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REVIEW ARTICLE

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Impact of hypoxia on the pathogenesis and therapy resistance in multiple myeloma

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Abstract

Multiple myeloma (MM) is a refractory plasma cell tumor. In myeloma cells, the transcription factor IRF4, the master regulator of plasma cells, is aberrantly upregulated and plays an essential role in oncogenesis. IRF4 forms a positive feedback loop with MYC, leading to additional tumorigenic properties. In recent years, molecular targeted therapies have contributed to a significant improvement in the prognosis of MM. Nevertheless, almost all patients experience disease progression, which is thought to be a result of treatment resistance induced by various elements of the bone marrow microenvironment. Among these, the hypoxic response, one of the key processes for cellular homeostasis, induces hypoxia-adapted traits such as undifferentiation, altered metabolism, and dissemination, leading to drug resistance. These inductions are caused by ectopic gene expression changes mediated by the activation of hypoxia-inducible factors (HIFs). By contrast, the expression levels of IRF4 and MYC are markedly reduced by hypoxic stress. Notably, an anti-apoptotic capability is usually acquired under both normoxic and hypoxic conditions, but the mechanism is distinct. This fact strongly suggests that myeloma cells may survive by switching their dependent regulatory factors from IRF4 and MYC (normoxic bone marrow region) to HIF (hypoxic bone marrow microenvironment). Therefore, to achieve deep remission, combination therapeutic agents, which are complementarily effective against both IRF4-MYC-dominant and HIF-dominated fractions, may become an important therapeutic strategy for MM.

KEYWORDS

HIF, hypoxia, IRF4, multiple myeloma, MYC

1 | INTRODUCTION

MM, a typical refractory hematopoietic tumor of plasmacytoid origin, has achieved a marked improvement in prognosis not only for newly diagnosed cases but also for relapsed and refractory cases due to the increase in therapeutic options.[1] New treatments such as proteasome inhibitors (PIs) target ER stress, cerebron modulators target transcription factors such as Ikaros and Aiolos, and

Abbreviations: BM, bone marrow; EPO, erythropoietin; ER, endoplasmic reticulum; HIF-PH, HIF proline hydroxylase; HIFs, hypoxia-inducible factors; HK2, hexokinase-2; IMiDs, immunomodulatory drugs; IRF4, interferon regulatory factor 4; lncRNAs, long noncoding RNAs; miRNAs, microRNAs; MM, multiple myeloma; PIs, proteasome inhibitors; SP, side population.

monoclonal antibodies (mAbs) target surface antigens such as SLAMF7 and CD38.

In normal hematopoiesis, interferon regulatory factor 4 (IRF4) is required in lymphocyte activation and plasma cell differentiation as the master regulator.[2] In MM, IRF4 plays a principal role in maintaining the disease phenotype.[3,4] In 1997, Dalla-Favera's group was the first to report that *IRF4* is the gene responsible for the development of MM with t(6;14)(p25;q32).[3] In 2008, Staudt's group reported that IRF4 is upregulated in every MM subtype.[4] They further demonstrated that IRF4 potentially possesses a variety of oncogenic capabilities, and c-MYC (MYC) forms a positive feedback loop with IRF4. This positive feedback induces a variety of oncogenic functions such as anti-apoptosis, cell proliferation, and metabolic activation, and maintains plasmacytoid phenotypes.[5,6] IMiDs such as lenalidomide and pomalidomide inhibit the Ikaros-IRF4 axis.[7,8] Moreover, PIs and mAbs are used based on the idea that MM is a malignancy possessing plasmacytoid phenotypes with high ER stress or similar surface antigens as well as normal plasma cells. Unfortunately, almost all patients experience disease progression.

Generally, cancer cells have inherent plasticity and can change their properties depending on the signals arising from their microenvironment and they, therefore, acquire stemness and therapy resistance.[9,10] Similarly, in myeloma cells, there is likely to be a switch back between dominant clones and stem-like cells, leading to treatment resistance via the effects of microenvironmental factors.[11] Therefore, BM microenvironmental factors are attractive therapeutic targets. These factors comprise not only stromal cells and immune cells, but also metabolic stresses such as hypoxia, nutrient starvation, and low pH.[12] Of these, hypoxia contributes to the maintenance of homeostasis in various cells including tumor cells. "Hypoxia" is generally defined as a condition in which a particular tissue has less oxygen supply than required. In solid tumors, hypoxia occurs due to the rapid growth of cancer cells and the aberrant angiogenesis of tumor blood vessels. Hypoxia also occurs when myeloma cells are present in the endosteum niches, where the cells are exposed to low blood flow and consume large amounts of oxygen.[13] The most important regulator of the hypoxic response is the hypoxia-inducible factor (HIF). The HIF-inducible genes exert an anti-apoptotic effect and promote drug resistance in a variety of cancer types under hypoxic conditions.[14] Interestingly, HIF-1α downregulates MYC expression, and the downregulation of MYC under hypoxic conditions is required for the precise regulation of energy metabolism.[15,16] Hypoxic stress causes the accumulation of HIF-1α with downregulation of IRF4 and MYC in myeloma cells.[17] This is interesting because the regulation of anti-apoptotic function might be induced by distinct pathways between normoxic and hypoxic conditions.

