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REVIEW

Cancer–stromal cell interactions mediated by hypoxia-inducible factors promote angiogenesis, lymphangiogenesis, and metastasis

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Interactions between cancer cells and stromal cells, including blood vessel endothelial cells (BECs), lymphatic vessel endothelial cells (LECs), bone marrow-derived angiogenic cells (BMDACs) and other bone marrow-derived cells (BMDCs) play important roles in cancer progression. Intratumoral hypoxia, which affects both cancer and stromal cells, is associated with a significantly increased risk of metastasis and mortality in many human cancers. Recent studies have begun to delineate the molecular mechanisms underlying the effect of intratumoral hypoxia on cancer progression. Reduced O_2 availability induces the activity of hypoxia-inducible factors (HIFs), which activate the transcription of target genes encoding proteins that play important roles in many critical aspects of cancer biology. Included among these are secreted factors, including angiopoietin 2, angiopoietin-like 4, placental growth factor, platelet-derived growth factor B, stem cell factor (kit ligand), stromal-derived factor 1, and vascular endothelial growth factor. These factors are produced by hypoxic cancer cells and directly mediate functional interactions with BECs, LECs, BMDACs and other BMDCs that promote angiogenesis, lymphangiogenesis, and metastasis. In addition, lysyl oxidase (LOX) and LOX-like proteins, which are secreted by hypoxic breast cancer cells, remodel extracellular matrix in the lungs, which leads to BMDC recruitment and metastatic niche formation.

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INTRODUCTION

Although considerable effort has been devoted to the identification of genetic alterations in cancer cells, it is clear that knowledge of changes at the DNA level is necessary but not sufficient to understand cancer biology or to develop effective cancer therapies. Complementing the analysis of DNA alterations are studies of changes at the RNA level, which reflect both alterations in the cancer cell genome and responses to the tumor microenvironment. The microenvironment within the primary tumor differs from that of the normal tissue from which the tumor arose in many critical aspects,[1] only two of which will be highlighted here. First, cancer cells recruit and interact with stromal cells[2,3] and these interactions play critical roles in (a) the establishment and growth of the primary tumor; and (b) the metastasis of cancer cells via lymphatic vessels and blood vessels to local lymph nodes and distant tissues throughout the body. Included among the tumor stromal cells are blood vessel endothelial cells (BECs), lymphatic vessel endothelial cells (LECs), bone marrow-derived angiogenic cells (BMDACs) and other bone marrow-derived cells (BMDCs), cancer-associated fibroblasts, and inflammatory cells. A second critical difference between the tumor microenvironment and that of the surrounding normal tissue is the presence of intratumoral hypoxia. In cancers in which it is possible to directly measure tumor oxygenation by Eppendorf microelectrode, such as cervical cancer[4] and soft tissue sarcoma,[5] the presence of severe intratumoral hypoxia (PO_2 <10 mm Hg [$\sim$1.5% O_2]) is associated with an increased risk of metastasis and decreased disease-free and overall survival. This review will summarize recent studies that have delineated molecular mechanisms by which hypoxic cancer cells functionally interact with BECs, LECs, BMDACs and other BMDCs, and thereby promote angiogenesis, lymphangiogenesis, and metastasis.

Reduced O_2 availability is a stimulus for the activation of hypoxia-inducible factor 1 (HIF-1), which is a heterodimeric protein that is composed of an O_2-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit.[6,7] HIF-1 activates the transcription of hundreds of target genes in hypoxic stromal cells and cancer cells.[8,9] The presence of increased HIF-1α protein levels in the primary tumor biopsy is associated with increased mortality in bladder, breast, cervical, colorectal, endometrial, esophageal, gastric, laryngeal, lung, oropharyngeal, ovarian, and pancreatic cancer.[10] In breast cancer, in which the median PO_2 is 28 mm Hg as compared to 65 mm Hg in normal breast tissue,[11] increased HIF-1α levels in the diagnostic biopsy are associated with increased metastasis and mortality even in patients with lymph node-negative breast cancer, who would otherwise have a good prognosis.[12,13] High HIF-1α levels in the primary tumor are also predictive of disease recurrence in breast and prostate cancer.[13,14]

