COMMENTARY

Hypoxia promotes tumor cell motility via RhoA and ROCK1 signaling pathways

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Blood vasculature provides tissues of the body with oxygen to support their growth. Tumors can outgrow their vascular supply, leading to regions of low oxygen, also known as hypoxia. Tumor cells that are within about 150–200 μm from a functional blood vessel may be hypoxic, and cells more distant are more susceptible to necrosis (1, 2). However, some of these oxygen-deprived tumor cells mobilize and retreat to less hypoxic microenvironments via a molecular axis dependent on activated hypoxia-inducible factors (HIF), including HIF1α. When deprived of oxygen, HIF1α is able to transactivate key promotility genes, such as *RHOA* and *ROCK1* (*Rho kinase 1*), as described by Gilkes et al. in PNAS (3). During such hypoxia-induced migration, it is conceivable that cancer cells can gain access to the circulation through tumor vasculature, which is often disordered and irregular (4, 5). Hence, hypoxia-induced tumor cell migration could be a major contributor to cancer cell intravasation, the first step of the metastatic cascade. As a result of poor vascularization of the tumor, hypoxia can give rise to significant changes in tumor cell gene-expression patterns, metabolism, and behavior (6), which are believed to work in concert to promote tumor cell motility. However, Gilkes et al. reveal that active HIF1α alone can promote motility by transactivating mRNA expression of *RHOA* and *ROCK1*, two major drivers of cell motility (3). The abundance of these two hypoxia-induced promigratory factors has dramatic feed-forward effects on other promotility pathways, resulting in increased focal adhesion kinase (FAK) and myosin light-chain kinase (MLCK) activity.

Targeting or minimizing tumor hypoxia would have several other benefits in addition to antagonizing the promotility effects generated by active RhoA/ROCK levels; tumor hypoxia is associated with decreased responsiveness to chemo- and radiation therapy (7, 8). Thus, tumor hypoxia is a major regulator of malignant properties of cancer, as well as being a barrier to successful therapy. Much research has been devoted to the study of tumor hypoxia (9), with recent progress revealing molecular details of how cells sense oxygen levels in their microenvironment and the subsequent events that follow such as global changes in gene-expression patterns and cellular behavior. Recent ChIP-seq data of activated HIF1α in hypoxic MCF7 breast cancer cells has provided a high-resolution genomic glimpse of what is actively transcribed under hypoxic conditions (10), although an equivalent analysis has yet to be performed on aggressive breast cancer cells capable of hypoxia-induced motility. Gilkes et al. provide compelling evidence on how breast cancer cells sense and respond to low levels of oxygen by immediately inducing *RHOA* and *ROCK* mRNA transcription, resulting in increased motility via increased FAK and MLCK activity (3). The hub of this oxygen-sensitive pathway is HIF1α, the most ubiquitous member of the oxygen-sensing protein family, which acts as a transcription factor when oxygen is no longer bound to its oxygen-binding site. The transcriptional activity of HIF1α requires hypoxic levels of oxygen but is also dependent on complex formation with other oxygen-sensitive factors, such as prolyl-4-hydroxylases PHD-1 and PHD-2 (11, 12). Indeed, attempts to partially disable the PHD-1/-2 portion of this oxygen-sensitive complex in host vasculature have been successful in inhibiting metastasis, improving intratumoral oxygen levels via increased functionality of tumor vasculature (11, 12). In contrast, studies that aim to block tumor growth via antiangiogenesis-targeted therapy have revealed

