Significance and Molecular Regulation of Lymphangiogenesis in Cancer

Mihaela Skobe and Bronislaw Pytowski

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Abstract

Lymphatic dissemination of tumor cells involves invasion into tumor-associated lymphatic vessels, seeding of metastases in the lymph nodes, and, ultimately, delivery into the blood circulation and to distant organs. Tumor lymphangiogenesis is induced by factors released by tumor or stromal cells, such as macrophages, and facilitates metastasis by

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providing pathways for cancer cell spread. Vascular endothelial growth factors VEGF-C and VEGF-D are the most specific lymphangiogenic factors that mediate signals for lymphatic endothelial cell growth and migration by binding to and activating VEGFR-3 receptors. Extensive preclinical data in mouse tumor models with specific inhibitors of lymphangiogenic signaling pathways provided the impetus for clinical trials of such agents in patients. In clinical practice, the presence of tumor cells in sentinel lymph nodes is an adverse prognostic factor for patients with solid tumors and constitutes a major consideration in tumor staging. Lymphovascular invasion, lymphatic vessel densities, and the expression of lymphangiogenic factors are also strongly correlated with poor prognosis. Although lymphatic and blood vascular endothelium share many molecular features, they are structurally and functionally distinct and play very different roles in tumors. Here, we discuss the distinct functions and significance of the lymphatic vascular system in cancer.

Keywords
Tumor lymphangiogenesis · VEGF-C · VEGF-D · VEGFR-3 · Lymphatic vessels · Lymph node · Metastasis

Introduction

This chapter discusses tumor lymphangiogenesis, a process by which solid tumors induce the formation of new lymphatic vessels into peritumoral and tumor tissue from pre-existing lymphatic vessels. Tumor lymphatic vessels are involved in draining the tumor interstitial space of fluid, while also providing conduits for the traffic of immune cells from the tumor to draining lymph nodes. Lymphangiogenesis has also been implicated in tumor progression, primarily by facilitating the dissemination of tumor cells. As few nonspecialists are familiar with the unique biology of the lymphatic system, the beginning of this chapter provides a general introduction to its structure, function, and development as a foundation for the subsequent discussion of tumor lymphangiogenesis.

Normal Lymphatic Structure, Function, and Molecular Regulation

Functions of the Lymphatic Vasculature

Lymphatic vessels carry out several important functions, which broadly fall into two different categories: transport and regulatory functions. Lymphatics transport fluid, macromolecules, and immune cells from tissues back into the blood circulation. The endothelial lining of blood vessels must provide sufficient barrier functions to prevent the significant loss of plasma into tissues. However, blood vessel walls, particularly in capillaries, must also maintain sufficient plasticity to permit an increase in permeability in response to injury or infection, during regeneration of damaged vessels and angiogenesis. Furthermore, the endothelial lining of blood capillary walls must be sufficiently permeant to allow the bidirectional transport of gases, nutrients, and waste products. These opposing requirements necessitate a compromise between the barrier and transport functions of blood endothelium. The hydrostatic fluid pressure of blood varies depending on the type of blood vessel, but, even at the capillary level, significantly exceeds that of tissue interstitial fluid. As a consequence, the circulation in all vertebrates must be able to accommodate a degree of continuous, low-level leakage of plasma and tissue-derived proteins that result in the formation of interstitial fluid (Moore and Bertram 2018; Wiig and Swartz 2012). Lymphatic vessels mediate the return of excess interstitial fluid into the blood in the form of lymph and thus play a central role in maintaining tissue fluid and pressure homeostasis. Lymphatics also perform the important function of returning solutes and macromolecules that have leaked into the tissues back into the blood circulation. In humans, 8–12 L of protein-rich fluid that would otherwise accumulate in tissues is transported by the lymphatic

system daily (Scallan et al. 2016; Wiig and Swartz 2012). In addition, a unique system of lymphatic capillaries called the lacteals plays a vital role in the absorption and transport of dietary lipids. Triglycerides, absorbed into the lumen of the small intestine and packaged into chylomicrons, are transported by lacteals in the form of a substance called chyle to lymph nodes in the mesentery, and eventually into the blood circulation (Dixon 2010).

Another key role of the lymphatic vasculature is to transport soluble antigens and antigen-presenting dendritic cells from the tissue periphery to secondary lymphoid organs, where they interact with naïve T and B lymphocytes to allow the initiation of adaptive immune responses. Distinct T-cell subsets also traffic through the lymphatics and lymphatic endothelial cells (LECs) directly interact with T cells and dendritic cells to modulate their function. Furthermore, lymphatic endothelial cells help regulate innate and adaptive immune responses through the expression of cytokines, inhibitory receptors, and adhesion molecules.

While the vital role of blood circulation is apparent even to nonscientists, the importance of efficient lymphatic functioning is only revealed when the system is compromised by genetic errors, infectious agents, trauma, or surgery. Dysfunction of lymphatics in the peripheral tissues and extremities manifests itself as tissue swelling, known as lymphedema (Rockson 2001; Rockson et al. 2019). Lymphedema commonly leads to disability by inducing irreversible tissue fibrosis, chronic inflammation, and susceptibility to infections and represents a significant clinical problem. Dysfunctional lymphatics in internal organs compromise their function, leading to serious, often fatal, medical conditions.

Structural Features of the Lymphatic System

Lymphatic vessels or lymphatic-like structures have been identified in almost all organs, including, most recently, in the brain and eye (Aspelund et al. 2014, 2015; Louveau et al. 2015; Park et al. 2014; Petrova and Koh 2018). Lymphatics possess structural features that are distinct from those of blood vessels (Fig. 1) and exhibit unique characteristics depending on their location along the lymphatic vascular tree. The uptake of interstitial fluid occurs in lymphatic capillaries, which are

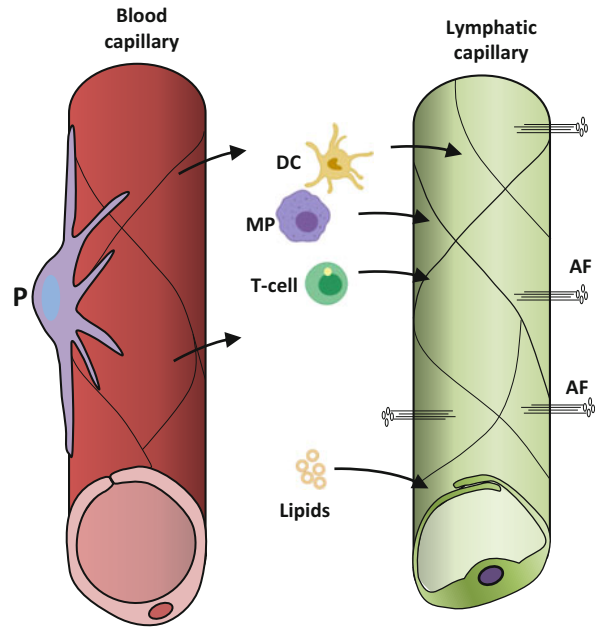

Fig. 1 Lymphatic capillary structure and function. Lymphatic capillaries have thin endothelium, overlapping junctions, irregular-shaped lumen and lack pericytes. Anchoring filaments (AF) connect LECs directly to the interstitial collagens. Lymphatic capillaries are uniquely adapted for the uptake of fluid, macromolecules, lipids, and cells from the interstitium. *DC* dendritic cell, *MP* macrophage, *P* pericyte

blind-ended initial lymphatic vessels typically found in close proximity to blood capillaries. Lymphatic capillaries generally possess a wider and more irregular lumen than blood capillaries, and their endothelium is extremely thin. Diameters of lymphatic capillaries vary depending on the tissue and range from 20 to 300 microns. In contrast to blood capillaries, lymphatic capillaries have an incomplete basement membrane and are not invested by pericytes (Skobe and Detmar 2000). Lymphatic capillaries are also characterized by oak leaf-shaped endothelial cells that partially overlap and form flaps at sites of fluid entry (Leak 1971; Schmid-Schonbein 2003). Endothelial cells of lymphatic capillaries have unique junctions composed of VE-cadherin and tight junction-associated proteins that connect two overlapping cells in a discontinuous pattern. Discontinuous junctions in initial lymphatics are referred to as "buttons" in contrast to conventional, continuous junctions in blood capillaries, i.e., "zippers" (Baluk et al. 2007; Leak 1971).