HIF-2α (also known as EPAS1), which shares 48% amino acid identity with HIF-1α,[15] is also induced by hypoxia, dimerizes with HIF-1β, and activates transcription of target genes, some of which are shared with HIF-1α and some of which are distinct.[16] In some breast cancer cell lines, such as MDA-MB-231, both HIF-1α and HIF-2α contribute to HIF target gene transactivation, primary tumor growth and metastasis.[17] However, in MDA-MB-435 breast cancer cells, although both HIF-1α and HIF-2α are expressed, only

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HIF-1α contributes to HIF target gene transactivation, tumor growth and metastasis.[18] In contrast, HIF-2α plays a critical role in the progression of renal carcinoma and neuroblastoma,[16] while in colon cancer HIF-2α expression is lost with advanced tumor stage.[19]

Several drugs have been shown to block HIF transcriptional activity and inhibit tumor growth, angiogenesis, and/or metastasis in mouse models, including (among others): acriflavine,[20,21] cetuximab and other EGFR inhibitors;[22] digoxin and other cardiac glycosides;[17,23] doxorubicin and other anthracyclines;[24] geldanamycin and other HSP90 inhibitors;[25,26] HDAC inhibitors;[27] rapamycin and other mTOR inhibitors;[28–30] selenium compounds;[31] and topotecan and other topoisomerase I inhibitors.[32,33] Translation to the clinic has begun: effective inhibition of HIF-1α and angiogenesis in tumors of patients treated with topotecan has been demonstrated[34] and a clinical trial of digoxin in patients with prostate cancer is ongoing (NCT01162135, ClinicalTrials.gov).

Recent studies have delineated molecular mechanisms by which intratumoral hypoxia promotes angiogenesis, lymphangiogenesis, and metastasis through the HIF-dependent production by cancer cells of secreted factors that mediate functional interactions with BECs, LECs, BMDACs, and other BMDCs. These functional interactions are summarized in Figure 1 and described in detail below. HIF target genes encode proteins that play critical roles in many aspects of cancer biology that will not be addressed in this review, including immortalization; metabolic reprogramming, epithelial-mesenchymal transition, stem cell self-renewal, and tissue invasion.[35]

HIF-MEDIATED BEC ACTIVATION AND BMDAC RECRUITMENT PROMOTE ANGIOGENESIS AND PRIMARY TUMOR GROWTH

HIFs have been shown to activate the transcription of multiple angiogenic growth factors and cytokines, including vascular endothelial growth factor (VEGF; also known as VEGF-A), stromal-derived factor 1 (SDF1; also known as CXCL12), angiopoietin 2 (ANGPT2), and stem cell factor (SCF; also known as kit ligand).[36–40] VEGF and ANGPT2 play direct roles in angiogenesis by binding to their cognate receptors (VEGFR2 and TIE2, respectively) on BECs, which activates pro-angiogenic signaling programs.[41] In addition to the local effects of these factors, angiogenic cytokines such as VEGF, SDF1, and SCF produced by hypoxic cells bind to their cognate receptors (VEGFR2, CXCR4, and CKIT [also known as CD117], respectively) located on the surface of bone marrow cells, which are released into the circulation; these mobilized BMDACs home to the tumor and stimulate vascularization.[20,24,42] When immunodeficient mice bearing human prostate cancer xenografts were treated with doxorubicin or acriflavine, the consequences of reduced HIF activity included: decreased VEGF, SDF1, and SCF mRNA levels within the tumor; impaired mobilization of $CXCR4^+Sca1^+$, $VEGFR2^+CD34^+$, and $VEGFR2^+CD117^+$ BMDACs into the circulation; and inhibition of tumor vascularization, thereby impairing tumor growth.[20,24]

Whereas treatment with VEGF inhibitors selects for cancer cells that induce angiogenesis by VEGF-independent mechanisms,[43] treatment with HIF inhibitors blocks all of the major pro-angiogenic signaling pathways. Furthermore, VEGF receptor tyrosine kinase inhibitors induce tumor hypoxia, increase the number of cancer stem cells,[44] and promote metastasis,[45] which may involve activation of many of the HIF-dependent pathways that are described below.