In this review, we aimed to evaluate the disease progression or therapy resistance induced by hypoxic response and assess the usefulness of complementary therapeutic approaches against IRF4-MYC- and HIF-regulated fractions in MM.

2 | POSSIBILITY OF EXISTING MYELOMA STEM CELL IN BONE MARROW MICROENVIRONMENT

A "cancer stem cell" is a cancer cell that has the potential to self-renew and differentiate into heterogeneous nontumorigenic cancer cells.[18] Other stem cell-specific characteristics including quiescence, resistance to metabolic stress, enhanced DNA repair capability, and anti-apoptotic capability lead the malignant cells to develop therapeutic resistance. In some hematopoietic tumors, such as acute myelogenous leukemia,[19] stem cell phenotypes have been identified. Some approaches have been previously used to detect myeloma cells that possess cancer stem cell characteristics (namely myeloma stem-like cells), although the phenotype of myeloma stem-like cells remains undetermined. In this paragraph, we discuss 2 approaches for identifying myeloma stem-like cells by analyzing the SP and hypoxia-subjected cells. In cancer stem cells, the activation of genes involved in drug efflux has been observed. Higher expression levels of these genes could be detected in the SP fraction than in the non-SP (major population: MP) fraction.[20,21] In our previous study, the SP cells of MM were in the proliferative phase with high expression of mitotic genes such as *AURKA* and oncogenes including *IRF4* and *MYC*, and CD138.[22] Considering these data, the majority of SP fraction are thought to be "activated stem-like cell fractions."[11]

In contrast, myeloma stem-like cells may exist in the treatment-resistant fractions in a hypoxic BM microenvironment, in which they remain dormant and undifferentiated.[23] In MM, hypoxic stress reduces the expression of plasmacytoid markers such as IRF4, BLIMP1, and XBP1, and surface antigens such as SLAMF7 and CD138.[24] By contrast, the progenitor markers of plasma cells, such as BCL6 and PAX5, and stem cell markers or signals, such as Oct-4, NANOG, SOX2, and TGF-β/Smad, are upregulated under hypoxic conditions.[24-26] Hypoxic stimulation also decreases expression levels of MYC, cyclin Ds, and AURKA, leading to cell cycle arrest.[17,25,27] Although both SP and hypoxia-subjected cells commonly show some stem cell characteristics, they have distinct phenotypes, as shown in Table 1. SP and hypoxic myeloma cells may be comprised of heterogeneous populations, however we believe that myeloma stem cells may exist in these populations.

3 | ROLE OF HIF FOR MYELOMA ONCOGENESIS IN HYPOXIC MICROENVIRONMENT

3.1 | Myeloma cell fraction adapting to hypoxic niche

The oxygen partial pressures of human arterial and venous blood are approximately 95 mm Hg and 40 mm Hg, respectively. Bone marrow

TABLE 1 Comparison of phenotype between SP and hypoxia-subjected myeloma cells

Characteristics	SP	Non-SP (MP)	Hypoxia	Normoxia
Transcription factor expression				
IRF4-MYC feedback	High	Low	Low	High
HIF	N/A	N/A	High	Low
Stem cell-like capability				
Quiescence	−	−	+	−
Differentiation	+	−	N/A	N/A
Repopulation	+	−	N/A	N/A
Others				
CD138	High	High	Low	High
Drug resistance	High	Low	High	Low

Abbreviations: HIF, hypoxia-inducible factor; IRF4, interferon regulatory factor 4; MP, major population; N/A, not applicable; SP, side population.

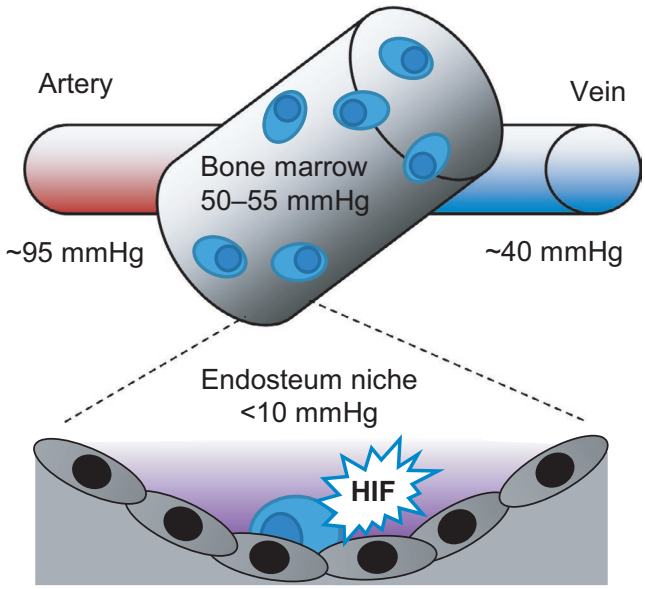

FIGURE 1 Oxygen partial pressure differences affect myeloma cells. HIF, hypoxia-inducible factor