Transient changes in pressure gradients across lymphatic vessel walls are thought to drive lymph formation (Breslin 2014; Moore and Bertram 2018; Wiig and Swartz 2012). An increase in interstitial fluid pressure causes the overlapping junctions to transiently open, thereby allowing the passage of fluid and particles into the vessel. As fluid enters the lumen, pressure differences across the vessel wall decrease, and the junctions begin to close, preventing retrograde flow back into the interstitium (Ikomi and Schmid-Schonbein 1996; Schmid-Schonbein 1990a). Lymphatic capillary function is critically dependent on its connections to the extracellular matrix. LECs are attached to interstitial collagen by anchoring filaments composed of elastic fibers (Gerli et al. 1990; Leak and Burke 1966), which allow lymphatics to directly sense biomechanical changes in the interstitium (Moore and Bertram 2018; Wiig and Swartz 2012). Lymphatic capillaries are frequently observed with closed or partially open lumina because intralymphatic fluid pressure is generally lower than the interstitial fluid pressure in the surrounding tissue (Aukland and Reed 1993; Schmid-Schonbein 1990b; Wiig and Swartz 2012).

Lymph is transported as a result of intrinsic and extrinsic pumping mechanisms (Moore and Bertram 2018; Scallan et al. 2016). From the initial lymphatics, lymph moves into collecting vessels, which are invested by smooth muscle and actively transport lymph. Intrinsic pumping involves the peristaltic contraction of smooth muscle that propagates along the lymphatic vessel wall, coordinated with the action of bicuspid luminal valves that prevent backflow. The segment of a collecting lymphatic vessel between two intraluminal valves is called a lymphangion. Contraction waves are coordinated over the length of a lymphangion, and lymph is transported in pulses from one lymphangion to the next. The driving force for extrinsic pumping includes the contraction of neighboring skeletal muscles or rhythmical pulsing of the adjacent artery. Together, these forces propel lymph along the coalescing branches of the lymphatic tree and into two great lymphatic ducts, the thoracic and right lymphatic duct, which exhibit an autonomous pumping motion and empty lymph into the blood circulation through the left and right subclavian veins in the neck. Since blood fluid pressure is greater than that of exiting lymph, specialized structures called lympho-venous valves at the lympho-venous junctions prevent the retrograde flow of blood into the ducts (Moore and Bertram 2018; Scallan et al. 2016; Zawieja 2009).

Molecular Regulation of Lymphangiogenesis: VEGF-C and VEGF-D

Physiological lymphangiogenesis, which occurs primarily during embryogenesis and postnatal development, is a tightly controlled process regulated by a number of sequential and cooperative molecular signals. Lymphangiogenesis in adults is largely restricted to wound healing and immune activation. However, lymphangiogenesis is also a major component of pathological processes such as chronic inflammation and cancer. Pathological lymphangiogenesis is mediated by highly perturbed signaling networks, leading

to the formation of lymphatic vessels with compromised organization and functional features.

The principal mediator of lymphangiogenesis is vascular endothelial growth factor C (VEGF-C). VEGF-C is the dominant ligand for the receptor tyrosine kinase (RTK) vascular endothelial growth factor receptor 3 (VEGFR-3), the only RTK whose expression in normal postnatal tissues is largely restricted to the lymphatic endothelium (Jeltsch et al. 1997; Joukov et al. 1996; Kaipainen et al. 1995). LECs can also express VEGFR-2, an RTK that is expressed by activated blood endothelium, and that, upon activation by the ligand VEGF-A, is a critical regulator of blood angiogenesis. The role of VEGFR-2, activated by either VEGF-A or VEGF-C in lymphangiogenesis, has been controversial (Tammela and Alitalo 2010). VEGF-C is expressed in all species possessing lymphatic vessels. The specificity and affinity of the binding of VEGF-C is dependent on tightly regulated proteolytic processing (Joukov et al. 1997). VEGF-C is secreted as a precursor protein in the form of an antiparallel dimer that must be processed in a highly conserved manner at both the N- and C-termini to acquire full function. Pro-peptides at the N- and C-termini are sequentially removed to yield active forms of VEGF-C. Partially processed VEGF-C homodimers are capable of activating VEGFR-3, but not VEGFR-2, and therefore specifically signal for lymphangiogenesis. Full proteolytic processing of VEGF-C enhances its affinity for VEGFR-3 and enables mature VEGF-C to also bind to VEGFR-2. Mature VEGF-C, therefore, has the ability to drive the growth of both lymphatic and blood vessels (Joukov et al. 1997; Sáinz-Jaspeado and Claesson-Welsh 2018; Zheng et al. 2014).

VEGF-D is a closely related ligand, whose processing and consequent receptor specificity parallels that of VEGF-C (Achen and Stacker 2012). The biological role of this cytokine has been difficult to elucidate, as in gene knockout experiments' VEGF-D is dispensable for the development of the lymphatic system (Haiko et al. 2008). The binding of VEGF-C or VEGF-D to VEGFR-3 induces receptor dimerization and leads to the phosphorylation of critical tyrosine residues on the cytoplasmic domain that, in turn, trigger downstream signaling events (Fig. 2). One key downstream event following

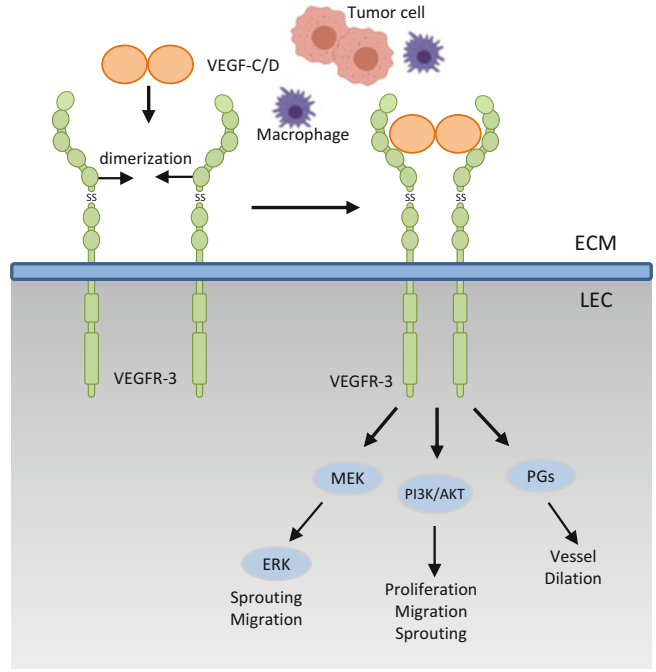

Fig. 2 VEGFR-3 signaling in lymphangiogenesis. VEGF-C and VEGF-D derived from tumor cells or inflammatory cells, mainly macrophages, activate VEGFR-3 and initiate signaling cascade leading to lymphatic endothelial proliferation, migration, and vessel dilation

VEGFR-3 activation is the phosphorylation of the serine/threonine kinases AKT and ERK, which mediate migration, survival, and proliferation of LECs (Davydova et al. 2016; Karaman et al. 2018).