HIF-MEDIATED LEC PROLIFERATION AND MIGRATION PROMOTE LYMPHANGIOGENESIS AND LYMPHATIC METASTASIS

Most cancer-related deaths are due to metastasis,[46] the systemic dissemination of cancer cells from the site of the primary tumor to one or more distant sites. Cancer cells disseminate systemically by invading blood vessels or lymphatic vessels. In the latter case,

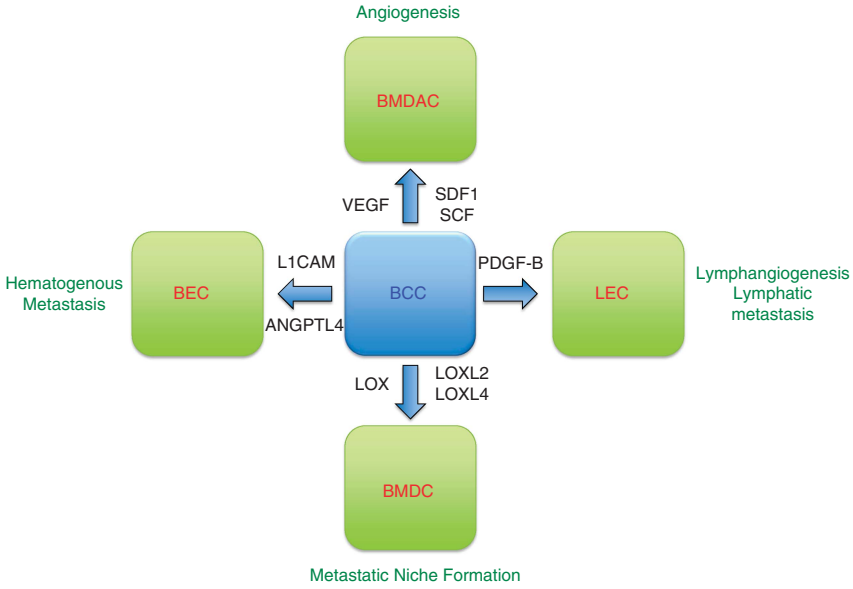

Figure 1. Functional interactions between breast cancer cells and stromal cells that are mediated by the HIF-dependent expression of secreted factors. In response to hypoxia, HIFs are induced and activate transcription of genes encoding angiopoietin-like 4 (ANGPTL4), L1 cell adhesion molecule (L1CAM), lysyl oxidase (LOX), LOX-like 2 (LOXL2), LOXL4, platelet-derived growth factor B (PDGF-B), stem cell factor (SCF), stromal-derived factor 1 (SDF1), and vascular endothelial growth factor (VEGF). These factors promote functional interactions with blood endothelial cells (BECs), lymphatic endothelial cells (LECs) bone marrow-derived angiogenic cells (BMDACs), and other bone marrow-derived cells (BMDCs). These interactions promote angiogenesis, lymphangiogenesis, lymphatic metastasis, metastatic niche formation, and blood vessel (hematogenous) metastasis.

cancer cells access blood vessels either within lymph nodes or through the lymphatic ducts, which enter the blood circulation at the junction of the jugular and subclavian veins. Just as cancer cells produce angiogenic factors that bind to BECs and stimulate angiogenesis, they also produce lymphangiogenic factors that bind to LECs and stimulate lymphangiogenesis. The greatest attention has been focused on VEGF-C and VEGF-D, which bind to VEGFR3 on the surface of LECs.[47] Other secreted factors that have been implicated in lymphangiogenesis include VEGF-A and members of the fibroblast growth factor (FGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) families.[47–49]

In the case of breast cancer, most if not all patients with distant metastases have lymph node involvement, which is the most clinically important predictor of patient mortality.[49] Although women presenting with lymph node-negative breast cancer have a good prognosis, those with high HIF-1α expression in the primary tumor biopsy have a greatly increased risk of mortality.[12] In addition, HIF-1α levels are correlated with VEGF-C expression and peritumoral lymphatic vessel density in breast cancer.[50] Taken together, these clinical studies suggest that HIF-1 may promote lymphatic metastasis of breast cancer cells.

