A DNA-binding Molecule Targeting the Adaptive Hypoxic Response in Multiple Myeloma Has Potent Antitumor Activity

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Abstract

Multiple myeloma is incurable and invariably becomes resistant to chemotherapy. Although the mechanisms remain unclear, hypoxic conditions in the bone marrow have been implicated in contributing to multiple myeloma progression, angiogenesis, and resistance to chemotherapy. These effects occur via adaptive cellular responses mediated by hypoxia-inducible transcription factors (HIFs), and targeting HIFs can have anticancer effects in both solid and hematologic malignancies. Here, it was found that in most myeloma cell lines tested, HIF1α, but not HIF2α expression was oxygen dependent, and this could be explained by the differential expression of the regulatory prolyl hydroxylase isofoms. The anti–multiple myeloma effects of a sequence-specific DNA-binding pyrrole-imidazole (Py-Im) polyamide (HIF-PA), which disrupts the HIF heterodimer from binding to its cognate DNA sequences, were also investigated. HIF-PA is cell permeable, localizes to the nuclei, and binds specific regions of DNA with an affinity comparable with that of HIFs. Most of the multiple myeloma cells were resistant to hypoxia-mediated apoptosis, and HIF-PA treatment could overcome this resistance in vitro. Using xenograft models, it was determined that HIF-PA significantly decreased tumor volume and increased hypoxic and apoptotic regions within solid tumor nodules and the growth of myeloma cells engrafted in the bone marrow. This provides a rationale for targeting the adaptive cellular hypoxic response of the O2-dependent activation of HIFs using polyamides.

Implications: Py-Im polyamides target and disrupt the adaptive hypoxic responses in multiple myeloma cells that may have clinical significance as a therapeutic strategy to treat myeloma engrafted in the bone marrow microenvironment.

Introduction

Multiple myeloma is an incurable disease of malignant plasma cells characterized by high rates of relapse and resistance to drug therapies (1–3). The reasons why this disease is so difficult to cure is unclear, but the bone marrow microenvironment is known to confer critical growth and survival advantages that protect tumor cells from apoptosis-inducing stressors (4, 5). The bone marrow is hypoxic (pO2 ~10–30 mm Hg) when compared with most other tissues (pO2 ~85–150 mm Hg; ref. 6), and paradoxically, although oxygen stress can kill tumor cells (7), low-oxygen conditions also promote tumor progression (8), angiogenesis (9), and resistance to chemotherapy (10). Thus far, prosurvival responses to low oxygen tension are regulated by adaptive cellular responses mediated by several oxygen-sensitive transcription factors; the most important of these being the hypoxia-inducible factors (HIFs) for review see ref. 11). HIFs are composed of a constitutively expressed β-subunit (HIF1β/ARNT) and inducible α-subunits (HIF1α, 2α, and 3α) that are responsive to oxygen levels and are regulated via proteasome-mediated degradation. Briefly, under “normoxic” conditions, the HIFα subunits are hydroxylated by a number of closely related prolyl hydroxylase domain proteins (PHD1–3) that results in recognition of the α-subunit by the von Hippel-Lindau tumor suppressor (VHL) and its subsequent ubiquitination and rapid degradation by the proteasome. This normally maintains the HIFα-subunits at very low levels in the cell. Under hypoxic conditions (pO2 < 50 mm Hg), the proline hydroxylase activity is inhibited, and HIFα degradation is not initiated (12). This allows dimerization of α and β subunits and the translocation and binding of HIF to the hypoxic response elements (HRE), thereby inducing gene transcription of hypoxia-related survival and angiogenic factors.

It has been shown that heightened expression of proangiogenic factors (such as VEGF) and increased microvessel density within myeloma tumors is strongly correlated with disease development and progression, as well as being predictive of poor patient prognosis (13–16). This provided a rationale for using VEGF-targeting drugs, such as bevacizumab (Avastin), to attempt to inhibit angiogenesis and increase hypoxic stress in multiple myeloma tumors, although only modest antitumor effects were observed (17). These results call into question the overall effectiveness of targeting angiogenesis as a monotherapeutic strategy for treating multiple myeloma in the clinic. The increase of hypoxia within the tumor bed following inhibition of VEGF results in the subsequent activation of the adaptive hypoxic...
response and induction of survival factors may provide an explanation for these underlying effects. Under this hypothesis, we would argue that these resultant hypoxic conditions are actually supportive of multiple myeloma progression (because low O2 represents a natural component of the bone marrow niche in which myeloma engraft) by maintaining hyperactive HIF activity. Thus, rather than killing the tumor cells, the inhibition of angiogenesis may actually facilitate a prosurvival adaptive hypoxic response through HIF activation. If true, then targeting the specific HIF-mediated response to hypoxia may be a more effective antimyeloma therapy than targeting VEGF or angiogenesis alone. In fact, there are a number of different hypoxia and HIF-targeting strategies currently being tested in multiple myeloma with varying levels of success (18).

In this study, we tested the effects of inhibiting the hypoxic adaptive response using a synthetically derived, sequence-specific DNA-binding Py-Im polyamide (PA) composed of the aromatic rings of N-methylpyrrole and N-methylimidazole amino acids designed to recognize and interfere with HIF binding to HRE sequences within the minor grove of the DNA helix (19). We found that under low oxygen-culturing conditions, most myeloma cell lines were relatively resistant to hypoxia-mediated apoptosis and that HIF1α, but not HIF2α, was upregulated in an oxygen-dependent manner. Treatment of multiple myeloma cells with our HIF-polyamide (HIF-PA) overcame resistance to hypoxia-mediated apoptosis in vitro as well as inhibited the transcription of multiple hypoxia-induced genes. We also found that combination treatment with HIF-PA polyamides (to inhibit gene transcription) and the mTOR inhibitor rapamycin (to inhibit gene translation) was markedly more effective at overcoming resistance to hypoxia-mediated apoptosis in multiple myeloma cells. In additional experiments, we used xenograft models to study the anti–multiple myeloma effects of Py-Im polyamide treatment on multiple myeloma tumors in vivo and found that Py-Im polyamides were well tolerated by the mice and had a marked antitumor effect characterized by a significant increase in hypoxia as well as concomitant increases in apoptotic and necrotic regions within solid tumor nodules as well as inhibition of myeloma growth in tumors engrafted in the bone marrow. Altogether, these data suggest that sensitivity of myeloma to polyamide therapy may be related to the inhibition of gene expression induced by the oxygen-dependent activation of HIF1α (but not necessarily HIF2α) and provides a rationale for targeting the adaptive hypoxic responses in multiple myeloma using these compounds.

