Microenvironment and Immunology

In vivo Dynamics and Distinct Functions of Hypoxia in Primary Tumor Growth and Organotropic Metastasis of Breast Cancer

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Abstract

Tumor hypoxia is known to activate angiogenesis, anaerobic glycolysis, invasion, and metastasis. However, a comparative analysis of the potentially distinct functions of hypoxia in primary tumor growth and organ-specific metastasis has not been reported. Here, we show distinct hypoxia kinetics in tumors generated by the MDA-MB-231 breast cancer sublines with characteristically different primary tumor growth rates and organotropic metastasis potentials. Hypoxia-induced angiogenesis promotes both primary tumor growth and lung metastasis but is nonessential for bone metastasis. Microarray profiling revealed that hypoxia enhances the expression of a significant number of genes in the lung metastasis signature, but only activates a few bone metastasis genes, among which DUSP1 was functionally validated in this study. Despite the different mechanisms by which hypoxia promotes organ-specific metastasis, inhibition of HIF-1α with a dominant-negative form of HIF-1α or 2-methoxyestradiol reduced metastasis to both lung and bone. Consistent with the extensive functional overlap of hypoxia in promoting primary tumor growth and lung metastasis, a 45-gene hypoxia response signature efficiently stratifies breast cancer patients with low or high risks of lung metastasis, but not for bone metastasis. Our study shows distinct functions of hypoxia in regulating angiogenesis and metastasis in different organ microenvironments and establishes HIF-1α as a promising target for controlling organotropic metastasis of breast cancer. Cancer Res; 70(10); 3905–14. ©2010 AACR.

Introduction

Metastasis to vital organs such as bone, lung, liver, and brain is responsible for the vast majority of breast cancer deaths (1). It has been well recognized that metastatic seeding and growth is determined by both the intrinsic genetic properties of tumor cells and the local characteristics of the stromal microenvironment (1, 2). Functional genomic analyses of the in vivo selected organotropic variants of breast cancer cell lines have led to the identification of distinct sets of organ-specific metastasis genes to bone, lung, and brain (3–5). Understanding the organ-specific functions of metastasis-related signaling pathways may provide new opportunities for therapeutically targeting metastasis in different organs.

Hypoxia has been increasingly recognized to play a central role in different stages of tumor progression (6). Signaling responses to hypoxia are mediated mainly through the hypoxia-inducible factors (HIF), including HIF-1, HIF-2, and HIF-3 (7). Among them, HIF-1 is best characterized as being responsible for the regulation of many hypoxia-inducible genes. HIF-1 is composed of the hypoxia-responsive subunit, HIF-1α, and the constitutively expressed subunit, HIF-1β. Under normoxia, the oxygen-dependent degradation (ODD) domain of HIF-1α is hydroxylated, facilitating the binding of the von Hippel-Lindau E3 ubiquitin ligase and subsequent proteosome-mediated degradation of HIF-1α. When oxygen levels become limited, HIF-1α is free of hydroxylation and escapes degradation. Accumulated HIF-1α enters the nucleus, forms a transcription complex with HIF-1β, and drives transcription of downstream genes (8).

HIF-1α overexpression is correlated with distant metastasis and poor prognosis for breast cancer patients (9). Furthermore, HIF-1α overexpression was more frequently observed in metastases than in primary tumor of breast cancer, suggesting an active role of HIF-1α in regulating metastatic progression (9). The functions of hypoxia and HIF-1α in promoting metastasis may overlap with its functions in primary tumor growth, for example, the enhancement of angiogenesis. However, hypoxia is also known to promote metastasis-specific functions, such as epithelial-mesenchymal transition, invasion, and metastatic seeding (10). The distinct functions of

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hypoxia in promoting metastasis to different organs have not been thoroughly investigated. Here, we integrate in vivo hypoxia imaging, functional inactivation of HIF-1α, and transcriptomic analysis to investigate the distinct functions of hypoxia in tumor growth and metastasis in the recently developed organotropic metastasis models of breast cancer.