Embryonic Lymphangiogenesis

Lymphatic vasculature develops primarily from veins during embryonic lymphangiogenesis (Makinen et al. 2007; Yang and Oliver 2014). Endothelial cells in the veins of the embryo express large amounts of VEGFR-3, in contrast to adult blood endothelium that does not express this RTK. During embryonic days 9.5–10.5 in mice, or approximately days 45–50 in humans, VEGFR-3-positive endothelial cells (ECs) of the cardinal vein begin to express the lymphatic vessel hyaluronan receptor-1 (LYVE-1), heralding the start of developmental lymphangiogenesis. The process is initiated when the expression of the transcription factor SOX18 is induced in the VEGFR-3/LYVE-1 positive ECs of the cardinal vein. SOX18 induces the expression of the transcription factor Prox1, a critical factor in determining lymphatic endothelial identity. The sprouting of lymphatic capillaries from the cardinal vein is initiated in response to VEGF-C produced by mesenchymal cells (Srinivasan et al. 2007). The crucial role of Prox-1 in this process is evidenced in embryos of Prox1-deficient mice that are not viable and completely lack lymphatic vasculature (Wigle and Oliver 1999). Concomitantly with the appearance of the first lymphatic endothelial precursor cells, VEGFR-3 expression is downregulated in embryonic blood vessels. The final step in developmental lymphangiogenesis is a separation of the blood and lymphatic vascular systems. This process is initiated when podoplanin, a mucin-type transmembrane glycoprotein expressed by newly differentiated LECs, binds to the C-type lectin receptor 2 (CLEC-2) on platelets, leading to platelet aggregation that blocks any remaining lympho-venous connections (Tammela and Alitalo 2010; Welsh et al. 2016). The lymphatic system subsequently undergoes several maturation steps, including the formation of a differentiated network of capillaries and collecting lymphatic vessels containing intraluminal valves and smooth muscle cells (Mauri et al. 2018; Tammela and Alitalo 2010; Ulvmar and Makinen 2016).

Other Regulators of Lymphangiogenesis

While VEGFR-3 signaling is indispensable for lymphangiogenesis, other cytokine/receptor systems also influence this process. Key among them are the angiopoietin (Ang) and fibroblast growth factor (FGF) families of ligands and their cognate receptors (Saharinen et al. 2017a, b; Sáinz-Jaspeado and Claesson-Welsh 2018; Zheng et al. 2014). In humans, the Ang family has three members: Ang1, Ang2, and Ang4. Mice express a related gene, Ang3, in lieu of Ang4. Angiopoietins function by activating a receptor tyrosine kinase denoted Tie2 (Tek) that is principally expressed on endothelial cells of blood and lymphatic vessels. Genetic experiments in mice have elucidated critical and complex roles of the Ang/Tie system in the development and maturation of lymphatic vessels. Blocking Ang2 or Tie2 disrupts the integrity of LECs, inducing leakage of lymphatic vessels. This has been linked to the observation that transmembrane form of Ang2 can bind Tie2 on adjacent endothelial cells and that the formation of this complex is crucial for lymphatic junctional stability. LECs also express two members of the FGF receptor tyrosine kinase family, FGFRs 1 and 3. Activation of these RTKs in LECs induces signaling through PKB/AKT and ERK1/ERK2 pathways that mediate proliferation, migration, and survival. There appears to be considerable redundancy in the pro-lymphangiogenic RTK signaling since the same pathways are triggered in LECs by the activation of the VEGF-C/VEGFR-3 system (Tammela and Alitalo 2010; Zheng et al. 2014). In addition, hepatocyte growth factor (HGF) is a lymphangiogenic factor that exerts its action directly and indirectly (Cao et al. 2006; Gibot et al. 2016; Kajiya et al. 2009). The HGF

receptor c-Met is constitutively expressed by LECs in the skin, where HGF promotes lymphangiogenesis directly by activating c-Met signaling. HGF strongly stimulates LEC proliferation and tubulogenesis, but is less effective in stimulating LEC migration (Gibot et al. 2016). The effects of HGF on lymphangiogenesis may be different in various tissues, since, in the model of corneal inflammation, c-Met is not expressed by LECs and HGF seems to stimulate lymphangiogenesis indirectly (Cao et al. 2006).

Tumor Lymphangiogenesis and Lymphatic Metastasis

Tumor Lymphangiogenesis

VEGF-C and VEGF-D are the two most specific lymphangiogenic factors and play a central role in tumor lymphangiogenesis and metastasis (Karaman and Detmar 2014; Podgrabinska and Skobe 2014; Stacker et al. 2014). VEGF-C and VEGF-D are primarily released by cancer cells, but may also be produced by stromal cells, in particular, by macrophages and fibroblasts. The initial discovery that lymphangiogenesis occurs in tumors was made in 2001, when three groups concurrently reported that the overexpression of VEGF-C or VEGF-D in experimental tumor models leads to intra- and peritumoral lymphangiogenesis and that the induction of lymphangiogenesis by the tumor facilitates metastatic spread (Mandriota et al. 2001; Skobe et al. 2001; Stacker et al. 2001). It is generally assumed that lymphangiogenesis promotes metastasis by facilitating tumor cell access to lymphatic vessels. In addition, VEGF-C and VEGF-D drive the remodeling of collecting lymphatic vessels that lead to the lymph nodes. The enlargement of collecting lymphatics and remodeling of smooth muscle cells result in an increased flow rate, which may promote metastasis by enhancing the delivery of tumor cells to the lymph nodes (Harrell et al. 2007; Hoshida et al. 2006; Karnezis et al. 2012).

Numerous studies using murine tumor models have shown that the inhibition of lymphangiogenesis by the neutralization of either VEGF-C or VEGFR-3 reduces lymph node metastases (Brakenhielm et al. 2007; Burton et al. 2008; Chen et al. 2005; He et al. 2005; Kawakami et al. 2005; Krishnan et al. 2003; Lin et al. 2005; Roberts et al. 2006). Importantly, VEGFR-3 inhibition does not reduce primary tumor growth, indicating that the consequence of tumor lymphangiogenesis is primarily an increase in tumor dissemination. Consistent with these findings, overexpression of VEGF-C or VEGF-D in epithelial cancers promotes metastasis, but does not change primary tumor growth rate. VEGF-C also facilitates metastatic spread to distant sites and, consequently, blocking VEGF-C or VEGFR-3 inhibits distant metastases in the majority of experimental models (Brakenhielm et al. 2007; Burton et al. 2008; Chen et al. 2005; Krishnan et al. 2003; Lin et al. 2005; Podgrabinska and Skobe 2014; Roberts et al. 2006).

Despite similarities in structure and receptor specificity, there are differences in the function of VEGF-C and VEGF-D in tumors that are just beginning to be elucidated (Davydova et al. 2016). For example, VEGF-C promotes the expression of COX-2 in the endothelial cells of collecting lymphatic vessels, whereas VEGF-D does not. COX-2 is an enzyme involved in the biosynthesis of prostaglandins and contributes to the dilation of collecting lymphatic vessels and metastatic spread. Similarly, although VEGFR-2 and VEGFR-3 are both expressed by LECs, the function of VEGFR-2 and VEGFR-3 in tumor metastasis is strikingly different. Studies in mouse models of cancer have demonstrated that while blocking VEGFR-3 significantly inhibits lymph node metastasis, the blocking of VEGFR-2 does not (Roberts et al. 2006).

