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Mini-review

Hypoxia and lymphangiogenesis in tumor microenvironment and metastasis

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ABSTRACT

Hypoxia and lymphangiogenesis are closely related processes that play a pivotal role in tumor invasion and metastasis. Intratumoral hypoxia is exacerbated as a result of oxygen consumption by rapidly proliferating tumor cells, insufficient blood supply and poor lymph drainage. Hypoxia induces functional responses in lymphatic endothelial cells (LECs), including cell proliferation and migration. Multiple factors (e.g., ET-1, AP-1, C/EBP-δ, EGR-1, NF-κB, and MIF) are involved in the events of hypoxia-induced lymphangiogenesis. Among them, HIF-1α is known to be the master regulator of cellular oxygen homeostasis, mediating transcriptional activation of lymphangiogenesis via regulation of signaling cascades like VEGF-A/-C/-D, TGF-β and *Prox-1* in experimental and human tumors. Although the underlying molecular mechanisms remain incompletely elucidated, the investigation of lymphangiogenesis in hypoxic conditions may provide insight into potential therapeutic targets for lymphatic metastasis.

1. Introduction

Hypoxia (low oxygen tension) is a critical feature of the tumor microenvironment that promotes invasion and metastasis, and resistance to therapy. The transcription factor hypoxia-inducible factor-1 (HIF-1) is a key regulator of the cellular response to hypoxia. Coordinate upregulation of HIF-1 and its target genes is involved in many aspects of tumor biology including anaerobic energy metabolism, angiogenesis, cell survival, invasion, and metastasis [1–3]. The role of vascular endothelial growth factors (VEGFs) in hypoxia-induced angiogenesis has been extensively studied in experimental tumor models and human cancers [4–8]. In recent years, it has been known that increased HIF-1α expression arises through the activation of oncogenes and/or inactivation of tumor suppressor genes in the hostile hypoxic microenvironment, leading to upregulation of lymphangiogenic factors. HIF-1α correlates with expression of VEGF-C and consequent lymphangiogenesis in wound healing, experimental lung carcinoma, and in human breast cancer [9–12], suggesting that the VEGF-C/VEGFR-3 signaling pathway, a key downstream target of HIF-1α stimulates proliferation and migration of lymphatic endothelial cells (LECs). Hypoxia may cause increased microvascular permeability and barrier dysfunction of LEC monolayers with abnormal intercellular junctions, affecting the expression of many endothelial genes and tumor cell adhesion. Understanding the role of hypoxia in tumor-associated lymphangiogenesis will provide us with new insights into mechanisms that modulate interactions of tumor cells with LECs and extracellular matrix (ECM) components in metastatic spread.

Intratumoral hypoxia is associated with a significantly increased risk of metastasis and mortality in many human cancers [13]. A number of recent reviews have focused on the molecular mechanisms involved in hypoxic signal transduction and potential factors that mediate functional interactions between cancer cells and stromal cells including LECs. However, most of the analyses have focused on the effect of blood vessels on tumor invasion and metastases. VEGF-induced changes in vascular integrity and permeability promote both intravasation and extravasation, while VEGF-induced angiogenesis in the secondary tissue is essential for cell proliferation and establishment of metastatic lesions in the hypoxic microenvironment [5,14]. The descriptions of the formation of functional lymphatic vessels are usually limited to involvement of single molecules or factors, e.g., VEGF-C [15], and lacking substantial evidence to illustrate hypoxia-induced lymphangiogenesis and to support its role in lymphatic metastasis. The present review has collated the available evidence of the interplay between lymphangiogenesis, HIF-1α expression and other crucial factors in hypoxic conditions, and emphasized the interrelation between lymphangiogenesis and cancer metastasis. In addition, the review has also summarized HIF-α expression and lymphatic metastasis in various types of human cancers, and some experimental therapies that target hypoxia-induced lymphangiogenesis for inhibiting metastasis.

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2. Hypoxia, endothelial cells and lymphangiogenesis

2.1. Hypoxia and endothelial cells

Hypoxia affects endothelial physiology *in vivo* and *in vitro* in a number of ways, including the transcriptionally regulated expression of matrix proteins that contribute to remodeling of the vasculature and surrounding tissues [16]. As reflected in cell proliferation and matrix synthesis, the endothelial cells take an active part in sensing various forms of pathophysiological stress, and have the potential to regulate their hypoxic responses in coordination with changes in the oxygenation of surrounding tissues. Hypoxia-driven vascular remodeling has been ascribed to an imbalance between vasodilators (nitric oxide, NO) and vasoconstrictors (endothelin-1, ET-1) [17,18]. Hypoxia regulates the reciprocal production and expression of ET-1 and NO in LECs, which affects leukocyte adhesion to lymphatic wall and extravasation, and alters lymphatic tone and contractile activity [19,20]. Blocking endothelial NO synthase can decrease lymphatic hyperplasia and prevent lymphatic metastasis [21]. ET-1 stimulates the growth and differentiation of endothelial cells through a mechanism mediated by the endothelin B receptor (ETBR) expressed in LYVE-1-positive LECs. ET-1-induced lymphatic formation *in vivo* can be blocked by an ETBR-selective antagonist [22]. Loss of the balance between ET-1 and NO is a prerequisite for endothelial cell pathology.