blood gas analysis of healthy individuals and MM patients revealed that the average partial pressure of oxygen was 50-55 mm Hg, which is within the ranges of arterial blood and venous blood values.[28,29] However, the partial pressure of oxygen in the endosteum under hypoxic conditions could not be assessed by a gas analysis. A study of the results of pimonidazole (a 2-nitroimidazole compound) administration analysis using an MM mouse model revealed that the partial pressure of oxygen in the hypoxic niche was <10 mm Hg.[30] Considering that hematopoietic stem cells also adapt to the hypoxic endosteal niche, myeloma tumor-initiating cells may exist in the hypoxic environment (Figure 1).[13]

3.2 | Role of HIFs in hypoxic microenvironment

HIF is comprised of 3 α-subunits and 2 β-subunits. HIF-1α, HIF-2α, and HIF-3α form heterodimers with HIF-1β (aryl hydrocarbon receptor nuclear translocator; ARNT) or ARNT2, activating as transcription factors in the nucleus.[31] The existence of these factors was initially predicted as an enhancer of erythropoietin (EPO) in response to hypoxia.[32] The expression of HIF-1α, which is immediately expressed under hypoxic conditions, triggers the activation of hypoxia-inducible genes.[33] HIF-2α, which is the main enhancer of EPO, is continuously activated after HIF-1α activation and contributes to the adaptation to chronic hypoxia via upregulation of its specific targets.[34] These 2 α-subunits are thought to play important roles in MM, however the association between tumorigenesis and HIF-3α remains largely unknown. Under normoxic conditions, the proline of the HIFα protein is hydroxylated, and the von Hippel-Lindau protein binds to this site. As a result, ubiquitination is induced, and HIFα protein is continuously degraded by the proteasome. However, under hypoxic conditions, proline hydroxylation does not occur and HIFα accumulates in the hypoxic cells; HIFα exerts its activity as a transcription factor in genes with hypoxic response elements.[35,36] HIF proline hydroxylase (HIF-PH) inhibitors are also used clinically as a treatment for renal anemia.[37] In this situation, erythropoiesis-stimulating agents are often used to treat anemia in patients with MM complicated by renal failure. However, because HIF-regulated genes are important not only for normal cells, but also for tumor cell survival and treatment resistance, it is controversial whether HIF-PH inhibitors, which appear to activate the HIF-EPO axis, should be used in patients with cancer, including MM.[38,39] We discuss the recent advances in the study on hypoxic response in MM (Table 2, Figure 2).

4 | IMPACT OF HYPOXIA IN MYELOMA ONCOGENESIS

4.1 | Hypoxia-inducible metabolism switching factors

Many of the glycolytic genes, which are dual regulated by MYC and HIF, are potentially promising therapeutic targets for various types of cancer.[40] Cancer cells depend on glycolysis rather than oxidative phosphorylation (oxphos), even in the presence of oxygen (Warburg effect).[41] Furthermore, hypoxia causes increased glycolysis by further activating the HIF.[42] By comprehensive analysis of glycolytic genes and the metabolites of hypoxia-exposed myeloma cell lines, it was found that hypoxia-induced lactate dehydrogenase A and hexokinase-2 (HK2) led myeloma cells to develop resistance to PIs.[43] However, the mechanism by which HK2, which is also a crucial enzyme that catalyzes the first step of glycolysis, contributes to the development of PI resistance remained unclear. MM with low HK2 expression were included in the FDG-PET false-negative (MRI-positive but FDG-PET-negative) group, while those with high HK2 expression were included in the FDG-PET-positive group.[44] It was found that the FDG-PET false-negative group with low HK2