Materials and Methods

Cell culture. The MDA-MB-231 sublines were maintained as described (11).

Tumor xenografts and analysis. All procedures involving mice, such as housing and care, and all experimental protocols were approved by the Institutional Animal Care and Use Committee of Princeton University. Intracardiac, i.v., and mammary gland injections were performed as described (3, 4). Development of metastases in bone and lung was monitored by firefly or Renilla luciferase bioluminescence imaging (BLI) as described (12–14).

2-Methoxyestradiol treatment. 2-Methoxyestradiol (2ME2; BIOMOL, Inc.) was suspended in 0.5% carboxymethylcellulose (Sigma) at 15 mg/mL for in vivo treatment. Mice were inoculated with tumor cells, and oral feeding commenced 10 days later with vehicle or 2ME2 daily at 75 mg/kg mouse weight. Primary mammary gland tumors were treated for 20 days. Metastases were treated for 30 days.

Microarray analysis. SCP2 or LM2 cells were cultured in duplicate under ambient O2 level (21%) for 24 hours or low level of O2 (1%) for 6 and 24 hours. RNA was extracted and microarrays were performed with the Agilent Whole Human Genome 4x44k platform as described (15). Microarray data were deposited at the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with the accession number GSE17188. Patient microarray samples with metastasis information were obtained from Minn and colleagues (4). Hierarchical clustering was performed using the GeneSpring GX 7.3 software (Agilent Technologies).
Statistical analysis. Results were reported as average ± SD or average ± SEM. Group comparisons were performed using either the nonparametric Mann-Whitney test or unpaired two-sided Student’s t test without equal variance assumption. Kaplan-Meier curve comparison was performed with the log-rank test.

Additional materials and methods, including construction of plasmids and stable cell lines, CoCl₂ treatment, Western and Northern blot analyses, histology and immunohistochemistry (IHC), detection of hypoxia, quantitative reverse-transcriptase PCR, DUSP1 knockdown, gene set enrichment analysis, and derivation of hypoxia response signatures, are listed in the Supplementary Information.

Results

Organotropic MDA-MB-231 variants SCP2 and LM2 display differential HIF-1α hypoxic response pattern in vitro and in vivo. MDA-MB-231 sublines with distinct metastasis organotropisms were previously isolated by in vivo selection in xenograft metastasis models in mice (3, 4, 16). We chose to use the bone-tropic subline SCP2 and the lung-tropic subline LM2 to perform our study because of their distinctive proclivities to metastasize to different target organs: Following intracardiac injection, SCP2 forms osteolytic bone lesions much more efficiently than LM2, whereas following i.v. injection, LM2 generates numerous lung metastasis nodules but much more efficiently than LM2, whereas following i.v. injection, LM2 generates numerous lung metastasis nodules but SCP2 rarely forms any (Fig. 1A; ref. 11). SCP2 and LM2 have similar growth rates in vitro (11). Interestingly, SCP2 and LM2 display significant differences in primary tumor growth when injected into mammary fat pads. Although LM2 forms rapidly growing and well-vascularized primary tumors, SCP2 gives rise to tumors of much smaller size (Fig. 1B). The distinct growth and metastatic properties of the two cell lines that share a closely related genetic background allow us to comparatively investigate the potential organ-specific contribution of hypoxia to the growth of primary tumor and metastases in different organs.

