (O_2) plays a key role in the production of the currency of cellular energy, adenosine triphosphate (ATP), in mitochondria. O_2 is inhaled and incorporated from lung to bloodstream, and is delivered to peripheral tissues via red blood cells through binding to hemoglobin. After reaching peripheral tissue, O_2 is released from hemoglobin and is passively diffused into every cell. Partial pressure of O_2 in the peripheral tissue is, therefore, lower than atmospheric oxygen, and varies by cell type [1]. Interestingly, stem cells, which occupy the top tier of the organ differentiation hierarchy, often localize at hypoxic microenvironments within tissues. Stem cells have two essential abilities: self-renewal and multilineage differentiation [2, 3]. These are maintained by a microenvironment suitable for stem cells (niche) by various factors, including cytokines, growth factors, adhesion molecules, and extracellular matrix derived from surrounding niche cells [4]. The hypoxic stem cell activates various molecular machineries to respond and adapt to the hypoxic environment [5–7].

In contrast to normal tissue, most tumor tissues are supported by the nutrient vessels generated by abnormal angiogenesis. Because the tumor vasculature differs significantly from functional, mature blood vessels, the tumor is poorly perfused and there are hypoxic areas in the tumor tissue [8]. Several tumors are thought to be maintained by a subpopulation called cancer stem cells [9]. The pool size, biological character and dynamics of cancer stem cells contribute to tumor development, progression, drug resistance, and metastasis in vivo. Accordingly, cancer stem cells are important therapeutic targets. A subset of the cancer stemcell population localizes at the hypoxic niche and maintains itself by the hypoxia response molecular

system [6]. In this article, we will overview the hypoxia-responsive molecular machinery and describe its involvement in the maintenance of normal and malignant hematopoietic stem cells.

Hematopoietic stem cell

The hematopoietic stem cell (HSC) is one of the best characterized tissue stemcell types. Mammalian adult HSCs are localized in bone marrow, where PO_2 and oxygen saturation are relatively low compared to other organs [10]. Low O_2, therefore, appears to be a niche component for HSCs. O_2 generates reactive oxygen species (ROS) through mitochondrial electron transport chain or cytosolic/phagosomal NADPH oxidase. Exposure to aberrant levels of ROS induces senescence dysfunction in stem cells [11], resulting in perturbed homeostasis at the organ (and whole body) level. Clearly it is advantageous for stem cells to localize at a hypoxic niche where the ROS source O_2 is limited. Recent research in stemcell biology has shed light on the precise molecular mechanism by which stem cells are maintained by the hypoxia response system.

HIF-1 as a pivot of cellular hypoxia response

HIF-1α is a bHLH-PAS-type transcriptional factor, which is essential for cellular and systemic hypoxic responses [12]. HIF-α family has three members, including HIF-1α, HIF-2α, and HIF-3α. Prolyl residues in the HIF-1α oxygen-dependent degradation domain (ODD) are hydroxylated by HIF prolyl hydroxylases (Phds) under normoxia [5, 12]. The hydroxylated ODD domain of HIF-1α is recognized by von Hippel-Lindau protein (VHL). VHL is an E3 ubiquitin ligase and is responsible for the autosomal dominant hereditary disorder von Hippel-Lindau disease, or the autosomal recessive disorder Chuvash polycythemia. If VHL is mutated, HIF-1α and HIF-2α proteins are over-stabilized by the impaired ubiquitin proteasome pathway, even under normoxic conditions. As a result, patients with von Hippel-Lindau disease or Chuvash polycythemia (mild stabilization of HIF-1α and extensive stabilization of JAK2 [13]) suffer from various symptoms due to the deregulated HIF-related pathways.

Under hypoxic conditions, Phds are enzymatically inactive and HIF-1α protein is stabilized by escaping from protein degradation [12]. Several niche factors for HSCs, such as thrombopoietin (TPO) and stemcell factor (SCF), also stabilize HIF-1α protein in hematopoietic cells even under normoxia [14, 15]. Stabilized HIF-1α forms a heterodimer with the oxygen-independent subunit HIF-β (aryl hydrocarbon receptor nuclear translocator; Arnt), translocates to the nucleus, and directly binds hypoxia response elements (HREs) in the promoter regions of HIF downstream genes, thereby activating these expressions [5, 12].

