Lymphatics in Malignant Tumors

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Abstract

The lymphatic system constitutes a one-way conduit returning filtered interstitial fluid back to the blood circulation and also performing immunosurveillance. As most other tissues, solid tumors have lymphatics, and inherent and draining lymphatics influence solid tumor development and progression. Tumor lymphatics are also associated with metastasis to regional lymph nodes and dissemination to distant organs. Recent insights indicate that the tumor-associated lymphatic vasculature does not merely serve as a passive conduit for metastasis but also shapes the immune microenvironment in various tumors. It is reasonable to expect that modulating the lymphatic vasculature in combination with immunotherapeutic strategies will improve treatment efficacy. There are studies implying that the development of new lymphatic vessels might be associated with resolution of an immune response and induction of an immune tolerance, which may explain why high lymphatic vessel density is often associated with poor prognosis. Therefore, it is likely that lymphatic vessels play multiple complex roles at different stages of cancer development, and that the research on the impact of lymphatics on cancer will continue to increase.

Take-Home Lessons

- The role of the lymphatic vascular system, in the setting of cancer, is relatively understudied compared to the blood vascular system.
- Tumor-associated lymphatic vasculature does not merely serve as a passive conduit for metastasis but also shapes immune microenvironment in various tumors.
- Modulating the lymphatic vasculature in combination with the immunotherapeutic strategies might improve treatment efficacy.
- Development of new lymphatic vessels might be associated with resolution of an immune response and induction of an immune tolerance, and thus explain why high lymph vessel density is often associated with poor prognosis.
- It is likely that lymphatic vessels play multiple complex roles at different stages of cancer development.

Introduction

In mammals, there are two circulatory systems, the blood vessels that form a closed circulatory system and the lymphatic vessels. The latter, which is the topic of this chapter, constitutes a one-way conduit returning filtered interstitial fluid (i.e., the fluid phase that baths and surrounds cells in the tissues) and leukocytes back to the blood circulation. Although parts of the lymphatic system were recognized in the early seventeenth century, it was not until the eighteenth century that William Hunter concluded that "lymphatic vessels are the absorbing vessels all over the body … they constitute one great and general system dispersed throughout the whole body for absorption" [1]. The lymphatic system has three main functions: (1) fluid balance preservation by returning capillary ultrafiltrate and escaped plasma proteins to the blood circulation, (2) absorption of digested fat via intestinal lymphatics, and (3) defense function. Filtered interstitial fluid contains foreign material such as antigens, which is transported to lymph nodes as part of the body's

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L. A. Akslen, R. S. Watnick (eds.), *Biomarkers of the Tumor Microenvironment*, https://doi.org/10.1007/978-3-030-98950-7_4

immunosurveillance. As most other tissues, solid tumors have lymphatics. The function of inherent and draining lymphatics has particular relevance in as much as solid tumor progression is associated with metastasis to regional lymph nodes and dissemination to distant organs. Moreover, because the immune system and immunosurveillance via lymphatics can be assumed to take part in control of tumor progression, it is of interest to discuss here. Importantly, lymphatic vessels represent a route for tumor cells to escape from the primary tumor and metastasize. In the present chapter, we will discuss the tumor interstitium (microenvironment) where the lymph originates, and lymphatics embedded in interstitium, their role in fluid transport and cancer cell dissemination, and finally place the regulation of tumor immune microenvironments by lymphatics in a translational perspective by considering the implications for immunotherapy. The biological functions of lymphatic vessels and their role in disease, notably those of solid tumors, have been extensively reviewed elsewhere, including papers from pioneers in the field, e.g., [2–4].