First, we evaluated the hypoxic response of SCP2 and LM2 in vitro. HIF-1α protein was not detectable by immunoblotting in either subline under ambient oxygen conditions (~21%), but became highly abundant under low-oxygen conditions (1%; Fig. 1C). LM2 showed a noticeably stronger response to hypoxia in vitro. To investigate the status of hypoxia and HIF-1α in tumor tissues, we detected hypoxia with Hypoxyprobe (Supplementary Fig. S1) and performed IHC of HIF-1α on primary tumors formed by LM2 and SCP2, lung metastasis formed by LM2, and bone metastasis formed by SCP2 (Fig. 1D). All tumor samples were harvested at the end of the in vivo experiments shown in Fig. 1A and B. Hypoxia was detected in primary tumors and metastases formed by both cell lines (Supplementary Fig. S1), suggesting hypoxia as the cause for in vivo HIF-1α activation, although we cannot rule out the influence of other signaling pathways on the HIF-1α level. In primary tumors, HIF-1α was positively stained in cell nuclei for both LM2 and SCP2 tumors with an appreciably stronger staining in SCP2 tumors (Fig. 1D, top). Considering the intrinsically weaker HIF-1α response in SCP2 in vitro (Fig. 1C), the more intense HIF-1α staining of SCP2 tumors suggested that these tumors experienced more hypoxia stress. Consistently, bone metastases formed by SCP2 have a stronger HIF-1α staining than lung metastases formed by LM2 (Fig. 1D, bottom). It was also noted that LM2 lung metastases contained prominent intratumoral blood vessels whereas SCP2 bone metastases contained very few.

SCP2 and LM2 display different intracellular hypoxia kinetics during primary tumor growth. HIF-1α immunostaining only shows tumor cell responses to the hypoxia condition at a static time point. To analyze dynamic changes in the hypoxia response by tumor cells longitudinally in vivo, we adopted a recently developed approach of real-time of HIF-1α prolyl hydroxylase activity using the reporter construct ODD-Fluc (17). When the ODD peptide is fused with firefly luciferase (Fluc), the stability of ODD-Fluc fusion protein mimics that of HIF-1α and serves as an accurate reporter of the intracellular HIF-1α level and an indirect reflection of hypoxia activity (17). Unlabeled SCP2 and LM2 were stably transfected with either a constitutively expressed Fluc reporter or the ODD-Fluc reporter. To test the sensitivity of the reporter in detecting hypoxia response in vivo, we used the hypoxia mimic CoCl₂ to create acute hypoxia-like conditions in tumors. We first detected dose-dependent and time-dependent stabilization of HIF-1α, as well as a dose-dependent increase of ODD-Fluc activity, by CoCl₂ treatment in vitro (Supplementary Fig. S2). Next, we determined the efficacy of the reporter system in vivo by treating mammary tumor-bearing mice with a single dose of CoCl₂. Tracking the Fluc activity with BLI after the injection showed peak luciferase signals at 20 hours posttreatment for ODD-Fluc sublines, indicating response kinetics similar to the in vitro treatment (Supplementary Fig. S3). As a negative control, the Fluc-labeled sublines showed little fluctuation of BLI signals after CoCl₂ treatment.

After validating the ODD-Fluc hypoxia reporter system in vivo, we characterized the dynamic changes of hypoxia activity during the primary tumor development of SCP2 and LM2. To quantify the unit cellular response to hypoxia, the ODD-Fluc sublines were further labeled with a constitutively expressed Renilla luciferase (Rluc; ref. 11). Previous work has shown no cross-reactivity of Rluc and Fluc to their noncorresponding substrates, α-luciferin and coelenterazine (14). The dual-luciferase imaging system allowed direct comparison of relative hypoxia activity of tumors with different sizes by normalizing Fluc (absolute hypoxia signaling strength) to Rluc (tumor size). When Fluc/Rluc ratio was plotted at different time points after tumor inoculation, distinct hypoxia response kinetics of SCP2 and LM2 were observed (Fig. 2A). At the early phase of tumor growth (day 18), LM2 cells experienced higher hypoxia stress as LM2 showed greater normalized Fluc signal than SCP2. However, at later time points (day 34 and 41), a more hypoxic environment was detected in SCP2 tumors than in LM2 tumors. This result is consistent with the end point HIF-1α IHC analysis (Fig. 1D).