An important factor that guides treatment decisions about adjuvant chemotherapy in stage I (lymph node-negative) breast cancer is the presence or absence of high-level expression of the estrogen (ER), progesterone (PR), and $HER2^{neu}$ receptors: women with ER/PR-expressing cancers (~70% of all breast cancers) are treated with tamoxifen or aromatase inhibitors, whereas women with $HER2^{neu}$-overexpressing tumors (~20%) are treated with the monoclonal antibody trastuzumab.[51] Women with triple-negative (*i.e.* $ER^-/PR^-/HER2^-$) breast cancer are treated with cytotoxic chemotherapy, but have early recurrence and a poor prognosis,[52] indicating a need for novel therapeutic approaches.

To identify lymphangiogenic factors that are induced by hypoxia in breast cancer cells, the triple-negative human breast cancer cell line MDA-MB-231 was incubated for 24 h in 20 or 1% O_2 and analyzed for the expression of mRNA encoding VEGF, PDGF, IGF, and FGF family members.[53] Despite the reported association between HIF-1α and VEGF-C levels,[50] hypoxia did not induce VEGF-C expression in MDA-MB-231 cells. VEGF-D expression is decreased in breast cancer as compared to normal breast, VEGF-D levels are inversely correlated with lymphatic metastasis[54] and, interestingly, VEGF-D mRNA levels decreased ~16-fold in hypoxic MDA-MB-231 cells.[53] In contrast, PDGF-B mRNA levels increased more than 4-fold in response to hypoxia in MDA-MB-231 and also in MDA-MB-435, another triple-negative breast cancer cell line,[53] which was consistent with a report associating HIF-1α and PDGF-B expression in breast cancer[55] and another clinical study linking expression of PDGF-B and PDGF receptor β to lymphatic metastasis in gastric cancer.[56] HIF-1 (but not HIF-2) was shown to bind directly to a hypoxia response element located in intron 3 of the human *PDGFB* gene and activate its transcription.[53]

Overexpression of PDGF-B in a mouse fibrosarcoma cell line was previously reported to stimulate lymphangiogenesis and lymphatic metastasis through effects on LEC migration and proliferation.[57] Conditioned medium from hypoxic breast cancer cells stimulated LEC migration and proliferation, an effect that was lost when HIF or PDGF activity was inhibited by genetic or pharmacologic means and these manipulations also significantly reduced lymphangiogenesis and lymph node metastasis following mammary fat pad implantation of MDA-MB-231 or MDA-MB-435 cells in immunodeficient mice.[53] Immunohistochemical analysis of human biopsy sections revealed a highly significant co-localization of HIF-1α and PDGF-B expression in grades 2 and 3, but not in grade 1, breast cancer. Podoplanin$^+$ lymphatic vessel area in the biopsies also correlated with HIF-1α and PDGF-B expression, and scoring for HIF-1α, PDGF-B, and podoplanin was sufficient to predict tumor grade. Taken together, the molecular, cellular, animal, and clinical studies indicate that HIF-1-dependent PDGF-B expression promotes lymphangiogenesis and lymphatic metastasis of hypoxic breast cancer cells. Treatment of tumor-bearing mice with digoxin to inhibit HIF-1α expression dramatically impaired primary tumor growth and lymph node metastasis,[53] suggesting that women with HIF-$1\alpha^+$/PDGF-B^+ grade 2–3 breast cancers may benefit from addition of a HIF inhibitor to their therapeutic regimen. Clinical trials are needed to test this hypothesis.

HIF-MEDIATED FUNCTIONAL INTERACTIONS BETWEEN BREAST CANCER CELLS AND BECS PROMOTE BLOOD VESSEL METASTASIS

The process of blood vessel metastasis can be deconvoluted into several discrete steps (Figure 2): (i) intravasation, in which cancer cells invade through the endothelium of a tumor blood vessel; (ii) circulation, during which cancer cells must survive shear stress and the lack of cell-cell and cell-matrix attachments; (iii) margination, in which a circulating tumor cell arrests at a distant site by adhering to the luminal surface of a BEC; (iv) extravasation, in which the marginated cancer cell invades through the endothelial wall of a capillary at a distant site of metastasis; and (v) colonization, during which a single extravasated cancer cell proliferates to form a metastatic focus. The molecular mechanisms by which intratumoral hypoxia modulates each of these steps, specifically within the context of breast cancer dissemination to the lungs, are considered below. HIF-1 also promotes breast cancer metastasis to bone,[58,59] but the underlying mechanisms have not been investigated.

