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Review

The hypoxic tumor microenvironment: A driving force for breast cancer progression

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ABSTRACT

Intratumoral hypoxia is a common finding in breast cancer and is associated with a significantly increased risk of metastasis and patient mortality. Hypoxia-inducible factors activate the transcription of a large battery of genes encoding proteins that promote primary tumor vascularization and growth, stromal cell recruitment, extracellular matrix remodeling, premetastatic niche formation, cell motility, local tissue invasion, extravasation at sites of metastasis, and maintenance of the cancer stem cell phenotype that is required to generate secondary tumors. Recent preclinical studies suggest that the combination of cytotoxic chemotherapy with drugs that inhibit hypoxia-inducible factors may improve outcome for women with triple-negative breast cancer.

1. Introduction

1.1. The tumor microenvironment has a major impact on cancer progression

Three general processes have major effects on cancer initiation and progression. First, discrete somatic mutations lead to tumor suppressor loss-of-function and oncoprotein gain-of-function [1]; second, epigenetic alterations change patterns of gene expression [2]; and third, alterations in the tumor microenvironment also lead to broad changes in gene expression [3]. Considerable attention has been paid to somatic mutations and epigenetic alterations, which are easily interrogated by high-throughput molecular techniques [4]. In contrast, changes in the tumor microenvironment are less easily assayed but have profound effects on cancer progression.

The tumor microenvironment can be subdivided into: the chemical microenvironment, which encompasses pH, PO_2 and the concentration of other small molecules (e.g. NO) and metabolites (e.g. glucose, glutamine, lactate); and the cellular microenvironment, which includes tumor cells, stromal cells, and the extracellular matrix (ECM) produced by these cells. Among the stromal cell types are vascular endothelial cells (ECs) and pericytes, lymphatic ECs, fibroblasts, myofibroblasts, and various bone marrow-derived cells such as macrophages, neutrophils, mast cells, myeloid-derived suppressor cells (MDSCs), and mesenchymal stem cells (MSCs). Many of the stromal cells recruited to the primary tumor promote primary tumor growth or metastasis [5].

Metastases are found in only 6% of women with breast cancer at the time of initial presentation, yet 30% of women with early stage disease at diagnosis will eventually progress to metastatic disease, which is responsible for 40,000 deaths annually [6]. This review will focus on recent advances in understanding the pathogenesis of breast cancer that demonstrate how one critical aspect of the chemical microenvironment, reduced O_2 availability (hypoxia), exerts major effects on the cellular microenvironment with profound consequences for cancer progression, i.e. acquisition of the invasive and metastatic properties that lead to patient mortality. Indeed, intratumoral hypoxia is a pathological stimulus that drives changes in gene expression, leading to alterations in cell

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signaling that in many cancers are comparable to those caused by somatic mutations or epigenetic changes.

1.2. Intratumoral hypoxia is commonly observed in breast and other cancers

Groundbreaking clinical studies, in which PO_2 measurements of breast cancers were obtained in situ by the use of Eppendorf microelectrodes, revealed a median PO_2 of 65 mm Hg (with all measurements > 10 mm Hg) in normal breast tissue; in contrast, a meta-analysis of 10 studies involving over 200 patients revealed that the median PO_2 in breast cancers prior to therapy was 10 mm Hg [7]. Remarkably similar results were obtained in meta-analyses of studies involving over 700 patients with cervical cancer and over 500 patients with head and neck cancers; in these cancers, multiple studies demonstrated an association between intratumoral PO_2 < 10 mm Hg and decreased disease-free survival [7]. The severe intratumoral hypoxia detected in these studies is a result of diffusion-limited O_2 delivery, in which rapid cancer cell proliferation leads to cells that are too far away from a blood vessel, and perfusion-limited O_2 delivery, in which structurally and functionally abnormal tumor vessels do not maintain constant blood flow, so that cancer cells even immediately adjacent to a blood vessel may be hypoxic [8]. Regions of necrosis, which are commonly observed in advanced solid tumors, reflect the consequences of prolonged periods at O_2 levels that are insufficient to maintain cell viability [9].

1.3. Expression and activity of hypoxia-inducible factors are increased in breast cancer

Cells respond to reduced O_2 availability through changes in gene expression that are mediated by hypoxia-inducible factors, which are composed of an O_2-regulated HIF-α subunit (HIF-1α, HIF-2α, or HIF-3α) and a constitutively expressed HIF-1β subunit [10,11]. The HIF-α subunits are subjected to O_2-dependent prolyl hydroxylation, ubiquitination, and proteasomal degradation, which are inhibited under hypoxic conditions [12], leading to stabilization and rapid accumulation of the proteins and transcriptional activity. Over 1500 HIF target genes have been identified thus far, although in any given cell, transcription of several hundred of these will increase significantly in response to hypoxia [13].