Materials and Methods

Cell lines and reagents

All cell lines were purchased from ATCC and maintained at 37°C and 5% CO2 (“normoxic” condition) unless noted. The cell lines were validated using the Johns Hopkins Genetic Core Research Facility (Baltimore, MD), and stock aliquots were stored under liquid nitrogen. Testing for mycoplasma was performed using a Mycoplasma PCR Detection Kit (Sigma-Aldrich). Py-Im polyamides were synthesized by solid-phase methods on Kaiser oxime resin (Novabiochem; ref. 20). The tested polyamide, HIF-PA, targets the sequence 5'-WTWCGW-3' (W = A or T) and modulates a subset of hypoxia-induced genes, while the control polyamide (CO-PA) recognizes the non-HRE sequence 5'-WGGWCW-3'. ELISA kits specific for human VEGF were purchased from R&D Systems. The Hypoxyprobe-1 Kit was purchased from HIPI Inc. Cellular apoptosis was measured by flow cytometry using a Cleaved Caspase-3 Kit (BD Biosciences). siRNA for HIF1α (silencer select siRNA ID# s6539, gene ID# 3091) and scrambled control RNA (silencer select negative control #1 siRNA) were purchased from Ambion. Cells were transfected with siRNA using Lipofectamine-2000 (Life Technologies).

Immunoblots

Protein was isolated and Western blot analysis was performed as described previously (21). Nuclear and cytoplasm fractions were isolated using the Thermo Scientific NE-PER™ Nuclear and Cytoplasmic Extraction Kit following the manufacturer's instructions. HIF1α antibody (clone 54/HIF1α) was purchased from BD Biosciences. β-tubulin (clone H-235). Lamin A/C (clone 14/LaminAC). BCL-XL (clone H-5), survivin (clone D-8), BNIP3 (clone ANa40). BCL-2 (clone C-2), goat anti-mouse, and goat anti-rabbit IgG horseradish peroxidase-conjugated antibodies were purchased from Santa Cruz Biotechnology. BID (rabbit polyclonal). BAX (rabbit polyclonal). AKT-total (clone C67E7), AKT-S473 (clone D9E), and AKT-T308 (clone C31E5E) and the P70-total, P70-T421/S424 and P70-T389 antibody kits were purchased from Cell Signaling Technology. MCL-1 (clone 542808) was purchased from R&D Systems. REDD1 (clone 1G11) was purchased from Bethyl Laboratories. The EGLN1/PHD2 (rabbit polyclonal). EGLN3/PHD3 (mouse polyclonal). EGLN3/PHD3–positive control (an EGLN3/PHD3–overexpressing lysate from HEK293T cells). HIF2α (rabbit polyclonal), and factor-inhibiting HIF1 FIH antibodies (clone 162C) were purchased from NOVUS Biologicals.

Hypoxia treatments

For the induction of hypoxia, MM cells were cultured in a humidified Hypoxxygen hypoxia chamber (Gradipair). Variable pO2 levels were established in the hypoxia chamber from 2–0.1% O2, and 5% CO2 at 37 °C. Oxygen levels were regularly tested and calibrated using the manufacturer’s protocol.

Generation of hypoxia response element luciferase-expressing (HRE-LUC) cell lines

HRE-LUC reporter multiple myeloma cell lines (8226, U266, and OPM-2) were generated by stably transducing cells using the Cignal Lentiviral Kit (Qiagen), followed by selection with hygromycin (350 mg/mL). For the orthotopic xenograft studies, other luciferase-expressing 8226 cells (8226-LUC) were stably transduced with the pGL4.5 Luciferase Reporter Vector (Promega) using an AMAXA Nucleofector Kit (Lonza), followed by selection with hygromycin (350 mg/mL). The in vitro luciferase activity was confirmed and measured using the Dual-Luciferase Reporter Assay Kit (Promega) in a luminometer. The in vivo luciferase activity was measured using the VivoGlo Luciferin Substrate (Promega).

Real-time PCR

Quantitative expression of VEGF was carried out by qPCR. RNA was isolated using TRIzol (Life Technologies), and cDNA was synthesized using cDNA Synthesis Kit (Life Technologies). VEGF mRNA was amplified using VEGF primers (Life Technologies) in an Applied Biosystems 7300 Real-Time PCR Machine (Life Technologies). The Applied Biosystems TaqMan Array Human Hypoxia 96-well Plate was used to test for changes in hypoxia signaling–associated genes.
Animals
Male NOD/SCID or NOG mice (4–6 weeks old) were obtained from The Jackson Laboratory. All animal studies were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Greater Los Angeles Veterans Administration Healthcare System (Los Angeles, CA).

Subcutaneous xenograft model
We used the murine myeloma xenograft model of Leblanc and colleagues (22) with minor modifications (21). The 8226 cells (10 × 10⁶ cells/mouse) were mixed with Matrigel (BD Biosciences) and then injected subcutaneously into the right flank (200 μL/mouse) of the mice. The mice were monitored and randomized into drug treated or control groups (10 mice/group) when the tumor volume reached approximately 300 to 500 mm³. The Py-Im polyamide solution was prepared as previously described (23). Mice were given HIF-PA injections intraperitoneally every other day for a total of 5 injections. The tumor volume was measured using calipers (width and length) using the formula \( W^2 \times (L/2) \) every other day during the course of the experiment.

Orthotopic xenograft model
8226-LUC cells were injected (10 × 10⁶ cells/mouse) through the tail vein (200 μL/injection). In our initial experiments, the localization of human 8226 myeloma cell lines in the bone marrow was determined by two methods: (i) flow cytometry staining of bone marrow aspirates from the femurs of mice using FITC-labeled anti-human CD45 antibodies (BD Biosciences) and (ii) IHC of femurs using anti-human CD45 antibodies. Real-time 8226-LUC engraftment in the bone marrow was measured in anesthetized mice given an intraperitoneal injection of VivoGlo Luciferin Substrate (100 mg/kg mouse) and then monitored for luciferase activity in the skeleton using a Perkin-Elmer IVIS XRS small-animal imaging system. Luciferase activity in the skeleton of mice challenged with 8226-LUC-Luc was typically observed between day +15 and day +20 post challenge. Once a positive bioluminescent signal was observed, the animals were randomized into groups (6–8 animals/group) and were treated with HIF-PA or vehicle control as described above, except a total of 6 injections would also exhibit resistance to hypoxia-mediated apoptosis

Statistical analysis
Data were screened for consistency and quality by both graphical (histograms and scatter plots) and analytic methods (descriptive statistics). Variables were analyzed using generalized linear models, such as ANOVA and t tests. The effect of combining HIF-PA with rapamycin on the induction of apoptosis was assessed by the median-effect method using CalcuSyn Software version 1.1 (Biosoft). Combination indices (CI) values were calculated using the most conservative assumption of mutually nonexclusive drug interactions. CI values were calculated from median results of apoptosis assays.

