

## The Role of Hypoxia and Exploitation of the Hypoxic Environment in Hematologic Malignancies

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### Abstract

Tumor hypoxia is a well-described phenomenon during the progression of solid tumors affecting cell signaling pathways and cell metabolism; however, its role in hematologic malignancies has not been given the same attention in the literature. Therefore, this review focuses on the comparative differences between solid and hematologic malignancies with emphasis on the role of hypoxia during tumorigenesis and progression. In addition, contribution of the bone marrow and angiogenic environment are also discussed. Insight is provided into the role of hypoxia in metastatic spread, stemness, and drug resistance in hematologic conditions. Finally, emerging therapeutic strategies such as small-molecule prodrugs and hypoxia-inducible factor (HIF) targeting approaches are outlined to combat hypoxic cells and/or adaptive mechanisms in the treatment of hematologic malignancies. *Mol Cancer Res*; 12(10); 1347–54. ©2014 AACR.

### Introduction

Hypoxia is a level of oxygen tension below the physiologic level; physiologic range varies due to diversified blood vessel network in different organs (1). Hypoxic conditions develop during cancer progression due to rapidly proliferating tumor cells that reduce O<sub>2</sub> diffusion as well as impaired perfusion of the abnormal blood vessels in the tumor. The oxygen level in hypoxic tumor tissues is found to be less than 1.3% O<sub>2</sub>, far below the physiologic oxygenation level (5%–10% O<sub>2</sub>; ref. 2).

Cellular adaptation to hypoxia is mediated through protein stabilization of hypoxia-inducible factor (HIF) subunits (HIF1 $\alpha$ , HIF2 $\alpha$  and HIF3 $\alpha$ ) that are regulated by prolyl hydroxylase domain (PHD) and factor-inhibiting HIF1 (FIH-1) enzymes. In oxygenated cells, HIF $\alpha$  subunits are hydroxylated by PHD and FIH-1, poly-ubiquitinated, and degraded by the proteasome; in hypoxia, the PHD enzymes lose their activity and the HIF degradation is halted. The stabilized, non-hydroxylated HIF $\alpha$  translocate to the nucleus, where they dimerize with constitutively expressed HIF $\beta$  subunit, and bind to DNA to initiate gene transcription of the adaptive pathways (3, 4). Cellular adaptations to hypoxia can also occur by HIF-independent mechanisms including mammalian target of rapamycin (mTOR) kinase, unfolded protein response (UPR) in response to endoplasmic reticulum (ER) stress

(5), p38 MAPK, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS; ref. 6), NF- $\kappa$ B (7), and alarmins (8).

Hypoxia in solid tumors is widely involved in solid tumor biology including angiogenesis, metastasis, and tumor resistance. The overexpression of HIF $\alpha$  subunits in solid tumors is associated with aggressive cancer cell behavior and is correlated with poor overall survival of patients (9). In this review, we focused on recent insights into the hypoxic aspect of tumorigenesis and therapy in hematologic malignancies.

### The Role of Hypoxia in Hematologic Malignancies

Physiologically, the bone marrow shows the average oxygen tension between 5% and 7% O<sub>2</sub> (10, 11). Because of the bone marrow tissue architecture, there is a heterogeneous O<sub>2</sub> level implied by the fact that the bone marrow consists of at least two different niches, the endosteal niche, the most hypoxic region of the bone marrow and closer to the bone, and the well-oxygenated vascular niche closer to the blood vessels (12). High levels of HIF1 $\alpha$  were demonstrated at the border of endosteum, whereas lower levels of HIF1 $\alpha$  were shown in the vascular niche (13). Interestingly, the hematopoietic stem cells (HSC) are localized mainly in the bone marrow with the endosteal niche, suggesting fundamental role of hypoxia in stem-cell function maintenance (14), such as homeostasis, low mitochondrial activity, and increased glycolysis (15).

### Development of hypoxia due to tumor progression

The development of hypoxic conditions during the progression of solid tumors was very well documented (16–19), and was shown to be mediated through a complex network of signaling including HIF-dependent and -independent pathways (3, 5). Severe hypoxic conditions (beyond the

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physiologic bone marrow conditions) were shown to develop during the progression of hematologic malignancies including multiple myeloma, lymphoma, and leukemia.