Both groups of primary tumors harvested at the end of the experiment contained necrotic centers as revealed by H&E
staining (Fig. 2B). However, LM2 tumors showed a much thicker growth zone than SCP2 tumors, suggesting the ability of LM2 tumors to sustain continuous growth. This was confirmed by Ki67 and terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) stainings (Fig. 2C). LM2 tumors contained significantly more Ki67–positive nuclei than SCP2 tumors (Supplementary Fig. S4). Neither of the tumors showed significant levels of apoptosis in the non-necrotic regions. Taken together, these results suggested that LM2 cells possess an overall proliferation advantage over SCP2 cells in primary tumors. In general, continuous tumor growth is dependent on adequate oxygen and nutrient supply provided through blood circulation. Therefore, we determined whether intratumoral blood vessel density was different in the two tumors. Both CD31 IHC and in vivo dextran labeling showed that vessels in LM2 tumors were more abundant and with larger inclusion areas, whereas those in SCP2 tumors were fewer and narrower (Fig. 2D). Because functional tumor angiogenesis is largely triggered by hypoxia stress, and hypoxia level, in turn, is alleviated by the successful formation of neovasculature, the hypoxia kinetics observed in SCP2 and LM2 primary tumors may reflect the stronger angiogenic response of LM2 cells to hypoxia.

**Differential dynamics of hypoxia in organ-specific metastases of SCP2 and LM2.** To investigate the potential differences in hypoxic activities in organotropic metastasis, SCP2 and LM2 cells harboring the dual-reporter system were subjected to in vivo bone and lung metastasis assays. During the progression of bone metastasis, we observed that the trends of normalized Fluc activity in SCP2 and LM2 were quite similar to those in the primary tumors: the Fluc/Rluc signal ratio decreased continuously in LM2 bone metastasis compared with a sustained high level of hypoxia in SCP2 bone metastasis (Fig. 3A). Again, the difference of the hypoxia reporter activity at the end of the experiment was consistent with the end point HIF-1α IHC analysis (Fig. 1D). Because SCP2 failed to generate any detectable lung metastasis signal, we could only analyze the hypoxia activity kinetics for LM2 lung metastasis. The normalized Fluc curve also showed a continuous decrease (Fig. 3B).

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**Figure 2.** Distinct hypoxia response kinetics and angiogenesis profiles of primary tumors produced by SCP2 and LM2. A, intracellular hypoxia dynamics in SCP2 and LM2 primary tumors as measured by Fluc/Rluc ratios. Points, mean (n = 10); bars, SEM. Right, representative animals. B, H&E staining of representative LM2 and SCP2 tumors. Average distance (arrows) between tumor margin and necrotic perimeter is 600.6 ± 62.8 μm for LM2 and 123.9 ± 5.16 μm for SCP2 (average ± SEM). Scale bar, 200 μm. C, Ki67 and TUNEL staining of representative LM2 and SCP2 primary tumors. Insets, the positively stained necrotic center. Scale bar, 50 μm. D, CD31 staining and fluorescent dextran labeling of representative LM2 and SCP2 primary tumors. In dextran labeling images: green, green fluorescent protein from tumor cells labeled with the GFP-Fluc fusion reporter; red, Texas red dextran; blue, 4,6-diamidino-2-phenylindole. Quantifications were performed using CD31-stained slides. Columns, mean; bars, SEM. Scale bar, 50 μm. ***, P < 0.001 with Student’s t test.
Because the trends of hypoxia kinetics were consistent between metastases and primary tumors formed by LM2 and SCP2, we hypothesized that the different angiogenic properties of the two cell lines might also explain the different hypoxic dynamics seen in metastases. Indeed, CD31 staining showed very few blood vessels in SCP2 bone metastases, whereas LM2 bone metastases, although much less frequent, possessed rich pools of vasculature (Fig. 3C). Similarly, LM2 cells recruited conspicuous blood vessels when forming lung metastases (Fig. 3D). SCP2 did not generate any appreciable tumor mass in the lung to allow for a similar analysis. It is unclear how SCP2 was able to generate fast-growing osteolytic bone metastases without necrosis despite the lack of hypoxia-induced angiogenesis. The relatively small size of bone metastases and the unique characteristics of the bone microenvironment may allow SCP2 tumors to sustain growth and avoid necrosis despite the lack of productive angiogenesis. Nonetheless, HIF-1α accumulation in SCP2 may have angiogenesis-independent functions in promoting bone metastasis. Likewise, hypoxia response in LM2 may have lung metastasis-promoting functions beyond the activation of angiogenesis. Inhibition of HIF-1α function in vivo was necessary to test the pleiotropic effect of hypoxia in tumor growth at different organs.

