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Role of hypoxia and vascular endothelial growth factors in lymphangiogenesis

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Abbreviations: ARE, AU-rich element; FGF2, fibroblast growth factor 2; HIF, hypoxia-inducible factor 1; HRE, hypoxia-responsive element; IRES, internal ribosome entry site; LEC, lymphatic endothelial cell; PDGF, platelet-derived growth factor; uORF, upstream open reading frame; UTR, untranslated region; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor

Hypoxia is known to be a major factor in the induction of angiogenesis during tumor development but its role in lymphangiogenesis remains unclear. Blood and lymphatic vasculatures are stimulated by the vascular endothelial family of growth factors – the VEGFs. In this review, we investigate the role of hypoxia in the molecular regulation of synthesis of the lymphangiogenic growth factors VEGF-A, VEGF-C, and VEGF-D. Gene expression can be regulated by hypoxia at either transcriptional or translational levels. In contrast to strong induction of DNA transcription by hypoxia-inducible factors (HIFs), the majority of cellular stresses such as hypoxia lead to inhibition of cap-dependent translation of mRNA and downregulation of protein synthesis. Here, we describe how initiation of translation of *VEGF* mRNA is induced by hypoxia through an internal ribosome entry site (IRES)-dependent mechanism. Considering the implications of the lymphatic vasculature for metastatic dissemination, it is crucial to understand the molecular regulation of lymphangiogenic growth factors by hypoxia to obtain new insights into cancer therapy.

The Lymphatic Network

The lymphatic vasculature consists of a network of lymph vessels whose main function is to return protein-rich interstitial fluid to the circulating blood. Fluid, macromolecules, and cells, such as leukocytes and activated antigen-presenting cells, enter the lymphatic system through the blind-ended lymphatic capillaries. From here, lymph is transported toward collecting lymphatic vessels and is returned to the blood circulation in the jugular area through the lymphaticovenous junctions.[1] On its way, lymph is filtered through the lymph nodes, where foreign particles taken up by antigen-presenting cells initiate specific immune responses.[2] In the small intestine, lacteal lymphatic vessels inside the intestinal villi absorb the dietary fat released by enterocytes in the form of lipid particles called chylomicron. In addition to these physiologic functions, the lymphatic system contributes to pathologic conditions such as lymphedema, inflammatory diseases, and tumor metastasis. Many studies have demonstrated the existence of proliferative peri- and intratumoral lymphatic vessels.[3] Additionally, tumoral lymphangiogenesis correlates with an increase in metastases,[4,5] and detection of lymphangiogenic growth factors is associated with poor prognosis in many human tumors.[6-8]

Similar to blood capillaries, lymphatic capillaries are thin-walled, relatively large vessels composed of a single layer of endothelial cells, but they are not covered by pericytes or smooth muscle cells and have an absent or poorly developed basement membrane.[9] In addition, they lack tight junctions and adherens junctions, which allows easy access for fluid, macromolecules, and cells to enter the vessel lumen.[10] Endothelial cells of lymphatic capillaries are oak leaf-shaped and are characterized by discontinuous vascular endothelial (VE)-cadherin–positive button-like junctions. Collecting lymphatic vessels downstream have continuous zipper-like junctions previously described in blood vessels.[9] Initial lymphatics combine to form larger vessels called precollectors and collectors, which in turn feed into four major groups of lymph nodes in the axillary and inguinal regions. Collecting lymphatic vessels have a smooth muscle cell layer, basement membrane, and valves.

Lymphatic Markers

Lymphatic vessels were first described in the beginning of the 17th century; however, the first growth factors and molecular markers specific for these vessels were discovered only 10 to 15 y ago. These growth factors include Prox1, the main transcription factor implicated in lymphatic vasculature development;[11] lymphatic vascular endothelial-cell hyaluronan receptor-1 (LYVE-1),[12] a new homolog of CD44 glycoprotein that is a lymph-specific receptor