Results
Myeloma cell lines are resistant to hypoxia-induced apoptosis in vitro
Because patient multiple myeloma tumor cells specifically engraft within the hypoxic bone marrow microenvironment, we anticipated that patient-derived multiple myeloma cell lines would also exhibit resistance to hypoxia-mediated apoptosis in vitro. This was confirmed by analyzing the sensitivity of a panel of multiple myeloma cell lines cultured under various pO₂ concentrations in increments from 2% down to 0.1% O₂ and for various time points up to 72 hours. We chose these experimental ranges because these values fell within the actual pO₂ ranges reported by Spencer and colleagues (6) from in situ measurement in mouse bone marrow. In their study, O₂ levels in the marrow were found to be <32 mm Hg, but in some bone marrow niches, it could be as low as 9.9 mm Hg (or about 1% O₂ with a range of 2%–0.6% measured in the extravascular spaces). We found that at O₂ levels greater than approximately 1%, only modest cytotoxicity was observed, even when cells were cultured out to 72 hours. Only at very low oxygen conditions (i.e., 0.5%–0.1% O₂), did we see significant levels of hypoxia-mediated apoptosis of the multiple myeloma cells tested when compared with the “normoxia-cultured” controls. As shown in Fig. 1A, 8226 and U266 cell lines were the most resistant to low pO₂ (~15%–20% apoptosis), whereas H929 and MM1.S were intermediately sensitive (~25%–35% apoptosis) to hypoxia-mediated apoptosis (all measured at 72 hours). In contrast, OPM-2 cells were the most sensitive (>50% apoptosis) to low O₂, and this effect occurred significantly earlier, by 48 hours.

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HIC
For studies in our subcutaneous model, 24 hours after the last injections, the mice were euthanized, and the tumor mass was excised. The tumor was bisected using a razor blade: one half of the tumor was immediately placed in 10% buffered formaldehyde overnight, and the other half was flash frozen for protein extraction. Formaldehyde-fixed tumors were embedded in paraffin and cut into 5-μm thick serial sections using standard histologic procedures. Immunohistochemical staining with cleaved caspase-3, and Hypoxyprobe (pimonidazole; HPI Inc) was conducted using standardized automated methods.

Morphometric analysis
Immunohistochemical analysis was performed on tissue sections with a Nikon Microphot-SA Microscope equipped with plan-apochromat lenses (20× and 40×). A Diagnostic Technologies digital camera, model SPOT-RT was used to capture images with a resolution of 1520 × 1080 pixels. Fields were selected by reviewing the slides at low power by a researcher (V.S. Mysores) blinded to the treatments. Multiple nonoverlapping fields were identified for analysis of regions of hypoxia/necrosis and apoptotic index at higher powers within these areas of interest. ImageJ software (NIH, Bethesda, MD) was used to measure the percentage of hypoxia-stained tumor sections. Final images for publication were prepared using Adobe Photoshop software.

Oncomine analysis
The expression level of PHD2/EGLN1 and PHD3/EGLN3 genes in normal, monoclonal gammopathy of undetermined significance (MGUS), and multiple myeloma tumors were analyzed using Oncomine, a cancer microarray database and web-based data-mining platform (25). To reduce our FDR, we selected \( P < 0.0001 \) as a threshold. We analyzed the results for \( P \) values and fold change of our genes of interest.

We anticipated that patient-derived multiple myeloma cell lines would also exhibit resistance to hypoxia-mediated apoptosis in vitro. This was confirmed by analyzing the sensitivity of a panel of multiple myeloma cell lines cultured under various pO₂ concentrations in increments from 2% down to 0.1% O₂ and for various time points up to 72 hours. We chose these experimental ranges because these values fell within the actual pO₂ ranges reported by Spencer and colleagues (6) from in situ measurement in mouse bone marrow. In their study, O₂ levels in the marrow were found to be <32 mm Hg, but in some bone marrow niches, it could be as low as 9.9 mm Hg (or about 1% O₂ with a range of 2%–0.6% measured in the extravascular spaces). We found that at O₂ levels greater than approximately 1%, only modest cytotoxicity was observed, even when cells were cultured out to 72 hours. Only at very low oxygen conditions (i.e., 0.5%–0.1% O₂), did we see significant levels of hypoxia-mediated apoptosis of the multiple myeloma cells tested when compared with the “normoxia-cultured” controls. As shown in Fig. 1A, 8226 and U266 cell lines were the most resistant to low pO₂ (~15%–20% apoptosis), whereas H929 and MM1.S were intermediately sensitive (~25%–35% apoptosis) to hypoxia-mediated apoptosis (all measured at 72 hours). In contrast, OPM-2 cells were the most sensitive (>50% apoptosis) to low O₂, and this effect occurred significantly earlier, by 48 hours.

Targeting the Adaptive Hypoxic Response in Multiple Myeloma Cells
Culturing cells under low pO2 had different effects on the HIFα-subunits expression in the nuclear and cytoplasmic fractions of the hypoxia-resistant (8226) and hypoxia-sensitive (OPM-2) cell lines cultured under “normoxic” conditions (~22% O2, 5% CO2) or hypoxic (0.1% O2, 5% CO2) conditions, except for OPM-2 cells, which were cultured for 48 hours. The cells were harvested on ice, and then the cytoplasmic and nuclear fractions were isolated, and the lysate was immunoblotted for indicated proteins.

