Review

Molecular mechanisms mediating metastasis of hypoxic breast cancer cells

Gregg L. Semenza

Vascular Program, Institute for Cell Engineering; Departments of Pediatrics, Medicine, Oncology, Radiation Oncology, and Biological Chemistry; and McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Breast cancers contain regions of intratumoral hypoxia in which reduced O_2 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.

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 O_2 leading to hypoxia (decreased O_2 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 O_2 is either diffusion-limited, due to the location of a cancer cell sufficiently distal to a blood vessel that O_2 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 O_2 (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 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 O_2 availability through the activity of the hypoxia-inducible factors HIF-1 and HIF-2, which are transcriptional activators that regulate the expression of over 1000 target genes [5]. HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β subunits [6,7]. HIF-1α is subjected to two O_2-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-1α rapidly accumulates, heterodimerizes with HIF-1β, 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 O_2 delivery to cells by stimulating angiogenesis, and intracellular proteins, such as the glycolytic enzymes, which allow cells to survive O_2 deprivation by reprogramming their metabolism [5]. HIF-2, a heterodimer consisting of HIF-1β and an O_2-regulated HIF-2α 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-1α and HIF-2α 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-1α

Corresponding author: Semenza, G.L. (gsemenza@jhmi.edu)
Keywords: acriflavine; angiopoietin-like 4; collagen crosslinking; digoxin; extravasation; HIF-1; lysyl oxidase; metastatic niche formation.

 1471-4914/$ – see front matter © 2012 Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.molmed.2012.08.001 Trends in Molecular Medicine, September 2012, Vol. 18, No. 9

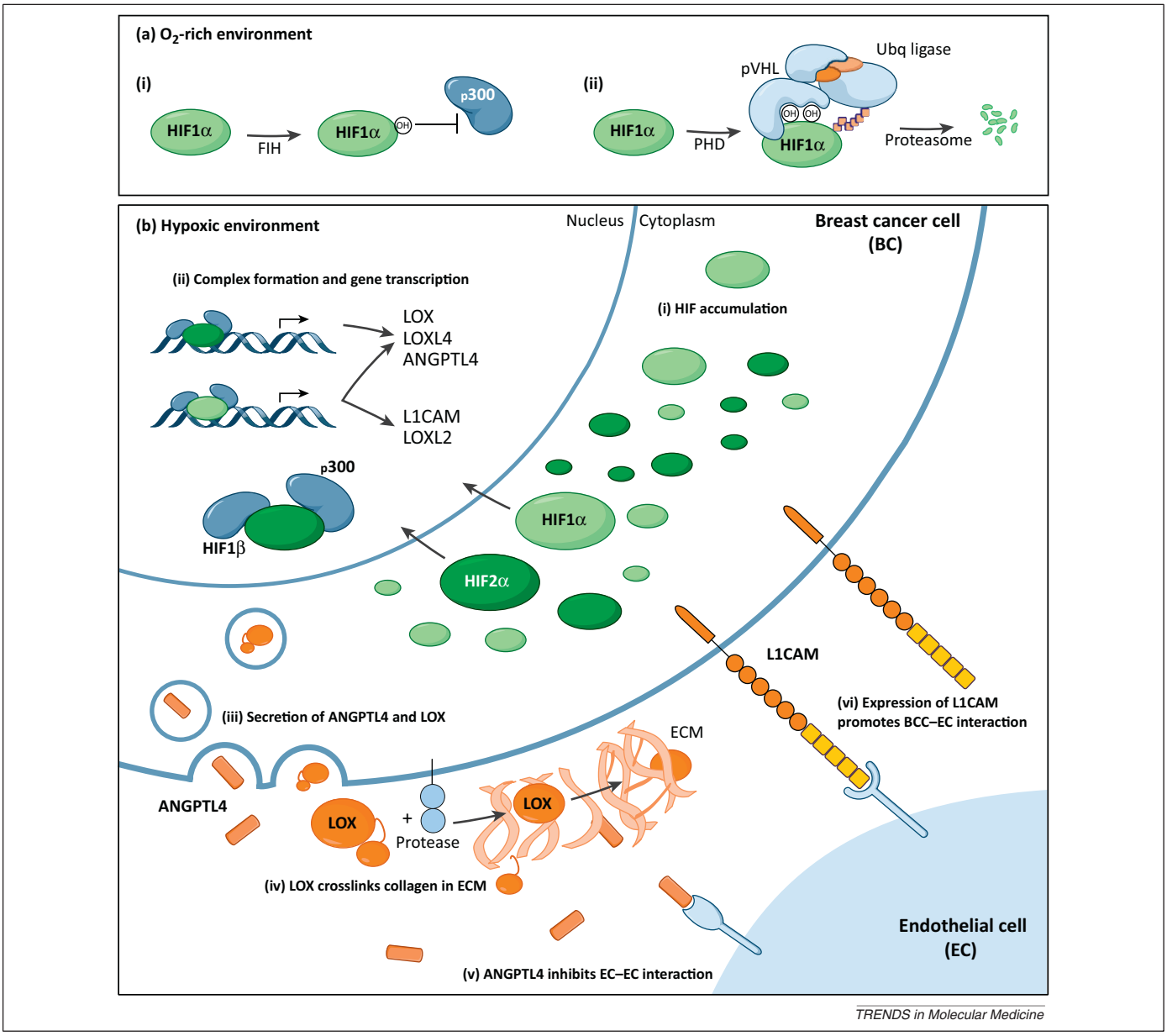

Figure 1. Mechanisms and consequences of HIF activation in hypoxic breast cancer cells. **(a)** In well oxygenated cells, HIF-1α (and HIF-2α) are subjected to two O_2-dependent hydroxylation reactions. **(i)** FIH-1 hydroxylates asparagine-803, which prevents HIF-1α from interacting with the coactivator 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).

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-1α in their diagnostic biopsies was significantly decreased. The percentage of HIF-1α overexpressing cells increased with disease progression, such that mortality was increased among node-negative patients with >5% HIF-1α overexpressing cells in biopsy sections [14] and among node-positive patients with >50% overexpressing HIF-1α cells [15]. HIF-1α overexpression also predicted adverse outcome in many subsequent studies of breast cancer patients [16–22]. HIF-1α 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-2α 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-1α and HIF-2α 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]).