There are several additional pleiotropic growth factors that mediate tumor lymphangiogenesis, including FGF2, HGF, IL-1, and TNFα. Because these factors bind to various receptors on non-vascular cell types, and are not selective for lymphatic endothelium, it is difficult to discern whether their action on lymphatics is direct or indirect, through the upregulation of VEGF-C. TNFα and IL-1, for example, promote lymphangiogenesis by recruiting inflammatory

cells that secrete VEGF-C and VEGF-D (Kataru et al. 2009; Kim et al. 2014). HGF and FGF2 seem less effective as sole drivers of tumor lymphangiogenesis, but they may exert an impact by cooperating with VEGF-C or VEGF-D. HGF has been shown to exert synergistic and FGF2 additive effects on lymphangiogenesis in the presence of VEGF-C (Cao et al. 2012; Gibot et al. 2016).

Many tumors induce lymphangiogenesis at the tumor periphery and promote the enlargement of the lymphatic vessel lumen (Podgrabinska and Skobe 2014; Sleeman et al. 2009). These enlarged, peritumoral lymphatics are considered a major site of tumor cell entry. Intratumoral lymphangiogenesis is induced in some, but not all, tumor types, and intratumoral lymphatics are typically seen in hot spots rather than uniformly distributed throughout the entire tumor (Fig. 3). While hot spots may be found in various locations within the tumor, there may be large tumor areas completely devoid of lymphatics. In contrast, blood vessels are typically present throughout the tumor, although their densities vary. This difference in the spatial organization of lymphatic and blood vessels in tumors relates to the differences in their function, which is drastically distinct despite the fact that the endothelial biology of these two vascular systems is shared on many levels. Angiogenesis is a requirement for tumors to grow, and therefore blood vessels are found in all tumors. Because lymphatics are not essential for tumor growth, they are not ubiquitously found in tumors. Furthermore, although tumor lymphangiogenesis profoundly increases metastatic spread, it is not required for metastasis as tumor cells can also disseminate using pre-existing lymphatic vessels.

Lymphogenous and Hematogenous Pathways of Tumor Metastasis

Metastasis, the escape of tumor cells from the primary tumor and the seeding of new tumor lesions in distant organs, is the primary cause of death in cancer patients. The metastatic process involves a sequence of key steps that need to be completed for the successful formation of metastases (Fidler 2003; Gupta and Massague 2006; Lambert et al. 2017). Among these steps are the entry and egress of cancer cells to and from the vasculature. Tumor cells may leave the primary site by entering either lymphatic vessels (i.e., lymphogenous spread) or the blood vasculature (i.e., hematogenous spread) (Fig. 4). Hematogenous metastasis is initiated by the intravasation of tumor cells into postcapillary

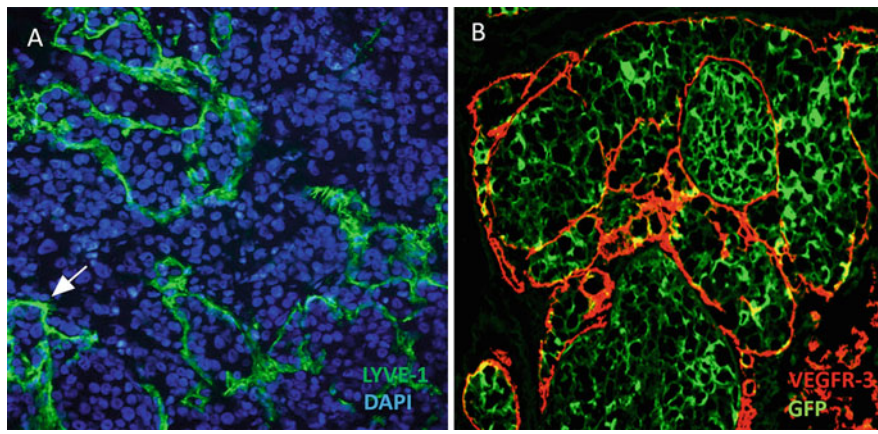

Fig. 3 Lymphangiogenesis in the primary tumor and in pulmonary metastases. (**a**) Immunostaining for lymphatic marker LYVE-1 (green, lymphatics) showing large lymphatics containing tumor cells in VEGF-C expressing primary tumors in a mouse xenograft model. (**b**) Spontaneous pulmonary metastasis from the same tumor. Immunostaining for lymphatic endothelial receptor VEGFR-3 (red, lymphatics) and GFP (green, tumor cell). Note that metastases are present exclusively within the lymphatic vessels. Arrow, lymphatic endothelium

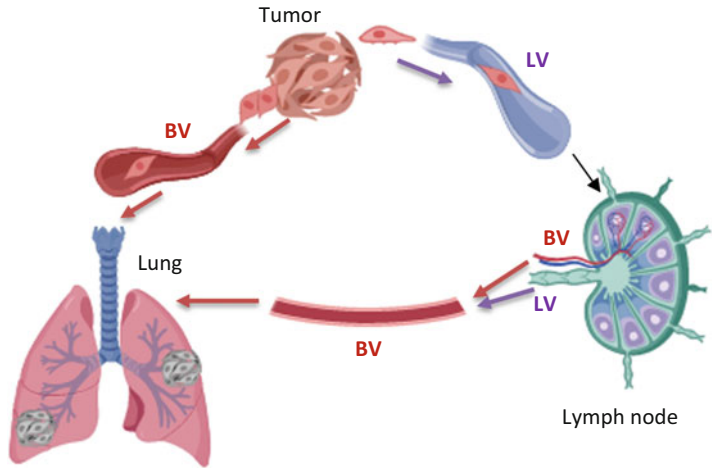

Fig. 4 Lymphatic and hematogenous pathways of metastasis. Tumor cells leave the primary tumor by entering into the lymphatic or blood vasculature. Lymphatic vessels deliver tumor cells first into the regional lymph nodes. Tumor cells can subsequently leave the lymph nodes through the blood or lymphatic vessels. Regardless of the initial path taken, lymphatic or blood, tumor cells ultimately reach distant organ by the blood vessels. *BV* blood vessel, *LV* lymphatic vessel

venules. Tumor cells are then transported via venous blood to a target organ that is a site of distant metastasis. Lymphogenous metastasis begins with the invasion of tumor cells into lymphatic capillaries and their subsequent transport into larger, collecting lymphatic vessels (Podgrabinska and Skobe 2014; Sleeman et al. 2009). Collecting lymphatics deliver the tumor cells into the draining lymph node through several afferent lymphatic vessels. Specifically, they converge onto the lymph node subcapsular sinus, which is lined by lymphatic endothelial cells. Tumor cells typically arrest and proliferate in the lymph node and may further disseminate by either lymph or venous blood. A single efferent lymphatic vessel transports the cells into the next lymph node in the regional cluster. The efferent lymph containing tumor cells eventually reaches great lymphatic ducts that deliver lymph contents into the venous blood. Therefore, cancer cells that first enter into the lymphatic vasculature at the primary tumor site are eventually delivered into the blood circulation and reach distant organs. It is important to recognize that circulating tumor cells detected in the blood may have originated from tumor cells that initially left the primary tumor by either the lymph or the blood. Thus, lymphogenous and hematogenous pathways of metastasis are intertwined and not mutually exclusive.

