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Review

Hypoxic pathobiology of breast cancer metastasis

Luana Schito*, Sergio Rey*

Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada

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ABSTRACT

Dissemination of breast cancer cells (BCCs) to distant sites (metastasis) is the ultimate cause of mortality in patients with breast cancer. Hypoxia (low O_2) is a microenvironmental hallmark of most solid cancers arising as a mismatch between cellular O_2 consumption and supply. Hypoxic selection of BCCs triggers molecular and cellular adaptations dependent upon hypoxia-inducible factors (HIFs), a family of evolutionarily conserved transcriptional activators that coordinate the expression of numerous genes controlling each step of the metastatic process. In this review, we summarize current advances in the understanding of HIF-driven molecular mechanisms that promote BCC metastatic dissemination and patient mortality. In addition, we discuss the clinical and therapeutic implications of HIF targeting in breast cancers.

1. Introduction

Metastatic breast cancer is the second-leading cause of deaths from malignancies in women within the United States and Canada [1,2]. Nearly all breast cancer cells (BCCs) originate in the mammary ductal or lobular *epithelia*; upon diagnosis, BCCs can be confined to the primary lesion (*in situ*), occupying structures beneath the epithelial basement membrane (*invasive*), or disseminated beyond the breast *parenchyma*, thereby compromising regional lymph nodes, bone, brain, liver or lungs *via* lymphatic and blood vessels (*metastatic*). Hypoxia (low O_2) is a microenvironmental common denominator throughout breast cancer progression, acting as a predictor of overall mortality independently of tumor size, stage and nodal status [3,4]. *In vitro* and *in vivo* studies show that hypoxia promotes a motile and invasive BCC phenotype through the activation of a transcriptional program controlled by hypoxia-inducible factors (HIFs); [5] consistently, HIF overexpression is frequently found in primary and metastatic breast cancers, behaving as an independent predictor of therapeutic failure and decreased survival [6,7]. In this review, we summarize current advances in the understanding of HIF-dependent mechanisms promoting breast cancer metastasis whilst discussing the therapeutic potential of HIF targeting in breast cancer patients.

2. Pathobiological drivers of hypoxia in breast cancer

BCCs present unconstrained proliferation and resistance to cell death signaling as a result of driver mutations and epigenetic changes [8]. The initial transformed BCC clones give rise to an expanding avascular mass that exclusively depends upon O_2 diffusion, a mechanism that becomes severely limited once the distance to the nearest vessel exceeds ≈ 100–200 μm. The development of multiple hypoxic 'cores' results in a heterogenous spatiotemporal distribution of hypoxic regions within the breast tumor microenvironment, which are dynamically regulated by intrinsic and extrinsic factors such as cellular O_2 consumption, vascular density, (lymph)angiogenesis and interstitial fluid pressure [9]. Clinical measurements using O_2-sensitive electrodes reveal that locally advanced breast cancers and normal breast tissues are exposed to median O_2 levels of ≈ 1.4% and ≈ 9%, respectively; [3] in addition, ≈ 50% of systematic measurements within a single breast tumor present O_2 levels ≤ 0.7%, whereas 100% of normal breast measurements are > 2% [3]. As a reference, adult arterial O_2 levels are ≈ 100 mm Hg (≈ 14% at sea level), [10] whilst cellular studies indicate that the half-maximal activation of HIFs occurs between 1.5 and 2% O_2 with a *maximum* at ≈ 0.5% [11]. These results are coherent with a clonally-mediated fine-tuning of the pathobiological responses to hypoxia in accord with O_2 levels found in human breast cancers. Indeed, the central role of hypoxia and HIFs in breast cancer progression can be illustrated through microarray analysis of public-domain data [12]. Specifically, transcript levels of HIF-1α and its paralog HIF-2α are respectively associated with shortened or lengthened relapse-free survival (RFS), independently of lymph node status (Fig. 1A); moreover, the median mRNA expression of four common HIF-1α (and -2α) transcriptional targets is consistently associated with shortened RFS (Fig. 1B), whereas HIF-1α (but not HIF-2α) overexpression is linked to increased risk for distant metastases (Fig. 1C). These data support a central pathobiological role of HIFs in breast cancer

* Corresponding authors at: Princess Margaret Cancer Centre, University Health Network, 101 College St., Rm. 12-401, Toronto, ON M5G 1L7, Canada.
E-mail addresses: luana.schito@ls2r.science (L. Schito), sergio.rey@ls2r.science (S. Rey).