HIF system in embryonic hematopoiesis

The Arnt subunit is reportedly required for hematopoietic cell generation during ontogeny. $Arnt^{-/-}$ mutation results in vascular and hematopoietic defects by 10.5 dpc [16, 17]. $Arnt^{-/-}$ embryos exhibit increased numbers of apoptotic hematopoietic cells, defective vasculogenesis and angiogenesis. Using para-aortic splanchnopleural (P-Sp) explant cultures [18], these defects are rescued by the addition of normal $Sca\text{-}1^{+}$ hematopoietic cells or VEGF protein [19]. These results suggest that HIF/Arnt heterodimer coordinates embryonic endothelial cell emergence and vessel maturation by promoting hematopoietic cell survival and paracrine VEGF production. The proliferation of embryonic multilineage hematopoietic progenitors is regulated by a hypoxia-mediated signaling pathway, as $Arnt^{-/-}$ embryoid bodies fail to exhibit progenitor proliferation by hypoxia [17]. Also, $Arnt^{-/-}$ embryos show decreased yolk sac hematopoietic progenitors. This phenotype is not cell autonomous, but rather cell extrinsic. A decreased level of in Arnt-dependent VEGF expression is the responsible mechanism. Therefore, hypoxia response system is essential for the proliferation and survival of hematopoietic progenitors during embryonic hematopoiesis. It is reported that an antagonist of aryl hydrocarbon receptor (AhR), a heterodimeric partner of Arnt, can maintain human HSCs in vitro [20]. It is interesting to see whether this is related to the HIF effect or not.

Hypoxic HSC niche in the bone marrow

Mammalian HSCs balance cell cycle quiescence and self-renewal division, and retain the capacity for multilineage differentiation, which generates all the lineages of hematopoietic cells. After birth, HSCs localizes to the endosteal zone of the bone marrow. The endosteal zone is highly vascularized by fenestrated sinusoidal endothelium, which is easily transited by hematopoietic cells. As a result, blood flow is slow and the perfusion rate of the bone marrow cells is thought to be low. Bone marrow is densely occupied by numerous hematopoietic cells, which actively consume O_2, and a simulation study suggests a 90 % reduction of PO_2 at the point 100 μm away from the bone marrow vasculature [21].

Intravital time-lapse observation of the bone marrow with a multi-photon microscopy revealed that a

transplanted HSC detach from bone marrow endothelium and penetrate deep into the bone marrow [22], which is thought to be hypoxic. Analysis of bone marrow hematopoietic cells following the injection of perfusion tracer into mice suggests that HSCs are poorly perfused compared to other bone marrow cells [23]. In parallel, a hypoxia probe pimonidazole is highly retained in murine HSC fraction of the bone marrow [23]. As transplanted human HSCs in immunodeficient mice reacquire the hypoxic status before they enter the quiescent steady state in cell cycle [24], the hypoxic nature of HSCs may be a common feature among species. A previous report showed that the average PO_2 in the human bone marrow is 55 mmHg and oxygen saturation about 87.5 % [10]. Considering a gradual decrease of PO_2 from the bone marrow vasculature, the actual PO_2 at the HSC niche distant from the bone marrow vasculature is low and hypoxic. Because hypoxic culture of human HSCs elevates HIF-1α protein [25], induces quiescence [26], and supports the transplantation capacity into immunodeficient mice [25, 26], HIF-1α-mediated hypoxia response is activated and plays roles in HSC maintenance in vivo.

Regulation of adult hematopoietic stem cells by VHL/HIF regulatory system

HIF-1α deficiency is lethal to the embryo. Until embryonic day 8.5, it shows drastic morphological defects including a lack of cephalic vascularization, reduced number of somites, abnormal neural fold formation and an enhanced tissue hypoxia [27]. Therefore, the role of HIF-1α in adult HSCs must be analyzed by a conditional knockout approach using HIF-1$\alpha^{flox/flox}$ mice harboring the Mx1-Cre transgene, which actuates the inducible Cre recombinase expression after synthetic double strand RNA poly I:C in various tissues including hematopoietic cells. Inducible knockout of HIF-1α by Mx1-Cre in mice (HIF-1$\alpha^{\Delta/\Delta}$ mice) results in a loss of stress resistance in HSCs during serial bone marrow transplantation, treatment using chemotherapeutic agents, and aging [28]. These stresses induce the loss of the HSC pool in the bone marrow. After the bone marrow transplantation assay, the expression levels of Ink4a locus products, $p16^{Ink4a}$ and $p19^{Arf}$ which contribute to cellular senescence are clearly elevated in HIF-1$\alpha^{\Delta/\Delta}$ HSCs. Because suppression of $p16^{Ink4a}$ and $p19^{Arf}$ restores the transplantation capacity of HIF-1$\alpha^{\Delta/\Delta}$ HSC, HIF-1α protects HSCs from stress-induced cellular senescence in vivo. Loss of cell cycle quiescence in HIF-1$\alpha^{\Delta/\Delta}$ HSCs suggests that an aberrant proliferation of HSC is a cause of senescence in HIF-1$\alpha^{\Delta/\Delta}$ HSCs.