TABLE 2 Hypoxia-inducible or inhibited elements and their functions in MM

Genes/noncoding RNAs	Functions in MM	Expression change by hypoxia	Upstream	Refs
HIF-1α	Hypoxic response	Upregulated	Hypoxia	24
HIF-2α	Hypoxic response	Upregulated	Hypoxia	24
IRF4	Plasma cell phenotype/oncogene	Downregulated	IRF4-MYC	4,17,24
MYC	Oncogene	Downregulated	IRF4-MYC	4,17
BLIMP1	Plasma cell phenotype	Downregulated	IRF4	4,24
XBP1	Plasma cell phenotype	Downregulated	N/A	24
SLAMF7	Surface antigen of monoclonal antibody target	Downregulated	Ikaros-IRF4	4,24
CD138	Plasma cell phenotype	Downregulated	N/A	17,24
BCL6	B-cell or plasma progenitor phenotype	Upregulated	N/A	24
PAX5	B-cell or plasma progenitor phenotype	Upregulated	N/A	24
Oct-4	Stemness	Upregulated	N/A	24
NANOG	Stemness	Upregulated	N/A	24
SOX2	Stemness	Upregulated	N/A	24
TGF-β/Smad	Stemness	Upregulated	N/A	26
AURKA	Cell cycle	Downregulated	IRF4	4,27
CCND1	Cell cycle	Downregulated	N/A	25
CCND2	Cell cycle	Downregulated	N/A	25
CCND3	Cell cycle	Downregulated	N/A	25
LDHA	Glycolysis, PI resistance	Upregulated	HIF-1α, MYC	4,43,46
HK2	Glycolysis, autophagy, and PI resistance	Upregulated	HIF-1α, MYC	4,43,46
CXCR4	Dissemination	Upregulated	HIF-1α	24,47
CCR1	Dissemination	Upregulated	HIF-2α	48
VEGFA	Angiogenesis	Upregulated	HIF-1α, IRF4, MYC	4,15,27,50
ADM	Angiogenesis	Upregulated	HIF-1α	51
IL32	Bone disease	Upregulated	HIF-1α	52
CREB	Transcription factor	Upregulated	p38	53
MMSET	Oncogene	Upregulated	HIF-1α	53
DKK1	Bone disease	Upregulated	CREB, MMSET	53
miR-210	Inhibition of IRF4	Upregulated	HIF-1α	17
DIMT1	Upregulation of IRF4	Downregulated	miR-210	17
miR-135b	Angiogenesis	Upregulated	HIF-1α	56
miR-17-92	Suppression of tumor suppressive genes	Downregulated	MYC	17
DARS-AS1	Proliferation	Upregulated	HIF-1α	65
H19	Dissemination	Upregulated	HIF-1α	66
KDM3A	Anti-apoptosis under hypoxia	Upregulated	HIF-1α	27

Abbreviations: lncRNAs, long noncoding RNAs; MM, multiple myeloma; PI, proteasome inhibitor, N/A, not available.

expression had a significantly favorable prognosis.[45] Furthermore, hypoxia-inducible HK2 acquired another oncogenic capability via activation of autophagy, leading to the development of PI resistance.[46] Altogether, we can conclude that even under normoxic conditions, glycolytic genes such as *HK2* are constantly regulated by IRF4-MYC. However, changes in the dependent regulator from IRF4-MYC to HIF led HK2 to exhibit an additional function that promotes the development of drug resistance. Therefore, we should pay attention to the additional functions of metabolic factors that are regulated not only by IRF4-MYC but also by HIF. This may lead to the development of novel therapeutic strategies.

4.2 | Hypoxia-inducible dissemination, neovascularization, and bone disease

Dissemination is important for the spread of myeloma cells. Under hypoxic conditions in MM cells, CXCR4 and CCR1 are upregulated by