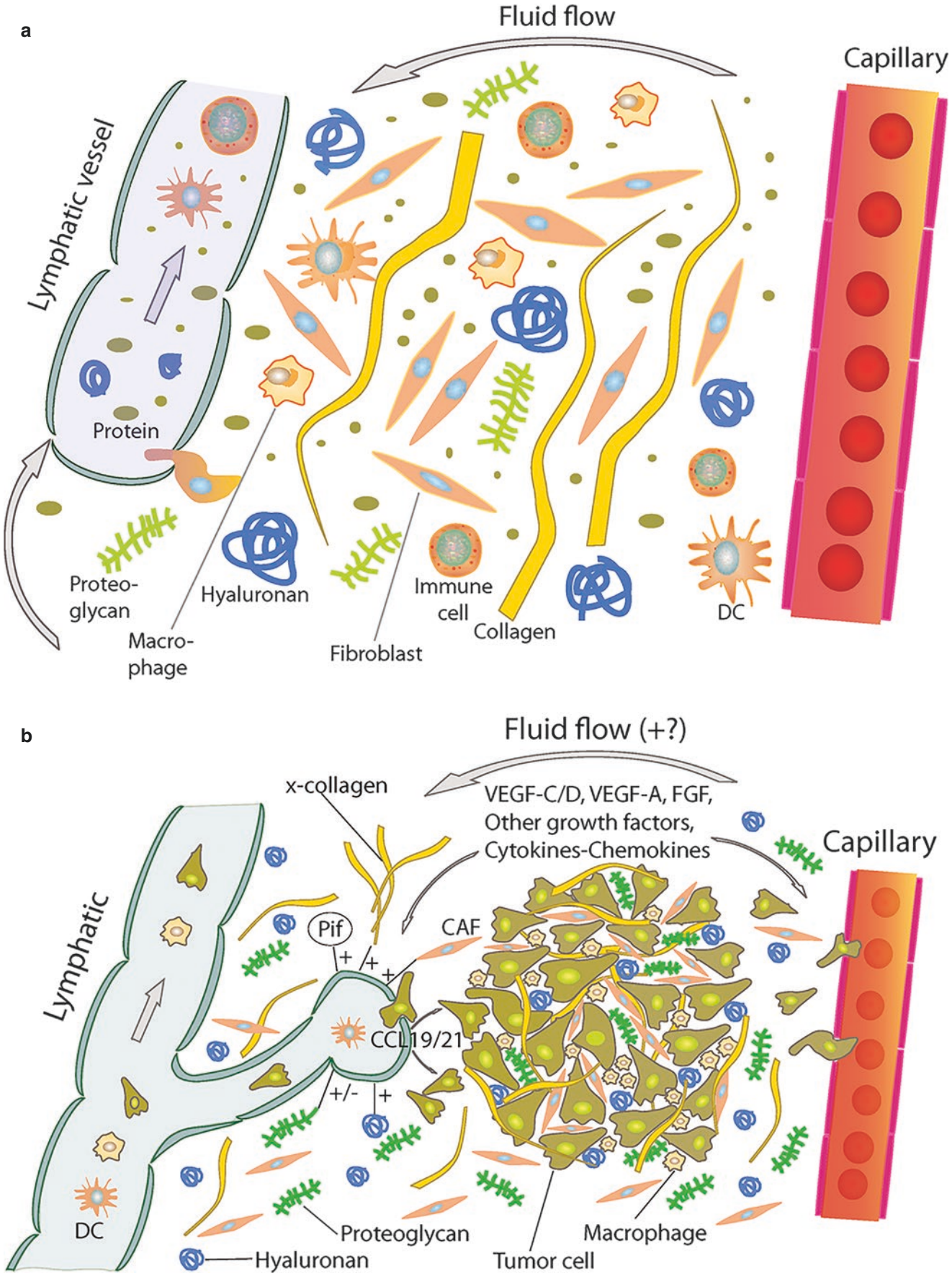
Tumor Interstitium (Microenvironment) and Lymphatics Embedded in Interstitium

Tumor lymph originates from the tumor interstitium or in the fluid phase of the extracellular matrix (ECM) where it is produced by filtration and thereafter finds its way to draining lymphatic vessels [5]. Because the interstitium represents the tumor microenvironment and is one of the determinants of lymph formation also hosting immune cells, which serves as a central element in this chapter, we will first briefly consider the interstitial structure and lymph formation in tumors. Normal interstitial tissue, as well as that of tumors, consists of a collagen fiber framework, a gel phase of glycosaminoglycans (GAGs), a salt solution, and plasma proteins [6]. The structure and composition of the tumor interstitium/stroma have been covered in many extensive reviews, e.g., Ref. [7–11], and therefore only salient features of particular relevance are discussed here. A schematic picture of the tumor interstitium is shown in Fig. 4.1. As described by Lu et al. [8], the ECM directly or indirectly regulates most cellular behavior and consequently also draining lymph composition. Notwithstanding the fact that the tumor interstitium consists of the same components as that of normal tissues as outlined in Fig. 4.1a, it has special features of relevance here. One of these is the stroma's "reactive" character [7], involving an increased number of inflammatory cells, endothelial cells, and fibroblasts, which evolve with and provide support to tumor cells during the transition to malignancy [13].

