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Molecular mechanisms mediating metastasis of hypoxic breast cancer cells

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Abstract

Breast cancers contain regions of intratumoral hypoxia in which reduced O₂ availability activates the hypoxia-inducible factors HIF-1 and HIF-2, which increase the transcription of genes encoding proteins that are required for many important steps in cancer progression. Recently, HIFs have been shown to play critical roles in the metastasis of breast cancer to the lungs through the transcriptional activation of genes encoding angiopoietin-like 4 and L1 cell adhesion molecule, which promote the extravasation of circulating cancer cells from the lung vasculature, and the lysyl oxidase family members LOX, LOXL2, and LOXL4, which promote invasion and metastatic niche formation. Digoxin, a drug that inhibits HIF-1 activity, blocks primary tumor growth, vascularization, invasion, and metastasis in *ex vivo* and *in vivo* assays.

Keywords

acriflavine; angiopoietin-like 4; collagen crosslinking; digoxin; extravasation; HIF-1; lysyl oxidase; metastatic niche formation

Many human cancers contain hypoxic regions

Cancers are characterized by dysregulated growth due to increased cell division and decreased cell death. The resulting increased numbers of cells consume increased amounts of O₂ leading to hypoxia (decreased O₂ availability), which stimulates angiogenesis (new blood vessel formation). However, the blood vessels that form within cancers are structurally and functionally abnormal, resulting in a marked spatial and temporal heterogeneity in the perfusion of the tumor tissue. Intratumoral hypoxia results when O₂ is either diffusion-limited, due to the location of a cancer cell sufficiently distal to a blood vessel that O₂ diffusing from the vessel is consumed by cells more proximal to the vessel, or perfusion-limited, due to reduced or absent blood flow through an abnormal tumor vessel [1]. As a result, hypoxic areas that are distributed heterogeneously throughout the tumor mass are observed in approximately half of all locally advanced cancers [1]. Clinical studies in which the partial pressure of O₂ (*PO*₂) in human breast cancers was measured directly by an Eppendorf microelectrode revealed a median *PO*₂ of 28 mm Hg, as compared to 65 mm Hg in normal human breast tissue; >50% of all breast cancers studied had a *PO*₂ of less than

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2.5 mm Hg [2]. In multiple types of human cancer, the presence of intratumoral hypoxia has been identified as an adverse prognostic factor for patient outcome and this effect is independent of established prognostic parameters such as clinical tumor stage, histological grade, and lymph node status [3]. Remarkably, despite the extensive clinical data linking intratumoral hypoxia to patient mortality, there are currently no approved drugs that target hypoxic cancer cells [4], indicating a major unmet clinical need in the effort to more effectively treat a major cause of mortality in Western societies.

Adaptive responses to hypoxia are mediated by HIFs

All human cells adapt to decreased O₂ availability through the activity of the hypoxia-inducible factors HIF-1 and HIF-2, which are transcriptional activators that regulate the expression of over 800 target genes [5]. HIF-1 is a heterodimer consisting of HIF-1 α and HIF-1 β subunits [6,7]. HIF-1 α is subjected to two O₂-dependent post-translational modifications (Figure 1a). First, hydroxylation of proline residue 402 and/or 564 targets the protein for ubiquitination and proteasomal degradation [8–10]; second, hydroxylation of asparagine residue 803 blocks the binding of the coactivator protein p300 [11]. In hypoxic cells, the prolyl and asparaginyl hydroxylation reactions are inhibited, HIF-1 α rapidly accumulates, heterodimerizes with HIF-1 β , binds to the consensus DNA sequence 5'-RCGTG-3' within hypoxia response elements located in target genes, and activates their transcription [12]. HIF-1 target genes encode secreted proteins, such as vascular endothelial growth factor (VEGF), that function to increase O₂ delivery to cells by stimulating angiogenesis, and intracellular proteins, such as the glycolytic enzymes, which allow cells to survive O₂ deprivation by reprogramming their metabolism [5]. HIF-2, a heterodimer consisting of HIF-1 β and an O₂-regulated HIF-2 α subunit, stimulates the expression of some (*e.g.*, angiogenic factors) but not all (*e.g.*, glycolytic enzymes) of the gene products that are regulated by HIF-1 [13].