Figure 1. Cytotoxic effects of low pO2 levels on myeloma cell lines. A, percentage of apoptosis (measured by cleaved caspase-3) following 72-hour culture of myeloma cells under “normoxic” conditions (~22% O2, 5% CO2) or hypoxic (0.1% O2, 5% CO2) conditions, except for OPM-2 cells, which were cultured for 48 hours. The cells were harvested on ice, and then the cytoplasmic and nuclear fractions were isolated, and the lysate was immunoblotted for indicated proteins. C, cytoplasm fraction; N, nuclear fraction. The data presented are representative immunoblots of at least 2 independent experiments. C, time course of CoCl2-mediated induction of HIF1α expression in OPM-2 cells. Cells were cultured with 100 mmol/L CoCl2 or vehicle control, and the cellular lysates were harvested at indicated time points and immunoblotted for HIF1α expression. The data presented are a representative immunoblots of at least 2 independent experiments. D, hypoxia-mediated induction of HIF1α and HIF2α in MM1S, H929, and U266 myeloma cell lines cultured under normoxic or hypoxic (0.1% O2, 5% CO2) conditions for 24 hours. The cells were harvested on ice, and the cell lysate was immunoblotted for HIF1α and HIF2α. E, immunoblots showing hypoxia-mediated changes in survival-related protein expression in 8226 and OPM-2 cells cultured under normoxic (N) or hypoxic (H) conditions (0.1% O2, 5% CO2) for 24 hours. The cells were harvested on ice, and the lysate was immunoblotted for indicated proteins. The data presented are representative immunoblots of at least 2 independent experiments.

Culturing cells under low pO2 had different effects on the HIFα-subunits expression in the nuclear and cytoplasmic fractions of the hypoxia-resistant (8226) and hypoxia-sensitive (OPM-2) cell lines cultured under standard normoxic (~22% O2) or hypoxic (0.1% O2) conditions. As shown in Fig. 1B (left), HIF1α was constitutively expressed in the nuclear fraction of 8226 cells as well as significantly upregulated under hypoxic conditions (measured at 24 hours). In contrast to 8226, baseline HIF1α was absent in OPM-2 cells but was strongly induced by low O2 (Fig. 1B, right). On the other hand, HIF2α was constitutively expressed in both 8226 and OPM-2 cells, and this was independent of O2 levels (Fig. 1B). The rapid upregulation of HIF1α in OPM-2 cells was confirmed using the hypoxia mimic, CoCl2, which induced HIF1α expression by 1 hour and reached a maximum by 18 hours (Fig. 1C). A strong O2-dependent induction of HIF1α was also noted in MM1S, H929, and U266 cell lines (Fig. 1D), whereas HIF2α expression was independent of O2 levels in H929 and U266 cells, but not in MM1S cells. These findings are generally similar to other reports describing HIF expression in multiple myeloma cells (3, 26). Low pO2 (0.1%, 24 hours) did not affect the expression of the prosurvival factor Bcl-2 but did inhibit Bcl-xL and MCL-1 in OPM-2 and 8226, while survivin was
only downregulated in OPM-2 cells (Fig. 1E). Survivin has been reported to play a role in HIF-regulated survival of multiple myeloma and may be an important target for future studies (27). Low pO2 also upregulated the proapoptotic factors, BNIP3 (a known HIF target) and BID in both lines tested, but BAX was only upregulated in OPM-2.

As we observed differential oxygen-dependent expression patterns for the HIFα subunits, we next asked if expression in the PHD isoforms responsible for regulating the α subunits could explain these results. As seen in Fig. 2A, expression of PHD2/EGLN1 was absent in 8226 but strongly expressed in OPM-2. It has been reported that PHD2/EGLN1

Figure 2. There is differential expression of HIF regulatory pathway in 8226 cells. A, immunoblots of PHD2/EGLN1, PHD3/EGLN3, and FIH in 8226 cultured under normoxic (N) or hypoxic (H) conditions (0.1% O2, 5% CO2) for 48 hours in the presence or absence of HIF-PA (10 μmol/L). Cells were harvested on ice, and the cell lysate was immunoblotted. A positive immunoblot control (an overexpression lysate from HEK293T cells) for PHD3/EGLN3 is included. The data presented are representative immunoblots of at least 2 independent experiments. B, boxplot results showing a meta-analysis of PHD2/EGLN1 and PHD3/EGLN3 in normal bone marrow, MGUS, multiple myeloma (MM), smoldering myeloma, and plasma cell leukemia. Analysis was performed using Oncomine and includes two datasets: Angelli and colleagues (N = 156) and Zhan and colleagues (N = 78). C, relative fold change in hypoxia-mediated expression of select genes compared with normoxic conditions. The 8226 cells were cultured under normoxic or hypoxic (0.1% O2, 5% CO2) conditions for 24 hours. The RNA was collected and gene expression was determined by qPCR analysis. The relative fold change in hypoxia-mediated gene expression compared with normoxic levels are shown. Values above the dotted line indicate upregulation of genes by hypoxia, and values below the dotted line indicate downregulation of genes by hypoxia. These data are representative of 2 independent experiments. Luc., luciferase.
preferentially hydroxylates HIF1α (28), and its absence would explain why 8226 cells express constitutive HIF1α under normoxic conditions. Along similar lines, we also found that PHD3/EGLN3, which preferentially hydroxylates HIF2α (29), was absent in both 8226 and OPM-2 cells, and that also explains the oxygen-independent expression of HIF2α in both these cell lines. To ensure that our antibodies could identify PHD/EGLN3 antigens, we included a positive internal EGLN3 control (EGLN3-overexpressing lysate from HEK293T cells purchased from NOVUS Biologicals) in our immunoblots. We also found that low pO2 (0.1%) significantly upregulated PHD2/EGLN1 protein expression, and probably, this likely acts as a negative feedback mechanism to return HIF back to basal levels when O2 levels return back to normal (30). Furthermore, hypoxia-mediated upregulation of PHD2/EGLN1 was unaffected by treatment with HIF-PA. We also examined the expression of another O2-dependent regulator of HIF1α, FIH, and found that there was no difference in expression under either normoxic or hypoxic conditions in the cell lines we tested.

To further characterize the expression patterns of PHD2/EGLN1, we used the Oncomine tool, a publicly available cancer microarray database to query if there were differences in gene expression in clinical specimens of multiple myeloma or MGUS versus normal controls within two datasets; Zhan and colleagues (N = 78 samples; ref. 31) and Agnelli and colleagues (N = 158; ref. 32). As shown in Fig. 2B, PHD2/EGLN1 (top) was significantly overexpressed in multiple myeloma specimens versus normal specimens (P < 0.05) in the Zhan and colleagues study (P < 0.05). But not in the Agnelli and colleagues study (P > 0.05). On the other hand, PHD3/EGLN3 (bottom) was not significantly different in multiple myeloma and MGUS versus normal specimens (P > 0.05) in either dataset, although there was a general downward trend of expression of this gene. Next, we used the TaqMan Array Human Hypoxia 96-well Assay System to analyze for changes in a subset of hypoxia-related genes in 8226 cells. As shown in Fig. 2C, hypoxia increased the relative expression of HIF1α by 6-fold but had only minor effects on HIF2α expression, mirroring the results shown in Fig. 1B. Other genes associated with the HIF regulatory pathway, such as PHD2/EGLN1, cullen-2, and EP300, were induced, while PHD3/EGLN3 and E3 ubiquitin were downregulated, which mirrored the results from Fig. 2A and B above. Not unexpectedly, VEGF and angiopoietin-4 gene expression, genes that are well known to be sensitive to hypoxia, were upregulated by hypoxia.