Among inflammatory cells, macrophages are probably the most abundant innate immune cells in the tumor microenvironment of most solid tumors. Macrophages are also, perhaps, the most plastic cells with tumor-associated macrophages (TAMs) serving as an example for their functional polarization. TAMs stimulate angiogenesis and enhance tumor invasion, and metastasis by secreting angiogenic and lymphangiogenic molecules (e.g., vascular endothelial growth factor (VEGF)-A and VEGF-C, respectively) as well as proteases, including cathepsins and matrix metalloproteinases (MMPs). Therefore, an abundance of TAMs in the tumor interstitium often portends a poor prognosis in numerous malignancies as revealed by pre-clinical and clinical data [5]. Importantly, some of the signaling molecules involved in macrophage polarization have already been defined in vitro. For example, classically activated (or M1) macrophages, which generally exert antitumoral functions, are induced following the stimulation with IFNγ alone or together with lipopolysaccharide (LPS), or TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF). On the other hand, IL-4, IL-13, and macrophage colony-stimulating factor (M-CSF) trigger an alternative (or M2) form of macrophage activation, which normally elicits tumor-promoting functions. In solid tumors, the crosstalk between macrophages and components of the tumor interstitium forges their phenotype. In response to numerous tumor- and stroma-derived signals, TAMs acquire an activation state that resembles a signature feature of M2 macrophages [5]. In contrast to macrophages, strong lymphocytic infiltration, particularly that of $CD8^+$ T cells correlates with good prognosis and is often associated with the presence of functional lymphatic vasculature [5], which will be discussed later in this chapter.

In the tumor interstitium there is an increased number of fibroblasts termed cancer-associated fibroblasts (CAFs) that have a profound role with respect to tumor ECM composition and function [8]. CAFs produce increased amounts of

Fig. 4.1 Schematic overview of the interstitium with some of its major extracellular matrix components in normal tissue and tumors. (**a**) Fluid containing plasma proteins and other solutes is filtered from the capillary percolates through the interstitium and is absorbed and thus returned to the circulation by lymph. In addition to proteins and solutes, immune cells migrate into lymphatic vessels and are transported to lymph nodes where they may initiate an immune response. Reproduced from Wiig et al. [12] with permission. (**b**) Role of the extracellular matrix and microenvironment in lymphangiogenesis in tumors. Growth factors and cytokines produced by tumor cells and stroma are transported by fluid flow and down a diffusion gradient to lymphatics and blood capillaries. Tumor and immune cells (expressing CCR7) are chemoattracted to and enter peritumoral initial lymphatics expressing CCL19/21. + (plus) and − (minus) denote stimulating and inhibiting lymphangiogenesis, respectively. *x-collagen* crosslinked collagen, P_{if} interstitial fluid pressure, *CAF* cancer-associated fibroblast. Reproduced from Wiig et al. [12] with permission


a
Fluid flow
Capillary
Lymphatic vessel
Protein
Proteo-
glycan
Hyaluronan
Macro-
phage
Fibroblast
Immune
cell
Collagen
DC
b
Fluid flow (+?)
x-collagen
VEGF-C/D, VEGF-A, FGF,
Other growth factors,
Cytokines-Chemokines
Capillary
Lymphatic
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CAF
+
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CCL19/21
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Proteoglycan
Hyaluronan
Tumor cell
Macrophage

collagen, proteoglycans, and GAGs, in particular hyaluronan and chondroitin sulfate [14]: For such reactive stroma formation, VEGF-A secreted by multiple cells of the tumor is a critical factor [15]. The resulting high levels of VEGF-A in tumors induce high-microvascular permeability, again resulting in extravasation of plasma proteins like fibrin, followed by attraction of fibroblasts, inflammatory cells, and endothelial cells [16]. Whereas it is well established that stromal cells and fibroblasts secrete angiogenetic factors [17], lymphangiogenic factors have received less attention. Secretion of such factors does take place, and immune as well as tumor cells are important sources for lymphangiogenic factors, notably VEGF-C and VEGF-D [18] that modulate the tumor stroma structure and function (Fig. 4.1b).