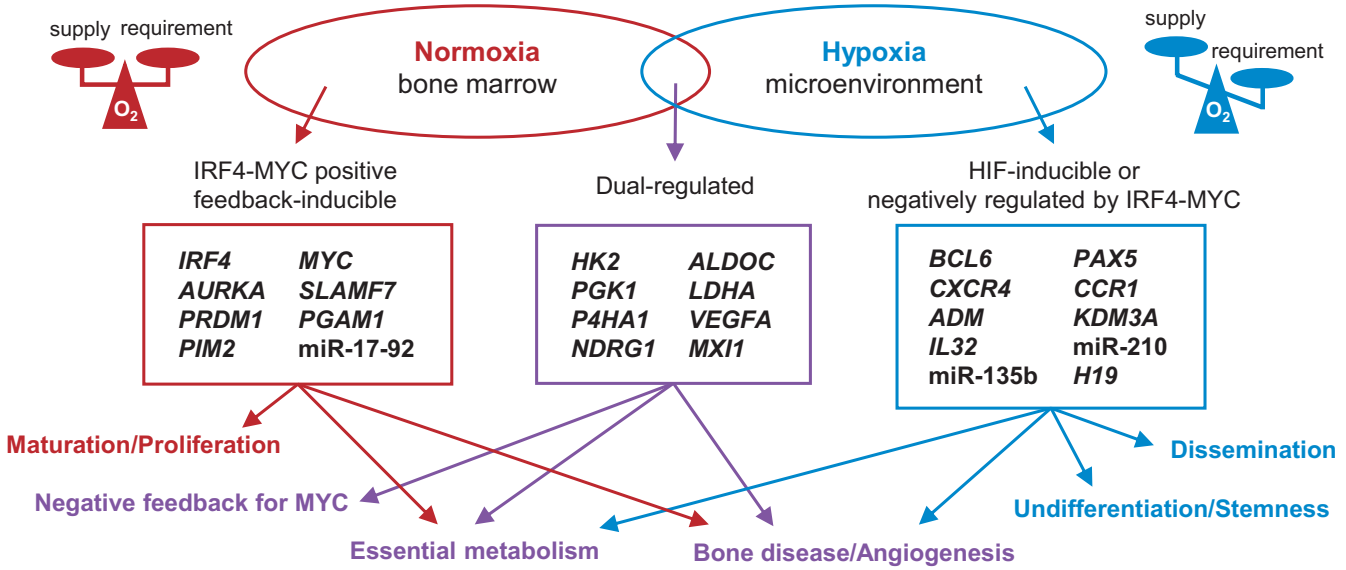

FIGURE 2 Schematic illustration of normoxia-inducible or hypoxia-inducible genes and their functions in MM. Genes were mainly identified from Shafer et al (Ref. 4) and Ikeda et al (Ref. 27). The other genes were from the main text. HIF, hypoxia-inducible factor; IRF4, interferon regulatory factor 4

HIF-1α and HIF-2α, respectively, and they disseminate to different bone marrow environments according to the concentration gradient of SDF-1 and CCL5, respectively.[47,48] This finding suggests that hypoxic conditions induce homing to a different bone marrow region, although myeloma cells proliferate in normoxic conditions via the IRF4-MYC pathway. This strategy of cancer cells is very rational for their own survival.[49] Importantly, fluid factors released by hypoxic myeloma cells alter the bone marrow microenvironment by neovascularization.[49] HIF regulates various angiogenic factors including VEGFA, bFGF, and HGF.[15,50] Furthermore, the HIF-inducible factor adrenomedullin is released from hypoxic myeloma cells and stimulates vascular endothelial cells expressing of its receptors, such as CRLR and RAMP2, to induce angiogenesis.[51] Osteoclast activation is also an important aspect of the BM microenvironment; interleukin-32, which is regulated by HIF-1α, is released from the myeloma cells by extracellular vesicles and taken up by osteoclasts to promote their differentiation.[52] Furthermore, the hypoxia-inducible p38-CREB-DKK1 axis and upregulation of HIF-1α-inducible MMSET contribute to inhibition of osteoblastic bone formation.[53] These reports indicate that fluid factors released from myeloma and stroma cells exposed to hypoxic stress create a favorable BM microenvironment for myeloma cell survival by regulating their chemotaxis, inhibiting the osteoblasts, and stimulating the osteoclasts and surrounding vascular endothelial cells.

4.3 | Hypoxia-inducible noncoding RNAs and their functions

Many studies have reported noncoding RNAs whose expression is also altered by the differences in oxygen partial pressures.[54] Small functional noncoding RNAs such as microRNAs (miRNAs) have a significant impact on the maintenance of normal cells as well as the molecular pathogenesis of cancer by regulating specific target messenger RNAs.[55] We recently reported that hypoxia-inducible miR-210 regulates the expression of ribosomal RNA methyltransferase DIMT1, leading to suppression of IRF4.[17] Moreover, miR-135b, which is present in exosomes, is transported from hypoxic myeloma cells to vascular endothelial cells, leading to angiogenesis via downregulation of the HIF inhibitor FIH-1.[56] These reports demonstrated the importance of HIF-inducible miRNAs in hypoxic environments. Our previous microarray study further showed that the miR-17-92 polycistron (known as an "oncomiR") was downregulated by exposure to hypoxia.[17] miR-17-92 polycistron was discovered in the 13q32 genomic amplification region in B-cell lymphoma and was subsequently upregulated by c-MYC.[57-60] Because miR-17-92 inhibits various tumor suppressors, it is considered a therapeutic target.[61,62] However, because hypoxia may downregulate the c-MYC-miR-17-92 cascade, targeting this cascade may not be a complete strategy.