HIF-PA inhibits the adaptive hypoxic response in multiple myeloma cells

We next tested the ability of HIF-PA to inhibit the cellular response to hypoxia using 8226 cells that had been stably transduced with a HRE-luciferase reporter construct (8226-HRE-LUC). As shown in Fig. 3A, culturing 8226-HRE-LUC cells under low pO2 (0.1% O2, 24 hours) resulted in approximately 2-fold induction of luciferase activity over the baseline “normoxic” controls. This hypoxia-mediated LUC induction was significantly inhibited (P < 0.05) by treatment with HIF-PA, but not by a CO-PA that recognized a non-HRE sequence. To provide further support for our model, we knocked down HIF1α expression in 8226-LUC cells using HIF1α siRNA (Fig. 3B top). As expected, and as shown in Fig. 3B (bottom), the hypoxia-mediated increase of LUC activity in 8226-LUC reporter cells transfected with HIF1α siRNA also demonstrated a significant inhibition of the hypoxia-induced LUC activity. These data support our hypothesis that HIF-PA can specifically inhibit the HIF-mediated cellular response to hypoxia in 8226 cells. Next, we showed that HIF-PA could inhibit the expression of HIF-mediated gene expression using real-time PCR to assay for the effect of HIF-PA treatment on the expression VEGF RNA. It is well known that hypoxia upregulates VEGF transcription in myeloma cells, and as expected, culturing 8226 cells under hypoxic conditions (0.1% O2, 24 hours) induced VEGF mRNA by approximately 3- to 4-fold (Fig. 3C). Treatment with HIF-PA significantly (P < 0.05) inhibited this hypoxia-mediated upregulation of VEGF mRNA in a dose-dependent manner. This effect on mRNA was also mirrored by HIF-PA-mediated inhibition of VEGF protein (measured by ELISA) in the supernatant of these cells (Fig. 3D). Altogether, these data demonstrate that HIF-PA specifically and effectively inhibits the adaptive hypoxic response in multiple myeloma cells.

HIF-PA treatment overcomes multiple myeloma cell resistance to hypoxia

We next asked if inhibiting the hypoxic response with HIF-PA could sensitize a panel of multiple myeloma cells to hypoxia-mediated killing. We assayed this using hypoxia-resistant 8226 and U266 cells and hypoxia-sensitive MM1.S and OPM-2 cells that were cultured under normoxic or hypoxic conditions (0.1% O2) in the presence of HIF-PA or a CO-PA. HIF-PA had little effect on 8226 cells (Fig. 4A) and U266 (Fig. 4B) cultured under standard conditions (white bars), but the treatment of 8226 and U266 cells cultured under hypoxic conditions resulted in significant and dose-dependent hypoxia-mediated killing (an increase from ~20%–60%; black bars; ANOVA *, P < 0.05). The hypoxia “sensitive” MM1.S and OPM-2 cells (Fig. 4C and D) were even more responsive to HIF-PA, (ANOVA *, P < 0.05), with a similar increase in apoptosis observed by only 24 hours when cultured under low O2 conditions. The CO-PA compound had little, if any, effect on the hypoxia-mediated apoptosis in any of the cell lines examined. These data support our hypothesis that inhibiting the adaptive hypoxic response with HIF-PA overcomes multiple myeloma resistance to hypoxia-mediated apoptosis in vitro.

As an in vivo correlate of the above in vitro data, we tested HIF-PA activity using a xenograft subcutaneous multiple myeloma tumor model in NOD/SCID mice (21, 24, 33). The mice were challenged on the flank with 10 × 106 8226 cells admixed with Matrigel. Once a palpable tumor developed (~15 days later), the mice were randomized into groups (N = 10 mice/group) to be given 5 intraperitoneal injections of either HIF-PA (100 nmol/injection) or vehicle control every other day (Fig. 5A, arrows on X-axis indicate days of treatment). The HIF-PA treatments were well tolerated, with only a small transient decrease in weight being observed, and induced a rapid and significant inhibition of tumor growth in the HIF-PA–treated mice compared with control mice (P < 0.05). To confirm uptake of HIF-PA, we treated an additional group of mice (N = 2 mice/group) with a FITC-conjugated HIF-PA and measured drug uptake by real-time fluorescent imaging. We observed some auto-fluorescence signal in the bladder and gut (Supplementary Fig. S1A, mice #1 and #2). However, in FITC–HIF-PA–treated mice, positive signals were also observed in the tumor nodules (Supplementary Fig. S1A, note arrow indicating
of 3 independent experiments. Values are presented as mean conditions were measured by ELISA. cultured under normoxic or hypoxic protein in supernatants of 8226 cells effect of HIF-PA treatment on VEGF hypoxic (0.1% O2, 5% CO2) conditions 8226 cells cultured under normoxic or inhibition of VEGF mRNA levels in C, HIF-PA (10°C) (ANOVA); NS, not signi¢ cant. HIF-PA reporter cells were cotransfected with HIFα siRNA or scrambled (sc) control and were then cultured under normoxic or hypoxic (0.1% O2, 5% CO2) 24 hours. Luciferase activity in cell extracts was measured using a luminometer. Values are presented as means ± 1 SD of 3 independent experiments. *, P < 0.05 (ANOVA); NS, not significant. B, 8226-HRE-LUC reporter cells were treated with either 10°C HIF-PA or 10°C mol/L CO-PA for 24 hours. Luciferase activity in treated 8226 cells. Values are representative of 3 independent experiments; bottom, change in LUC activity in treated 8226 cells. Values are presented as means ± 1 SD of 3 independent experiments; *, P < 0.05 (ANOVA); NS, not signi¢ cant. C, HIF-PA (10°C mol/L)–mediated inhibition of VEGF mRNA levels in 8226 cells cultured under normoxic or hypoxic (0.1% O2, 5% CO2) conditions for 24 hours. VEGF mRNA levels were measured by qPCR of 3 independent experiments; *, P < 0.05. (ANOVA). D, effect of HIF-PA treatment on VEGF protein in supernatants of 8226 cells cultured under normoxic or hypoxic conditions were measured by ELISA. Values are presented as mean ± 1 SD of 3 independent experiments; *, P < 0.05 (ANOVA).

