Review Article

Pathophysiological and anatomical basis of lymphatic transit of cancer cells and role of the lymphatic system: a review of published literature

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Abstract: Cancer metastasis involves dissemination of malignant cells from the primary tumor, via local lymphatic vessels to gain access to systemic circulation, while evading the destruction by immune cells, followed by successful extravasation and initiation of malignant growth in the distant organ. Despite the obvious contribution of the lymphatic system to the metastatic spread of cancer, basic research regarding the mechanisms leading to tumor dissemination via lymphatic vessels has been limited for years. Recent identification of molecular growth factors of lymphangiogenesis have led to new advances in our understanding of the underlying mechanisms of tumor metastasis. These growth factors have enabled scientists to better identify peritumoral lymphatics and to visualize precisely the ingrowth of tumor cells into the lumen of lymphatic capillaries. Moreover, it has been shown that these molecular markers secreted from a primary tumor can induce lymphangiogenesis in close regional lymph nodes, even prior to tumor cells arrival, which may facilitate metastasis spread. Comprehensive research of the multiplex interactions between tumor cells, lymphatics and the immune system will be crucial to further enhance the development of therapeutic and prognostic approaches to cancer. This review presents the ontogeny and anatomy of the lymphatic vasculature, discuss the immunological, molecular and physiological control of lymphatic vessel function, and explore the contribution of the lymphatic system to the development of metastases.

Keywords: Lymph node metastasis; lymphangiogenesis; lymphatic vessels; lymphatic metastasis

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Introduction

Cancer metastasis, the main cause of cancer mortality, involves the dissemination of malignant cells from the primary tumor, via local blood or lymphatic vessels (LVs) to gain access to systemic circulation, while evading the destruction by immune cells, followed by successful extravasation and initiation of malignant growth in the distant organ (*Figure 1*) (1). Numerous studies have shown that most epithelial cancers primarily develop metastatic growth by propagating via LVs to the draining lymph nodes (LNs) and then via the bloodstream to distant organs. This explains the correlation between the detection of metastases within the LNs and the prognosis, that is at the basis of many therapeutic decisions (2). Despite the obvious clinical relevance of LN metastasis and its impact on survival, the mechanism leading to tumor spread via LVs is the result of a very complex and controlled system, that has remained

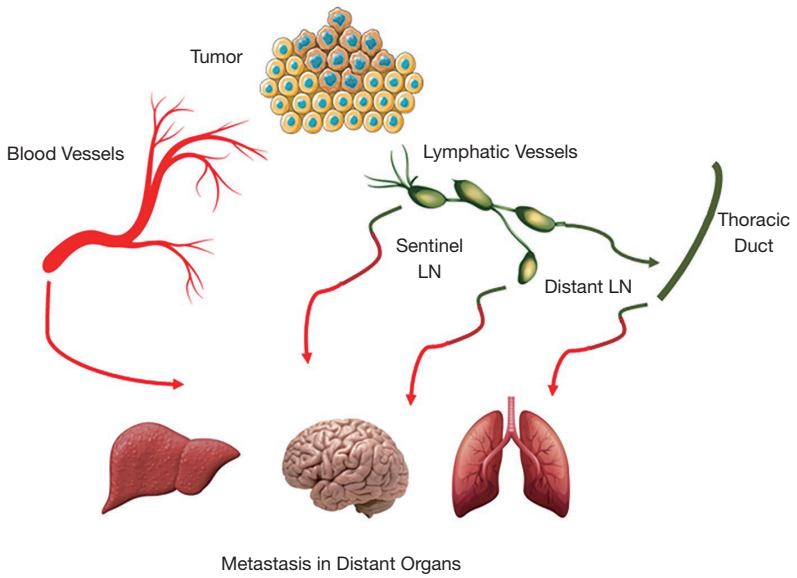

Figure 1 Current concepts of lymphatic and blood metastasis. Different pathways can result in cancer spread. Cancerous cells can invade the intra or peritumoral LVs and establish metastases in the nearby sentinel LN. Further metastatic dissemination occurs either from the sentinel LN to distant LN or through the thoracic duct to distant sites. Additionally, cancerous cells potentially spread through blood vessels with or without dropping by the sentinel LN to form metastases in distant organs.