Although lymph vessels were described early in the seventeenth century, growth factors and molecular markers for such vessels have only been identified in the past two decades. In this time period, lymphatic vascular biology in all areas, including that of tumors, has advanced rapidly through the discovery of lymphangiogenic factors, identification of lymphatic vascular markers, isolation of lymphatic endothelial cells, and the development of animal models to study lymphangiogenesis [3, 4]. These lymphatic vessel markers, which can be used to distinguish lymphatic from blood vessels, have been instrumental for recent progress in understanding tumor lymphatic biology.

There are several growth factors that induce growth of lymphatic vessels, although most important are VEGF-C and VEGF-D [2], which both bind to VEGFR-3 (flt-4) on lymphatic endothelial cells and result in downstream signaling as illustrated in Fig. 4.2. Many of the lymphangiogenic factors are also angiogenic factors, due to the common embryonic origins of lymphatic and blood vessels [2]. Tumors overexpressing these factors induce sprouting of lymphatic vessels, enlargement of collecting vessels, and lymph node lymphangiogenesis in the draining lymph nodes, apparently making the primary tumor more prone to developing lymph node metastases [2]. There are several other lymphatic growth factors such as VEGF, fibroblast growth factors, platelet derived growth factor-B, hepatocyte growth factor, and insulin-like growth factor-1 that can induce lymphangiogenesis and metastasis, but then via more indirect pathways like inflammation and induction of VEGF-C and VEGF-D expression [2].

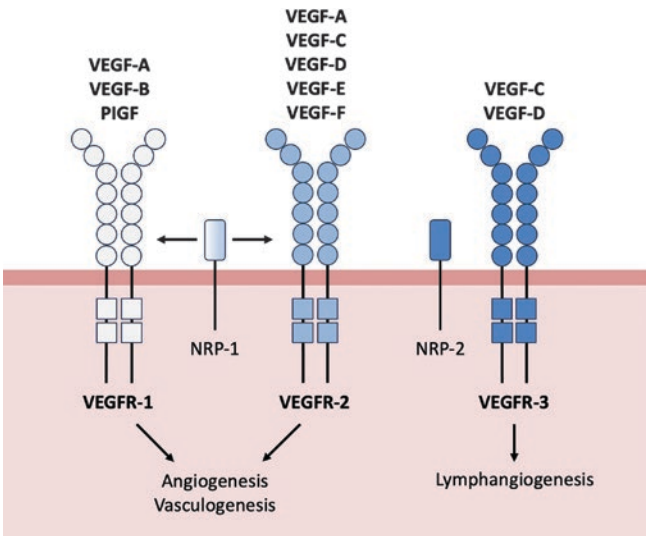

Fig. 4.2 Lymphangiogenesis. The formation of new lymphatic vessels is triggered by the binding of VEGF-C and VEGF-D to the VEGFR3 that is present on the surface of lymphatic endothelial cells

Lymphangiogenesis induced by VEGF-C secreted by stromal and tumor cells affects the tumor formation process in several ways. One might think that tumor growth would increase lymphatic drainage or lead to lymph flow in collaterals if the lymphatics are impinged upon by the expanding tumor. Whereas tumor lymphangiogenesis has been extensively studied, there are comparatively fewer studies in which lymphatic function is experimentally assessed. In a classical study addressing this issue, Padera et al. [19] investigated whether intratumoral lymphatic vessels generated by overexpression of VEGF-C in mice were functional. They found that although VEGF-C overexpression increased the lymphatic vessel surface area in the tumor margin and lymphatic metastasis, these tumors contained no functional vessels as evaluated by several independent assays. Their data suggested that intratumoral transport of injected (and thereby also filtered) fluid did not occur through lymphatic vessels but rather through preferential channels in the tumor interstitium. As an explanation for the lack of lymphatic vessel function they suggested that this could be due to: a lack of valve structure in newly formed lymphatics; mechanical forces such as an elevated interstitial fluid pressure could collapse the lymphatic vessels rendering them nonfunctional; or that invading tumor cells could destroy the lymphatic network. Lymphatics in the tumor margin were, however, functional and sufficient for lymphatic metastasis [19]. These studies should remind us that increased tumor lymphangiogenesis does not necessarily lead to increased lymphatic function.