HIF-1 α and HIF-2 α levels are increased in breast cancer

Cancer cells co-opt the adaptive responses to hypoxia that are mediated by HIFs (Figure 1b). Given the degree of intratumoral hypoxia that has been demonstrated in human breast cancer, it is not surprising that immunohistochemical studies have demonstrated increased HIF-1 α protein levels in the majority of biopsies analyzed from both lymph node-negative [14] and lymph node-positive [15] breast cancer patients. In both cohorts, the survival of patients with the highest levels of HIF-1 α in their diagnostic biopsies was significantly decreased. The percentage of HIF-1 α overexpressing cells increased with disease progression, such that mortality was increased among node-negative patients with >5% HIF-1 α overexpressing cells in biopsy sections [14] and among node-positive patients with >50% overexpressing HIF-1 α cells [15]. HIF-1 α overexpression also predicted adverse outcome in many subsequent studies of breast cancer patients [16–22]. HIF-1 α levels are not increased in benign fibrocystic disease but are already increased in ductal carcinoma *in situ*, particularly in poorly differentiated lesions [23]. High HIF-2 α levels in breast cancer biopsies are also associated with mortality [24]. Finally, increased expression of mRNAs encoded by HIF target genes is associated with breast cancer mortality [25], thereby linking the overexpression of HIF-1 α and HIF-2 α with increased HIF transcriptional activity. Among the more than 1000 known HIF target genes are many encoding proteins that play critical roles in angiogenesis, metabolic reprogramming, autocrine growth/survival signaling, epithelial-mesenchymal transition, immortalization, invasion, metastasis, stem cell maintenance, and resistance to radiation and chemotherapy (Table 1; the list is intended to be illustrative rather than comprehensive; for literature citations, see [12]).

Although it is not possible to see hypoxia in a standard hematoxylin- and eosin-stained biopsy section, one can clearly see its consequences. The presence of necrosis in cancer sections indicates regions of severe intratumoral hypoxia in which oxygenation was insufficient to maintain cell viability. HIF-1 α levels are usually highest within the viable cells surrounding areas of necrosis in breast cancers. Although it is commonly believed that hypoxia is only present in the central regions of large tumors, necrosis – and perinecrotic HIF-1 α expression – are seen within ductal carcinoma *in situ* [23], *i.e.*, severe hypoxia may be present even prior to the development of an invasive carcinoma.

HIFs promote lung metastasis by stimulating extravasation of hypoxic breast cancer cells

Virtually all patients who die from breast cancer have metastatic disease. Even at the time of initial cancer surgery, metastasis may have already occurred in many patients who eventually die of breast cancer [26]. Metastasis may occur by dissemination of breast cancer cells via either the lymphatic or vascular circulation and it is vascular metastasis that accounts for widely disseminated, malignant disease [27]. Less than 0.1% of cancer cells that enter the vascular system establish a metastatic lesion [28], indicating that extravasation of cancer cells from the circulation into a tissue microenvironment that is favorable for survival and proliferation is a limiting step in the metastatic process. Increased expression of several genes implicated in breast cancer metastasis to the lungs has been reported in primary tumors of women with lung metastases and in breast cancer subclones selected for lung metastasis in mice [29,30].