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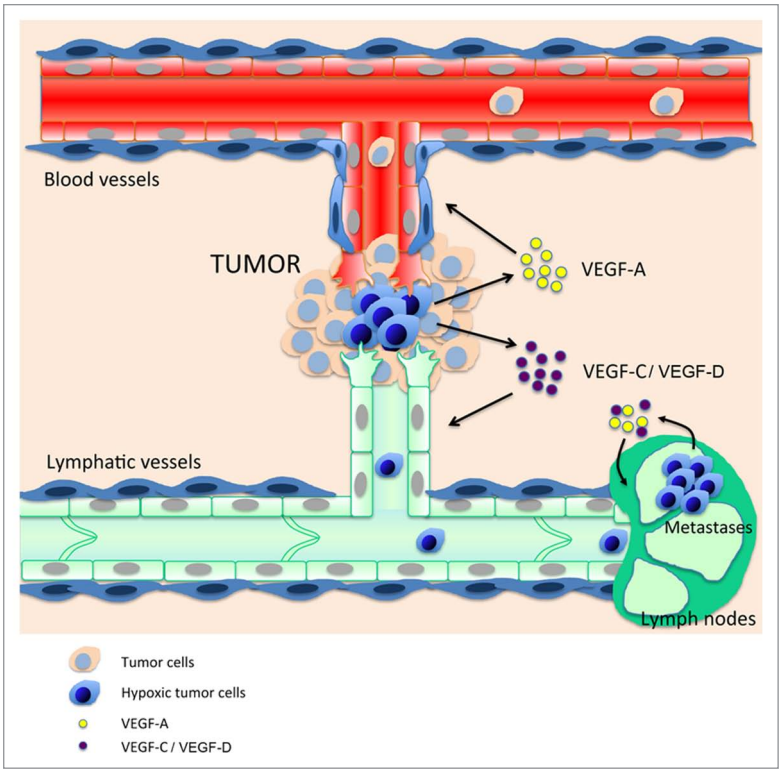

Figure 1. Crosstalk between tumor hypoxia and the lymphatic and blood vasculatures. Hypoxic tumor cells (blue) near pre-existing blood and lymphatic vessels secrete angiogenic and lymphangiogenic growth factors such as VEGF-A, -C, and –D. Blood vessels bring oxygen and nutriments to tumor cells, whereas lymphatics drain debris and provide new routes for tumor metastasis. Lymphatic metastatic tumor cells maintain lymphangiogenic growth factors synthesis in this poorly oxygenated environment to promote lymph node lymphangiogenesis and establish the "metastatic niche."

for hyaluronan;[13] and podoplanin, a transmembrane glycoprotein molecule.[14] Although the blood and lymphatic vascular systems are structurally related and function in concert, these lymphatic-specific markers have allowed investigation of the specific features of lymph vessels. Vascular endothelial growth factor receptor 3 (VEGFR-3, also known as FLT-4) has primarily been described as an major marker of lymphatics[15,16] because its expression in adults becomes restricted to the lymphatic endothelium.[17-19] However, recent studies have shown that VEGFR-3 is also upregulated on vascular endothelial cells in angiogenic sprouts and is present on vessels in tumors and wounds.[20,21]

Lymphangiogenesis in Pathology

In adult organisms, lymphangiogenesis takes place only in certain pathologic conditions. Abnormal function of the lymphatics is implicated in certain disease states, such as lymphedema, inflammation, immune diseases, and tumor metastasis.

Lymphedema is a disorder of the lymphatic vascular system characterized by impaired lymphatic return and swelling of the extremities. When the lymphatic system has been damaged during surgery or radiation treatment, its capacity to absorb excess water and cells from the interstitial space is reduced. If the transport capacity of the lymphatic system is reduced such so that it cannot handle this increase in lymphatic load, an insufficiency of the lymphatic system may occur. Lymphedema can be an unfortunate side effect of cancer treatment and is a chronic condition that, if ignored, can lead to disfigurement, immobilization, and severe infections. Without treatment, the swelling may continue to increase.

Inflammation is thought to contribute to the development and progression of various cancers, including lung,[22] breast,[23] gastrointestinal,[24-26] ovarian,[27] prostate,[28] skin,[29] and liver cancers.[30] Inflammatory breast cancer exhibits increased angiogenesis and lymphangiogenesis and has a higher metastatic potential than noninflammatory breast cancer.[31] Blocking lymphangiogenesis in chronic inflammatory diseases may be an important means of ameliorating the severity of some of these pathologies.