The heterogeneity among endothelial cells is manifested as differential responses to changes in oxygen tension. However, currently limited information exists on the genetic response of endothelial cells to hypoxia. Acute hypoxia rapidly activates the endothelial cells to release inflammatory mediators and to increase the expression of specific genes for the VEGF family [23], and the transcriptional induction of these genes is known to be mediated through HIF-1α activation. In an *in vitro* study, upregulation of lymphangiogenic genes, VEGF-C/-D and VEGFR-3, was found in hypoxic vein endothelial cells [24], suggesting the possibility that hypoxia may stimulate lymphatic formation. In malignant neoplasms, low oxygen tension associated with tumor necrosis induces increased expression of HIF-1α transcription factor. The activation of HIF-1α in turn stimulates the proliferation of endothelial cells by upregulating expression of VEGF family members, in which VEGF-A and VEGF-C genes, the best characterized hypoxia-regulated targets, are potent inducers of tumor angiogenesis, lymphangiogenesis and lymph node metastasis [25–27].

The biochemical and molecular responses of the endothelial cells in hypoxic conditions may account for the features observed in pathological situations, such as alteration of cell morphology and intercellular junctions. Hypoxia increases breast cancer cell-induced LEC migration [28], and HIF-1α promotes tumor metastasis through the regulation of endothelial cell function [29]. Loss of HIF-1α inhibits a number of important parameters of endothelial cell behavior, e.g., permeability, chemotaxis and ECM penetration, which are linked to a decreased level of VEGF expression [30]. In chronic obstructive lung disease, hypoxia causes irreversible loss of vessels and thickening of the vascular muscular coat [31]. Targeted disruption of the HIF-1α gene in mice results in embryonic lethality at midgestation that is associated with dramatic vascular regression due to extensive endothelial cell death [32]. In response to hypoxia, HIF-1α induces and activates transcription of genes encoding platelet-derived growth factor-B (PDGF-B), prospero-related homeobox gene-1 (*Prox-1*) and CCAAT/enhancer-binding protein-δ (C/EBP-δ) [33–35]. These factors involve interplay between endothelial cells and stromal cells for the maintenance of vascular structure and integrity. Hypoxia also increases endothelial cell stiffness and regulates cellular-ECM adhesion [36,37]. The ECM effect is mediated by surface receptors called integrins that trigger different cellular responses. Altered reactive oxygen species levels affect integrin-mediated signaling in regulation of cell proliferation and differentiation. The transforming growth factor-β (TGF-β)-induced protein, an integrin adaptor molecule, accumulates in ECM of hypoxia-exposed LECs, and mediates LEC adhesion and migration by its binding to the β_3 integrin [38]. The molecular interactions of endothelial cells with tumor cell surface receptors and adhesion molecules may contribute to tumor cell arrest and extravasation in lymphatic dissemination.

2.2. HIF-1 and lymphangiogenesis

2.2.1. HIF-1 function or expression

All nucleated cells have the ability to sense and respond to changes in oxygen concentration. The transcriptional responses to hypoxia are primarily mediated by hypoxia-inducible factors (HIFs), which are composed of three α subunits (HIF-1α, HIF-2α or HIF-3α) and one HIF-1β subunit [2]. Of the three HIF-α-subunits, HIF-1α is the most ubiquitously expressed and functions as a key regulator of oxygen homeostasis in many cell types [39].

Under normoxic conditions, HIF-1α is modified by prolyl hydroxylation leading to its ubiquitination and rapid proteasomal degradation, and thus cytoplasmic HIF-α protein remains low [40–42]. Under hypoxic conditions, stabilized HIF-1α protein accumulates and translocates to the nucleus and binds to HIF-1β, forming an active HIF-1 heterodimeric complex. In the nucleus, the HIF-1 complex recognizes and binds to core DNA sequences at the hypoxia response element (HRE), thereby transactivating multiple target genes (Fig. 1). The activated VEGF downstream pathway is involved in proliferation, differentiation, tumor invasion and metastasis, and angiogenesis [11,24]. Hypoxia is a strong stimulator of angiogenesis during embryonic development and in pathological conditions like tumor growth. In response to intratumoral hypoxia, angiogenic factors produced by tumor cells induce neovascularization from the preexisting vessels, which increases the supply of O2, glucose and nutrients to promote proliferation and survival of tumor cells in a hostile microenvironment [2]. As a result of deprivation of oxygen and nutrients, the growth and viability of cells are reduced. HIF-1α helps to restore oxygen homeostasis by inducing glycolysis, erythropoiesis and angiogenesis. Although HIF-1α expression is associated with the degree of hypoxia, it is additionally overexpressed by hypoxia-independent pathways, such as glucose deficiency and oncogene activation [43]. Consequently, hypoxia-regulated genes involved in controlling the cell cycle are either HIF-1α-dependent or HIF-1α-independent, suggesting that there are at least two different adaptive responses to being deprived of oxygen and nutrients.

Loss of HIF-1α reduces hypoxia-induced expression of VEGF, prevents formation of large vessels in embryonic stem-derived tumors, and impairs vascular function, resulting in hypoxic microenvironments within the tumor mass [1]. Hypoxia during tumorigenesis induces the expression of HIF-1α that functions as a master switch to regulate the expression of growth factors and cytokines, e.g., VEGF, NO synthase, and placental growth factor (PlGF) [29,44,45]. HIF-1α is also an important marker of tumor malignancy, and high levels of HIF-1α have been positively correlated with tumor progression and poor prognosis in patients [46,47]. Thus, cellular changes induced by HIF-1α are promising targets for cancer therapy.

2.2.2. HIF-1 and VEGF

HIF-1α upregulation in response to hypoxia is one of the most powerful stimuli of lymphangiogenesis by induction of VEGF-A/-C/-D, but the expression of VEGF can also be induced independent of hypoxia. VEGF-A expression in large osteoclasts is induced by a