To characterize the in vivo effect of HIF-PA, we excised the tumor nodules 24 hours after the last injection and performed IHC on serial sections stained either for hypoxia (pimonidazole staining, left) or apoptosis (cleaved caspase-3, right; Fig. 5B). Both vehicle control– (top left) and HIF-PA–treated (bottom left) tumors had regions of hypoxia (brown stained areas), but the extent of hypoxia was signi¢cantly greater in the HIF-PA–treated tumors (quantified in Fig. 5C). We also noted there were signi¢cantly greater areas of necrosis within the tumor bed in HIF-PA–treated tumors compared with control tumors as well as a strong physical correlation between areas of hypoxia and presence of apoptosis in the HIF-PA–treated tumors (see Fig. 5B, bottom right). In contrast, apoptotic cells were evenly distributed throughout the tumor bed in the control tumors (Fig. 5B, top right) and were not localized to a speci¢c geographic region in the tumor. As shown in Fig. 5C, we found that the area of positive hypoxia staining in tumor sections (10 tumors/group, 10 ¢elds/tumor) was approximately 35% in nodules harvested from the HIF-PA–treated mice, compared with about 18% in the tumors harvested from mice treated with vehicle control (P < 0.05). The apoptotic index (AI) in both the normoxic or hypoxic regions of the tumor was determined by counting the number of apoptotic cells in these regions using corresponding serial sections (10 tumors/group, 10 ¢elds/region) stained with pimonidazole to identify the speci¢c geographic regions of interest. As shown in Fig. 5D, there was an approximate 3- to 4-fold increase in apoptotic cells located within the hypoxic regions of tumors from the HIF-PA–treated mice compared with hypoxic regions of the control tumors (P < 0.05), whereas there was no difference in apoptotic index within “normoxic” regions of the tumor bed. Finally, we examined the expression of VEGF in tumor lysates by ELISA (Fig. 5E). As with our in vitro data (see Fig. 3D), HIF-PA signi¢cantly inhibited VEGF expression by approximately
50% when compared with control tumors. Altogether, these data support our hypothesis that HIF-PA can overcome resistance to hypoxia-mediated apoptosis in vivo by inhibiting the adaptive hypoxic response.

As a more physiologically relevant model, we developed an orthotopic, “disseminated” bone marrow–engrafted xenograft model based on the model developed by Miyakawa and colleagues (35) using LUC2-transfected 8226 (these cells use a different p4.5 LUC2 plasmid to drive luciferase expression and thus are different than the 8226-HRE-LUC cells described above), allowing us to perform real-time longitudinal studies on myeloma tumors engrafted in the bone marrow. As shown in Supplementary Fig. S1A, NOG mice challenged with 8226-LUC cells developed bone marrow–engrafted multiple myeloma tumors that could be observed by bioluminescence (top) and X-ray (bottom) analysis. Approximately 20% to 50% of the bone marrow cells from inoculated mice were positive for human CD45, confirmed by flow cytometry using FITC-conjugated anti-huCD45 antibody (Supplementary Fig. S2B) and by IHC of in situ huCD45+ 8226 cells in the mouse femurs (Supplementary Fig. S2C). Gross histologic analysis of the mice did not show tumor formation in other tissues (i.e., liver, lung, spleen, or kidney).

We then asked if treatment with HIF-PA had an anti–multiple myeloma effect on tumor cells in the bone marrow. As shown in Fig. 6A, approximately +20 days post challenge with 8226-LUC cell, engraftment of LUC2+ cells in the skeleton was confirmed, and mice were then randomized into treatment groups (6–8 mice/group). The mice were then given intraperitoneal injections of 100 nmol/injection of HIF-PA every other day (arrows indicate days of injection on day +27, +29, +31, +34, +36, and +38) or vehicle control. The average radiance (photons/sec/cm²/steradian) was measured at various time points using a Perkin Elmer Lumina XRMS small-animal imager (out to day +40). The treatment with HIF-PA significantly inhibited multiple myeloma tumor growth in the marrow (data are presented as average radiance ± 95% CI) starting after the third injection and reaching statistical significance by the last HIF-PA injection (on day +38). It should be noted that this particular HIF-PA treatment regimen was not able to delay the development of hind limb paralysis (the main endpoint criteria) when compared with control animals, with approximately 50% of the animals reaching their endpoint on day +59 (control mice) and day +62 (HIF-PA–treated mice). Representative images of some of the mice are shown for day +22, +35, and +40. We noted a decrease in the luciferase activity as well as a general shrinkage of individual tumor foci in the mice. Specifically, in control mice, tumor foci tended to grow and merge over time, whereas in HIF-PA–treated mice, the foci remain relatively small and isolated.