mysterious for decades. Several factors lead to the limited comprehension of the importance of the lymphatic system in metastasis spread: (I) the paucity of molecular markers that reliably differentiate LVs from blood vasculature within and surrounding the primary lesion, (II) the shortage of suitable experimental models to study and quantify LN metastasis, (III) the limited scientific interest in LVs system as compared to blood vessels system. The structure, development, and function of the lymphatic vasculature has been the subject of extensive studies over the past two decades, fueled by detection of the primary lymphatic growth factors (3) and markers that help recognize LVs in tissue (4,5). These seminal findings have allowed the investigation of the lymphangiogenesis in the embryo (4), the growth, maturation, and function of the lymphatic system in the adult (6), the role of lymphatics in malignancy development (7,8) and this might ultimately lead to the identification of several molecules in the lymphangiogenic pathway as therapeutic targets (9). In this review, we aimed to explore the current understanding of the highly controlled and complex processes of the pathophysiology and the anatomical basis of the lymphatic system in normal and malignant processes.

The importance of the tumor microenvironment in metastasis

The genotypic and phenotypic make-up of a tumor is a major determinant of its metastatic potential, and a receptive microenvironment is necessary for successful tumor growth in the original cancer site and in the metastatic site (10,11). In 1889, Stephen Paget was the first to report that metastatic dissemination of malignancy to various organs is not a random process. Paget evaluated data from 735 postmortem patients with breast cancer, and recognized that metastasis is not due to chance events and he proclaim the "seed and soil" theory: tumor cells (the "seed") grow preferentially in the microenvironment of selected organs (the "soil") and that metastases resulted only when the appropriate seed was implanted in its suitable soil (12). In the subsequent years, numerous studies has supported Paget's assertion that the tumor microenvironment has an essential impact on regulating the growth of metastases (13,14). It has been shown that tumor invasion and metastatic dissemination are dependent on the cooperation of multiple congruent adhesion signals and molecular networks in the tissue. Cellular behavior and activation or inactivation of genes are influenced heavily by

Table 1 Differences in metastatic dissemination of gynecologic cancers

Type of cancer	Subtypes	Loco-regional spread pattern
Vulvar, endometrial and cervical cancers	Arise typically from pre-malignant lesions (18)	Loco-regional dissemination occurs principally by local invasion into the lymphatic system
		Process is faster for some high risk subtypes (19)
"Ovarian" cancer*	High-grade serous subtype (15)	Originates in the fallopian tube
		Shed in the peritoneal cavity causing carcinomatosis (20)
	Clear cell and low-grade endometrioid	Common precursor in the endometrium
		Up to 40% have somatic ARIDIa mutations (21)
	Low-grade serous ovarian cancers	Appear to initiate in the normal surface epithelium of the ovary (22)

*, the ovary and the fallopian tube are "inside-out" organs, where the epithelium faces the peritoneal cavity. Shed malignant tubal cells find a welcoming "soil" on the vascular and dynamic ovarian surface (20,23). The microenvironments of the abdominopelvic serosa, peritoneal mesothelium, and omentum are similarly favorable (16).

the local tumor microenvironment. This reinforces the idea that a complex interplay of molecules and signals is needed for metastasis development (11).

The microenvironment of the tumor sites has a high metabolic demand requiring adequate blood supply for nutrients, removal of waste, influx of immune and stromal cells, and ultimately as a conduit for lymph-hematogenous spread (15-17). Tumors have developed mechanisms to sustain themselves such as angiogenesis (the formation of new blood vessels), and lymphangiogenesis (the emergence of new lymphatic vessels from pre-existing lymphatics) (17).

Angio-regulatory factors are secreted by elements of the tumor microenvironment, leading to new vasculature that supports tumor survival and progression (16). Invasion, angiogenesis, lymphangiogenesis, and metastasis are thus controlled within the tumor microenvironment through a dynamic interaction between the tumor cells, the extracellular matrix (ECM), stromal and immune cells, and secreted chemokines and growth factors (16,17).

Differences in metastatic dissemination of gynecologic cancers are listed in *Table 1*.