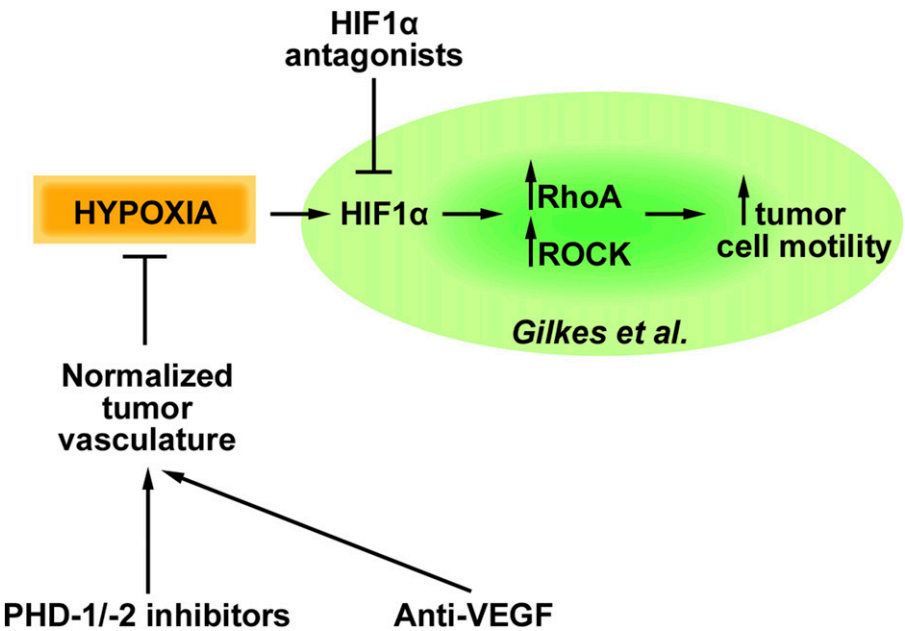

Fig. 1. Overview of therapeutic opportunities to counteract the malignancy-promoting effects of hypoxia. Hypoxia can directly induce the activation of HIF1α, which in turn transactivates *RHOA* and *ROCK*, leading to increased tumor motility. Normalizing tumor vasculature and antiangiogenic approaches may ameliorate tumor hypoxia, mitigating the effects of tumor cell motility. The study by Gilkes et al. (3) provides molecular details that may lead to therapeutic strategies to directly block the activation of *RHOA* and *ROCK* by HIF1α.

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sometimes deleterious consequences in which anti-VEGF therapy promoted metastasis and malignancy because of increased hypoxia throughout the tumor (13, 14). These unexpected failings fuel the current attempts to understand hypoxia-induced tumor cell migration, which endows cancer cells with the ability to migrate away from hypoxic areas of the tumor, via HIF1α-induced transactivation of *RHOA* and *ROCK*.

Targeting tumor hypoxia or its consequences has emerged as a leading strategy to combat cancer progression. Studies that normalized tumor vessel functionality ultimately improved oxygen delivery to tumors, mitigating the promotility effects of hypoxia, resulting in decreased metastasis (11, 12). Improved tumor perfusion could also increase tumor responses to chemo- and radiation therapy, mitigating the malignant effects of hypoxia. Clinically, a significant barrier for chemotherapy is intratumoral bioavailability, with much of the body exposed to cytotoxic levels of systemic chemotherapy, whereas poorly vascularized hypoxic tumors are not, thus limiting therapeutic efficacy. Targeting factors such as RhoA and ROCK, which are induced by active HIF1α, may not offer therapeutic gain in the first-line setting, particularly because systemically delivered specific RhoA and ROCK inhibitors may be unable to gain access to oxygen-deprived tumor cells. However, inhibition of these factors may be helpful during chemotherapy of normoxic tumors. When administered in combination with chemotherapy, both cell proliferation and cell migration could be minimized, preventing metastatic dissemination during treatment. This approach is plausible given that the *RHOA* and *ROCK1* gene loci are seldom amplified or mutated, as noted by Gilkes et al. (3), making targeting more straightforward. The opportunity to also target other downstream transcriptional targets of activated HIF1α requires high-resolution genomic mapping of HIF1α binding sites via ChIP-seq, as performed in aggressive or high-grade breast cancer cells (10). It is conceivable that activated HIF1α induces the expression of other transcripts that are the primary factors driving motility and are also specifically expressed in cancer cells. Identification of these hypoxia cancer-specific factors would offer novel avenues of targeting hypoxic cancer cells that threaten to migrate away from the primary tumor. The binding sequences for HIF1α and HIF2 in the *RHOA* and *ROCK1* genes identified by Gilkes et al. (3) may pave the way for strategies to therapeutically block these interactions, thus counteracting hypoxia-mediated increases in tumor cell motility and aggressiveness.

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In summary, although mutations to oncogenes and tumor-suppressor genes contribute to cancer progression, there is a growing appreciation that the tumor microenvironment can have profound regulatory effects on tumor behavior (15, 16), especially under hypoxic conditions. This knowledge opens up novel therapeutic opportunities to specifically counteract microenvironmental factors, such as hypoxia and nutrient deprivation (Fig. 1). Relieving tumor hypoxia ultimately will require strategies that "normalize" tumor vasculature, which may even include low-grade inhibition of angiogenesis to form stable vessels, as opposed to forming more vessels: quality versus quantity (17). Alternate approaches, as suggested by the work of Gilkes et al. (3), now invoke targeting the hypoxia-mediated downstream signaling cascade. The molecular details of these pathways provided by this study will be key to developing therapeutic measures that block the HIF1α regulation of *RHOA* and *ROCK*, which could be effective anticancer agents even in the face of ongoing tumor hypoxia.

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