Similar to solid tumors, bone marrow samples from patients with multiple myeloma were reported hypoxic by using endogenous hypoxic markers, HIF1 $\alpha$  and HIF2 $\alpha$  (11, 20–23). In animal models, the expression of exogenous (pimonidazole) and endogenous (HIF1 $\alpha$ ) hypoxia markers was significantly increased in the bone marrow of mice with high involvement of myeloma compared with the bone marrow of naïve mice or in areas with low tumor involvement (9, 18, 24). In addition, a direct correlation was found between the tumor burden and the levels of hypoxia in the bone marrow of mice with multiple myeloma (18). In addition to the bone marrow, circulating multiple myeloma cells were shown to have hypoxic phenotype (18). In multiple myeloma cell lines, including KMM1, U266, RPMI8226, JLN3, U266, H929, and MM1s, HIF1 $\alpha$  was constitutively expressed and was further increased when the cells were exposed to hypoxia (9, 18, 21, 23). In addition to hypoxia in the multiple myeloma cells, the multiple myeloma bone marrow microenvironment also showed hypoxic response, in which strong HIF1 $\alpha$  and HIF2 $\alpha$  expression was detected in CD68<sup>+</sup> macrophages (20, 22) and in stromal cells in areas with multiple myeloma progression (18).

HIF1 $\alpha$  and HIF2 $\alpha$  were detected in 54% of patients with Hodgkin lymphoma, and in 71% of patients with non-Hodgkin lymphoma; and the expression of HIF $\alpha$  and HIF2 $\alpha$  was found to play a role in tumor progression (25). In diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, and mantle cell lymphoma (MCL), HIF1 $\alpha$  and HIF2 $\alpha$  proteins were highly expressed in 70%, 73%, and 73.5% of the patients, respectively (26, 27).

High expression of HIF1 $\alpha$  was detected in 67% of patients with acute myelogenous leukemia (AML; ref. 28), 66.7% of patients with pediatric acute lymphoblastic leukemia (ALL; ref. 29) with hypoxic areas widely present in ALL leukemic bone marrow (30), and in 100% (10 of 10) of patients with B-cell chronic lymphocytic leukemia (CLL; ref. 31). It was demonstrated that during leukemogenesis in rat xenograft model of AML, the bone marrow became more hypoxic, detected by nitroimidazol, in which hypoxia was found in 80% of AML cells in the bone marrow and 40% of circulating cells (32). Other reports showed that hypoxic regions also expanded in the bone marrow in the mice xenograft models of ALL, AML, and chronic myelogenous leukemia (CML; refs. 30, 33, 34). In CML model, hypoxia was shown to develop in the bone marrow in correlation with the tumor burden, while no hypoxia was found in circulating CML cells (34).

Hypoxia was shown to develop during the progression of most types of solid tumors; however, the development of hypoxia in hematologic malignancies was variable. In myeloma and lymphoma, which have a more solid nature in the bone marrow, hypoxia was shown to develop in the bone marrow of the patients in correlation with the tumor progression in the bone marrow, while in myeloma also circulating tumor cells had hypoxic phenotype. Leukemia

involves both the bone marrow and circulation but the number of circulating cells is way much higher, and the dependency of leukemia cells on the bone marrow is less critical. Accordingly, hypoxia in the bone marrow was developed in different types of leukemia but less evidence was found for hypoxic phenotype in the circulating leukemic cells. We suggest that hypoxia plays a major role in the development of all three types of hematologic malignancies, but to a lower extent in leukemia.

### The role of hypoxia in angiogenesis

In solid tumors, tumor neovasculogenesis plays a crucial role in the growth, progression, and metastasis of cancer (16). New blood vessels provide oxygen and nutrients, entrance for the immune cells, and exit of tumor cells into the circulation. Uncontrolled cancer cell proliferation causes hypoxia, which induces proliferation and sprouting of endothelial cells from preexisting vessels, supports vessel maturation (1), and contributes to the recruitment of endothelial progenitor cells from the bone marrow to tumor areas and induction of their differentiation into endothelial cells (35).