Pharmacologic and dominant-negative inhibitions of HIF-1α compromise growth of primary tumors and metastases. 2ME2 downregulates HIF-1α, inhibits the HIF-induced vascular endothelial growth factor (VEGF) activation and angiogenesis, and has been used in vivo to block HIF-1α activity (18–20). Treating cells under hypoxia with 2ME2 abolished the induction of HIF-1α in vitro (Fig. 4A). Therefore, 2ME2 was tested for its effect on primary tumors and metastases. 2ME2 significantly reduced the growth of LM2 primary tumors, but had little effect on SCP2 tumors (Fig. 4B). Because SCP2 tumors generally stop expanding after reaching a certain size (<100 mm³), we cannot directly determine whether 2ME2 would inhibit SCP2 tumor growth if SCP2 grew to a similar size as LM2. Nevertheless, this result indicated that 2ME2 could inhibit the growth of well-vascularized tumors. IHC confirmed lower HIF-1α levels in LM2 and SCP2 tumors after treatment with 2ME2 (Supplementary Fig. S5A–B). Microvessel density and architecture analysis by CD31 IHC showed narrower vessels in LM2 tumors by 2ME2 treatment, although 2ME2 did not seem to significantly affect
vessel density (Supplementary Fig. S5C; Fig. 4B). Consistently, 2ME2 did not affect the already compressed vessels in SCP2 tumors (Fig. 4B). Overall, these results suggest that the primary effect of 2ME2 on primary tumor growth is through targeting the function of HIF-1α in angiogenesis.

If angiogenesis were also the primary contribution of HIF-1α to promote metastasis development, we would expect that 2ME2 could reduce LM2 lung metastasis burden but have relatively little effect on SCP2 bone metastasis. To our surprise, both LM2 lung metastasis and SCP2 bone metastasis were significantly inhibited by 2ME2 treatment (Fig. 4C–D), suggesting angiogenesis-independent functions of HIF-1α in promoting bone metastasis. Likewise, we also cannot rule out the possibility of nonangiogenic functions of HIF-1α and hypoxia in promoting lung metastasis.

One potential caveat of using 2ME2 to inhibit HIF-1α is the potential nonspecific effects. To block the function of HIF-1α more specifically, a FLAG-tagged dominant-negative (DN) form of HIF-1α (21) was stably expressed in SCP2 and LM2 (Fig. 5A), and the cell lines were subjected to in vivo analysis of tumor growth and metastasis. We observed similar effects of DN-HIF-1α on primary tumor and metastasis growth as 2ME2 treatments (Fig. 5B–D). LM2 primary tumor growth was inhibited by DN-HIF-1α, whereas SCP2 growth was unaffected within the tested period (Fig. 5B). LM2 lung metastasis and SCP2 bone metastasis were both significantly inhibited (Fig. 5C and D). These results further confirm that inhibition of HIF-1α reduces tumor growth in a site-independent manner.