Traditionally, metastasis has been viewed as a unidirectional process, whereby tumor cells leave a primary tumor and seed metastases in regional lymph nodes or distant sites. Recent data, however, indicates that metastasis is multidirectional. This novel view, the self-seeding paradigm, implies that tumor cells may leave distant sites and reseed established metastases, as well return to their tumor of origin (Comen et al. 2011). Tumor cells in distant organ may further disseminate via lymph to form secondary metastases in lymph nodes or reenter into the blood to recirculate. Either scenario may lead to the establishment of novel metastatic foci. Migratory patterns of tumor cells, therefore, resemble the trafficking of leukocytes and hematopoietic stem cells through both the lymph and blood compartments.

Mechanisms of Lymph Node Metastasis

The invasion of tumor cells into lymphatic vessels is the first step on the path towards the lymph

node. Tumor cells can be guided into lymphatic vessels by subverting the process through which lymphatic endothelium guides leukocytes into these vessels (Ben-Baruch 2008; Das and Skobe 2008). For example, chemokine CCL21, a ligand for the chemokine receptor CCR7, is constitutively expressed by lymphatic capillaries (Kerjaschki et al. 2004; Podgrabinska et al. 2002; Shields et al. 2007a). CCL21 is immobilized by binding to sulfated proteoglycans within the extracellular matrix and forms steep gradients within the interstitium (Haessler et al. 2011; Schumann et al. 2010; Weber et al. 2013). These gradients induce a directed migration of dendritic cells towards lymphatics and may also attract tumor cells expressing the CCR7 receptor (Houshmand and Zlotnik 2003; Muller et al. 2001). CCR7 overexpression in melanoma has indeed been shown to increase lymph node metastasis in mouse tumor models (Takeuchi et al. 2004; Wiley et al. 2001; Zlotnik et al. 2011), and the correlation between CCR7 expression on tumor cells and lymph node metastasis has been demonstrated in various human tumors (Cabioglu et al. 2005; Ishigami et al. 2007; Mashino et al. 2002).

CXCL12 is another chemokine that has been shown to facilitate lymph node metastasis of tumor cells that express its receptor CXCR4 (Muller et al. 2001; Uchida et al. 2007; Zlotnik et al. 2011). A large body of literature provides evidence for the importance of CXCL12 in directing the homing of $CXCR4^+$ cancer cells to the bone and lung (McAllister and Weinberg 2014; Zlotnik et al. 2011). CXCL12 is upregulated on lymphatic vessels in the primary tumor and has been shown to promote the recruitment of $CXCR4^+$ melanoma cells into the proximity of lymphatic endothelium. Several studies have demonstrated a correlation between CXCR4 expression by tumor cells and lymph node metastases. However, direct evidence for the role of CXCL12 in directing tumor cells into the lymphatic capillaries has not been demonstrated.

In addition to producing chemokines that recruit tumor cells positioned in the vicinity of lymphatics into the lymphatic vessels, LECs can also help generate chemokine gradients around tumor cells that help direct them towards lymphatic vessels from greater distances. By draining fluids from the tumor tissues, lymphatics generate interstitial flow at velocities of 0.1–0.8 micron/s. This slow flow creates steep gradients of the CCL21 around the tumor cell that is secreting this chemokine. The same tumor cell expressing the corresponding chemokine receptor migrates along this chemokine gradient and is thereby directed towards the lymphatics. This mechanism, where interstitial flow creates and amplifies autocrine chemokine gradients to direct cells towards lymphatics, is termed autologous chemotaxis (Shields et al. 2007b). These findings underscore the importance of the biophysical microenvironment, created by the normal lymphatic function of transporting fluids, for homing of tumor cells to lymphatics.

The cellular mechanism of tumor cell intravasation into lymphatic vessels remains elusive. Although it has been assumed that tumor cells enter through intercellular lymphatic junctions, there is no direct evidence to support that concept. Furthermore, there has been a long-standing misconception that lymphatic capillaries are highly permeant and thus more easily penetrated by tumor cells compared to blood capillaries. On the contrary, studies indicate that the entry of cells into the lymphatic vessels is a process tightly controlled by LECs themselves and by signals in the microenvironment.

Conventional wisdom suggests that tumor cells are delivered into the sentinel lymph nodes passively, with the flow of lymph. This has indeed been demonstrated for cell transport within large, collecting lymphatic vessels (Dadiani et al. 2006; Hayashi et al. 2007), where flow velocities of up to several mm/min have been recorded (Dadiani et al. 2006; Swartz 2001). Lymph flow velocities in lymphatic capillaries, however, are much slower, ranging from 60 to 180 μm/min (Berk et al. 1996; Swartz et al. 1996). Interestingly, dendritic cells have been shown to crawl along the luminal side of LECs in lymphatic capillaries (Tal et al. 2011), opening up the possibility that tumor cells may exhibit a similar behavior.

Afferent collecting vessels deliver lymph content into the LN. The subcapsular sinus (SCS) of the LN, which is lined by LECs, is the first port of entry into the LN and first site of lymph node metastasis (Carr 1983; Carr et al. 1985; Dadiani et al. 2006; Das et al. 2013; Dewar et al. 2004). The presence of tumor cells induces the dilation of the SCS, which begins at the junction with the afferent lymphatic vessel (Fig. 5). Sinus dilation precedes the arrival of tumor cells (Das et al. 2013) and may be a prerequisite for the entry of tumor cells into the SCS. Indeed, when tumor cells are injected directly into the lymphatic system of a mouse in the absence of a primary tumor, the tumor cells arrest at the junction of the afferent lymphatic vessel and the LN (Hayashi et al. 2007). Scanning electron microscopy analysis revealed that the SCS is divided vertically and horizontally into smaller compartments, resulting in passages 5–15 microns wide (Das et al. 2013; Ohtani and Ohtani 2008). Since the diameter of a single circulating tumor cell is at least 15 microns (Vona et al. 2000), it has been concluded that the small dimensions of the sinus prevent the passive flow of tumor emboli into the SCS (Das et al. 2013). The chemokine CCL1 has been shown to be important for the entry of tumor cells into the LN. CCL1 is produced by the LECs of the subcapsular sinus and facilitates the entry of $CCR8^+$ tumor cells into the sinus and across the lymphatic endothelium into the LN cortex. Conversely, blocking CCR8, which is expressed in a large subset of melanomas, leads to the arrest of tumor cells at the junction of the afferent lymphatic vessel and the LN (Das et al. 2013). These studies demonstrate that the LECs of the SCS regulate the entry of tumor cells into the lymph node and thereby identify entry into the lymph node as another rate-limiting step in the metastatic process.

Lymphangiogenesis in the Lymph Nodes

The activation of the lymphatic vasculature is not restricted to the microenvironment of the primary tumor. Lymphangiogenesis has also been observed within sentinel LNs (SLNs) in many cancer types, including breast cancer and melanoma. It has also been documented within metastases in the sentinel and more distal lymph nodes (Kerjaschki et al. 2011). Interestingly, lymphatic remodeling and expansion in sentinel lymph nodes has been shown to precede lymph node metastasis (Harrell et al. 2007; Hirakawa et al. 2005, 2007; Ruddell et al.