An increase in vascular density in the bone marrow, regulated at least, in part, by the HIF pathway, was demonstrated in patients during the monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma progression. A positive correlation was demonstrated between hypoxic markers, such as HIF1 $\alpha$  and HIF2 $\alpha$  expression, proangiogenic factors (VEGF) and its receptors, circulating chemokine SDF-1, and the level of bone marrow angiogenesis in patients with multiple myeloma (20, 22). Culturing multiple myeloma cell lines and CD138<sup>+</sup> cells from myeloma patients in hypoxia increased CXCL2, VEGF, TGF $\beta$ , and IL8 secretion, induced expression of HIF1 $\alpha$  or HIF2 $\alpha$ , and augmented angiogenesis *in vivo* (11, 22). Subcutaneous injection of CD138<sup>+</sup> multiple myeloma cells with overexpressed HIF1 $\alpha$  and HIF2 $\alpha$  into the nude mice induced a number of proangiogenic molecules, which resulted in multiple myeloma-induced angiogenesis *in vivo* (22). On the other hand, silencing HIF1 $\alpha$  in multiple myeloma cells reduced new vessels formation both *in vivo* (23) and *in vitro* (11).

Angiogenesis was shown to be involved in the pathogenesis and progression of non-Hodgkin lymphomas; matched lymph node biopsies at diagnosis and recurrence of relapsed/refractory indolent non-Hodgkin lymphomas patients showed increased vascular network and HIF1 $\alpha$  protein expression in the second biopsy, providing direct evidence that hypoxia-induced angiogenesis is an essential process for disease progression (37). In anaplastic large-cell lymphomas, secretion of VEGF and angiogenesis was shown to be mediated through hypoxia-induced downregulation of microRNA-16 (miR16) (38).

In leukemia, it was demonstrated that hypoxia contributes to angiogenesis through increased exosomes production by K562 leukemia cell line. This study has shown that exosomes derived from hypoxic leukemia cells increased tube formation when cultured with endothelial cells, partially via augmented exosomal miR210 expression (39). In addition,

silencing of HIF1 $\alpha$  in leukemic bone marrow stromal cells (BMSC) from AML and ALL decreased production of proangiogenic factor (such as VEGF), and decreased angiogenesis (40).

These results suggest that, similar to solid tumors, angiogenesis in hematologic malignancies is induced by tumor hypoxia and targeting one of the hypoxic pathways, namely HIF, shows promising results in controlling formation of the blood vessels. Hypoxia-induced angiogenesis in hematologic malignancies was shown to be mediated mainly through local secretion of angiogenic factors and sprouting of local vessels; unlike solid tumors, there is no evidence that hypoxia affects cell trafficking of endothelial progenitors in hematologic malignancies. The effect of tumor hypoxia on endothelial progenitors and their contribution to angiogenesis in hematologic malignancies is yet to be explored. We suggest that the inhibition of hypoxic response can provide an alternative approach to inhibit angiogenesis in which it will cause downregulation of multiple angiogenic factors at once, rather than inhibition of a specific factor.

#### The role of hypoxia in metastasis and spread

Hypoxia was shown to induce invasive behavior of epithelial cancer cells via epithelial-to-mesenchymal transition (EMT) in which cells acquire plastic and migratory abilities (17, 18). Tumor hypoxia was shown to activate EMT transcription factors (41, 42) and molecules responsible for polarization, adhesion, invasion of cells, such as vimentin, fibronectin, CXCR4, E-cadherin, MMP-2, and MMP-9 (17, 18).

Multiple myeloma is present at multiple sites of the bone marrow and the tumor cells spread throughout by continuous trafficking in and out of the bone marrow (18, 43–45). Steps of cell dissemination include: (i) cell detachment and acquisition of EMT-like phenotype (ii) invasion, (iii) egress to the bloodstream, (iv) circulation in the blood, (v) homing to the predetermined tissues, (vi) formation of a new tumor foci, and finally (vii) growth of micrometastasis (colonization; ref. 46). The selective choice is believed to be triggered by multiple factors such as chemoattractants (SDF-1 and its ligand, CXCR4), growth factors (IL6, TGF $\beta$ , IGFI, IGFII, FGF, PDGF, and VEGF), and calcium (46). Although not of an epithelial origin, Azab and colleagues (18) showed that increasing bone marrow tumor volume caused tumor hypoxia that induced multiple myeloma cell dissemination via hypoxia-induced EMT-like phenotype (increased expression of Snail1, FOXC2, and TGF $\beta$ ). It was observed that, with increasing hypoxia, multiple myeloma cells seemed to be less adherent to bone marrow microenvironment (through downregulation of E-cadherin and downregulation of secretion of SDF1 from the stroma), and consequently, a higher number of circulating multiple myeloma cells in the peripheral blood was observed in mouse xenograft model (18). At the same time, hypoxic multiple myeloma cells (in the bone marrow and in the circulation) expressed high CXCR4 through increased expression of HIF1 $\alpha$  and facilitated migration and homing of multiple myeloma cells to new bone marrow niches (18).