The extent of lymph node metastasis is a major determinant of staging and prognosis of most human malignancies. Although the clinical significance of lymph node involvement is well documented, the molecular mechanisms that promote tumor spread into the lymphatic or blood vascular systems and widespread dissemination are not well understood. Recent studies have provided a large body of evidence indicating that newly visualized lymphatics facilitate the formation of metastases. High tumor interstitial fluid pressure is thought to promote tumor cell entry into lymphatic vessels that have lower fluid pressure.[32,33] Intratumoral lymphatic vessel growth often correlates with metastasis of human melanoma, breast, or head and neck cancers[34-36] where tumor cells can be observed within lymphatic vessels, demonstrating that lymphatic vessel growth is important for tumor spread (**Fig. 1**).

Tumor Growth, Hypoxia, and Lymphangiogenesis

As solid tumors grow, the cells within the expanding mass frequently become hypoxic because of the increasing distance from the nearest blood vessels. Without an adequate vascular supply, solid tumors can grow only to a critical size of 1–2 mm (approximately 10^6 cells), primarily due to a lack of oxygen and nutrients.[37] A number of studies have been performed to characterize and ultimately inhibit tumor angiogenesis. However, since hypoxia also regulates the expression of lymphangiogenic factors, it is crucial to consider tumor hypoxia and tumor lymphangiogenesis as two tightly interlocked phenomena. In contrast to blood vessels, the lymphatic vasculature does not promote tumor growth by providing key elements for cell survival (i.e., oxygen and nutrients), but allows metastatic dissemination of solid tumors through lymph nodes and finally to distant organs.[38,39] The lymphatic network is not merely an alternative vehicle to blood vessels for dissemination, but actually constitutes the main

vascular system implicated in dissemination because lymphatic vessels have an optimal structure for tumor cell invasion. Indeed, the main difference between blood and lymphatic networks is the structure and permeability of their capillaries: whereas lymphatic capillaries are thin-walled, relatively large vessels, composed of a single layer of endothelial cells, lymphatic capillaries are not ensheathed by pericytes or smooth muscle cells and have little or no basement membrane.[3] As a result of this high permeabilty, tumor cells can spread more easily in lymphatics than in blood vessels. Moreover, this invasion is also not just a passive process as tumors induce growth of new lymphatic vessels in draining lymph nodes and enlargment of lymphatic endothelium before metastasis. This remodelling of lymph nodes potentially contributes to the migration, implantation, or survival of metastatic tumor cells by inducing a specific tumor microenvironment. Thus, a hypoxic tumor will not only ensure its survival through activation of angiogenesis but will also become more aggressive. This dual regulation of blood and lymphatic vasculature by hypoxia during tumor growth impairs therapeutic efficacy. First, there is real crosstalk between the tumor and blood and lymphatic endothelial cells. Blood vessel endothelial cells produce lymphangiogenic factors such as vascular endothelial growth factor C (VEGFC), fibroblast growth factor 2 (FGF2), and platelet-derived growth factors (PDGFs) to facilitate tumor-induced lymphangiogenesis.[40] Both endothelial cell types produce matrix metalloproteinases that promote tumor spreading. Lymphatic endothelial cells (LECs) also express the CCL21 chemokine that is physiologically implicated in dendritic cell mobilization[41] and interacts with the CCR7 receptor expressed by many tumors to stimulate lymphatic dissemination.[42] Several treatments have been developed specifically to inhibit tumor angiogenesis (e.g., Avastin) and therefore suppress tumor oxygenation and destroy tumor cells. However, these drugs also target lymphangiogenesis and would generate severe tumor hypoxia, which induces overexpression of lymphangiogenic factors and increased tumor dissemination. This cross talk between hypoxia and the two vascular systems and the resultant spread of the tumor can in part explain the failure of antiangiogenic drugs in cancer treatment (**Fig. 1**).