**Hypoxia activates organ-specific metastasis genes.** To identify hypoxia target genes that may play a role in promoting organ-specific metastasis, LM2 and SCP2 were cultured under ambient (21%) or low-oxygen (1%) condition for 6 or 24 hours before microarray profiling. Gene set enrichment analysis was used to examine the enrichment for gene sets representing the general HIF-1 target genes (22), lung metastasis gene signature (LM5; ref. 4), and bone metastasis gene signature (3) by the hypoxic condition. The bone metastasis gene signature and LM5 include genes that are upregulated in the highly metastatic cells (candidate metastasis enhancers) and those downregulated in highly metastatic cells (candidate metastasis suppressors). The upregulated and downregulated subsets were tested separately. With an
FDR q-value of 0.2 as the significance cutoff (more stringent than the recommended 0.25), hypoxic LM2 and SCP2 both were enriched for the general HIF-1α targets (Supplementary Fig. S6A–C), such as VEGF (Supplementary Fig. S6D), indicating that both cells were able to turn on the canonical HIF-1α transcription programs. Although VEGF is activated by hypoxia at a slightly stronger level in LM2 than in SCP2, this could not fully account for the dramatic differences in angiogenesis in the two tumors. SCP2 may possibly express a strong antiangiogenic factor(s) that buffers the proangiogenic activity of HIF-1α-induced factors. When metastasis gene signatures were tested in gene set enrichment analysis, the upregulated subset of the LMS, but not the downregulated subset, was found to be significantly enriched in hypoxic LM2 transcriptome (Supplementary Table S1; Supplementary Fig. S6A–C). This finding suggests that hypoxia may have lung metastasis–promoting functions beyond the activation of angiogenesis. Among genes in the LMS, ANGPTL4, an essential gene in lung metastasis formation (23), has the strongest response to hypoxia (Supplementary Fig. S6D).

The hypoxic SCP2 gene profile did not enrich for either the upregulated or the downregulated subset of the bone metastasis gene signature (Supplementary Table S2; Supplementary Fig. S6A–C). Nevertheless, several putative bone metastatic genes were activated by hypoxia, including CXCR4 and DUSP1 (Supplementary Fig. S6D). Hypoxic responses of these two genes were much weaker or absent in LM2 cells. The functional importance of CXCR4 in bone metastasis has been established before (24, 25). DUSP1 is overexpressed in breast carcinomas and implicated in breast cancer chemoresistance (26). We previously identified DUSP1 as one of the 11 mostly upregulated genes in the bone-metastatic sublines of MDA-MB-231 (3). Northern blot analysis confirmed the dramatic overexpression of DUSP1 in bone-tropic cells (Supplementary Fig. S7A). However, the functional importance of DUSP1 in bone metastasis had not been investigated. Using shRNA-mediated gene silencing, we knocked down DUSP1 expression in SCP2 and observed a significant decrease in bone colonization (Supplementary Fig. S7B and C). Furthermore, when we examined the potential clinical importance of DUSP1 in bone metastasis by analyzing its
expression level in a published microarray data set (4), higher DUSP1 level in primary tumors was detected in patients with bone metastases but not lung metastasis (Supplementary Fig. S7D). Taken together, these results suggest that hypoxia has an organ-specific metastasis-promoting function in addition to its well-appreciated role in eliciting angiogenesis.