In lymphoma, HIF1 $\alpha$  and HIF1 signaling were increased during liver metastasis of T-lymphoma cells *in vivo*, and HIF1 $\alpha$  knockdown significantly impaired Met expression and phosphorylation and inhibited scattered liver metastasis (47).

In leukemia, Azab and colleagues (34) demonstrated that hypoxia regulated trafficking of CML cells. Hypoxia decreased the expression of E-cadherin in CML cells in the bone marrow and *in vitro*, which resulted in decreased adhesion to bone marrow stroma. In addition, hypoxia increased CXCR4 expression *in vivo* and *in vitro* and enhanced chemotaxis. These results suggest that hypoxia in the bone marrow induced an EMT-like phenotype in CML cells (34). It was also reported that in AML, hypoxia increased CXCR4 expression, thus augmented migration and survival of leukemic cells (48). Moreover, silencing HIF1 $\alpha$  applied in BMSCs, resulted in VEGF and SDF-1 reduction, and as a consequence, decreased leukemia cell migration and adhesion (40). No changes were observed in the expression of CXCR4 and E-cadherin in circulating CML cells as a result of hypoxia in the bone marrow (34).

To sum up, these results show that metastatic spread of multiple myeloma is very similar to the metastatic spread of solid tumors, which is based on the fact that myeloma cells both in the bone marrow and in the circulation show hypoxic phenotype that facilitates metastasis through the EMT-like phenotype. Inhibition of the hypoxic response as a therapeutic strategy to prevent metastasis in multiple myeloma is yet to be explored. Although the literature in lymphoma is relatively limited, it seems that hypoxia plays an important role in the spread of lymphoma; more research to explore this role and its therapeutic applications is warranted. In leukemia, hypoxia seems to play a critical role of the progression of the disease in the bone marrow and it displays EMT-like phenotype, but not when the cells are circulating (in CML). However, the role of hypoxia in other leukemia types is still not clear.

#### The role of hypoxia in stemness and drug resistance

Hypoxia confers treatment resistance in solid tumors by regulating processes such as (i) cell-cycle arrest (quiescence), a state of reduced cell proliferation that protects the cells from external stress (49–51), (ii) inhibiting apoptosis and senescence of cells, (iii) controlling autophagy, ER-stress, and p53 and mitochondrial activity (50, 52). Hypoxia induces de-differentiated and immature phenotype of cells, as shown in neuroblastoma and breast cancer cells (53), as well as maintaining stem cell-like phenotype in both HSCs and brain stem cells (14, 54, 55). This was confirmed by activation of stemness-related genes and pathways, such as Oct4, HIF, Notch (56, 57), Wnt, and Hedgehog (58, 59). Hypoxic multiple myeloma cells were shown to undergo de-differentiation, which was demonstrated by decreased expression of plasma cell-specific markers (including CD138 and CS1) and transcription factors (IRF4, PRDM1, and XBP1), increased expression of B-cell-specific CD markers (CD20) and transcription markers (BCL6 and PAX5), and increased stem cell-like transcriptional program

including Oct-4, SOX2, and NANOG (60). Drug resistance is one of the main features of stem cell-like phenotype that are associated with hypoxia in solid tumors. It was demonstrated that by inhibiting HIF1 $\alpha$  in multiple myeloma cells using echinomycin or siRNA against HIF1 $\alpha$ , myeloma cells were sensitized to melphalan, which happened through reduction of one of the most important antiapoptotic proteins, survivin (21).