Recent studies have demonstrated that long noncoding RNAs (lncRNAs), which comprise >200 nucleotides, have diverse functions and contribute to the survival of not only normal but also cancer cells.[63] PVT1, which is an lncRNA associated with MYC and is encoded near the c-*MYC* gene, is functionally associated with cell proliferation and protects the MYC protein in MM with 8q24 abnormality.[64] In contrast, several studies have reported lncRNAs whose expression is upregulated by HIF. For example, the HIF-1α-inducible lncRNA DARS-AS1 inhibits the ubiquitination of RNA-binding motif protein 39, leading to the activation of mTOR signaling and consequently contributing to myeloma tumorigenesis.[65] An lncRNA, H19, is also HIF-1α-inducible and contributes to dissemination by regulating CXCR4 and snail expression.[66] Moreover, the lncRNA MALAT1 is regulated by the hypoxia-inducible H3K9 demethylase KDM3A.[27] MALAT1 is thought to protect the expression of HIF-1α and regulate

the expression of glycolytic genes, leading to cell survival under hypoxic conditions. These lncRNAs may also be therapeutic candidates.

5 | IMPACT OF HYPOXIA ON TREATMENT RESISTANCE

5.1 | Proteasome inhibitors

Various studies have been conducted to detect the effects of PIs, such as bortezomib, in myeloma cells exposed to hypoxic stress. When we consider that HIF-1α degradation occurs in proteasomes, it is likely that PIs upregulate the expression of transcriptional targets of HIF-1α via their accumulation. However, bortezomib can decrease the transcriptional activity of HIF-1α by inhibiting the recruitment of coactivator CBP/p300.[67] Therefore, PIs might accumulate HIF-1α but suppress its transcriptional activity, resulting in the reduced expression of downstream targets of HIF-1α. However, little information is known about the effects of PIs on the transcriptional activity of other HIFs. Several studies have experimentally shown that the effect of PIs is attenuated in hypoxic environments in vitro.[17,43] ER stress might be reduced in hypoxic environments due to the following reasons. Generally, the degradation of unfolded proteins of the cell occurs by proteasomal degradation and autophagy. In particular, autophagy acts as an alternative pathway to proteasomal degradation, leading to a reduction in ER stress. Its activation may be responsible for the occurrence of PI resistance.[68] Interestingly, we previously reported that HIF-inducible HK2 activates autophagy during hypoxia through the inhibition of mTOR signaling, resulting in PI resistance.[46] Therefore, a multifunctional factor such as HK2, which is involved in glycolysis and autophagy under hypoxia, is a promising therapeutic target. Ultimately, it may be necessary to avoid attenuation of the effects of PI under hypoxia. It may also be necessary to combine PI with hypoxia-targeted therapy to increase the therapeutic efficacy.

5.2 | Immunomodulatory drugs

IMiDs act as cereblon modulators and alter the targets of the ubiquitin ligase cereblon. They exert their anti-myeloma effects by degrading Ikaros and Aiolos, which are upstream activators of IRF4 and MYC.[7,8,69] Therefore, IMiDs might be effective under normoxic conditions in which the expression levels of IRF4 and MYC are higher than those under hypoxic conditions. However, as there have been only a few studies reporting the effects of IMiDs in hypoxia, we aimed to discuss the effects of IMiDs in terms of developmental stages. Interestingly, lenalidomide, a representative cerebron modulator, is effective against immature preplasmablasts, which represent CD38-negative and CD20-low or CD20-negative phenotypes.[70,71] Furthermore, this effect on preplasmablasts was demonstrated to be independent of Ikaros and Aiolos. Therefore, IMiDs may be effective even in myeloma cells with an immature phenotype and low IRF4 expression under hypoxic conditions. Recently, it was reported that the combined use of lenalidomide and HIF inhibition induced a synergistic effect in a myeloma-xenografted model.[72] This study suggested that treatment with IMiDs alone may have incomplete efficacy against myeloma cells that express high levels of HIF. Therefore, future studies must involve a detailed examination to determine the relationship between cereblon modulators and HIFs.

5.3 | Monoclonal antibodies

At this time, antibody drugs against SLAMF7 and CD38 are available for clinical use in MM. Therefore, it is important to determine whether the expression of these surface antigens is altered by hypoxia. The expression of SLAMF7 was downregulated under hypoxic conditions in myeloma cells.[24] However, whether hypoxia attenuates the effect of SLAMF7 antibody has not been investigated. A SLAMF7 antibody, elotuzumab, has the capability to exert an anti-myeloma effect by neutralizing soluble SLAMF7.[73] Therefore, to investigate the effect of elotuzumab in the BM microenvironment, we should not only take into account its decreased expression mechanism, but also the existence of its soluble fraction under hypoxia.