**Hypoxia response signature for lung metastasis prognosis.** HIF-1α overexpression and hypoxia-induced gene expression changes have been associated with poor prognosis and metastasis of breast cancer patients (27, 28). However, the prognostic value of the hypoxia gene signature in organ-specific metastasis has not been investigated. To test this, we compared the expression profiles of LM2 or SCP2 in normoxic or hypoxic conditions and identified differentially expressed genes as hypoxia response signatures for each cell line (see gene lists in Supplementary Tables S3 and S4). LM2- and SCP2-specific hypoxia response signatures were used to perform unsupervised hierarchical clustering of tumor samples in the Minn and colleagues data set (4), which contains organ-specific metastasis status of all patients in a 5-year follow-up period. Both the LM2 and SCP2 hypoxia signatures were able to segregate patients into two major clusters with distinctively different outcomes in lung metastasis, whereas the same signatures were not able to identify patients with high risk for bone metastasis (Supplementary Figs. S8 and S9). This result suggests that different MDA-MB-231 variants may share a common hypoxia transcriptomic program that can be used to predict organ-specific metastasis to lung. Indeed, using the overlapped hypoxia gene signature containing 45 genes with 70 probe sets (Supplementary Table S5), we successfully classified patients into two subgroups with distinct incidence rates for lung metastasis but not bone metastasis (Fig. 6A). Kaplan-Meier curve analyses further confirmed that patients with tumor samples harboring positive

![Figure 6](cancerres.aacrjournals.org)
It is interesting to note the apparent lack of extensive angiogenesis in the aggressive bone metastases generated by SCP2 confirmed by CD31 IHC, von Willebrand factor IHC, or dextran labeling (data not shown). This finding is in contrast to previous studies showing the positive CD31 staining in bone metastases formed by MDA-MB-231 and linking the HIF-1α activity in bone metastasis directly to angiogenesis (20, 21). We believe the difference can be explained by the heterogeneous nature of the parental MDA-MB-231 cell line. By using the relatively homogenous organotrophic sublines, we were able to discover pleiotropic functions of hypoxia in promoting bone- and lung-specific metastasis beyond regulating angiogenesis.

Intriguingly, although the inhibition of HIF-1α reduces both bone and lung metastases, the hypoxia response signature is prognostic of lung but not bone metastasis in breast cancer patients. This paradox could be explained by the different mechanisms through which hypoxia contributes to tumor growth at the different organs. Development of lung metastasis and the growth of the primary tumors both rely on productive angiogenesis in response to hypoxia. Such a function could be selected for in the primary tumor during tumor progression and consequently promote lung metastasis after tumor dissemination. Furthermore, the LMS genes are highly enriched in hypoxic conditions. As HIF-1α expression has been found to progressively increase during human breast carcinogenesis (31), elevated HIF-1α may induce the activation of LMS genes, such as ANGPTL4, in highly aggressive breast carcinoma. Such activation may subsequently prime tumor cells to effectively generate pulmonary metastases. Thus, it is not surprising that the hypoxia response signature correlates with higher risk of lung metastasis. On the other hand, only a limited number of genes (such as CXCR4 and DUSP1) among the hypoxia response signature contribute directly to bone metastasis, whereas many other common hypoxia-regulated genes, including various angiogenic factors, may have relatively little direct influence on bone metastasis. Therefore, the overall hypoxia response gene signature was not able to stratify patients for risk of bone metastasis based on the gene expression profiles of their primary tumors. Nevertheless, this finding does not exclude the targeting of HIF-1α as a viable strategy for the treatment of bone metastasis, as our current study clearly indicated the inhibitory effect of DN-HIF1α or 2ME2 on bone metastasis.

Overall, we showed the pleiotropic functions of hypoxia/HIF-1α at different stages of breast cancer progression and metastasis (Fig. 6D). In the primary tumor, hypoxia-induced angiogenesis promotes neovascularization and continuous expansion of the tumor mass. Furthermore, hypoxia transcriptional program may enhance the expression of LMS genes, which empowers departing tumor cells with the ability to effectively colonize the lung microenvironment. On the other hand, although hypoxia-induced angiogenesis seems to be noncritical for the aggressive growth of bone metastasis in the cell system studied, hypoxia does contribute to bone metastasis by activating the expression of a few bone metastasis genes, such as DUSP1 and CXCR4. Therefore, HIF-1α in breast cancer may be targeted to control the metastasis in multiple distant organs.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Correction: Online Publication Dates for Cancer Research April 15, 2010 Articles

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