Heterozygous deletion of VHL ($VHL^{+/\Delta}$) shows enhanced cell cycle quiescence in HSCs [28]. Moreover, hematopoietic progenitor fraction, which is rapidly proliferating in the steady state, enters cell cycle quiescence. Therefore, hematopoietic progenitors normally do not express HIF-1α protein but are sensitive to over-stabilized HIF-1α-mediated quiescent induction. These observations suggest that the VHL/HIF-1α regulatory system plays a key role in the cell cycle quiescence of hematopoietic stem/progenitor cells in vivo. The maximized expression of HIF-1α protein in HSCs by homozygous deletion of VHL ($VHL^{\Delta/\Delta}$) also induces quiescence in HSCs [28]. However, in clear contrast, $VHL^{\Delta/\Delta}$ HSCs completely lose the reconstitution ability of the bone marrow upon transplantation. These over-quiescence and defective transplantation phenotypes are reversed by the co-deletion of HIF-1α and VHL. Thus, these phenotypes in $VHL^{\Delta/\Delta}$ HSCs are HIF-1α dependent. A decreased homing capacity to bone marrow in $VHL^{\Delta/\Delta}$ hematopoietic stem/progenitor cells may explain the loss of stemcell capacity phenotype of $VHL^{\Delta/\Delta}$ HSCs. In addition to the protein level regulation of HIF-1α by VHL, transcriptional activation of HIF-1α by Meis1 transcription factor is involved in the anaerobic metabolic phenotype in hypoxic HSCs [29]. Because the moderate increment of HIF-1α by heterozygous deletion of VHL sustains the HSC pool during transplantation or aging [28], a modest induction of HIF-1α protein in HSCs by modulation of transcriptional or post-transcriptional methodology is thought to be useful for the ex vivo manipulation of HSCs without senescence.

Although the means by which HIF-1α maintains quiescence and metabolic features in HSCs remains unknown, several potential downstream candidates are reported (Fig. 1). First, Vegfa(δ/δ) mouse, a mutant mouse model with a mutated HRE in VEGF promoter region, shows a defective HSC phenotype in vivo [30]. HRE is recognized and bound by HIF-1 complex for the transcriptional activation. Decreased expression of Vegfa is observed in HSCs but not their progeny cells. Loss of hypoxia-regulated Vegfa expression in Vegfa(δ/δ) mouse increases the numbers of phenotypic HSCs. However, HSC function is clearly defective upon transplantation. Also, Miharada et al. [31] proposed a model that HIF-1α induces Cripto/GRP78 signaling for HSC maintenance. A subfraction of HSCs expressing GRP78, a heat shock protein, localizes in the hypoxic endosteal region and is quiescent in cell cycle, and shows a lower mitochondrial potential compared with $GRP78^-$ HSCs. Inhibition of GRP78 by a neutralizing antibody resulted in a reduction of $GRP78^+$ HSCs in the endosteal area. Cripto, a ligand for GRP78, is expressed in various niche cells including osteoblasts, mesenchymal stem cells, and HSCs themselves. Cripto/GRP78 signaling in the hypoxic HSC niche regulates HSC quiescence by inducing high glycolysis activity in HSCs and maintains HSCs in hypoxia. The promoter region of Cripto gene has HRE. In HIF-1$\alpha^{\Delta/\Delta}$ mice, decrease in $GRP78^+$ HSCs and

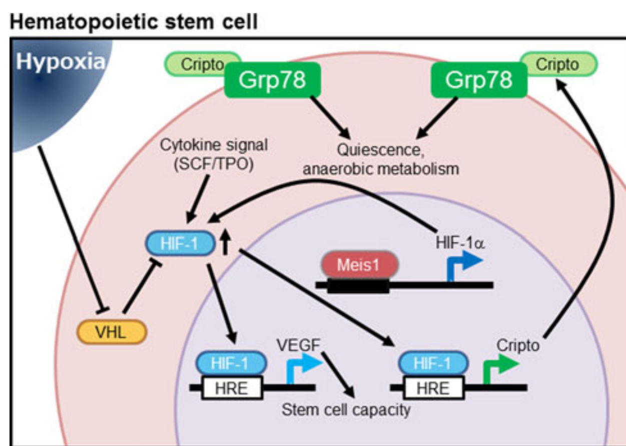

Fig. 1 Roles of HIF-1 in hematopoietic stem cells. Schematic representation of HIF-1 action on hematopoietic stem cells. Hypoxia or cytokine signaling by SCF or TPO enhances HIF-1-mediated transcriptional activation. These stimuli activate directly HIF-1 downstream target including VEGF or Cripto. Autocrine (hematopoietic stemcell-derived) or paracrine (osteoblast or mesenchymal stemcell-derived) Cripto binds to membrane GRP78 for maintaining quiescence. In addition, HIF-1α transcription is maintained by Meis1