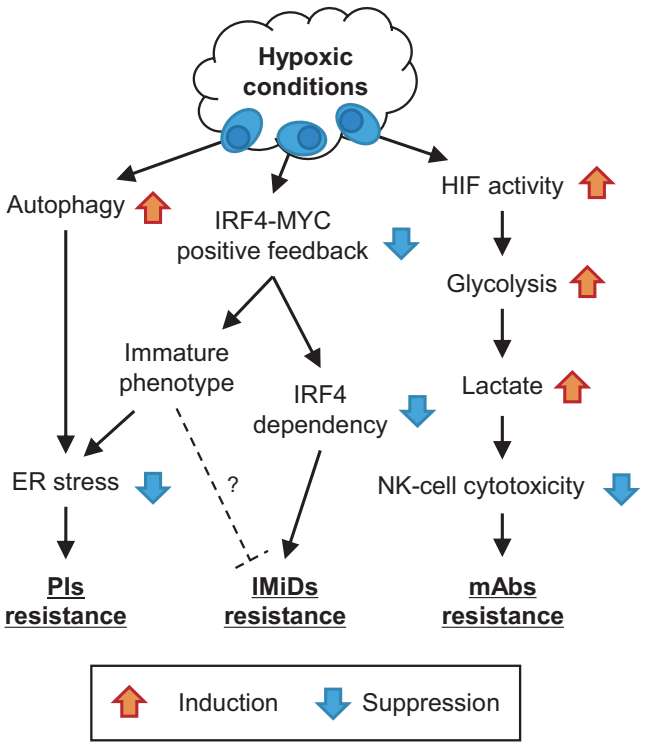

FIGURE 3 Effects of hypoxic response on drug resistance in patients with MM. Myeloma cells exposed to hypoxia may acquire resistance to PIs, IMiDs, and mAbs through autophagy, decreased IRF4 expression, and activation of glycolysis, respectively. However, hypoxia-induced undifferentiation may partially enhance the antitumor effects of IMiDs. ER, endoplasmic reticulum; HIF, hypoxia-inducible factor; IMiDs, immunomodulatory drugs; IRF4, interferon regulatory factor 4; mAbs, monoclonal antibodies; PIs, proteasome inhibitors

In contrast, CD38, which is also expressed in mature B cells and plasma cells, does not decrease even under hypoxic conditions.[17,24,74] This could be one of the reasons why CD38 mAbs (daratumumab and isatuximab), rather than other antibody drugs, are more widely used in clinical settings. To examine the occurrence of resistance to therapeutic mAbs, we also needed to consider immunity in the microenvironment, such as the activity of NK cells. Although there have been limited studies on hypoxia-induced immunosuppression in MM, NK cell activity may be reduced due to the presence of lactate, which accumulates due to hypoxia-induced glycolysis.[74] Moreover, hypoxic stress decreases the expression levels of NKG2D, CD16, perforin, and granzyme B, leading to a reduction in cytotoxicity.[75] Fortunately, even in these reduced CD38-expressing NK cell activity states, the CD38 mAb exerts antibody-dependent cellular cytotoxicity (ADCC) activity in vitro, however the effect appears to be attenuated due to the presence of lactate.[74] Detection of the mechanism for avoiding hypoxia-induced immunosuppression and the surface antigen whose expression is specifically upregulated in the BM microenvironment may be an important challenge. Overall, we need to pay attention to effects of hypoxic response on drug resistance in patients with MM (Figure 3).

6 | CONCLUSIONS

This review focused on the function and significance of genes and their products whose expression is induced or reduced via hypoxic response in MM. In the normoxic regions of the BM, myeloma cells are mainly regulated by IRF4-MYC, which has the following abilities: differentiation, proliferation, and anti-apoptosis. By contrast, HIFs, which may be activated in the BM microenvironment, strongly promote the transcription of genes involved in stemness, glycolysis, dissemination, angiogenesis, and autophagy, leading to quiescence, drug resistance, and anti-apoptosis. Notably, the anti-apoptotic capability is controlled by distinct upstream transcription factors under normoxic (IRF4 and MYC) or hypoxic conditions (HIF). Interestingly, both IRF4-MYC- and HIF-inducible genes include genes that regulate glycolysis and angiogenesis, as well as genes that negatively affect MYC function (ie, MXI1 and NDRG1).[16,76,77] In addition to these genes, hypoxia-inducible elements reported by our group, such as miR-210 and KDM3A, might play a role in regulating the maintenance of balance and switching between IRF4-MYC and HIF, leading to anti-apoptosis (Figure 4A).