The lymphatic system

The lymphatic system is a network of LVs, lymphoid organs, and lymphoid tissues that assist the organism to get rid of toxins and undesired materials (24). The primary function of the lymphatic system is to transport lymph, a fluid containing immune cells, proteins and excess interstitial fluid, throughout the body (25). Although the lymphatic and blood vascular systems are structurally two different systems, they are functionally interconnected and act in harmony to maintain tissue homeostasis. The blood vasculature contains a basal membrane, pericytes surrounding the endothelial cells, and smooth muscle cells in larger vessels (26). On the other hand, the lymphatic system consists of the lymphoid organs such as spleen, thymus, LNs, bone marrow, and Peyer's patches that are connected by LVs (24). Unlike blood vessels, LVs are consisting of a single layer of lymphatic endothelial cells (LECs), which is not surrounded by a basement membrane, pericytes, or smooth muscle cells. Instead, these blinded ending vessels are lined with a single layer of overlapping endothelial cells that form loose intercellular junction. These lymphatic capillaries are highly permeable to migrating cell, macromolecules, and different pathogens (27).

The lymphatic system in numerous ways complements functions of the blood vascular system by enhancing interstitial protein transport, regulating tissue fluid balance, and implementing immunological functions. Generally, fluid and plasma proteins that leak out of the venules to the tissue, and can not be reabsorbed directly, are returned back into the circulation as an act of the large lymphatics. Lymph returns to the venous circulation through the thoracic duct draining into the subclavian vein. Lymphatic flow is driven mainly by arterial pulsations, contraction of smooth muscle cells lining the large collecting LVs, and the action of neighboring skeletal muscles (28) (*Figure 2*).

Ontogeny of the lymph vessels

Historically, two hypotheses have been proposed on the embryonic origins of lymphatic development: the first

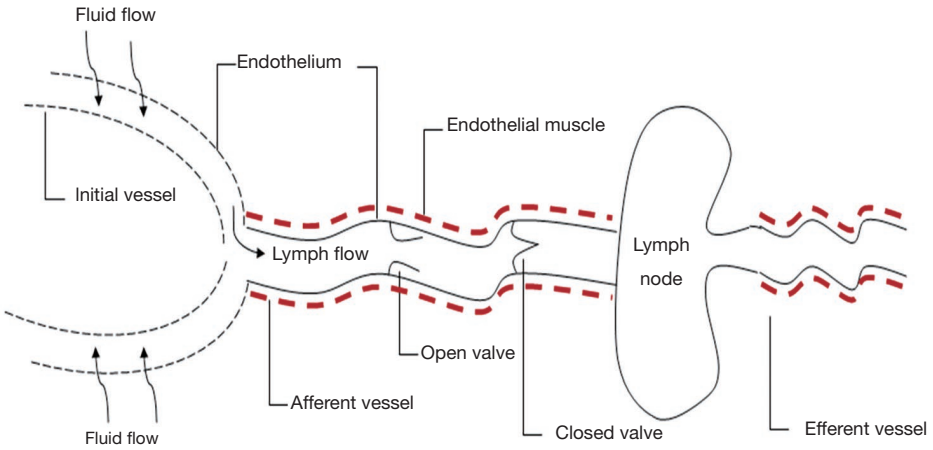

Figure 2 Anatomy of the lymphatic network. The lymphatic network is composed by initial LVs where the interstitial fluid is collected to create lymph, collecting afferent LVs which carry fluid to LN and efferent LVs which carry fluid out of the LN either to the next node in the chain or to the blood system. The collecting LVs are surrounded by a single layer of smooth endothelial muscle cells that contract to drive flow. Lymph backflow is prevented by intraluminal valves in the LVs.

based on lymphangioblasts, the second on embryonic veins. In the first model the primary lymphatics develop from specific precursor cells—lymphangioblasts, independently from veins, and only later linkage with the venous system is established (29). The alternative and most widely agreeable hypothesis is that the lymphatics develop from embryonic veins. This theory was proposed in 1902, by Florence Sabin (30) who speculated that the peripheral lymphatic system develops from the primary lymph sacs, originating from vascular endothelial cells, and then spreads by endothelial sprouting to form capillaries.