The immunohistochemical data from clinical studies described above link high intratumoral HIF-1 α and HIF-2 α levels to metastasis and patient mortality. Breast cancers arising in conditional knockout mice lacking HIF-1 α expression in mammary epithelial cells showed significantly reduced lung metastasis compared to breast cancers arising in wild type mice, demonstrating that HIF-1 promotes breast cancer metastasis [31]. To delineate the role of specific human HIF-1 target genes in defined steps of the metastatic process, we utilized a model system involving orthotopic implantation into the mouse mammary fat pad of MDA-MB-231 cells, which are derived from human breast cancer cells that had metastasized to the lungs [32]. MDA-MB-231 cells exemplify the class of triple-negative breast cancers, which lack expression of the estrogen (ER), progesterone, and HER2 receptors. Whereas ER⁺ breast cancers respond to anti-estrogen therapies, such as tamoxifen and aromatase inhibitors [33], and HER2⁺ cancers respond to trastuzumab, a monoclonal antibody directed against HER2 [34], there are no similarly targeted and effective therapies for triple-negative breast cancers, which have a poor prognosis with early relapse after chemotherapy [35].

We stably transfected MDA-MB-231 cells with expression vectors encoding short hairpin RNAs (shRNAs) targeting HIF-1 α and HIF-2 α and designated the subclone as MDA-MB-231-DKD (for double knockdown of HIF-1 α and HIF-2 α expression). This subclone and a control subclone, which was transfected with empty vector (designated MDA-MB-231-EV), were injected into the mammary fat pad of severe combined immunodeficiency (SCID) mice. Loss of HIF activity in the DKD subclone was associated with significantly decreased primary tumor growth and an even more dramatic reduction in spontaneous metastasis of cancer cells from breast to lungs [36].

In order to metastasize, breast cancer cells must intravasate into a blood vessel within the primary tumor and then extravasate out of the blood vessel at a distant metastatic site, such as the lungs. The hypoxia-induced and HIF-dependent expression of VEGF has been shown to increase vascular permeability and thereby promote breast cancer intravasation [37]. To determine whether HIFs specifically promote the extravasation of hypoxic breast cancer

cells from pulmonary capillaries into the lung parenchyma, we exposed EV and DKD cells to 20% O₂ (PO_2 = 140 mm Hg) or 1% O₂ (PO_2 = 7 mm Hg) for 48 h, injected the cells by tail vein (in order to bypass all steps that are required for cancer cells to access the vasculature), and one week later performed histological analysis to enumerate breast cancer cells that had extravasated out of pulmonary blood vessels into the lung parenchyma. Exposure to 1% O₂ (to simulate hypoxia within the primary tumor) increased the extravasation of EV cells, whereas extravasation of DKD cells was significantly impaired [36].

HIF-dependent expression of L1CAM increases the interaction of hypoxic breast cancer cells with endothelial cells

Two critical requirements for extravasation are that the cancer cell must first adhere to ECs in the lung vasculature (margination) and then disrupt the tight interactions between endothelial cells (ECs) in order to migrate from the luminal to the abluminal side of the blood vessel (extravasation). Exposure of EV cells to 1% O₂ for 48 h increased their adherence to ECs, whereas adherence of DKD cells to ECs was significantly impaired. We screened a microarray of metastasis-related genes to identify a cell surface receptor that is induced by hypoxia in MDA-MB-231 cells and might mediate the interaction of breast cancer cells with ECs. Expression of the L1 cell adhesion molecule (L1CAM) was induced by hypoxia in EV but not in DKD cells. Depending on the cell types involved, L1CAM has been shown to mediate cell–cell adherence by homophilic interactions and by heterophilic interactions with integrins, neuropilin 1, and CD24 (Figure 2). Further analysis revealed that HIF-1 α , but not HIF-2 α , was required for hypoxia-induced L1CAM expression (Figure 1b).