A key feature of the lymphatic system is its hypoxic environment as lymphatic vessels do not transport red blood cells. Lymph vessels are often located in remote areas away from oxygen-carrying blood vessels and are therefore exposed to a milieu with very low oxygen levels. Tumor cells have to adapt to this hostile hypoxic environment in order to spread to the lymph nodes.[43-45]

Normoxia is defined as a milieu where the O_2 concentration is sufficient to ensure aerobic metabolism of cells, the basis of eukaryotic physiology.[46] In contrast, hypoxia is an environment where the aerobic metabolism of cells is inhibited due to a lack of oxygen. The major cellular response to hypoxia is stabilization of hypoxia-inducible factor 1(HIF1). HIF1 is a transcription factor that controls the expression of a battery of more than 40 target genes.[47-49] It is composed of an α subunit that is constitutively expressed and a β subunit that is subject to rapid ubiquitination and proteasomal degradation under normoxic conditions.[50] The molecular basis for this regulation is the O_2-dependent hydroxylation of proline residues 402 and 564 in HIF-1α by any one of three enzymes in mammals that have been designated prolyl hydroxylase-domain proteins or HIF-1α prolyl hydroxylases.[51,52] Prolyl hydroxylation of HIF-1α is required for binding of the von Hippel–Lindau tumor suppressor protein (VHL), which is the recognition component of an E3 ubiquitin-protein ligase that targets HIF-1α for proteasomal degradation.[53,54] Hypoxia has been shown to regulate not only angiogenesis, but also lymphangiogenesis by promoting overexpression of specific lymphangiogenic factors (e.g., VEGF-C) and growth factors that are shared by the vascular and the lymphatic vasculature (e.g., VEGF-A, FGF2). In this review we provide an overview of the link between lymphangiogenic factors and hypoxia and the consequences of this relationship in a well-known hypoxic pathology: the development and dissemination of solid tumors.

Hypoxia-Induced Molecular Regulation of VEGFs

The VEGF family is composed of growth factors involved in vascular development. This family includes VEGF-A, -B, -C, -D, and -E, and placental growth factor.[55] All members of this family stimulate proliferation and migration of endothelial cells in vitro. These proteins bind and activate specific receptors on the endothelial cell surface: VEGF recognizes VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1); placental growth factor and VEGF-B recognize VEGFR-1; and VEGF-C and VEGF-D recognize VEGFR-2 and VEGFR-3 (Flt-4). Lymphangiogenesis is induced by VEGFs that promote angiogenesis (VEGF-A) or angiogenesis and lymphangiogenesis (VEGF-C and VEGF-D).

Hypoxia-induced gene expression was first described as a transcriptional mechanism mediated by hypoxia-responsive elements (HREs) present in the promoter region of different genes that are targets of the hypoxia-induced transcription factors (HIFs). In particular, a functional HRE has been identified within the 5′ flanking region element of the human *VEGFA* gene[56,57] that is the target of both HIF1 and HIF2.[58] This HRE allows transcriptional induction of *VEGFA* by hypoxia in several physiologic (i.e., wound healing, inflammation) and pathologic (i.e., ischemia, tumor development) states.

In addition to its transcriptional effects, hypoxia also regulates gene expression post-transcriptionally at the levels of mRNA stability and translation. An important class of mRNAs is stabilized by hypoxia: the so-called AU-rich mRNAs that bear AU-rich elements (AREs) in their 3′ untranslated regions (3′UTR).[59,60] AREs are found in mRNAs of most genes coding for cytokines, growth factors, and proto-oncogenes (7–8% of the transcribed genome), indicating that the stabilization of such mRNAs in hypoxic conditions has important consequences regarding cell pathophysiology. In particular, angiogenic cytokines including VEGF-A are regulated by this mechanism.[61] mRNA stabilization is controlled by the binding of HuR protein to the ARE in cooperation with polyA-binding protein interacting protein 2 (PAIP2).[62] One proposed mechanism is that HuR acts in competition with destabilizing proteins such as AUF1 or tristetraprolin (TTP) to bind to the ARE.[61] Another emerging concept is that HuR counteracts the binding of microRNAs to mRNA