Minimal progress had been achieved since then, due to the paucity of molecular markers that reliably differentiate lymphatics from blood vasculature. A major breakthrough was the discovery of a lymphatic-specific marker in 1999, named the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) (4). It provided a precise tool to specifically recognize LVs in a variety of tissues, leading to investigations of tumor-associated lymphatics (31,32). Another step forward was the detection of the lymphangiogenic vascular endothelial growth factors VEGF-C and VEGF-D and later the discovery of their receptor VEGFR-3 (32,33). Additionally, the identification of the membrane glycoprotein "podoplanin" (34) and the transcription factor prospero-related homeobox 1 (PROX1) (5) which are highly expressed by LVs but not blood vessels, and the discovery of an antibody that specifically detects human podoplanin (35), expedited robust research of LVs in the cancer field (*Table 2*).

Embryologically, lymphangiogenesis starts with the expression of Lyve-1 by lymphatic capillaries in venous endothelial cells of the cardinal vein (43), leading endothelial cells to become responsive to lymphatic signals such as Prox-1 (39), podoplanin (34) and VEGF-C (44,45) (secreted mostly by immune cells such as macrophages, dendritic cells, and neutrophils), giving rise to the lymphatic vasculature. Remodeling and maturation of the initial LV network into lymphatic capillaries and collecting LVs, are mediated by multiple factors, such as the transcription factor forkhead box protein C2 (Foxc2) (46) , the non-kinase receptor neuropilin-2 (47), the growth factor angiopoietin-2 (48), and the Eph receptor ligand ephrinB2 (49). This process requires the growth of new lymphatic capillaries from pre-existing ones, the acquisition of mural cell coverage, and the development of valves within collecting LVs walls.

Lymphatic metastasis

LVs represent the routes for trafficking through the body of reabsorbed immune cells, but malignant cells are exploiting these routes to travel via to the nearby sentinel LN, regional distal LN, and then to distant organs (1). The extent of LN involvement is a key prognostic factor for the outcome of the disease and often guides therapeutic decisions (50).

Lymphatic metastasis, historically thought to be a passive process, is now known to be a highly regulated process at

Table 2 Essential discoveries in lymphatic vessel development and molecular markers that facilitate differentiating between blood and lymphatic vessels

Marker/Factor	Function	Year	Reference
VEGF-C- VEGF-D	Stimulates both, VEGFR-2 and VEGFR-3, leading to angiogenesis and lymphangiogenesis	1996	Paavonen (36)
LYVE-1	Modulates the transport of hyaluronan by LECs	1999	Baneji (4)
LyP-1, Nrp2	Coreceptors for VEGF-C in lymphatic vessels	2002	Laakkonen (37)
			Alitalo (38)
PROX-1	Essential for embryonic lymphatic development	2002	Wigle (39)
PODOPLANIN	Co-expressed with VEGFR-3 in initial lymphatic vessels	1999	Breiteneder-Geleff (34)
COX-2	Enhances the expression of sema7a leading to the activation of β1-integrin receptors leading to increased lymphangiogenesis	2016	Black (40)
TNF-α	Stimulates lymphangiogenesis depending on the VEGF-C/VEGFR-3 induced LEC tip formation which is required	2002	Ji (41)
CCL19 and CCL21	Modulates migration of dendritic cells to the regional LNs	1998	Baggiolini (42)

different levels, including the movement of cancerous cells towards LVs and the efficient penetration to the lymph system (51).

Most malignant epithelial tumors are surrounded by peritumoral lymphatics (52). The hydrostatic pressure differences following the cancer cells invasion to the ECM, result in fluid flow that transport cells to the peritumoral lymphatic capillaries. Cancer cells invasion, motility surrounded by ECM, and movement toward the lymphatic capillaries are driven by several factors including proteolytic enzymes' secretion, expression of adhesion receptors, and chemokinesis (17). Upon tumor cells arrival to the adjacent lymphatic capillaries, fine cytoplasmic processes mobilize the cancer cells along the external surface of the endothelium. Cells' migration toward the lymphatics and invasion into the lumen are carried out either by inducing the opening of closed gaps or through open inter-endothelial gaps (19,53).