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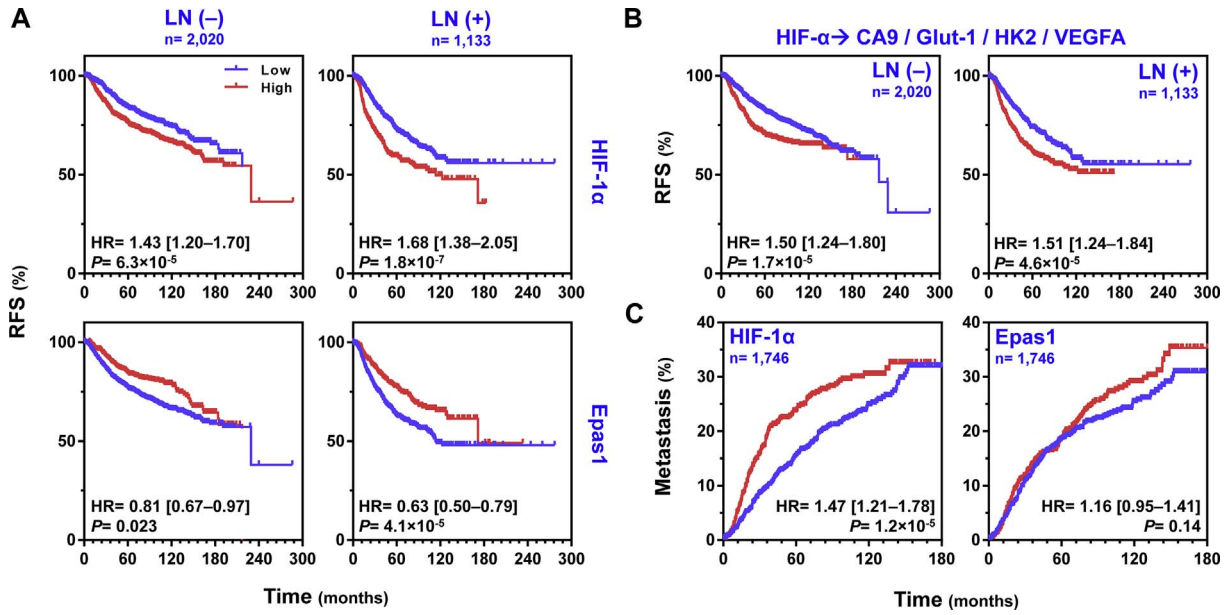

Fig. 1. Association between HIFα transcriptional activation, breast cancer recurrence and metastasis using publicly available microarray and clinical data. Expression levels were classified as low (*blue*, bottom three quartiles) or high (*red*, upper quartile). (A) Recurrence-free survival according to nodal status stratified by HIF-1α (*top*) or HIF-2α (Epas1, *bottom*). (B) Recurrence-free survival according to nodal status, stratified by the median expression of the HIFα targets CA9, Glut-1, HK2 and VEGFA. (C) Distant breast cancer metastases stratified by HIF-1α or HIF-2α (Epas1) transcript levels. *HR*, hazard ratio (95% confidence interval indicated in *brackets*); *LN*, lymph node; *RFS*, recurrence-free survival; *CA9*, carbonic anhydrase-9; *Glut-1*, glucose transporter-1 (encoded by *Slc2a1*); *HK2*, hexokinase 2; *VEGFA*, vascular endothelial growth factor-A.

dissemination, independently of O_2 level stratification. In addition, multiple studies have confirmed that intratumoral hypoxia and HIF-1α overexpression independently predict poor response to (chemo)radiotherapy in breast as well as in other solid cancers including bladder, breast, *cervix*, colon, endometrial, gastric, head and neck, nasopharyngeal, ovarian and pancreatic carcinomas, osteosarcomas, glioblastomas [13–24] and hematological malignancies (reviewed in [25]).