Tumor Lymphatics in Fluid Transport and Cancer Cell Dissemination

Interstitial fluid that percolates the tumor tissue and eventually enters initial lymphatics to become lymph is formed in a similar manner as in normal tissues. Such formation is determined by properties of the capillary wall, hydrostatic pressures, and protein concentrations in the blood and interstitium according to basic principles for fluid exchange described by Starling more than a century ago. Starling proposed, based

on his own experiments, that capillaries are semipermeable membranes, and that transcapillary fluid filtration is determined by the imbalance between oncotic (colloid osmotic) exerted by proteins in plasma (COP_p) and interstitium (COP_{if}) and the hydrostatic pressures in the capillaries (P_c) and the interstitial fluid (P_{if}).

Although similar in many ways to normal tissue like skin and muscle, solid tumors have special features. In particular, this applies to P_{if} that is elevated compared with normal tissues [20]. Skin and muscle P_{if} are slightly subatmospheric, whereas tumor P_{if} is above atmospheric pressure in experimental animals as well as in humans, observed to be the range of 10–40 mm Hg in humans [20]. The high intratumoral P_{if} represents a counterpressure against filtration from capillaries and thus affects the formation of tumor interstitial fluid and thereby lymph per se. It may also negatively influence the transport of therapeutic substances from blood to the tumor [21], thereby acting as a potential target in tumor therapy.

The high tumor P_{if} counteracting lymph production is a result of several specific features of the tumor microenvironment, notably its vasculature [20]. Because of the increased production of angiogenetic factors, notably VEGF-A, tumor vessels are convoluted, irregular, and highly permeable [22]. These vascular changes result in low restriction of protein and transcapillary water transport and tissue "counterpressure" equal to P_{if} [21]. An additional effect of the permeable tumor vessels is an increased transcapillary protein transport that will result in an increased interstitial fluid colloid osmotic pressure again contributing the high tumor P_{if}. Additionally, direct effects of growth factors such as VEGF-A, PDGF, and TGF-β may also drive tumor P_{if} upwards [20]. Knowledge of interstitial fluid and lymph formation is critical when attempting to overcome microenvironmental obstacles in therapy and to improve drug delivery to solid tumors [21].

The lymphatic system consists of lymphatic vessels and lymphoid organs. With the exception of avascular tissues such as epidermis, cartilage, and cornea and a few vascularized organs like the retina and brain (proper), all organs have blind-ended lymphatic capillaries [12]. These are known as initial lymphatics and transport lymph to larger collecting lymphatic vessels, again returning lymph to the general circulation in lymphatic-vascular junctions in the cervical area [2–4].

Before entering into the blood stream, lymph passes through the following conduits with increasing size, lymph capillaries (also called initial lymphatics), collecting vessels, lymph nodes, trunks, and ducts [3]. Accordingly, lymphatics are a transport route where metastatic cells can reach the blood circulation. The initial lymphatics are thin-walled, relatively large vessels compared with blood capillaries composed by a single layer of endothelial cells. These vessels are not ensheathed by pericytes and smooth muscle cells, have little or no basement membrane, and are the site of interstitial fluid absorption. From the initial lymphatics, lymph moves centrally via collecting lymphatics lined with smooth muscle propelled by spontaneous contractions. Moving centrally, lymph in collecting vessels passes through lymph nodes, and is accordingly classified as prenodal or postnodal (or afferent or efferent) depending on whether lymph is carried to or from the nodes, respectively. Importantly, the lymph composition as well as immune cells can be affected by the passage through the lymph nodes [23]. Moreover, the lymph nodes may determine whether disseminating tumor cells enter the blood via high endothelial venules or through the lymph system and thus whether there is lymphatic or hematogenic metastasis as will be discussed below. Eventually, the lymph enters the blood circulation through the thoracic duct that connects to the subclavian vein.