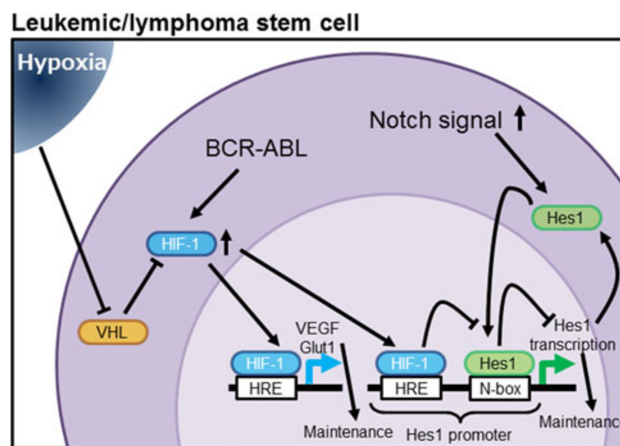

Fig. 2 Mechanisms of HIF-1-mediated maintenance of stem cell in hematological malignancy. Schematic representation of HIF-1 action on leukemic/lymphoma stem cells. Hypoxia or BCR-ABL enhances HIF-1-mediated transcriptional activation. These stimuli activate directly HIF-1 downstream target including VEGF or Glut1, or inhibits negative feedback loop of Notch1-Hes1 regulatory system to enhance Hes1-mediated transcription. As a result, leukemic stemcell generation and maintenance are promoted.

reduction of Cripto in the endosteal niche cells are observed. As Cripto is activated by other stimulus and HIF-1 activates various downstream regulators, it would be interesting to analyze the detailed function of HIF-1α/Cripto/GRP78 pathway in HSC and identify HIF-1α downstream regulator in HSCs.

Roles of HIFs in leukemic stem cells

In various patient samples from various hematological malignancies, HIF-1α activation is observed. Therefore, HIF-mediated signaling can play a pivotal role in maintenance of their stemcell fraction. There are several O_2-dependent and -independent modulation mechanisms of HIF-1α protein in leukemic cells. Notably an O_2-independent mechanism by isocitrate dehydrogenase (IDH) has been reported. IDH has two family members: IDH1 and IDH2. IDH catalyzes the interconversion of isocitrate and 2-oxoglutarate (2-OG) and is frequently mutated in human brain tumors and leukemia [32, 33]. IDH mutants have the neomorphic enzymatic ability to convert 2-OG to the (*R*)-enantiomer of 2-hydroxyglutarate ((*R*)-2HG). (*R*)-2HG, but not (*S*)-2HG, stimulates Phd enzymatic activity, resulting in decreased HIF protein levels [34]. Because IDH mutants block the differentiation of primitive hematopoietic stem/progenitor cells [35], these biochemical reactions are thought to promote transformation and generation of leukemic stem cells.

Although it is unclear whether the HIF-1α protein expression, therapeutic outcome, and prognosis are correlated, recent studies suggest that murine lymphoma model and human acute myeloid leukemia (AML) stem cells activate HIF-1α signal even under normoxia and maintain the tumor stemcell capacity (Fig. 2) [36]. Knockdown of HIF-1α and HIF inhibitor or overexpression of VHL suppresses the colony-forming capacities of murine lymphoma and human AML. The engraftment of these models in immunodeficient mice is also suppressed by the treatment of HIF inhibitor. In these models, the putative lymphoma stemcell fraction activates HIF-1α to suppress the negative feedback loop of Hes1 and increased the impact of Notch signals [36]. Retroviral transduction BCR-ABL in Vav-Cre-mediated HIF-1$\alpha^{-/-}$ hematopoietic stem/progenitor cells also showed that the generation of chronic myeloid leukemia stem cells by BCR-ABL is defective under HIF-1α deficiency (Fig. 2) [37]. Although both the IDH-mediated destabilization [34] and the aberrant stabilization [36, 37] of HIF-1α are potentially involved in the pathogenesis of leukemia/lymphoma stem cells, therapeutic modulation of HIF-1α levels in leukemic stem cells may represent a potential target for the treatment of hematological malignancies.

Conclusion

In this review, we have outlined the effects of hypoxia response system on hematopoietic and leukemic stem cells. Classical concept of tumor hypoxia is now connected to the hierarchical normal and leukemic stemcell system by the growing evidences. A deeper understanding of the

similarities and differences of hypoxia response and oxygen metabolism between hematopoietic and leukemic stem cells may provide a novel approach to the therapeutic targeting of leukemic stem cells. Such knowledge may also contribute to new technologies for in vitro manipulation, maintenance, and expansion of normal hematopoietic stem cells.

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Conflict of interest The authors declare that they have no conflict of interest.

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