3. Hypoxia-inducible factors

One of the best delineated hypoxic molecular effectors in humans is the HIF family, an evolutionarily conserved group of transcription factors exerting a pivotal role upon O_2 homeostasis [5]. HIFs are heterodimers composed of an O_2-regulated subunit (HIF-1α, -2α, or -3α; henceforth collectively referred to as HIFα) and a constitutively expressed HIF-1β subunit [5,10]. In well-oxygenated cells (> 5% O_2), human HIFα undergoes enzymatic hydroxylation at proline residues (Pro^{402}/Pro^{564} in HIF-1α or Pro^{405}/Pro^{531} in HIF-2α) by prolyl-4-hydroxylases (PHDs) that utilize O_2, α-ketoglutarate, Fe^{2+} and ascorbate as co-substrates [26–28]. O_2-dependent hydroxylation allows recognition of HIFα by the *von Hippel–Lindau* (pVHL) tumor suppressor, which recruits E3-ubiquitin protein ligases targeting HIFα for proteasomal degradation [29–31]. In contrast, HIFα hydroxylation is substrate-limited under hypoxia resulting in HIFα stabilization, dimerization with HIF-1β, and transcriptional activation of target genes through binding to *cis*-acting hypoxia-responsive elements (HREs), bearing the DNA *consensus* sequence 5′–[A/G]CGTG–3′ [32]. HIFα undergoes additional O_2-dependent hydroxylation at a conserved asparagine residue (Asn^{803} in HIF-1α or Asn^{851} in HIF-2α) within the C-terminal transactivation domain (CTAD) [33,34]. This post-translational modification impedes the recruitment of the transcriptional coactivators p300/CBP thereby abrogating HIFα activity under non-hypoxic conditions. Interestingly, the CTAD domain of HIF-2α is hydroxylated less efficiently than its HIF-1α counterpart, an effect attributed to differences in the amino acids adjacent to the hydroxylated asparagine, therein providing a molecular mechanism for the higher basal HIF-2α transcriptional activity often observed under non-hypoxic conditions [35,36].

Whereas PHD/pVHL is an established O_2-dependent mechanism for HIFα regulation, a number of additional O_2-independent pathways modulating HIFα stability and transcriptional activity have been identified. These pathways include, but are not limited to cytokines, growth-factor stimulation and oncogenic mutations that interfere primarily with ubiquitylation and proteasomal degradation of HIFα (reviewed in [37]). Of relevance for this review, activation of the human epidermal growth factor receptor (HER)-2, overexpressed in ≈ 15–20% of breast cancers, [38] results in increased HIF-1α mRNA translation *via* the PI3K → AKT → mTOR pathway under non-hypoxic conditions [39]. In triple-negative breast cancer (TNBC), a particularly aggressive molecular subtype characterized by lack of HER-2, estrogen (ER) and progesterone receptors, the transcription factor BHLHE41 (also known as SHARP1) binds to HIFα thus promoting proteasomal degradation independently of O_2 levels [40]. As a result, low SHARP1 expression is associated with the clinical induction of a HIFα-dependent gene signature and increased metastatic risk, whilst repressing BCC migration and lung metastases in preclinical models of TNBC [40].

In addition to translation, HIF-1α mRNA is subjected to transcriptional regulation *via* NF-κB, a transcription factor complex that acts as a major integrator of the inflammatory responses in malignant and non-malignant diseases [41,42]. Upon activation, it has been shown that NF-κB binds to a DNA *consensus* sequence (κB element: 5′–GGG[A/G]N$[C/T]_4$CC–3′) located 197 bp upstream of the *Hif1a* transcription start site, thus enhancing HIF-1α mRNA levels under non-hypoxic conditions, [42] whereas in non-tumorigenic BCCs, the NF-κB → HIF-1α axis stimulates the acquisition of an invasive phenotype independently of O_2 levels [42–44]. Subsequent studies revealed a positive feedback loop between HIF-1α and NF-κB that provides a mechanistic basis for the crosstalk between the hypoxic and inflammatory signaling pathways in the tumor microenvironment [45–48]. Consistently, the expression of a number of NF-κB-driven proinflammatory genes is blunted by HIF-1α loss-of-function *in vitro* and in patients bearing invasive breast carcinomas, despite the absence of systemic inflammation [49,50]. Conversely, NF-κB loss-of-function impaired the hypoxic induction of proinflammatory cytokines, HIF-1α protein levels and transactivation in non-malignant mouse cells and tissues [51]. Taken together, these data suggest that the HIF-1α ↔ NF-κB crosstalk supports a local pro-inflam-