Intravasation

In many cancers, VEGF produced by hypoxic cancer cells play critical roles in promoting increased permeability of tumor blood vessels, thereby facilitating the intravasation of large numbers of cancer cells into the blood circulation.[60] Hypoxia-induced ANGPT2 expression may also promote intravasation by reducing the coverage of BECs by pericytes in tumor vessels.[40,61] Interactions between tumor-associated macrophages, cancer cells, and BECs appear to be critical for intravasation,[62] but it is not known whether hypoxia modulates these interactions.

Figure 2. Deconvolution of steps involved in hematogenous metastasis of cancer cells. The process includes the invasion of a cancer cell into a blood vessel within the primary tumor (intravasation); survival of the cancer cell in the circulation; adherence of the cancer cell to the endothelium of a blood vessel in a distant tissue (margination); migration through the endothelium (extravasation); and cancer cell survival and proliferation at the distant site (colonization), which requires prior metastatic niche formation.

Circulation (and survival)

Epithelial cells require survival signals generated by interaction of integrins with the extracellular matrix (ECM) and, in the absence of such signals, cells undergo a form of apoptosis known as anoikis.[63] Circulating cancer cells acquire resistance to anoikis by a variety of molecular mechanisms.[64] Inhibition of ANGPTL4 expression in a skin cancer cell line resulted in increased susceptibility to anoikis and ANGPTL4 was shown to bind and activate integrins, which function as ECM sensors.[65] As described below, ANGPTL4 expression is induced by hypoxia in human breast cancer cells and is required for hematogenous metastasis to the lungs following implantation into the mammary fat pad of severe combined immunodeficiency (SCID) mice.[17] However, additional studies are required to determine whether ANGPTL4 protects hypoxic breast cancer cells from anoikis. CD24 is a HIF-1 target gene that was recently shown to promote the survival and metastasis of bladder and prostate cancer cells to the lungs after orthotopic, subcutaneous, or intravenous injection into immunodeficient mice,[66] but it is not known whether CD24 specifically increases the survival of circulating tumor cells, nor whether it mediates cancer survival and metastasis in breast cancer. Expression of the hyaluronic acid receptor CD44, which is required for the survival of breast cancer cells that have invaded into the lung parenchyma,[67] is also induced by hypoxia in a HIF-1-dependent manner.[68]

One potential source of confusion in understanding the impact of intratumoral hypoxia on vascular metastasis is that once a cancer cell enters the circulation, it is no longer in a hypoxic environment. However, although HIF activity is lost within minutes after reoxygenation,[69] the proteins encoded by HIF target genes are much more stable. As a result, the hypoxic phenotype will persist for hours to days after the cancer cell has left the hypoxic microenvironment of the primary tumor and therefore a window exists during which the circulating cancer cell has an increased propensity for metastasis based on its prior exposure. HIF-1α expression can also be driven via O_2-independent pathways that are activated by tumor suppressor loss-of-function or oncoprotein gain-of-function,[10] which may explain why circulating tumor cells from over half of all breast cancer patients analyzed had detectable HIF-1α protein expression.[70]

Margination

In order to invade distant tissues, circulating cancer cells must first adhere to the luminal surface of a capillary by interacting with a BEC. This is analogous to margination, the process by which inflammatory cells arrest in the circulation. Exposure of human MDA-MB-231 triple-negative breast cancer cells to hypoxic culture conditions increased their ability to adhere to BECs, due to the HIF-1-dependent expression of the L1 cell adhesion molecule (L1CAM).[17] When L1CAM expression was knocked down in these cells, adherence to BECs was inhibited *ex vivo* and *in vivo*, and spontaneous metastasis to the lungs following mammary fat pad implantation was dramatically reduced.