Stable transfection of DKD cells with an expression vector encoding L1CAM (*i.e.*, L1CAM gain-of-function) increased the number of extravasated cancer cells in the lung after tail vein injection. Conversely, stable transfection of MDA-MB-231 cells with expression vector encoding shRNA targeting L1CAM (*i.e.*, L1CAM loss-of-function) dramatically impaired the spontaneous metastasis of cancer cells from breast to lung, whereas primary tumor growth was only modestly reduced [36]. Thus, HIF-1-dependent L1CAM expression in hypoxic breast cancer cells increases their interaction with ECs, thereby facilitating margination, which leads to extravasation, and lung metastasis.

HIF-dependent ANGPTL4 expression inhibits EC–EC interactions

To investigate whether HIF activity promoted the ability of hypoxic breast cancer cells to invade through an EC monolayer, we exposed ECs to conditioned medium generated by culturing the MDA-MB-231-EV and -DKD subclones in the presence of either 20% or 1% O₂ for 48 h. When naïve breast cancer cells were tested for their ability to invade through these conditioned EC monolayers, more breast cancer cells invaded through EC monolayers that were exposed to conditioned medium from hypoxic, as compared to non-hypoxic, EV cells. This hypoxia-induced effect was lost when DKD cells were used as the source of conditioned medium. Transendothelial electrical resistance is a biophysical measure of EC–EC interactions and conditioned medium from hypoxic EV cells decreased transendothelial resistance, an effect that was lost in DKD cells. These data suggested that a secreted factor, which was produced by hypoxic breast cancer cells in a HIF-dependent manner, inhibited EC–EC interactions [36].

Screening of the microarray of metastasis-related genes for a secreted factor that was induced by hypoxia in MDA-MB-231 cells identified angiopoietin-like 4 (ANGPTL4), which is expressed at increased levels in the primary breast cancers of women with lung metastases [38]. ANGPTL4 mRNA and protein expression were hypoxia-inducible in EV

but not in DKD cells and a hypoxia-response element containing a HIF binding site was identified 1.5 kb 5' to the *ANGPTL4* gene transcription start site. Both HIF-1 and HIF-2 contributed to *ANGPTL4* expression (Figure 1b). Conditioned medium from MDA-MB-231-DKD cells stably transfected with an *ANGPTL4* expression vector (*i.e.*, *ANGPTL4* gain-of-function) inhibited EC-EC interactions as measured by transendothelial electrical resistance and breast cancer invasion assays. When injected via tail vein, DKD cells with forced expression of *ANGPTL4* manifested increased extravasation in the lungs. When MDA-MB-231 cells stably transfected with vector encoding shRNA against *ANGPTL4* (*i.e.*, *ANGPTL4* loss-of-function) were exposed to hypoxia, the conditioned medium no longer inhibited EC-EC interactions. Following mammary fat pad implantation of *ANGPTL4* knockdown cells, primary tumor growth was unaffected but metastasis to the lungs was almost completely eliminated [36]. Thus, HIF-dependent *ANGPTL4* expression in hypoxic breast cancer cells inhibits EC-EC interactions (Figure 3b), thereby facilitating extravasation and lung metastasis.

Breast cancer metastatic niche formation is a HIF-1-regulated process

A major advance in our understanding of the metastatic process occurred with the discovery that the metastasis of cancer cells from the primary tumor to the lungs is preceded by, and requires, the recruitment of bone marrow-derived cells (BMDCs) to sites in the lungs that are subsequently colonized by metastatic cancer cells [39]. A second major advance was the discovery that hypoxic breast cancer cells produce lysyl oxidase (LOX), an enzyme that crosslinks extracellular matrix proteins such as collagen (Figure 4a) and promotes breast cancer metastasis [40]. Whereas the role of LOX in metastasis was initially attributed to its remodeling of ECM in the primary tumor (Figure 4b), subsequent studies revealed that LOX can remodel ECM at sites distant from the primary tumor (Figure 4c). Specifically, breast cancer cells in the primary tumor secrete LOX into the circulation, leading to collagen crosslinking in the lungs, which is required for the recruitment of BMDCs (Figure 3a) and, subsequently, cancer cells to the metastatic niche [41].