Inhibition of HIF1 $\alpha$  using echinomycin in mouse lymphoma stem cell population (characterized as c-Kit<sup>+</sup>Sca-1<sup>+</sup>) significantly reduced the colony formation of the lymphoma stem cells, indicating clear involvement of hypoxia in lymphoma tumor initiation (61). Interestingly, VHL protein (involved in HIF $\alpha$  degradation) transcript was undetectable in c-Kit<sup>+</sup>Sca-1<sup>+</sup> lymphoma cells, which suggests the importance of HIF $\alpha$  in maintaining stem cell-like phenotype in lymphoma (61). In addition, HIF $\alpha$  protein stabilization was correlated with high expression of antiapoptotic protein, Bcl(xL), indicating that HIF $\alpha$  is involved in apoptosis resistance of lymphoma cells conferring drug resistance in non-Hodgkin lymphoma (62).

In leukemia, using a mouse model of CML, it was demonstrated that HIF1 $\alpha$  is a key player in maintaining the survival of leukemia stem cells that present the phenotype of Lin<sup>-</sup>Sca-1<sup>+</sup>c-Kit<sup>+</sup>. HIF1 $\alpha$  deletion hindered propagation of CML by inhibiting cell-cycle progression and inducing apoptosis. Interestingly, when compared with normal HSCs, leukemia stem cells seem to be more HIF1 $\alpha$ -dependent (63). HIF2 $\alpha$  was also demonstrated to be strongly involved in the long-term repopulating ability of human CD34<sup>+</sup> HSCs as well as in survival and engraftment ability of primary human AML cells (64). Inducible HIF1 $\alpha$  in AML cells changed the expression of 19 miRNAs, among which miR17 and miR20a were downregulated by repressing c-Myc expression. These two miRNAs alleviated hypoxia and HIF-induced AML cell differentiation. Studies *in vivo* revealed that miR20a contributes to HIF1 $\alpha$ -inducible differentiation of AML cells (65). Overexpression of HIF1 $\alpha$  in hypoxic AML cell lines conferred chemoresistance to antimycin due to quiescence induced by cell-cycle arrest (66). Coculture of ALL cells with stromal cells in hypoxia induced HIF1 $\alpha$  and AKT/mTOR signaling; inhibition of mTOR decreased HIF1 $\alpha$  and led to chemosensitization of ALL cells (33).

Altogether, in solid tumors the contribution of hypoxia to the drug-resistant stem-cell-like phenotype is well established. However, the results which suggest that hypoxia plays an important role in development of cancer stem cells (CSC) and drug resistance in hematologic malignancies are still preliminary and need further confirmation to provide direct evidence for the involvement of hypoxia in these processes. We suggest to further investigate the role of hypoxia in the development of drug-resistant stem cell-like phenotype; in hematologic malignancy this will have a huge impact on our understanding of the development of resistance to therapy, development of minimal residual disease, and recurrence in hematologic malignancies. Exploring hypoxia will open new therapeutic windows for enhanced

treatments in the relapsed/refractory stages of these diseases. Special emphasis should be given to tumors involving stem cells and blasts.

### Other effects induced by hypoxia

It was demonstrated that HIF $\alpha$  plays an important role in bone biology and bone disease, because the bone is physiologically a hypoxic tissue (67). Lytic lesions affect more than 80% of patients with multiple myeloma and are caused by imbalance between proliferation and activity of overstimulated osteoclasts and suppressed osteoblasts. Receptor activator of NF- $\kappa$ B ligand (RANKL) produced by multiple myeloma cells and activated osteoclasts was shown to be increased in patients with multiple myeloma (68). Recent reports suggest that osteoclast activity contributing to the osteolytic bone disease in multiple myeloma is hypoxia-regulated, because the increase in number and size of osteoclasts was, in part, regulated by HIF1 $\alpha$  (23). Stable inhibition of HIF1 $\alpha$  in multiple myeloma cells halted proosteoclastogenic cytokines secretion including IL7 and CCL3/MIP-1 $\alpha$ , reduced the weight and tumor size *in vivo*, and significantly decreased bone destruction (23). Osteolytic lesions were reduced together with tumor burden by HIF inhibition that can be explained by the fact that active osteoclasts support multiple myeloma cell proliferation *in vivo*.