Extravasation

Cancer cells must migrate between BECs in order to exit from the capillary in which they have marginated. Exposure of breast cancer cells to hypoxic culture conditions leads to the HIF-mediated expression of ANGPTL4, which inhibits interactions between BECs as determined by measurement of transendothelial electrical resistance.[17] Knockdown of ANGPTL4 expression inhibits transendothelial migration of cancer cells *ex vivo* and extravasation *in vivo*, and completely blocks spontaneous metastasis to the lungs following mammary fat pad implantation, while having no effect on primary tumor growth or intravasation.[17,71] A HIF-1 binding site was identified in the 5′-flanking region of the *ANGPTL4* gene and a 56-bp sequence spanning the site functioned as a hypoxia response element in transcriptional reporter assays.[17] *ANGPTL4* gene expression is significantly increased in the primary breast cancers of women with lung metastases as compared to those who are metastasis-free.[71,72] Hypoxia-induced ANGPTL4 also contributes to the *ex vivo* transendothelial migration and pulmonary metastasis of hepatocellular carcinoma cells.[73] It should be noted that the ANGPTL4 polypeptide has a remarkable range of metabolic, vascular, and cytoprotective (as noted above) functions depending upon whether it undergoes oligomerization or cleavage and depending upon the particular proteins with which it interacts.[74] Because these processes are likely to be regulated in a cell-specific manner, the functional consequences of ANGPTL4 expression must be determined in a case-by-case manner.

HIF activity in BECs also plays an important role in extravasation. The formation of tumor foci in the lungs following tail vein injection of cancer cells is decreased in mice lacking HIF-1α expression in BECs, whereas loss of HIF-2α expression has the opposite consequence.[75] The molecular basis for these effects appears to involve differential regulation of nitric oxide production by BECs: HIF-1 activates transcription of the *NOS2* gene encoding inducible nitric oxide synthase, whereas HIF-2 activates transcription of the *ARG1* gene encoding arginase, an enzyme that metabolizes arginine, which is one of the substrates required for production of nitric oxide by NOS2. Production of NO by BECs promotes transendothelial migration of cancer cells. Differential HIF-1 → NOS2 and HIF-2 → ARG1 signaling also occurs in macrophages,[76] where it may promote intravasation (see above). These results suggest that even in cancers where HIF-2α overexpression in cancer cells is driving progression, proposed treatment with a HIF-2α-selective inhibitor[77] may not be desirable, since it might stimulate pro-metastatic properties of BECs.

Colonization (and the role of metastatic niche formation)

Primary breast tumors secrete factors that remodel tissue at the site of subsequent metastases, a process known as metastatic niche formation because the remodeling is required to create a microenvironment that allows the survival and proliferation of metastatic cancer cells. These changes include alterations of the ECM (fibronectin deposition) and the recruitment of bone marrow-derived cells (BMDCs).[78] Studies in xenograft models of melanoma and lung cancer indicated that production of VEGF-A, transforming growth factor-α, and tumor necrosis factor-α by the primary tumor induced the expression of the inflammatory proteins S100A8 and S100A9 in the lung parenchyma, which served as a signal for recruitment of $Mac1^+$ ($CD11b^+$) BMDCs.[79] The effect of S100A8 and S100A9 was later shown to be indirect: they induced the pulmonary expression of serum amyloid A3, which bound directly to toll-like receptor 4 on BMDCs.[80] Metastatic cancer cells homed specifically to areas of the lung that were populated by BMDCs.[78]

In addition to providing homing signals for the cancer cells, BMDCs in the metastatic niche may also serve as a source of growth/survival factors, angiogenic factors, and immunosuppressive factors, which are all required for clonal expansion of the cancer cell into a secondary tumor. It is likely that a functionally heterogeneous population of BMDCs is recruited to the metastatic niche, either in parallel or in series.