The demonstration that LOX played a critical role in lung metastasis suggested that a monoclonal antibody or small molecule inhibitor directed against LOX might be useful for breast cancer therapy [40]. However, in addition to LOX, four LOX-like (LOXL) proteins are encoded in the genome and LOX, LOXL2, and LOXL4 were all expressed, in a HIF-dependent manner, when different metastatic human breast cancer cell lines were subjected to hypoxia [42]. Various combinations of LOX, LOXL2, and LOXL4 were also overexpressed (relative to surrounding normal tissue) in different primary human breast cancers [42]. In MDA-MB-231 cells, in which LOX and LOXL4 expression were induced by hypoxia, knockdown of HIF-1 α , HIF-2 α , or both blocked hypoxia-induced expression of LOX and LOXL4 and collagen remodeling *ex vivo* and blocked collagen remodeling and BMDC recruitment in the lungs of tumor bearing mice, which was similar to the effect of knocking down LOX or LOXL4 expression [42].

MDA-MB-435 is another metastatic triple-negative breast cancer cell line [43]. In MDA-MB-435 cells in which only LOXL2 expression were induced by hypoxia, knockdown of HIF-1 α , or both HIF-1 α and HIF-2 α (but not HIF-2 α alone), blocked hypoxia-induced expression of LOXL2 and collagen remodeling *ex vivo* and blocked collagen remodeling and BMDC recruitment in the lungs of tumor bearing mice, which was similar to the effect of knocking down LOXL2 (but not LOX) expression [42]. These results showed that different LOX family members were expressed in different breast cancer lines but that inhibition of HIF-1 α in each of these lines was sufficient to dramatically inhibit metastatic niche formation and subsequent breast cancer metastasis to the lungs.

Drugs that inhibit HIF-1 activity block cancer growth and lung metastasis in mice

Multiple drugs have been shown to inhibit HIF-1 activity, block tumor growth and vascularization, and improve responses to chemotherapy and radiation therapy in a variety of mouse models, through different molecular mechanisms of action (Table 2; the list is intended to be illustrative rather than comprehensive). Digoxin was shown to inhibit the growth of human prostate cancer xenografts and its effect was dependent on its inhibition of HIF-1 α expression [44]. In the orthotopic model involving implantation of human MDA-MB-231 and MDA-MB-435 cells into SCID mice, treatment of tumor-bearing mice with acriflavine (4 mg/kg) or digoxin (2 mg/kg) by daily intraperitoneal injection significantly inhibited the following: expression of ANGPTL4, L1CAM, LOX, LOXL2, and LOXL4 in the primary tumor; primary tumor growth; collagen remodeling and BMDC recruitment in the lungs (*i.e.*, metastatic niche formation); and metastasis of cancer cells from breast to lungs [36,45]. Most strikingly, treatment of mice bearing MDA-MB-435 primary breast tumors with digoxin reduced the lung metastatic burden by >96% [36]. In contrast to the ability of digoxin to block LOX/LOXL activity in both MDA-MB-231 and MDA-MB-435 cells, the small molecule LOX inhibitor β -aminopropionitrile blocked LOX/LOXL activity in MDA-MB-231 cells, which express LOX and LOXL4, but not in MDA-MB-435 cells, which express LOXL2 [45].