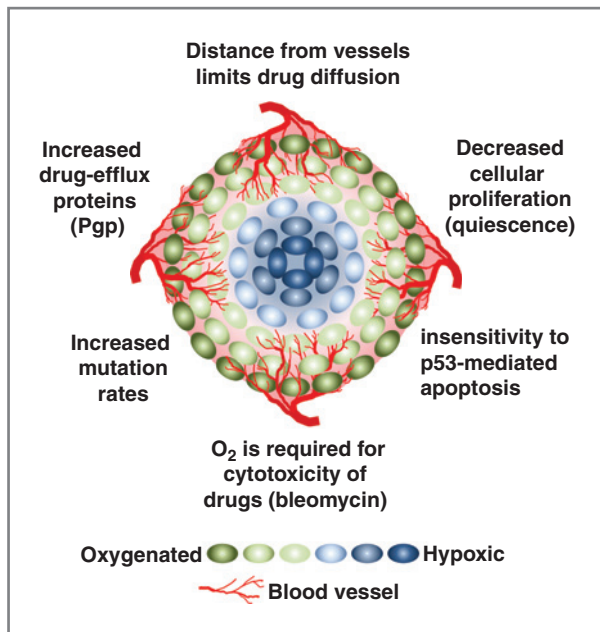
### Therapeutic Strategies Targeting Tumor Hypoxia in Hematologic Malignancies

Targeting hypoxia is an emerging strategy for the treatment of hematologic malignancies, in which hypoxia-induced cellular adaptation mechanisms and hypoxic cells are being used as therapeutic targets for prevention of angiogenesis, metastasis, stemness, and drug resistance.

Targeting hypoxia is an emerging approach to target tumor tissue and microenvironment, mainly due to the fact that hypoxia induces resistance to anticancer therapies. Some of the mechanisms of therapy-resistance include (i) increased expression of drug efflux pumps (such as P-glycoprotein, Pgp), (ii) oxygen-dependent cytotoxicity of some drugs (such as bleomycin), (iii) mutations conferring resistance, (iv) selection of cells insensitive to p53-mediated apoptosis, (v) decreased cell proliferation rate in hypoxic regions, and (vi) drug diffusion limits due to the distance from the blood vessels and thus inadequate exposure of cells to anticancer drugs (see Fig. 1).

### Targeting hypoxic cells with prodrugs

Small-molecule prodrugs discovery in the 1970s was a result of an attempt to overcome the problem with hypoxia-induced radiation resistance. It led to the finding of nitroimidazoles that can mimic the effect of oxygen and enhance the effect of radiation. Recently, a paradigm has shifted and hypoxia-activated prodrugs are currently used as compounds that target hypoxia and HIF-mediated signaling pathways. This strategy is intended to penetrate the tissue and deliver cytotoxic agents to the hypoxic nutrient-starved regions of tumor areas with minimal toxicity for the surrounding



**Figure 1.** Mechanisms of resistance to anticancer drugs developed by hypoxic cells. Hypoxia-associated mechanisms conferring therapy-resistance include (i) increased expression of drug efflux pumps (such as Pgp), (ii) oxygen-dependent cytotoxicity of drugs (such as bleomycin), (iii) mutations providing apoptosis resistance, (iv) selection of cells insensitive to p53-mediated apoptosis, (v) decreased cell proliferation rate in hypoxic regions, and (vi) inadequate exposure of cells to anticancer drugs due to diffusion limit.

normoxic cells (69). In principle, non-toxic prodrug requires one- or two-electron reduction to a far more toxic radical, or further to a downstream product.

In solid tumors, some of the first prodrugs such as metronidazole, misonidasole, etanidazole, and tirapazamine were shown to have a very high toxicity. Combination of these drugs with other chemotherapies enhanced the effectiveness of the treatment *in vitro* and *in vivo* (70–73). The combination of tirapazamine with cisplatin shows prolonged survival in phase III clinical trial; however, dosing is limited because of neutropenia and other toxicities (74). No literature was found describing the use of these compounds in hematologic malignancies.