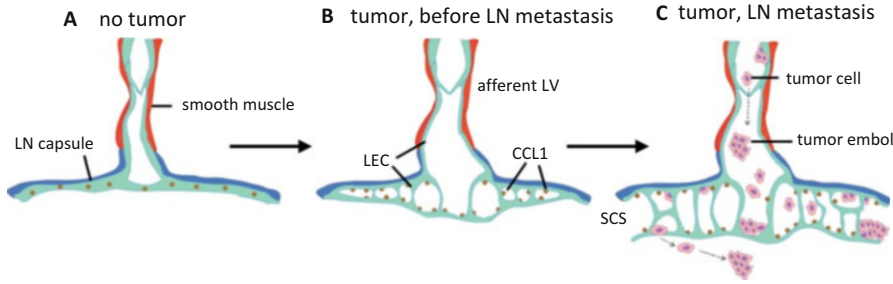

Fig. 5 Steps of tumor cell entry into the lymph node. (**a**) Afferent lymphatic vessel (LV) delivers lymph into the subcapsular sinus (SCS) of the lymph node (LN). In the normal setting, without a tumor present, SCS appears closed. (**b**) In the presence of a tumor, afferent lymphatic vessel leading into the sentinel lymph node becomes dilated, and SCS begins to open starting at the junction of the afferent LV and the LN. (**c**) In the collecting lymphatic vessels, tumor cells are carried passively with the flow of lymph towards the LN, both as single cells and clusters (emboli). Active cell migration is required for tumor cell entry into the sinus, which is wide open, and subsequently across the floor of the sinus into the lymph node cortex. For tumor cells expressing chemokine receptor CCR8, entry into the SCS and LN cortex is regulated by the CCL1 chemokine expressed constitutively by the LECs of the SCS. Tumor cells can also attach to the luminal side of the lymphatic vessel and to the LECs of the sinus and continue to grow and form intravascular lesions.

2008; Van den Eynden et al. 2006, 2007). Expanded lymphatic networks in the LNs have been suggested to represent a pre-metastatic niche that promotes the colonization of LNs by metastatic cells. However, because the selective inhibition of lymph node lymphangiogenesis is difficult to achieve experimentally, this conclusion is largely based on correlative studies. The extent to which LN lymphangiogenesis plays a role in cancer metastasis remains to be elucidated.

The mechanisms mediating lymphatic vessel expansion within tumor-draining LNs are not completely understood. Lymph node lymphangiogenesis may be, in part, driven by VEGF-C produced by cells of the immune system in the LNs, primarily B cells (Jones et al. 2018). Lymphangiogenic factors produced by the primary tumor may be transported into the SLNs via lymph and act directly on pre-existing lymphatic sinuses (Hirakawa et al. 2005, 2007). Once metastases have formed in the LNs, tumor cells may represent a major source of lymphangiogenic factors. Of note, LN lymphangiogenesis is not unique to cancer, as it also occurs during inflammation and is a component of a normal immune response (Angeli et al. 2006; Kim et al. 2014).

Lymph Node Metastasis Is an Important Prognostic Indicator

Clinical data has unequivocally established that lymph node metastases correlate with poor outcome. The status of regional lymph nodes is, therefore, an important parameter used for determining the stage of disease progression and treatment options. The first lymph node to receive lymph from the primary tumor is defined as a sentinel lymph node (SLN). Sentinel lymph node biopsy procedure involves the removal and examination of SLNs for the presence of tumor cells and is a standard of care for most cancers. SLNs are typically identified by preoperative lymphoscintigraphy and by intraoperative staining after blue dye injection at the primary tumor (Cochran et al. 2000; Morton et al. 1992). The SLN biopsy is performed based on the assumption that if sentinel lymph nodes are free of metastases, other lymph nodes will also be unaffected and their removal is not medically indicated. Indeed, if the SLNs do not contain cancer cells, other regional lymph nodes are almost certainly free of metastases, and the risk of having distant metastasis is low, indicating an excellent prognosis. Alternatively, if the biopsy shows the presence of metastases in the SLN, additional lymph nodes may be removed for diagnostic purposes. The number of lymph nodes involved and the presence of micro- versus macro-metastases influence decisions about the choice of postsurgical (adjuvant) therapy. Sentinel lymph node biopsy is the standard of care for melanoma, breast, prostate, and colon cancer, among others, and the presence of tumor cells in the SLN continues to be one of the most important prognostic indicators of patient survival.

Lymphovascular invasion (LVI), detected histologically by the presence of tumor cells within lymphatic vessels in the tumor microenvironment, is another important parameter in assessing the risk for tumor metastasis. LVI in a primary tumor indicates a significantly increased risk of lymph node involvement, distant metastases, and disease relapse and thus leads to an unfavorable prognosis in melanoma, breast, gastric, bladder, prostate, and many other cancer types (Dicken et al. 2006; Hoda et al. 2006; Lee et al. 2006; Lotan et al. 2005; May et al. 2007; Straume and Akslen 1996). In lymph node-negative cancers, LVI is an independent adverse prognostic factor for metastases to regional and distant sites.

Historical Perspective on Lymph Node Metastasis

French surgeon Henry LeDran (1684–1770) was the first to recognize the importance of cancer dissemination to the lymph nodes. He noted that cancer begins as a local disease that first spreads to lymph nodes and subsequently to distant organs. LeDran also observed that a surgical cure was much more likely when lymph nodes did not contain cancer cells. His theory offered the hope

that there may be a cure for the disease if surgery is performed sufficiently early (Rayter 2003). Building on this premise, William Halsted, an American surgeon, believed that the removal of the whole breast and associated lymph nodes would prevent malignant spread of breast cancer. He introduced radical mastectomy and lymphadenectomy as standard surgical treatments for breast cancer. This approach was later abandoned because the majority of patients relapsed despite the extremely aggressive surgery and suffered from significant morbidity. Lymphadenectomy – the partial or complete removal of regional lymph nodes – has continued to be a standard surgical practice for cancer management. Whether a complete regional lymphadenectomy in patients with metastatic disease in lymph nodes provides benefits in terms of patient survival, however, remains controversial. Many clinical studies have shown that patients with melanoma, gastric, colon, and prostate cancer who undergo extensive lymph node dissections have higher survival rates (Morton et al. 2006; Wu et al. 2006), yet other studies contradict these results (Bembenek et al. 2007; Hartgrink et al. 2004). Furthermore, lymphadenectomy is associated with a significantly increased risk of developing lymphedema, which is a chronic and disabling morbidity. Today, complete lymph node dissection is rarely performed.

Lymph Node Metastasis as a Source of Distant Metastases

While the presence of lymph node metastases is undoubtedly a strong negative prognostic indicator, the reason behind this association is unclear and remains a subject of significant controversy. This has led to the proposal of two alternative models. One model argues that lymph nodes are the first and critical site of metastasis, and the site from which tumor cells will further spread to distant organs. An alternative model posits that distant metastases arise independently of lymph node metastases and that the presence of metastases in lymph nodes only indicates that tumors have acquired the ability to metastasize. Indeed, distant organ metastases are occasionally detected despite the absence of tumor cells in the lymph nodes, although this clinical scenario is relatively rare.

Many studies have examined the evolutionary relationship between the primary tumor, lymph node, and distant metastases by phylogenetic analysis. If distant metastases are genetically more closely related to the clones in the primary tumor than to clones in the lymph nodes, distant metastases most likely originated directly from the primary tumor and independently of lymph node metastases. Conversely, if distant metastases are derived directly from lymph node metastases, they would be genetically more closely related to lymph node metastases than to the primary tumor. This would support a linear model, where tumor cells from the primary tumor first spread into the lymph nodes and subsequently to distant sites.