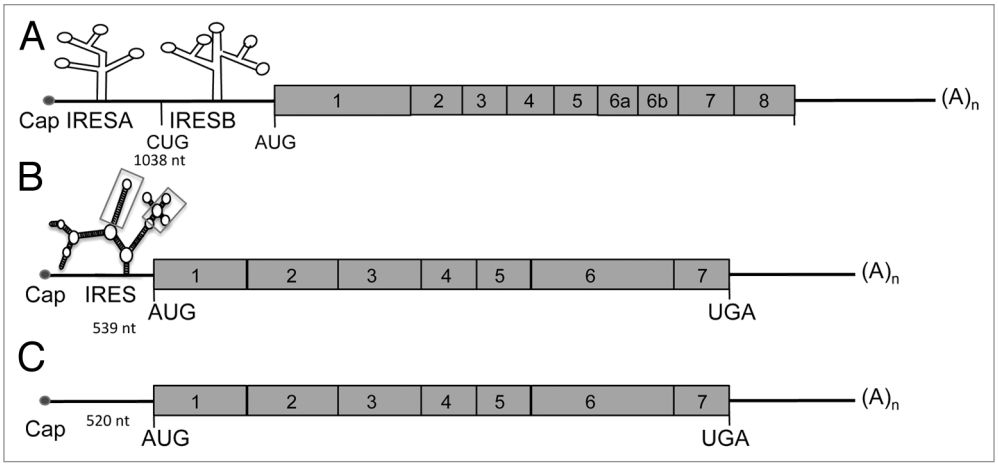

Figure 2. Schematic representation of *VEGF-A*, *-C*, and *-D* mRNAs. (**A**) *VEGF-A* mRNA is characterized by a long 5′UTR (1038 nt) containing two internal ribosome entry sites (IRESs) (A and B). The *VEGF-A* gene encodes multiple isoforms generated by mRNA splicing of four constitutive and four alternative exons. (**B**) *VEGF-C* mRNA possesses a GC-rich 5′UTR containing one IRES. The secondary structure of VEGF-C IRES has been determined by shape analysis and contains 2 motifs (indicated by boxes) showing a similar reactivity pattern between human and mouse mRNA. (**C**) Similar to *VEGF-C*, the *VEGF-D* mRNA is generated from 7 exons.

3′UTRs.[63] In the case of *Vegfa* mRNA, the HuR binding site overlaps with the binding site of miR-200b, thus HuR antagonizes the suppressive effect of this microRNA.[63]

Hypoxia also strongly regulates gene expression at the translational level. First, it silences global cell translation by inhibiting mRNA cap-dependent translation through inactivation of mTOR kinase. This results in hypophosphorylation of the 4E-BP protein, which then sequesters the cap-binding factor eIF4E.[64,65] In addition, hypoxia induces phosphorylation of the initiation factor eIF2a by activation of PERK kinase, which also generates translational blockade.[66] Two major alternative mechanisms are able to overcome this global inhibition of translation that is induced by hypoxia: upstream open reading frames (uORFs) and internal ribosome entry sites (IRESs). uORFs are a key element of translational control in response to stress. These elements precede the initiation codon of the mRNA main coding regions and are present in approximately 40–50% of mRNAs. They are primarily translational inhibitors when eIF2a is dephosphorylated and the complex of initiator tRNA with eIF2 and GTP is available for translation initiation. In contrast, they allow the ribosome to scan and reach the initiation codon of the main coding sequence in conditions of stress when eIF2a is phosphorylated.[67]

IRESs are RNA structural elements present in the 5′ nontranslated regions of a small number of mRNAs that allow recruitment of the ribosome to a site that is a considerable distance from the cap structure, most frequently in the presence of trans-acting factors.[64,68] The majority of identified IRESs are found in mRNAs of proteins associated with the control of cell growth and death, including growth factors, proto-oncogenes, and proteins required for apoptosis.[65,69] IRES-dependent translation is cap-independent and, in the case of cellular mRNAs, independent of eIF2a phosphorylation; this allows translation to occur in stress conditions.[64,70] Notably, *HIF1*α mRNA possesses an IRES, suggesting that these structure are crucial for translational regulation occurring under hypoxia. IRESs have also been identified in the mRNAs of three major lymphangiogenic growth factors, FGF2, VEGF-A, and VEGF-C.[71,72] Interestingly, these IRESs are activated in hypoxic conditions, resulting in translational induction of these factors.[45,73] The regulation of VEGF-A and -C expression and their relationship with hypoxia is discussed below.