Intra-tumoral interstitial fluid pressure increases as tumors grow in size (19,50,54), leading to lymph flow to be in the direction of the peritumoral lymphatics, resulting in an increase in the volume of the interstitial fluid. Once access to the lymphatic lumen is gained, smooth muscles in the walls of the lymphatic vessels pump lymph rhythmically, with the assistance of the placed valves that prevent backflow (19,55), leading the cancer cells to embolize alone or in clusters to the sentinel LNs (*Figure 2*). Through afferent lymphatics, tumor cells pass the subcapsular sinus of the LN and then either cells pervade into the cortex of the node, bypass it via lymphatic-venous connections (56), or bypass the node completely and progress directly into the efferent lymphatics to the next LNs (57,58).

Patterns of lymphatic metastasis

Apparently, the "seed" and the "soil" process is likely determined by several factors including molecular alterations within the tumor microenvironment, acquired mutations promoting invasion, molecular interplay between tumor cells and blood and lymphatic vasculature and ultimately the anatomical and molecular characteristics of the target tissue (19,59,60). While direct hematogenous spread can possibly happen, the robust association between tumor lymphangiogenesis and distant metastasis formation favors a sequential model of metastasis evolution where tumor cells spread first to the lymphatic system and from there into blood system either directly or ultimately through the thoracic duct (19,61) (*Figure 2*).

Brown *et al.* (62) recently confirmed this sequential model in mice. He showed that metastatic tumor cells migrate to the sentinel LNs and then disseminate to distant sites by gaining access to blood vasculature. Using genomic analyses to reconstruct clonal evolution of primary tumor and metastasis, from a single prostate cancer patient, Hong *et al.* (59) showed complex pattern of metastatic dissemination that include recolonization of the primary tumor and cross-colonization between different metastases. In melanoma, when the timing of metastatic

seeding was examined in relation to tumor thickness and specific genomic alterations, it was shown that lymphatic involvement takes place shortly after the dermis is invaded by the primary tumor and that typical driver mutations can be acquired by tumor cells within the LN. Therefore, melanoma cells can leave primary tumors early and then grow in parallel at different sites such as the LN (63). Unlike early metastasis that may result in an independent simultaneously growth of the cancer in different locations, late metastasis may evolve in a more linear pattern and gradual acquisition of additional genetic mutations (59).

The metastatic spread through the LVs to regional LNs in many cancers is considered the first distant development beyond local advancement, and therefore represents an important sign of the metastatic potential of the tumor (64,65). In some tumors such as colorectal cancers, LN dissection appears to have therapeutic value and provide overall survival benefit, and LN removal remains an important determinant for prognosis despite that most of these tumors spread directly to the liver via the venous system (66). The impact of lymphadenectomy on prognosis is still subject of debate in most cancers, including gynecologic cancers, failing to show any survival advantage of the removal of LNs (67).

Molecular regulation of LN metastasis

Numerous signaling molecules cooperate in the highly complex regulation of lymphangiogenesis (3,68) (*Table 2*).

(I) The first major family of signaling pathways that dominate the lymphatic endothelial cell biology is ANG-TIE (angiopoietin-tyrosine kinase with immunoglobulin and epidermal growth factor homology domain). Angiopoietin molecules have an essential role in stimulating postnatal vessel growth, remodeling, and maturation (37,69).

(II) The second is the VEGF-VEGFR (vascular endothelial growth factor-VEGF receptor) family. VEGF-C (36) was the first growth factor that was found to induce lymphangiogenesis, in addition to its important role in angiogenesis (70,71). The "mature" form of VEGF-C activates VEGFR-2 as well as VEGFR-3, leading to angiogenesis and lymphangiogenesis, whereas the "immature" form preferentially binds and activates VEGFR-3 only, and specifically induces lymphangiogenesis (72). Structurally, VEGF-C resembles vascular endothelial growth factor-D (VEGF-D), which also binds to and stimulates VEGFR-2 and VEGFR-3 in a similar manner and induces angiogenesis and lymphangiogenesis (33,73). VEGF- C expression has been reported in several studies to be correlated with metastatic spread to regional lymph nodes including in breast (74), colon (75), lung (76), and gynecologic malignancies (77-81).