In this context, a recent study of colorectal cancer examined the origin of liver metastases through phylogenetic analysis (Naxerova et al. 2017). The authors found that in 35% of cases, lymph node and liver metastases shared a common clonal origin, indicating that liver metastases were derived from lymph node metastases. However, in 65% of the cases, lymph node and liver metastases arose from independent clones in the primary tumor, indicating that the seeding of lymph node and distant metastases developed in parallel and independently of each other (Naxerova et al. 2017). This study demonstrated that lymph node and liver metastases may have a common origin as well as arise independently from each other, thus reconciling the two seemingly opposing concepts. The relative contribution of lymph node metastases to the formation of distant metastases may be different in different types of cancer and for the specific distant organ. For example, liver is the most frequent distant organ site for metastasis of colorectal cancer. Since venous blood from the intestines reaches the liver directly through the portal vein, it is possible that liver metastases are preferentially seeded hematogenously (Naxerova et al. 2017). In contrast, cancer cells that egress from lymph nodes ultimately enter into the subclavian vein,

and the first capillary beds these cells encounter are in the lung. It is therefore plausible that lung metastases are more frequently seeded from the lymph nodes.

Recent studies of human cancers provide further evidence that tumor cells derived from metastatic foci in lymph nodes indeed contribute to distant organ metastases (Greaves and Maley 2012; Hunter et al. 2018; Marusyk et al. 2014; Nowell 1976). Furthermore, studies in mouse models have also shown that metastatic tumor cells can spread to distant organs from sentinel lymph nodes. These studies specifically demonstrate that tumor cells may exit lymph nodes via blood vasculature (Brown et al. 2018; Pereira et al. 2018).

Together, these studies suggest that the two models of metastatic dissemination likely represent extremes on a biological continuum. Even within the same patient, hematogenous spread may be a preferred pathway to certain organs (e.g., liver), whereas lymph nodes may be important hubs for spread to another organ (e.g., lung). The relative frequency and importance of the different pathways of metastasis in different tumor types will need to be established through additional large-scale studies of patient-matched primary tumors and metastases (Hunter et al. 2018).

Lymphangiogenesis in Target Organs for Metastasis

From a therapeutic perspective, it is critically important to understand what role lymphatics play in the formation and progression of distant metastases. Lymphangiogenesis can be induced in a distant organ that is a site of metastasis, such as the lung, and, in some patients, metastatic disease is characterized by the extensive involvement of lung lymphatics with cancer. This type of metastasis, referred to as pulmonary lymphangitic carcinomatosis, denotes the growth of metastases within pulmonary lymphatic vessels. Lymphangitic carcinomatosis has been observed primarily in patients with epithelial cancers, including breast, lung, gastric, pancreatic, and prostate cancer (Goldsmith et al. 1967; Janower and Blennerhassett 1971; Thomas and Lenox 2008; Tomashefski and Dail 2008). It is invariably associated with extremely poor prognosis, and most patients succumb to the disease within several months of diagnosis.

Lymphangitic carcinomatosis has a diffuse presentation and is very difficult to diagnose in patients using current imaging techniques. Approximately half of the cases of histologically proven pulmonary lymphangitic carcinomatosis present with normal radiographs (Janower and Blennerhassett 1971; Trapnell 1964). Because of these imaging limitations in patients and because an immunohistological evaluation of lung metastases is not commonly performed, the true incidence of lymphangitic carcinomatosis may be greatly underestimated. Indeed, imaging studies reported the incidence of this type of pulmonary metastases to be as low as 6%, whereas studies by pathologists reported it to be as high as 56% (Tomashefski and Dail 2008).

Data from one mouse model of spontaneous metastasis revealed that the overexpression of VEGF-C in tumor cells induced lymphangiogenesis in the lung and changed the pattern of metastases to pulmonary lymphangitic carcinomatosis (Das et al. 2010). The expansion of the pulmonary lymphatic network was accompanied by a dramatic increase in the size of metastases, which grew within the constraint of lymphatic vessel walls. VEGF-C was necessary for the manifestation of lymphangitic carcinomatosis, but not sufficient, since its overexpression alone did not induce lymphangitic carcinomatosis in all cancer cells tested. In agreement with these findings, another study using a mouse model with inducible VEGF-C expression in the lung found that increased pulmonary lymphangiogenesis promoted growth of metastases in the lung and dissemination to other organs (Ma et al. 2018). Together, with clinical observations, these experimental data demonstrate an unappreciated role of lymphatics in facilitating lung colonization.

Recent studies using the VEGFR-3 luciferase reporter mouse, which enables noninvasive whole-body imaging of lymphovascular niches,

revealed systemic lymphangiogenesis in lymph nodes and distant organs in tumor-bearing mice (Olmeda et al. 2017). Systemic induction of lymphangiogenesis preceded organ colonization, consistent with the role of lymphangiogenesis in the creation of pre-metastatic niches. Tumor cells at the primary site were the main source of the factors inducing systemic lymphangiogenesis, and this ability was attributed mainly to the pleiotropic factor midkine. Notably, different tumors showed preference for inducing lymphangiogenesis in different organs, suggesting that organotropism may also be influenced by the remodeling of distant vascular microenvironments by the tumor. Importantly, the metastatic capability of melanoma correlated with systemic lymphangiogenesis. Together, these findings provide evidence for the importance of systemic lymphangiogenesis in facilitating tumor metastasis.

Clinical Implications of Lymphangiogenesis

Prognostic Significance of Lymphangiogenesis in Human Tumors

Prognostic biomarkers are typically used to establish a statistical correlation between the levels of a particular marker in a patient's blood or tumor and the probability of disease progression, relapse, or overall survival, irrespective of treatment. Prognostic relevance of tumor lymphangiogenesis, as evidenced by an increase in lymphatic vessel density, has been investigated retrospectively in many types of human solid tumors. The availability of specific antibodies that recognize several lymphatic markers in immunohistological assays has made it possible to identify and quantify lymphatic vessels in tissues (Van der Auwera et al. 2006).

Podoplanin, also known as gp38, is a mucin-type glycosylated transmembrane protein that has been widely used as a marker for the identification of lymphatic vessels in many human tissues. Podoplanin is specifically expressed by lymphatic and not by blood vascular endothelium, but its expression is not restricted to the lymphatic vasculature. It is expressed by many other cell types, most notably on kidney podocytes, fibroblast-type reticular stromal cells in the lymph node, and by some tumor cells (Breiteneder-Geleff et al. 1997, 1999; Wicki et al. 2006). Another widely used lymphatic marker is LYVE-1 (lymphatic vessel endothelial hyaluronan receptor-1), a transmembrane glycoprotein ubiquitously expressed by lymphatic endothelium (Banerji et al. 1999; Jackson 2004). LYVE1 is also expressed by specialized blood endothelial cells such as liver sinusoids and by a subset of macrophages, and it can be downregulated by inflammation (Johnson et al. 2007; Lim et al. 2018b; Mouta Carreira et al. 2001). PROX1 (the prospero homeobox protein 1) is a nuclear transcription factor that defines lymphatic endothelial identity and is not expressed by blood endothelial cells (Escobedo and Oliver 2016; Wigle and Oliver 1999). The above markers accurately differentiate lymphatics from the blood vasculature, but because of their expression by certain non-endothelial cells, antibodies to lymphatic endothelial markers are typically combined with the pan-vascular marker CD31 to ensure endothelial identity.