Clinical trials of HIF inhibitors

Digoxin has been used for decades to treat heart disease and a daily oral dosing regimen that is safe and effective (in the context of heart disease) is well established, as are methods for determining serum drug levels. A clinical trial is currently underway in which men with prostate cancer and biochemical evidence of recurrence (rising PSA levels) will be treated with a daily oral dose of 125 mcg or 250 mcg per day of digoxin to achieve target serum levels of 0.8–2.0 ng/mL (ClinicalTrials.gov Identifier: NCT01162135). It should be noted that there are currently no data demonstrating that administration of digoxin to humans at these doses inhibits HIF-1 activity within cancer cells. A pilot clinical trial of topotecan in patients with advanced cancer and HIF-1 α overexpression demonstrated on tumor biopsy was recently reported in which HIF-1 α protein levels were undetectable in the post-treatment biopsies from 4 of 7 patients studied [46]. HIF-1 inhibitors block the expression of multiple pro-angiogenic factors (including VEGF, stromal-derived factor 1, and stem cell factor) and tumor vascularization in mouse models [45,46], and serial DCE-MRI scans demonstrated reduced tumor perfusion in 7 of 10 patients treated with topotecan [46].

Concluding Remarks

Intratumoral hypoxia induces HIF-1 activity within many primary breast cancers, which may lead to the expression of multiple gene products that play critical roles in metastasis to the lungs by promoting establishment of the metastatic niche and the subsequent extravasation of circulating tumor cells into the lung parenchyma (Figure 5; see also Box 1). Clinical trials are needed using available drugs (Table 2) to immediately test the hypothesis that the addition of one or more HIF inhibitors to current therapeutic regimens will increase the survival of breast cancer patients, particularly those with triple negative disease, and high HIF-1 α levels in their tumor biopsies, by impairing metastasis as well as other critical steps in cancer progression that are mediated by HIF-1 and HIF-2 (Table 1). The National Cancer Institute should create a funding mechanism specifically designed to support clinical trials involving off-patent drugs, which are unlikely to be performed by pharmaceutical companies but have the potential to establish safe, effective, and inexpensive new therapies for cancer patients.

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Box 1. Beyond Breast Cancer - Other known activities of L1CAM and LOX family proteins

Along with the functions described here, both L1CAM and the LOX family of proteins have been shown to have additional functions in other cell types. In addition to mediating cell-cell interactions, L1CAM-mediated signaling via interactions with surface proteins promotes proliferation and migration, and is associated with an aggressive, metastatic phenotype in many cancers (Figure I). Although it does not have intrinsic kinase activity, L1CAM acts by binding several proteins to favor proliferation and cell migration, including integrins, growth factor receptors, and other protein kinases (represented here by a pair of receptor tyrosine kinases, RTKs). Cell behavior is modified to promote metastasis through, among others, the MAP kinases ERK1 and ERK2, FAK, and NFkappaB. Finally, proteolytic processing of L1CAM at the cell surface produces two soluble domains, the L1 intracellular domain (L1-ICD) and the L1CAM ectodomain, which act independently to modulate cell behavior. After cleavage, the L1-ICD can translocate to the nucleus and modulate gene expression, whereas the soluble ectodomain can interact with surface receptors, similar to the membrane-spanning form of the protein, to stimulate signal transduction. Similar to L1CAM, the LOX family of proteins have been shown to possess functions beyond those observed in the context of breast cancer (Figure II). In addition to remodelling the ECM, the LOX proteins can have both paracrine and autocrine functions that derive from being imported into cells. Imported, processed LOX enzymes can then modify cytoplasmic proteins or localize to the nucleus and participate in transcriptional regulation.

Figure 1.

Mechanisms and consequences of HIF activation in hypoxic breast cancer cells. (a) In well oxygenated cells, HIF-1α (and HIF-2α) are subjected to two O₂-dependent hydroxylation reactions. (i) FIH-1 hydroxylates asparagine-803, which prevents HIF-1α from interacting with the coactivator p300 and renders it transcriptionally inactive. (ii) PHD2 hydroxylates proline-402 and/or proline-564, which allows HIF-1α to interact with VHL and renders it subject to ubiquitination and proteasomal degradation. (b) In hypoxic cells, the hydroxylation reactions are inhibited, HIF-1α and HIF-2α accumulate, dimerize with HIF-1β, recruit p300, bind to target genes, and activate their transcription, leading to the expression of L1 cell adhesion molecule (L1CAM), angiopoietin-like 4 (ANGPTL4), lysyl oxidase (LOX), and

LOX-like proteins 2 and 4 (LOXL2, LOXL4), which promote breast cancer metastasis by effects on endothelial cells (ECs) and the extracellular matrix (ECM).