Figure 4. HIF-PA treatment sensitizes the hypoxia-resistant 8226, U266, and hypoxia-sensitive MM1S and OPM-2 cells to hypoxia-mediated apoptosis in vitro. A, 8226 were cultured under normoxic or hypoxic (0.1% O2, 5% CO2) conditions, with indicated concentration of HIF-PA or CO-PA for 72 hours. B, U266 were cultured under normoxic or hypoxic (0.1% O2, 5% CO2) with indicated concentration of HIF-PA or CO-PA for 72 hours. C, MM1S cells were cultured under normoxic or hypoxic (0.1% O2, 5% CO2), with indicated concentration of HIF-PA or CO-PA for 48 hours. D, OPM-2 cells were cultured under normoxic or hypoxic (0.1% O2, 5% CO2), with indicated concentration of HIF-PA or CO-PA for 24 hours. At the end of the treatment, cells were harvested and the percentage of apoptosis was measured by flow cytometry for cleaved caspase-3. Values are mean ± 1 SD of 3 independent experiments. Asterisks indicate significant change (*, P < 0.05; ANOVA) of treatment compared with control.
Figure 5. HIF-PA (100 nmol/kg mouse) inhibits 8226 tumor growth in a subcutaneous myeloma xenograft model (N = 10 mice/group). A, change in 8226 tumor volume in HIF-PA–treated NOD/SCID mice. Mice were challenged subcutaneously with 10 × 10^6 cells admixed in Matrigel. When the tumor volume was ~500 mm^3 (~day 15 post challenge), the mice were randomized into treatment and control groups (10 mice/group). The mice were given 5 intraperitoneal injections at indicated concentration (arrows indicate days of treatment), and the tumor volume was measured using calipers. Values represent mean volume ± 95% CI. *P < 0.05. B, photomicrographs of subcutaneous tumor sections harvested from mice treated with HIF-PA or vehicle control–treated mice (N = 10 mice/group). Tumors were harvested 24 hours after last the intraperitoneal injections. Serial tumor sections were stained for hypoxia (by pimonidazole; brown stained areas) or apoptosis (cleaved caspase-3). Asterisks indicate corresponding geographic regions in the sections. Arrow indicates areas of hypoxia and associated apoptosis. C, percent area of hypoxic tumor bed measured by staining with pimonidazole (measured in 10 fields/tumor, 10 tumors/group) in 8226 tumors harvested from HIF-PA or vehicle control–treated mice. Area was measured using MetaMorph computer software. Values are presented as mean ± 1 SD. *P < 0.05 (ANOVA). D, apoptotic index (the number of apoptotic cells/unit area) was measured by counting cells staining for cleaved caspase-3 in 8226 tumors harvested from HIF-PA or vehicle control–treated mice. Area was measured using MetaMorph computer software. Values are presented as mean ± 1 SD. *P < 0.05 (ANOVA). D, apoptotic index (the number of apoptotic cells/unit area) was measured by counting cells staining for cleaved caspase-3 in 8226 tumors harvested from HIF-PA or vehicle control–treated mice. Values are presented as mean ± 1 SD. *P < 0.05 (ANOVA). E, tumor lysate was collected, and VEGF was analyzed by ELISA (10 tumors/group). Values are presented as mean ± 1 SD. *P < 0.05 (t test).
Effect of targeting the mTOR pathway on regulation of HIF-PA sensitivity

As we have previously demonstrated that mTOR inhibitors kill multiple myeloma cells in vivo, and this anti–multiple myeloma effect is mediated, at least in part, by inhibiting VEGF translation and de novo angiogenesis (21, 24, 33, 36), we asked whether there would be a synergistic effect if we combined HIF-PA–mediated inhibition of hypoxia-inducible gene transcription in combination with rapamycin-mediated inhibition of mTOR-mediated regulation of protein translation. As shown in Fig. 7A, treatment...
of 8226 cells with the mTOR inhibitor rapamycin had a modest effect on apoptosis in multiple myeloma cells cultured under normoxic or hypoxic conditions (0.1% O₂, 72 hours). However, under low pO₂ conditions, using median-effect CI analysis for apoptosis induction, we found that the combination treatment with rapamycin and HIF-PA resulted in synergistic drug interactions with CI < 1 across several concentrations tested. Asterisk indicates significant change (P < 0.05) of treatment compared with control (ANOVA). The effect of combining HIF-PA with rapamycin on induction of apoptosis was assessed by the median-effect method using CalcuSyn Software version 1.1 (Biosoft). CI values were calculated using the most conservative assumption of mutually nonexclusive drug interactions. CI values were calculated from median results of apoptosis assays. B, immunoblots of mTOR-related signaling proteins in 8226 and OPM-2 cells cultured under normoxic (N) or hypoxic (H) conditions for 24 hours. The data presented are representative immunoblots of at least two independent experiments. C, time course of p70S6 kinase phosphorylation in OPM-2 cells following reoxygenation. Cells were cultured under hypoxic (H) conditions (0.1% O₂, 5% CO₂) for 24 hours and then cultured under normoxic (N) conditions (22% O₂, 5% CO₂). Cell lysates were harvested at indicated time points and immunoblotted for HIF-1α and phospho-P70S6K (389) expression. The data presented are representative immunoblots of at least two independent experiments.

Discussion

There is increasing evidence that low oxygen conditions are supportive of multiple myeloma growth, progression, and the development of resistance to chemotherapy and that this occurs via a cellular-adaptive hypoxic response mediated, at least in part, by the action of HIFs. Because of this and the development of more resistant tumor phenotypes associated with hypoxia, there has been increasing interest in targeting HIF-mediated gene transcription to overcome these effects (18). Hypoxia induces the expression of approximately 100 to 200 genes mostly related to metabolism, angiogenesis, and apoptosis (37, 38), and in this study, we found that a sequence-specific DNA-binding oligomer (HIF-PA) that is capable of binding to the HRE and inhibiting HIF-mediated gene transcription (19, 39) can overcome multiple myeloma resistance to hypoxia-mediated apoptosis in vitro and...
in vivo. We also found that treating MM cells with a combination of HIF-PA, to inhibit gene transcription, and rapamycin, to inhibit gene translation, had a strong synergistic cytotoxic effect against multiple myeloma tumor cells cultured under hypoxic conditions. Our results provide a strong preclinical rationale for using polyamides to target the adaptive hypoxic response in multiple myeloma and may prove to be efficacious in treating myeloma tumors engrafted in the hypoxic bone marrow microenvironment. We also note that the ability of HIF-PA to recognize and bind to HRE sequences enables them to block both the HIF1α/HIF1β and HIF2α/HIF1β dimers.