(III) Another group of chemoattractant cytokines (CC) called "Chemokines" is involved in the lymphangiogenesis process. "Chemokines" is a family of more than 40 CC that bind to G-protein–coupled receptors on target cells to enhance cytoskeletal rearrangement, firm adhesion to endothelial cells, and directional migration (42,82). Chemokines have an important role in the regulation of both, physiologic and pathologic lymphocytic cell traffic (83). Chemokine ligand (CCL19) is secreted by dendritic cells. Majority of T-cells that present chemokine receptor 7 (CCR7) are responsive to CCL19 secretion and follow a gradient in its concentration. CCR7 receptor and its ligands CCL19 and CCL21 are crucial in the travel of dendritic cells to the regional LNs and when their interaction is blocked, dendritic cells fail to migrate (84).

(IV) Tumor necrosis factor-alpha (TNFα) is a cell signaling protein involved in systemic inflammation and is one of the cytokines that make up the immunologic acute phase reaction. It is produced mainly by activated macrophages (85). VEGF-C secretion by tumor-associated macrophages is triggered by the interaction between TNFα and its TNF receptor 1 (TNFR-1), leading to amplification of lymphatics expansion and metastasis (41). Furthermore, TNFα induced lymphangiogenesis significantly relies on the VEGF-C/VEGFR3-induced LEC tip formation which is mandatory to activate fibroblast growth factor (FGF2) that stimulates lymphangiogenesis and promotes tumor metastasis (86). On the other hand, proangiogenic factors such as angiopoietins (87) and platelet derived growth factor B (88) serve as direct lymphaniogenic factors by binding to their specific receptors expressed by LECs.

(V) Cyclooxygenase-2 (COX-2) is an enzyme that is encoded by the prostaglandin-endoperoxide synthase 2 gene (89). It is involved in the conversion of arachidonic acid to prostaglandin H2, an important precursor of prostacyclin, which is expressed in inflammation. Black *et al.* has demonstrated

Table 3 Immunomodulation by lymphatic epithelial cells (LEC)

Action	Mechanism	Outcome
Present exogenous antigens on MHC I without costimulatory molecules	Inhibit T cell proliferation & function	Apoptosis of $CD8^+$ T cells
Present endogenous tissue-restricted antigens on MHC-I	Inhibit T cell proliferation & function	Eliminates autoreactive $CD8^+$ T cells
Produce nitric oxide		Inhibits T cells
ICAM-1 upregulation		Inhibits dendritic cell maturation
IDO production	Depletion of tryptophan	Inhibits T cell proliferation
Release of S1P		Egress of activated T cells from LN
		Promotes survival of naïve T cells

LEC cells play an important role in many immunomodulatory functions (detailed description in text). MHC-I, major histocompatibility complex class I; ICAM-1, intercellular adhesion molecule 1; IDO, indoleamine 2,3-dioxygenase; S1P, sphingosine 1-phosphate.

recently the pro-lymphangiogenic effect of COX-2 that stimulates semaphorin 7a expression in breast cancerous cells resulting in activation of β1-integrin receptors on adjacent cancerous cells and LECs, to eventually increase lymphangiogenesis and metastasis spread (40). Additionally, semaphorin 7a gene is highly expressed in breast cancer cells and correlates with poor prognosis and metastatic disease (40). Moreover, Elder *et al.* has shown that semaphorin 7a stimulates gp38 upregulation in breast cancer by tumor-infiltrating macrophages, and this leads to promote their adhesion to LVs and this leads to lymphangiogenesis induction and metastasis spread (90).

Immune interactions in lymphatics

LECs, the main components of lymphatics, undergo active modifications that promote metastatic spread during tumor development, and enhance immunoregulation. It has been shown that LECs which are found in the cancer microenvironment can serve as an immunoregulator of the T cell response against tumoral cells (91). To overcome the immune system defenses and enhance the metastatic potential, tumors express immunosuppressive ligands and recruit a variety of immunosuppressive leucocyte subtypes to the primary tumor site, sentinel LN and metastatic sites as well (1,92). Recently, evading this immunosuppression is the principal rationale of several new immunotherapy approaches and holds promise for controlling established metastatic cancer (92,93).