Subsequent studies demonstrated that the crosslinking of collagen fibers in the lungs by lysyl oxidase (LOX), which was secreted by primary breast tumors, was required for the recruitment of BMDCs.[81] Expression of LOX was induced by hypoxia in breast cancer cells in a HIF-1-dependent manner,[82] providing another mechanism by which intratumoral hypoxia promotes metastasis. LOX was found to colocalize with fibronectin in the lungs of tumor-bearing mice, prior to the arrival of $CD11b^+$

BMDCs.[81] Metastatic niche formation in the lungs of SCID mice showed a strikingly ordered and rapid progression following implantation of MDA-MB-435 breast cancer cells in the mammary fat pad: collagen crosslinking was observed by day 8, BMDC recruitment by day 16, and metastatic cancer cells were detected by day 24.[18] This process is not an artifact of implanting human cancer cells into immunodeficient mice, as it was also observed after implantation of 4T1 mouse breast cancer cells into syngeneic immunocompetent hosts.[81]

In addition to LOX, a family of LOX-like (LOXL) proteins also participate in metastatic niche formation. In different metastatic breast cancer cell lines, the expression of LOX, LOXL2, or LOXL4 was induced by hypoxia: in MDA-MB-231 cells HIF-1 and HIF-2 activate transcription of the *LOX* and *LOXL4* genes, whereas in MDA-MB-435 cells HIF-1 alone activates *LOXL2* expression.[18] Analysis of human breast cancer biopsies also revealed heterogeneous expression of LOX/LOXL family members. Knockdown of LOX or LOXL4 in MDA-MB-231 cells and knockdown of LOXL2, but not LOX, in MDA-MB-435 cells blocked metastatic niche formation.[18,81] Treatment of tumor-bearing mice with digoxin or acriflavine, which are drugs that inhibit HIF activity, blocked metastatic niche formation.[21] Although studies in mouse models described above implicated HIF-dependent expression of LOX/LOXL family members in breast cancer cells, analysis of the Oncomine database revealed increased expression of LOXL2 and LOXL4 within stromal cells of invasive breast cancer as compared to normal breast tissue.[21] Finally, it should be noted that in addition to the effects of LOX family members on ECM in the lung, these same proteins also remodel the ECM within the primary tumor and thereby facilitate the local invasion of cancer cells.[21,82,83]

CLINICAL IMPLICATIONS

In the case of breast cancer, intratumoral hypoxia exerts a profound effect on cancer cells through the HIF-mediated production of secreted factors that mediate functional interactions with BMDACs, BECs, LECs, and BMDCs, which drive key aspects of cancer progression including angiogenesis, lymphangiogenesis, lymphatic metastasis, metastatic niche formation, and blood vessel metastasis (Figure 1). These recent findings provide direct molecular mechanisms underlying the clinical observation that intratumoral hypoxia promotes metastasis and patient mortality. The demonstration that HIFs play a master regulatory role in activating the transcription of the genes encoding all of the secreted factors involved in these processes provides a scientific foundation for the inclusion of HIF inhibitors in cancer therapy regimens, particularly in women with breast cancer who do not (yet) have lymph node involvement but have high HIF-1α levels in their primary tumor biopsy, which places them at greatly increased risk of cancer death.[12] In addition, it should be noted that many of the studies described above were performed with the triple-negative breast cancer cell lines MDA-MB-231 and MDA-MB-435. Triple-negative breast cancer is currently treated with cytotoxic chemotherapy with rapid development of drug resistance and poor long-term survival.[52] Combined treatment with digoxin and anthracycline chemotherapy increases primary tumor control in mouse xenograft models,[17] suggesting that patients with triple-negative breast cancer may also benefit from the addition of digoxin or another HIF inhibitor to their therapeutic regimen.

PERSPECTIVE AND COMMENTARY

There are many other stromal cell types that interact with cancer cells to promote metastasis, including fibroblasts, immune cells (lymphocytes, macrophages, mast cells, neutrophils), and mesenchymal stem cells.[3] Additional studies are required to investigate whether hypoxia induces HIF-mediated functional interactions between these stromal cells and cancer cells that also promote metastasis. It is striking that $>90\%$ of cancer deaths are attributable to metastasis, yet $<3\%$ of the National Cancer Institute (NCI) budget is devoted to investigating the cellular and molecular mechanisms of cancer metastasis.[84] The profusion of clinical and experimental data linking intratumoral hypoxia to metastasis and patient mortality also stands in striking contrast to the paucity of NCI-funded initiatives that specifically target hypoxic cancer cells for therapy.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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