Numerous clinical studies have reaffirmed the positive correlation between VEGF-C or VEGF-D, lymphangiogenesis, and adverse patient outcome. Lymphatic vessel density has emerged as a promising indicator of patient prognosis, showing high concordance with the incidence of regional and distant metastases, as well as poor survival in breast, lung, head and neck, colorectal, gastric, and endometrial cancers, among others (Beasley et al. 2002; Furudoi et al. 2002; Kyzas et al. 2005; Mohammed et al. 2007; Nakamura et al. 2005; Shields et al. 2004; Stacker et al. 2014; Van der Auwera et al. 2006; Zhang et al. 2017). The quantification of intratumoral and peritumoral lymphatic vessels in primary human malignant melanomas of the skin revealed that the extent of lymphangiogenesis was the most significant predictor of the presence of SLN metastases at the time of surgery and held higher

significance than tumor thickness (Dadras et al. 2005; Shields et al. 2004). Thus, the quantification of lymphangiogenesis as part of a routine pathological evaluation of tumor tissue has the potential of providing an important early prognostic marker that would be particularly beneficial for patients presenting with primary tumors, but without lymph node involvement.

Although VEGF-C and VEGF-D have both been correlated with adverse prognosis, these factors may exhibit different expression patterns in various human tumors types. For example, VEGF-C is highly expressed in head and neck cancer, whereas VEGF-D is not (Beasley et al. 2002; Pornchai et al. 2001). Conversely, VEGF-D, but not VEGF-C, was reported to be an independent predictor of poor outcome in epithelial ovarian cancer (Yokoyama et al. 2003). In breast cancer, both VEGF-C and VEGF-D are expressed and both are negative prognostic indicators (Nakamura et al. 2005; Wang et al. 2012). Overall, VEGF-C appears to be the more dominant lymphangiogenic and pro-metastatic factor as more studies reported a correlation of VEGF-C to poor prognosis.

Therapeutic Targeting of Lymphangiogenesis

Recognition of the importance of the lymphatic system in the pathology of cancer has raised interest in the possibility of developing anti-lymphangiogenic therapies. Preclinical studies strongly suggest a therapeutic utility of blocking lymphangiogenesis to down-modulate the rate of tumor spread. Conceptually, it is possible to delineate distinct clinical scenarios where such therapies may be useful: prevention of metastasis, slowing down the spread of existing metastases, and treatment of metastatic lesions within the lymphatic bed. These distinct, if related, scenarios are discussed below.

A subclass of tyrosine kinase inhibitors (TKIs) that are in clinical use as anticancer agents has been shown to inhibit the VEGFR-3 kinase. Typically, these compounds also inhibit other closely related RTKs such as VEGFR-2 and the PDGF receptors, and it is impossible to determine what clinical benefits, as well as toxicities, seen in cancer patients treated with RTK inhibitors stem from the anti-lymphangiogenic activity of these molecules. A more promising approach involved the development of targeted biologics. VEGF-C and VEGF-D emerged as initial targets based on the wealth of preclinical studies showing that the targeted inhibition of these molecules potently inhibited tumor lymphangiogenesis and lymphatic metastasis. In mouse tumor models, this initially involved the use of either monoclonal antibodies (mAb) to VEGF-C and VEGF-D or a trap macromolecule such as soluble VEGFR-3 (sVEGFR-3). Enhanced tumor lymphangiogenesis and accelerated metastasis induced by overexpression of VEGF-C or VEGF-D in human tumor xenografts were reversed by treatment with sVEGFR-3 or by the use of neutralizing mAbs to these growth factors. Further studies showed that metastasis could also be significantly reduced by downregulating VEGF-C either by co-expression of sVEGFR-3 as a transgene in human tumor cell lines, by injection of sVEGFR-3 cDNA in a viral vector, or by the use of small interfering RNAs(siRNA) (Stacker et al. 2014).

In the clinic, this approach was attempted with VGX-100, a selective anti-VEGF-C antibody. In the phase 1 trial (NCT01514123), VGX-100 was combined with the anti-VEGF-A antibody bevacizumab in the hope of a synergistic effect of a simultaneous blockade of VEGFR-2 and VEGFR-3 activation. Although treatment with VGX-100, either alone or in combination with bevacizumab, was well tolerated, major responses in patients with solid tumors were not observed (Falchook et al. 2014). Further development of VGX-100 in cancer has not been reported.

A more specific approach to selectively target VEGFR-3 required the development of antagonist mAbs to this RTK. In a preclinical model, treatment with an anti-murine VEGFR-3 antibody potently inhibited lymphangiogenesis in a wound regeneration model (Pytowski et al. 2005) and markedly reduced tumor lymphangiogenesis and lymphatic metastasis (Burton et al. 2008; Roberts et al. 2006). Based on these encouraging data, a fully human antagonist mAb to human VEGFR-3 was developed (LY3022856/IMC-3C5) (Persaud

et al. 2004). A phase 1 trial of this antibody was conducted in patients with advanced solid tumors (NCT01288989). The mAb was found to have an acceptable safety profile and limited activity in a subgroup of patients with colorectal cancer (Saif et al. 2016). However, clinical development of IMC-3C5 was discontinued.

Certain types of cancer exhibit high lymphatic vessel densities and prominent lymphovascular invasion and may be particularly amenable for treatment with anti-lymphangiogenic agents. Inflammatory breast cancer (IBC) is the most aggressive subtype of breast cancer, characterized by rapid, diffuse growth, extensive lymph node involvement, and frequent distant metastases. Skin edema and erythema are typically observed and related to extensive lymphovascular emboli in the dermal lymphatics. Intralymphatic tumor emboli are found in virtually all cases of IBC, and elevated levels of VEGF-C have been reported (Lim et al. 2018a). IBC has been hard to approach experimentally largely because of the lack of good animal models. However, it is tempting to speculate that anti-lymphangiogenic therapy may benefit IBC patients, but this remains unclear at this time.

Another tumor characterized by lymphangiogenesis, extensive intralymphatic emboli, and poor prognosis is cutaneous melanoma. Melanoma can also form cutaneous metastases, so-called "in-transit" metastases, which are clusters of tumor cells growing within the skin lymphatic vessels. The blockade of lymphangiogenesis in melanoma with VEGFR-3 antagonists has been attempted in murine tumor models with some success (Alitalo and Detmar 2011). Head and neck cancer also shows prominent lymphatic remodeling and lymphovascular invasion and may be particularly appropriate for future clinical trials of anti-lymphangiogenic therapy (Beasley et al. 2002; Kyzas et al. 2005).

Conclusions

A rapidly growing understanding of the biology of the lymphatic system and its role in cancer has catalyzed efforts to develop novel anti-lymphangiogenic therapies aimed at reducing metastatic tumor spread. While the specific targeting of lymphatic vessels in rodent tumor models of metastasis has shown promise, the critical difference between such models and the reality of human cancer imposes a formidable challenge to the design of clinical studies that, to date, have not progressed beyond phase 1 testing. Such hurdles notwithstanding, one can envision the use of anti-lymphangiogenic biologics, most likely in conjunction with chemotherapy or with other targeted agents, as part of neoadjuvant or adjuvant therapy, especially in patients whose tumors are not amenable to complete resection. Alternatively, an exciting possibility lies in combining pro- or anti-lymphangiogenic therapy with immunotherapy. Lymphatics play important roles in regulation of immune response and in preclinical models exhibit immunosuppressive as well as immune-activating functions. The answer to which combination approaches may be beneficial must await further research and clinical testing.

Cross-References

- ▶ Angiopoietins and TIE Receptors in Lymphangiogenesis and Tumor Metastasis
- ▶ Mechanisms of Tumor Angiogenesis
- ▶ The Role of the VEGF Signaling Pathway in Tumor Angiogenesis

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