VEGF-A

VEGF-A, also called vascular permeability factor, is a homodimeric glycoprotein with a molecular weight of approximately 45 kDa. At least 9 VEGF isoforms exist as a result of alternative patterns of splicing.[74] Three of these, containing 121, 165, and 189 amino acids respectively, are preferentially expressed by VEGF-A producing cells[75-77] Each of these isoforms contributes to formation of a VEGF-A gradient essential for the proper migration of ECs/LECs during angiogenesis or lymphangiogenesis. The larger species, VEGF-165, VEGF-189, and VEGF-206, are basic and bind to isolated heparin and heparin proteoglycans distributed on cellular surfaces and extracellular matrices whereas the smaller species, VEGF-121, is acidic and is freely diffusible.[78] Although VEGF-A is mainly known as a growth factor that plays an essential role in physiologic and pathologic angiogenesis during both development and adulthood,[79] it also has prolymphangiogenic properties.[80,81] The proangiogenic activity of VEGF-A is mediated by interaction with a high-affinity VEGFR2 receptor, whereas the prolymphangiogenic activity is promoted by binding to the VEGFR2/R3 heterodimeric receptor.

In addition to its transcriptional upregulation during hypoxia, is probably the most highly post-transcriptionally regulated factor.[74] The gene structure of *VEGF-A* has been predicted in silico (**Fig. 2A**). *VEGFA* mRNA contains two IRESs[72] located upstream of the alternative initiation codons CUG and AUG that are responsible for synthesis of alternative isoforms of VEGF-A.[74]

Both IREs are activated by hypoxia.[73] *Vegfa* IRESs are differentially regulated by an upstream ORF and by binding of Mir16 to the 3′UTR.[82,83] A study of VEGF-A IRES trans-acting factors revealed tight regulation by both positive regulators activated by hypoxia (e.g., MAPK3 kinase) and negative regulators that are inhibited during this stress (e.g., DEAD-box RNA helicase 6).[84] Another mechanism implicated in translation regulation of VEGF-A is riboswitch. Riboswitch refers to the ability of mRNAs to alter their folding structure and hence rate of translation in response to an environmental modification. During hypoxia, intracellular accumulation of heterogeneous nuclear ribonucleoprotein L (hnRNP L) promotes an active conformation and increases the rate of translation of *VEGF-A* mRNA.[85] VEGF-A expression is strongly regulated at the level of mRNA stability, a process primarily mediated by the AREs present in the *Vegfa* mRNA. Indeed, the *Vegfa* mRNA is destabilized by several proteins including AU-rich element RNA-binding protein 1 (AUF1, also known as hnRNPD) and tristetraprolin (TTP), which target the AREs.[61] Destabilization of *Vegfa* mRNA by TTP is responsible for its antiangiogenic activity.[86] In contrast, *Vegfa* mRNA is stabilized by hypoxia.[60] This process is mediated by binding of the RNA stabilizing protein HuR and its partner PAIP2 to the AREs, which prevents binding of the destabilizing proteins.[60,61] Interestingly, the MDM2 protein, which is translocated from the nucleus to the cytoplasm under hypoxic conditions, increases *Vegfa* mRNA stabilization.[87] *Vegfa* mRNA stability is thus controlled by interplay between stabilizing and destabilizing proteins that compete for binding to the AREs. Moreover, it has been proposed that export of *VEGF-A* mRNA from the nucleus and its loading onto ribosomes can be increased during hypoxia by extranuclear shuttling of mRNA-binding proteins such as hnRNP L and A1, which also regulate *VEGF-A* mRNA stability.[88] These mechanisms, combined with transcriptional regulation induced by HIFs, allow fast and massive overexpression of VEGF-A in response to hypoxia.