Immunohistochemical studies of tumor biopsies have linked increased HIF-1α protein levels with increased risk of metastasis [14] and mortality in lymph node-positive [15], lymph node-negative [16], HER2-positive [17], estrogen receptor-positive [18], and unselected [14,19] breast cancer patients. Increased expression in primary breast cancers of a panel of 99 [20] or 16 [21] mRNAs encoded by HIF target genes is also associated with increased patient mortality.

2. Consequences of HIF activity in mouse models of breast cancer

2.1. HIF-1α promotes primary breast tumor growth and vascularization

Studies in many cancer cell types have established that HIF-1α promotes tumor xenograft growth and vascularization [22–24], which was also observed in an autochthonous model of breast cancer driven by expression of polyoma middle T antigen (PyMT) from the mouse mammary tumor virus (MMTV) promoter [25]. HIF-1 activates transcription of the *VEGF* gene encoding vascular endothelial growth factor [26] and HIF-1α levels in breast cancers are associated with VEGF expression and with microvessel density, even in ductal carcinoma in situ, the pre-invasive stage of breast cancer pathogenesis [27]. HIFs mediate expression by cancer cells of angiogenic factors, such as VEGF, stromal-derived factor 1 (also known as CXCL12), and stem cell factor (also known as kit ligand), which induce the mobilization into the circulation of bone marrow-derived angiogenic cells that express the cognate receptors (VEGFR2, CXCR4, and CKIT, respectively) and home to the tumor where they promote vascularization [28].

2.2. HIF-1α promotes metastasis of breast cancer to axillary lymph nodes

In breast cancer, the most important clinical finding that predicts distant metastasis is the degree of axillary lymph node involvement; conversely, most women with distant metastases have lymph node involvement and it is likely that, at least in some cases, breast cancers access the peripheral circulation via the lymphatic system [29]. In an orthotopic mouse model, in which human MDA-MB-231 [30] breast cancer cells (BCCs) were implanted into the mammary fat pad of immunodeficient mice, expression of short hairpin RNA (shRNA) to inhibit HIF-1α, HIF-2α, or both led to decreased lymphatic vessel density in the primary tumor and decreased metastasis to the ipsilateral axillary lymph node [31]. Conditioned medium, which was collected from BCCs cultured under hypoxic conditions, increased the migration and proliferation of lymphatic ECs, whereas the effects of hypoxia were lost when HIF-1α and HIF-2α shRNA was expressed [31].

HIF-dependent expression of platelet-derived growth factor B (PDGF-B) was required for, and PDGF-B knockdown eliminated, the effects of hypoxia on lymphatic EC migration and proliferation in vitro; PDGF-B knockdown also decreased lymphatic vessel density and lymph node metastasis in vivo [31]. Hypoxia induced the binding of HIF-1 to a hypoxia response element located within intron 3 of the *PDGFB* gene. Remarkably, immunohistochemistry revealed that there was no association between HIF-1α and PDGF-B levels in biopsies from grade 1 breast cancers but an association was observed in grade 2 and grade 3 cancers, suggesting that HIF-1α was necessary but not sufficient for *PDGFB* gene expression during breast cancer progression [31].

2.3. HIF-1α promotes bone colonization by breast cancer cells

Injection of MDA-MB-231 cells into the left ventricle of immunodeficient mice results in the formation of osteolytic bone metastases. Compared to an empty vector subclone, expression of a constitutively active or dominant negative form of HIF-1α increased or decreased, respectively, the tumor area and blood vessel density in long bone sections [32]. HIF-1α knockdown by shRNA also reduced the radiographic area of osteolytic lesions, decreased vessel density in bone metastases, and increased survival time after injection of BCCs [33]. The bone metastases that formed after injection of MDA-MB-231 cells contained hypoxic regions and exposure of spleen cells to hypoxia in vitro inhibited osteoblast differentiation and stimulated osteoclast differentiation [32]. Because BCCs were injected directly into the circulation, these studies do not provide compelling evidence that HIF-1 is required for spontaneous metastasis from breast to bone as opposed to a more limited requirement for bone colonization by circulating tumor cells.

2.4. HIF-1α is required for metastasis of breast cancer to the lungs

Exposing cancer cells to hypoxia ex vivo is known to increase lung colonization after intravenous injection [34]. Conditional knockout of HIF-1α in mammary epithelial cells increased survival of *MMTV-PyMT* mice and decreased the number of spontaneous lung metastases by ~50% [25]. Knockdown of HIF-1α, HIF-2α, or both in human MDA-MB-231 BCCs significantly decreased the number of spontaneous lung metastases and total lung metastatic burden in immunodeficient mice after mammary fat pad injection [35]. In this orthotopic transplantation model, a large battery of HIF target genes that mediate specific steps in the metastatic process have been delineated, as described in the following section, providing a detailed molecular basis for the effect of intratumoral hypoxia on breast cancer metastasis.

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