Recently, clinical trials have been conducted on drugs that target the hypoxic response.[78] For instance, evofosfamide (TH-302), a

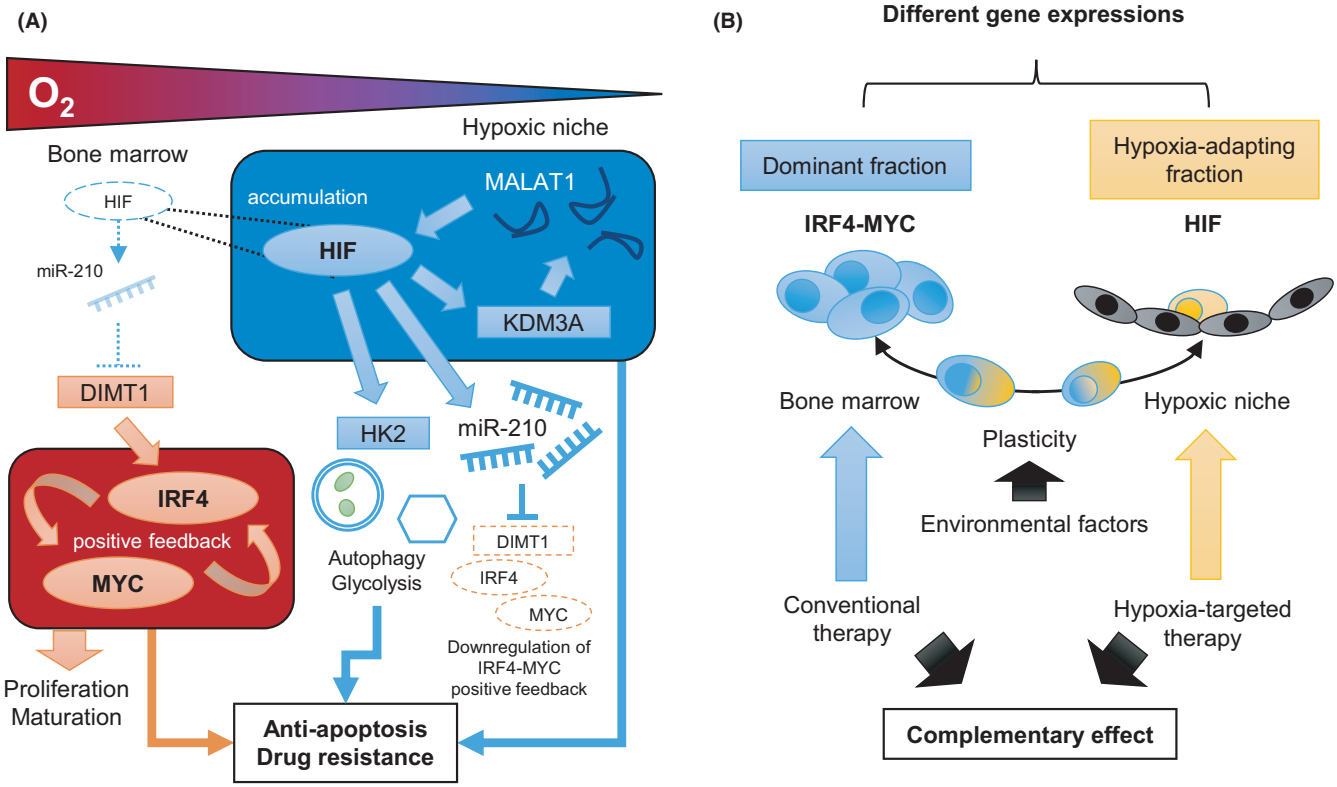

FIGURE 4 Schematic illustration of master transcription factor switch. A, Under normoxic conditions, inactivation of HIF leads to the activation of a IRF4-MYC-positive feedback. By contrast, under hypoxic conditions, HIF suppresses IRF4-MYC and contributes to the occurrence of drug resistance. In this switch, hypoxia-inducible KDM3A, HK2, and microRNA-210 play critical roles. B, Importance of the complementary effects of hypoxia-targeted therapy and conventional therapy. Gene expression fluctuates because of environmental factors, and different fractions must be killed at the same time. DIMT1, dimethyladenosine transferase 1; HIF, hypoxia-inducible factor; HK2, hexokinase 2; IRF4, interferon regulatory factor 4; KDM3A, lysine demethylase 3A; MALAT1, metastasis associated in lung adenocarcinoma transcript 1; miR-210, microRNA-210

hypoxia-activated prodrug, induces apoptosis by releasing alkylating agents under hypoxic conditions. Evofosfamide has been tested in clinical trials for MM as well as for hypoxic tumors such as pancreatic cancer and soft tissue sarcoma.[79] However, treatment with hypoxia-adapted fractions alone is not sufficient. This is because, even if it is possible to kill the quiescent fraction that developed an adaptive response to hypoxia, the plasticity of myeloma cells may allow a shift from the dominant fraction to the quiescent clone via the effect of microenvironmental factors.[11] Therefore, the development of complementary therapies that are effective on different gene expression populations is needed. Previous experiments have supported the idea that the combined use of evofosfamide and bortezomib has synergistic effects on myeloma cells.[80] Combining effective therapeutic agents for the IRF4-MYC-dominated fraction and the HIF-dominated fraction may be a rational and important therapeutic strategy for MM as a way to cure this disease (Figure 4B).

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DISCLOSURE

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