VEGF-C

The VEGF-C/VEGFR3 signaling pathway is the major pathway implicated in lymphangiogenesis. First identified in 1996,[89] VEGF-C is produced as a precursor protein that is activated by intracellular proprotein convertases.[89,90] The secreted disulphide-linked VEGF-C subunits only bind VEGFR-3, but the factor is further proteolyzed in the extracellular environment by plasmin and other proteases to generate non–disulfide-linked homodimeric proteins with high affinity for both VEGFR-2 and VEGFR-3.[3,90] VEGF-C is crucial for the induction of proliferation, migration, and survival of endothelial cells.[91] VEGF-C is also an essential chemotactic and survival factor during embryonic lymphangiogenesis; homozygous deletion of *VEGF-C* leads to complete absence of lymphatic vasculature in mouse embryos whereas VEGF-C$^{+/-}$ mice display severe lymphatic hypoplasia. In *VEGF-C* null mice, lymphatic endothelial cells initially differentiate in the cardinal veins but fail to migrate and form primary lymph sacs.[92] Although several studies have shown positive correlations between HIF-1α and VEGF-C in various cancers,[93-95] for a long time the molecular mechanisms of hypoxia-induced regulation of VEGF-C remained poorly understood. The likelihood of direct transcriptional regulation of VEGF-C by HIF1α is low because the *VEGF-C* promoter does not contain a HRE sequence.[96] Our recent work demonstrated the existence of a single IRES in the 5′UTR of both murine and human *VEGF-C* mRNA (**Fig. 2B**). We have demonstrated that VEGF-C IRES activity is upregulated in vivo during tumor growth in three murine models of carcinoma, similar to the IREs of FGF2 and VEGF-A.[45] Strikingly, we also observed that VEGF-C IRES activity increases under hypoxia in vitro, but the presence of HIF-1α is not required in cultured cells.

VEGF-D

Binding of VEGF-D, also called c-fos induced growth factor, to its receptor VEGFR-3 promotes lymphangiogenesis. The *VEGF-D* gene encodes 7 exons (**Fig. 2C**). Maturation of VEGF-D is similar to that of VEGF-C and occurs by protein cleavage in N and C-terminal regions. VEGF-D has been poorly studied because of the lack of a phenotype resulting from its depletion in mice. Recent reports have shown that overexpression of VEGF-D induces tumor lymphangiogenesis and promotes lymphatic metastasis in mouse tumor models.[97] However, few clinical studies have investigated the association between the expression of VEGF-D and lymphatic metastasis. VEGF-D overexpression correlates with an increase in lymphatic vessel growth and lymphatic metastasis.[39] Recent studies suggest that VEGF-D is necessary for for the entry of tumor cells into the lymphatic system that results in metastasis.[98] VEGF-D promotes structural changes in tumor-draining lymphatic vessels and induces vasodilatation. VEGF-D also increases the endothelial response to prostaglandin E2 (PGE2) by inhibiting the prostaglandin dehydrogenases (PGDH).[99,100]

The role of hypoxia in the promotion of VEGF-D expression has not been clearly established. Recent studies have demonstrated correlations between VEGF-D and HIF-1α expression in invasive breast ductal carcinoma[101] and in resected esophageal squamous cell carcinoma.[102]

These findings revealed that expression of lymphangiogenic factors is tightly linked to hypoxia, which activates their expression at both transcriptional and translational levels. It is now well known that, at least in solid tumors, hypoxia is a major component of the tumor microenvironment and induces critical changes in tumor cell metabolism, angiogenesis, and lymphangiogenesis.

Concluding Remarks and Perspectives

The lymphatic vasculature has long been considered the poor relation of the blood vasculature. Compared with the vascular network, which provides both oxygen and nutrients and is therefore obviously necessary for life, the lymphatic system appeared to be a less important vascular network. In addition, until

recently it was challenging to differentiate lymph from blood vessels due to lack of a specific marker. Recently, however, the lymphatic system has emerged as a crucial player during development and in adulthood. Although it is implicated specifically in chronic inflammatory and vascular pathologies (such as psoriasis and lymphedema), it is also able to interact with blood vessels in cancer. Indeed, recent studies have highlighted hypoxia-induced regulation of lymphangiogenic factors in the tumor microenvironent. Understanding the molecular regulation of lymphangiogenesis in a wide range of organs and pathologies might lead to new therapeutic solutions for diseases such as cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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