We surveyed a panel of multiple myeloma cell lines and found that the expression of the HIF1α-subunit was generally absent under normal culturing conditions but was rapidly increased by low pO2 levels. The exception to this was 8226 cells, which constitutively expressed HIF1α under normoxic conditions, although it was further upregulated by hypoxia. In contrast to the HIF1α subunit, HIF2α was constitutively expressed in all the cell lines studied (except MM15), and the expression levels were independent of O2 levels. Of note, we did not observe a correlation between the expression of the HIF1α subunit and sensitivity to hypoxia-mediated cytotoxicity in the cells we examined. These results are interesting because they suggest that the HIFα subunits are differentially regulated by low pO2 levels in multiple myeloma cells and that mechanisms other than just the expression of HIF1α regulate sensitivity to hypoxia. In fact, despite their similarities, it is known that the α subunits can mediate different responses to hypoxia and tumorogenesis. HIF1α acts as a tumor suppressor gene and inhibits tumor growth, whereas HIF2α acts as an oncogene and promotes tumor growth (3, 40). It has also been demonstrated that the different α subunits have varying affinities for hydroxylation by the different PHD isoforms, with PHD2/EGLN1 showing the highest affinity for HIF1α (28) and PHD3/EGLN3 showing the highest affinity for HIF-2α (29). In our study, we found that the expression patterns of PHD/EGLN isoforms could explain HIFα-subunit expression in both 8226 and OPM-2 cells. Specifically, PHD2/EGLN1 was expressed at very low levels in 8226, but not in OPM-2, which can explain why HIF1α was constitutively expressed in the former but not the latter. It is also likely that the absence of PHD3/EGLN3 in multiple myeloma cells results in the inability to hydroxylate HIF2α, thereby rendering its expression independent of O2 levels. Thus, it is likely that the expression and ability of PHD isoforms to regulate the expression of the different α subunits may have important ramifications for disease progression and pathology in multiple myeloma, such as what is observed in patients with VHL cancer syndrome (41). In support of this, PHD3/EGLN3, but not PHD2/EGLN1 silencing, has been reported at a relatively high frequency for patients with multiple myeloma, Waldenström macroglobulinemia, and MGUS and that these patients have a poorer prognosis (42), suggesting that PHD/EGLN activity is as important as HIF activity in regulating the adaptive hypoxic responses in multiple myeloma and is something that we will pursue in future experiments.

There is a dynamic physiologic process that exists between the metabolic needs of tumor cell growth and the sufficiency of the vascular networks that are required to support these needs. On one hand, the development of oxygen stress within the tumor inhibits cell division and growth and induces cell death, but on the other hand, hypoxia activates the HIF-mediated cellular responses that provide protective growth and survival advantages, and that can foster the development of tumor resistance to radiation and chemotherapy (27, 43). In this study, we found that HIF-PA was well tolerated by mice, localized to the nuclei of the tumor xenografts and could inhibit tumor growth. Histologic analysis of HIF-PA-treated subcutaneous xenografts indicated that tumor cytotoxicity was remarkably colocalized with regions of ischemic stress in the tumor nodules and that VEGF expression was significantly inhibited. Although VEGF is clearly a factor in the induction of the HIF-PA–mediated killing of multiple myeloma tumor cells, we believe that VEGF is probably only one of many genes affected by targeting the adaptive hypoxic response using HIF-PA (39). Although our subcutaneous model demonstrated some efficacy of HIF-PA in reducing solid multiple myeloma xenograft tumors, we recognize that this particular model may not be physiologically relevant to clinical myeloma, which engraft in the bone marrow. Therefore, we also present data using an orthotopic multiple myeloma xenograft model. In this model, 8226 tumor cells localized to the mouse bone marrow demonstrate a similar antitumor response to HIF-PA treatment that mirrored the effects seen in our subcutaneous model. Specifically, we noted a decrease in luciferase activity measured by the change in average radiance, as well as a general decrease in the size of the multiple myeloma tumor foci engrafted in the bone marrow. Although HIF-PA had a statistically significant effect on orthotopic multiple myeloma tumors, this did not translate to a longer survival in the mice. We believe that this may be because our initial HIF-PA treatment strategy was not the most effective dosing regimen for establishing and maintaining long-term anti–myeloma responses in our orthotopic model. For example, HIF-PA availability and clearance in the bone marrow still need to be established. This, in fact, has important ramifications for addressing the known limitations of these antiangiogenesis therapies (44) because HIF-PA directly targets the hypoxic response at the level of gene transcription, rather than targeting a single downstream effector of hypoxia, such as VEGF.

Because of our previous in vivo studies (21, 24, 33, 36) that demonstrated a correlation between the antiangiogenic effects of the mTOR inhibitor, temsirolimus, we initially hypothesized that hypoxic stress alone would be sufficient to kill multiple myeloma cells. However, we found that multiple myeloma cell lines are generally resistant to low O2, suggesting that hypoxia-induced physiologic stress alone cannot fully explain our results. In fact, myeloma cells that are the most resistant to mTOR inhibition (e.g., 8226 and U266), are also the most the resistant to hypoxia, whereas cells that are more sensitive to rapalogs, such as OPM-2 are the most sensitive to hypoxia-mediated killing (45). It is also known that hypoxia regulates the activity of mTOR via the induction of REDD1, (46) although the relationship between hypoxia, mTOR activation, and HIF expression/activation remains complex and is not fully understood (47). This is further confounded by the presence of cap-independent, IRES-mediated translational pathways (48, 49) that may allow tumor cells to escape mTOR-targeting therapies. Interestingly, we find that the combination of polyamides and rapamycin can effectively synergize with each other to overcome resistance to hypoxia-mediated killing of 8226 cells in vitro. Along another line of reasoning, we noted that in a recent study by Maiso and colleagues (10), hypoxic conditions conferred a striking resistance to bortezomib-mediated apoptosis in multiple myeloma cell lines and, critically, inhibiting HIF1α expression could restore sensitivity. If true, then we hypothesized that HIF-PA would likely have a similar effect in
bortezomib-treated cells by inhibiting the adaptive hypoxic response and overcoming any hypoxia-mediated resistance to this drug. However, despite our best efforts, we were unable to replicate this phenomenon and instead found that our results were more similar to those reported by Hu and colleagues (50), which found instead that bortezomib killed multiple myeloma cell lines cultured under hypoxic conditions. It is unclear why our experiments differed from those of Maiso and colleagues but could reflect variations in how hypoxic conditions were established. Therefore, at least in our hands, it remains to be shown if hypoxia confers resistance to chemotherapeutic drugs and if HIF-PA can overcome and sensitize multiple myeloma cells by inhibiting HIF-activity.

In summary, HIF and related hypoxic response factors are frequently upregulated in multiple myeloma tumors and has been implicated in contributing to the development and progression of multiple myeloma (15). The induction of proangiogenic, proliferative, metastatic, and glycolytic genes by HIF1 may also be involved in the development of chemotherapy-resistant phenotypes (10, 27). In this sense, HIF1, and in particular, the expression of the different α subunits may play dual roles in the survival and progression of multiple myeloma, through the differential α-subunit expression patterns and the genes that they activate. Thus, we argue that understanding the role and mechanisms HIF1-mediated adaptive hypoxic response at the level of HIF1/DNA binding could be clinically relevant for developing novel therapies against patient multiple myeloma engrafted in the hypoxic bone marrow environment.

References


Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Targeting the Adaptive Hypoxic Response in Multiple Myeloma Cells


A DNA-binding Molecule Targeting the Adaptive Hypoxic Response in Multiple Myeloma Has Potent Antitumor Activity

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