Lymphatics display either positive or negative effects on tumor immunity; on one hand lymphatics have been implicated in tumor immunosuppression, but there is also evidence that lymphatics have a role in the induction of anti-tumor immune responses.

Generally, LECs are critical for the transport of immune mediators from peripheral organs to LNs leading to the immune response initiation (94,95). In addition to their role in tissue drainage and immune cell migration, LECs mediate T cell responses via various mechanisms (*Table 3*) (96):

(I) LECs can cross-present exogenous antigens on major histocompatibility complex class I (MHC I) in the presence of PD-L1, which signals through PD-1 to suppress the proliferation and function of the T cells leading to apoptosis of antigen-specific CD8+ T cells (97,98).

(II) LECs play a role in peripheral T cell tolerance by exposing endogenously expressed tissue-restricted antigens (94) via MHC I molecules and discarding autoreactive $CD8^+$ T cells (98,99).

(III) T cell activation and proliferation in a negative regulatory feedback process can be prevented by LECs. This can be achieved by producing nitric oxide in response to inflammatory signals (Interferon γ (IFNγ) and TNFα) secreted by T cells, resulting in T cell inhibition (100).

(IV) LECs, stimulated by IFNγ and TNFα, suppress dendritic cell maturation through ICAM-1 upregulation (101); suppress T cell proliferation via enzymatic depletion of tryptophan by indoleamine

2,3-dioxygenase (IDO) (102); and also upregulate MHC II (103).

(V) The impact of LECs on peripheral CD4+ T cell responses in various immunological settings still debatable. LECs were shown in Rouhani *et al.* study to be unable to load MHCII molecules with antigenic peptides owing to their H2-M deficiency at steady-state (103). On the other hand, Dubrot *et al.* showed that surface MHCII could be expressed on LN stromal cells as a result of the combination of both acquired and endogenous molecules (104).

(VI) LECs, by releasing the sphingosine 1-phosphate (S1P), are essential in releasing of activated T cells from LNs (105) and maintaining naïve T cells (106).

Lymphatics can also enable anti-tumor immune responses (107,108), as indicated by transgenic mice with defects in dermal lymphatic drainage, that displayed a defective immune responses to implanted cancerous cells (109).

High serum VEGF-C has been correlated with response to immunotherapy in melanoma patients and could be used as a biomarker for immunotherapy, despite its association with lymphatic metastasis (93), and VEGF-C resulted in an enhanced immune response against glioblastoma tumors (110). VEGF-C/VEGFR-3 signaling increased the number of activated T cells within primary melanoma lesions. In colorectal cancer decreased LV presence at the invasive margin of the specimen was associated with reduced infiltration of cytotoxic T cells and both were correlated with higher rate of distant metastasis (111). Furthermore, serum VEGF-C levels in cancer patients was found to be positively correlated with immunotherapy responses and even with survival outcome (93). One will need to balance the value of anti-lymphangiogenesis therapy with the risk that it might affect the effectiveness of immunotherapy.

Conclusions and future directions

The role of the lymphatic system in cancer development is currently receiving extensive scientific and clinical interests; the identification of molecular lymphangiogenic factors and receptors and the implications of their activity in normal physiology and pathology have improved our comprehension of the underlying mechanisms of tumor metastasis. It is now clear that tumor lymphangiogenesis is crucial in tumor development and blocking this process might inhibit metastasis to LNs. Moreover, lymphatic vascular markers may be useful as a prognostic indicator of metastatic risk. Novel targets have been identified, supporting biologically based therapeutic opportunities, and one such lymphatic-targeted therapy is already being used to preclude corneal grafts rejection (7). Further knowledge in the area of lymphagiogenesis will enable researchers and clinicians to investigate and treat tumors in a targeted and efficient fashion, being careful not to interfere with anti-tumor immunity. Additionally, a molecular understanding of factors that predict the likelihood of LN metastasis, i.e., a molecular signature, could replace the need for regional lymph node sampling.

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Footnote

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Ethical Statement: All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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