Figure 2. Mechanisms by which L1CAM mediates cell–cell interactions. L1CAM-expressing cells have been reported to interact with other cells via homophilic interactions (a) or by heterophilic interactions with neuropilin 1 (NRP1), integrins (b), or CD24 (c).

Figure 3.

Pro-metastatic effects of proteins secreted by hypoxic breast cancer cells. Breast cancer cells in the primary tumor are subjected to hypoxia, which induces the expression of LOX and ANGPTL4. (a) In the primary tumor, LOX is secreted into the circulation (i) and crosslinks collagen in the lungs, leading to the recruitment of bone marrow-derived cells that together define the pre-metastatic niche (ii). (b) A circulating tumor cell from the hypoxic primary tumor that has adhered to endothelial cells (ECs) in a pulmonary blood vessel secretes ANGPTL4, which inhibits EC-EC interaction, thereby facilitating the extravasation of the breast cancer cells into the lung parenchyma.

Figure 4.

Local and distant effects of LOX. (a) LOX (and LOXL proteins) crosslink collagen proteins, thereby altering the physical properties of the ECM. (a) Crosslinking of ECM proteins by LOX in the primary tumor promotes local cancer cell invasion. (b) Secretion of LOX into the circulation results in ECM remodeling at distant sites such as the lungs, which promotes metastasis.

Figure 5.

Molecular and cellular mechanisms by which HIF-1 promotes metastasis of hypoxic breast cancer to the lungs. Hypoxia in the primary tumor induces the activity of HIF-1 (and HIF-2) in breast cancer cells, leading to increased transcription of genes encoding proteins that subsequently promote metastasis. The expression of L1CAM on the cell surface of circulating breast cancer cells increases their adherence to the endothelial cells (ECs) of pulmonary blood vessels (margination), whereas the secretion of ANGPTL4 decreases EC–EC interactions, which promotes the extravasation of breast cancer cells into the lung parenchyma. Prior to the arrival of breast cancer cells, a pre-metastatic niche forms in the lung through the activity of LOX/LOXL proteins, which are secreted by hypoxic breast

cancer cells in the primary tumor and crosslink collagen fibers in the lung, thereby promoting the retention of bone marrow-derived cells, which in turn promote the recruitment of metastatic breast cancer cells. Digoxin inhibits the synthesis of HIF-1 α (and to a lesser extent, HIF-2 α) and thereby blocks all of the downstream steps described above.