Two new prodrugs, 2-nitroimidazole of the phosphoramidate mustard, namely TH-302 and PR-104, also show potential in the clinical studies due to better dose potency compared with tirapazamine. TH-302 was shown to be cytotoxic against a number of human hypoxic cancer cell lines *in vitro* and *in vivo* (75–79). TH-302 is being clinically tested in solid tumors as a therapy in combination with chemotherapy (69, 75), and has completed phase I clinical trial. TH-302 and PR-104 have been tested in myeloma and leukemia and their activity was demonstrated both *in vivo* and *in vitro*. Preclinical studies on myeloma model (5T33MMvv) showed that TH-302 induced multiple myeloma cell apoptosis and decreased paraprotein secretion *in vivo*, and combination of TH-302 and bortezomib significantly improved survival of mice

(24, 75). TH-302 also effectively inhibited onset, progression of AML, and extended the overall survival, both in systemic AML xenograft models and mice with advanced AML disease. *In vitro*, TH-302 decreased HIF1 $\alpha$  protein expression and reduced ROS production, which resulted in decreased proliferation, increased cell-cycle arrest, and induced double-stranded DNA breaks (80). TH-302 is currently evaluated in phase I/II study in patients with relapsed/refractory multiple myeloma combination with dexamethasone, with or without bortezomib (NCT01522872). Dose-limiting toxicity with most common adverse events, such as leukopenia, thrombocytopenia, anemia, and stomatitis, was described. TH-302 in combination with dexamethasone scheduled twice weekly showed initial clinical activity with benefit rate in 33% patients who were relapsed/refractory to both bortezomib and thalidomide/lenalidomide regimens.

PR-104 is being evaluated in treating patients with refractory/relapsed AML in phase I/II study (NCT01037556), because leukemic bone marrow was reported to be sufficiently hypoxic to activate PR-104 to its active metabolite PR-104H and PR-104M. The final report demonstrated that PR-104 had moderate toxicity such as myelosuppression and enterocolitis (81).

The hypoxic prodrugs are showing promising results in preclinical and clinical studies in hematologic malignancies as well as solid tumors; however, their toxicity and pharmacokinetics appear to be a limiting factor in these treatments. We suggest further studies for the development of a new generation of hypoxic prodrugs with greater hypoxic selectivity, lower toxicity, and better pharmacokinetics, as well as more efficient vector for enhanced delivery of these drugs to hypoxic regions.

### Targeting HIF pathway

HIF $\alpha$ -targeted therapy is another promising approach to overcome hypoxia-induced progression, metastasis, and drug resistance. Although hypoxia does not equate HIF $\alpha$  expression, because the HIF $\alpha$  pathway is also regulated in normoxic conditions, HIF $\alpha$  is strongly intertwined with almost every aspect of cancer biology due to its powerful potential of regulating gene transcription. Recently, a high-throughput screening of FDA-approved drugs disclosed digoxin, acriflavine, anthracyclines (doxorubicin), vincristine, paclitaxel, and many more as HIF $\alpha$  inhibitors, which suggest that currently used drugs are already inhibiting HIF $\alpha$ . However, based on their primary therapeutic functions, these inhibitors have also HIF $\alpha$ -independent effects, thus their usage is not specific (82).

Silencing HIF1 $\alpha$  with shRNA successfully reduced multiple myeloma-induced new vessel formation and tumor burden (11, 23). In addition, inhibition of HIF $\alpha$  was proposed to target therapy-resistant stem cell-like phenotype in hematologic CSCs (83). Promising study *in vivo* demonstrated that HIF1 $\alpha$  inhibition using echinomycin in lymphoma (61) and leukemia (63) stem cells, efficiently decreased tumor-initiating activity of these cells and improved survival of mice. It was also shown that blocking

HIF $\alpha$  enhances the antiproliferative effect of lenalidomide (23) and melphalan (21).

Further studies are warranted to investigate the therapeutic applications of inhibition of HIF $\alpha$  in prevention of tumor progression, metastasis, and drug resistance in hematologic malignancies. Furthermore, the development of more specific small-molecule HIF $\alpha$  inhibitors is urgently needed in the field to test these therapeutic applications.

## Conclusions

Tumor-hypoxia was shown to develop in correlation with the progression of hematologic malignancies and is associated with aggressive tumor behavior. Exploiting the distinctive features of hypoxia and hypoxia-regulated

molecules in hematologic malignancies is a promising strategy to improve the treatment of hematologic malignancies; however, its full potential is yet to be determined. We hypothesize that targeting hypoxia in hematologic malignancies, especially in multiple myeloma and lymphoma, will improve the treatment in these diseases through inhibition of angiogenesis, metastasis, and drug resistance.

## Disclosure of Potential Conflicts of Interest

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