Figure I.
Functions of L1CAM observed in other cell types

Figure II.
Functions of LOX observed in other cell types

Table 1

Examples of HIF target genes that play key roles in cancer biology

Gene	Encoded protein	Role in cancer biology
<i>ABCB1</i>	Multidrug resistance protein 1	Chemotherapy resistance
<i>ADM</i>	Adrenomedullin	Autocrine growth/survival signaling
<i>ALDOA1</i>	Aldolase 1	Metabolic reprogramming
<i>ANGPT2</i>	Angiopoietin 2	Angiogenesis
<i>BNIP3</i>	BCL2/adenovirus E1B interacting protein 3	Metabolic reprogramming
<i>CAR9</i>	Carbonic anhydrase 9	Metabolic reprogramming
<i>CTSD</i>	Cathepsin D	Invasion and metastasis
<i>CXCL12</i>	Stromal-derived factor 1	Angiogenesis
<i>EDN1</i>	Endothelin 1	Autocrine growth/survival signaling
<i>ENO1</i>	Enolase 1	Metabolic reprogramming
<i>EPO</i>	Erythropoietin	Autocrine growth/survival signaling
<i>PGF</i>	Placental growth factor	Angiogenesis
<i>PGK1</i>	Phosphoglycerate kinase 1	Metabolic reprogramming
<i>PTGS2</i>	Cyclooxygenase 2	Angiogenesis; Invasion and metastasis
<i>GPI</i>	Glucose phosphate isomerase	Immortalization; Metabolic reprogramming
<i>HK1</i>	Hexokinase 1	Metabolic reprogramming
<i>ID2</i>	Inhibitor of differentiation 2	Epithelial-mesenchymal transition
<i>IGF2</i>	Insulin-like growth factor 2	Autocrine growth/survival signaling
<i>KITLG</i>	Kit ligand/stem cell factor	Angiogenesis
<i>L1CAM</i>	L1 cell adhesion molecule	Invasion and metastasis
<i>LDHA</i>	Lactate dehydrogenase A	Metabolic reprogramming
<i>LOX</i>	Lysyl oxidase	Invasion and metastasis
<i>MET</i>	Hepatocyte growth factor receptor	Invasion and metastasis
<i>MMP14</i>	Matrix metalloprotease 14	Invasion and metastasis
<i>NHE1</i>	Na ⁺ /H ⁺ exchanger 1	Metabolic reprogramming
<i>PDGFB</i>	Platelet-derived growth factor B	Angiogenesis
<i>PDK1</i>	Pyruvate dehydrogenase kinase 1	Metabolic reprogramming
<i>PGM</i>	Phosphoglucomutase	Immortalization; Metabolic reprogramming
<i>PKM2</i>	Pyruvate kinase M2	Metabolic reprogramming
<i>PLAUR</i>	Urokinase plasminogen activator receptor	Invasion and metastasis
<i>POU5F1</i>	Octamer binding protein 4	Stem cell maintenance
<i>SLC2A1</i>	Glucose transporter 1	Metabolic reprogramming
<i>SLC16A3</i>	Monocarboxylate transporter 4	Metabolic reprogramming
<i>SNAIL</i>	Snail	Epithelial-mesenchymal transition
<i>SNAIL2</i>	Slug	Epithelial-mesenchymal transition
<i>TERT</i>	Telomerase	Immortalization
<i>TGFA</i>	Transforming growth factor α	Autocrine growth/survival signaling

Gene	Encoded protein	Role in cancer biology
<i>TCF3</i>	Transcription factor 3	Epithelial-mesenchymal transition
<i>TWIST1</i>	TWIST1	Epithelial-mesenchymal transition

Table 2

Drugs that inhibit HIF activity and tumor growth in mouse models

Drug	Drug class	Mechanism of action	Refs
Acriflavine	Antimicrobial	Inhibits dimerization of HIF-1 α or HIF-2 α with HIF-1 β	[47]
Ascorbic acid	Antioxidant	Induces HIF-1 α and HIF-2 α degradation	[48]
AFP-464	Aminoflavone	Inhibits HIF-1 α mRNA stability and translation	[49]
Bortezomib	Proteasome inhibitor	Inhibits HIF-1 transactivation	[50]
Digoxin	Cardiac glycoside	Inhibits translation of HIF-1 α and HIF-2 α mRNA	[44]
Doxorubicin	Anthracycline	Inhibits binding of HIF-1 and HIF-2 to DNA	[50]
Echinomycin	DNA intercalator	Inhibits binding of HIF-1 to DNA	[52]
LAQ824	HDAC inhibitor	Induces HIF-1 α degradation	[53]
MSC	Selenium compound	Induces HIF-1 α degradation	[54]
Rapamycin	mTOR inhibitor	Inhibits translation of HIF-1 α mRNA	[55]
Topotecan	Topoisomerase inhibitor	Inhibits translation of HIF-1 α	[46]
2-ME2	Microtubule inhibitor	Inhibits translation of HIF-1 α mRNA	[56]
17-AAG	HSP90 inhibitor	Induces HIF-1 α degradation	[57]