Tumor Dissemination via Lymphatic Vessels

The role of tumor lymphatics in relation to cancer progression and metastasis is an area of ongoing research. One might imagine that tumor cells could disseminate via lymphatics, and this was actually shown by Skobe et al. [24]. They found that lymphatic vessels support metastasis and interpreted their findings to suggest that the lymphatic vessels support the development of a route for the tumor cells to escape from the primary tumor to enter the lymph node and beyond. Moreover, lymphatic vessel density at the tumor margin has been demonstrated to correlate with poor prognosis of patients with melanoma, breast, colorectal, and lung cancer [25]. Presence of lymphatic vessels at the tumor margin, using mouse melanoma as a model, is shown in Fig. 4.3. However, it has been found that removal of sentinel lymph nodes only incrementally improves patient prognosis [25]. Moreover, for colorectal cancer metastases, different sites of metastases arise from different clones in the primary tumor and 65% of lymph node metastases are unrelated to distal metastases [26]. The lack of correlation between lymph node and distal metastases suggests that the role of lymphatic vasculature and associated lymph nodes in distal metastasis is not straightforward. This notwithstanding, one third of liver and lymph node metastases arise from tumors having passed lymph nodes [26]. Metastatic tumor cells escape the tumor via afferent lymphatic vessels led into lymph node subcapsular sinus. From there, the tumor cells can invade the lymph node stroma and enter the blood circulation via high endothelial venules or alternatively pass through a series of lymph nodes and enter the thoracic duct feeding into the subclavian vein and thereby the systemic circulation [27, 28]. As reviewed by Oliver et al. [4], the great concern that lymphat-

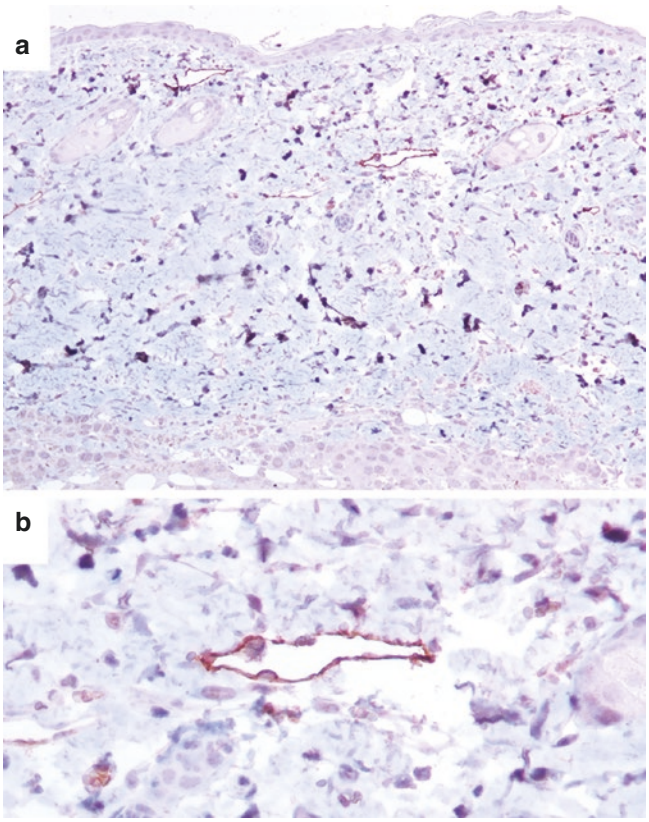

Fig. 4.3 Presence of lymphatic vessels in the peritumoral tissue. Analysis of a section of mouse melanoma (B16F10; skin cancer) reveals (via specific anti-LYVE1 antibody staining) that lymphatic vessels (arrows and asterisks) are present in the normal tissue above the tumor tissue (dotted line) but are absent within the tumor tissue itself. Size bars: 100 μm (**a**) and 50 μm (**b**)

ics might promote cancer progression solely via enhanced metastasis is now shifting toward a view that induction of lymphangiogenesis might modulate the tumor immune microenvironment. As discussed below, inhibition of lymphangiogenesis together with T cell-mediated immunotherapy may be efficacious for treating cancer patients without increasing the risk of metastasis.

Immune Microenvironment and Tumor Progression

Apart from serving as a route for tumor cells to escape from the primary tumor, lymphatic vessels together with lymphoid organs (e.g., lymph nodes) along with tissues in mucous layers of the body provide the structural basis for the proper function of the immune system. The field of cancer immunology has been faced with a number of challenges and controversies. The idea that the immune system plays a role in controlling the development of tumors or shaping the nature of the diseases has been a long-term debate that continued throughout the past century. Paul Ehrlich, in the early nineteenth century, was the first to propose the concept that "cancer would be quite common in long-lived organisms, if it was for a properly functioning or a fully competent immune system" [29]. However, at that time, there was little known about the components of the immune system and their function and thus no effective way of validating or testing this hypothesis. The idea of cancer immunosurveillance re-emerged five decades later due to the work of Lewis Thomas [30] and Macfarlane Burnet [31]. They proposed that the immune system, particularly an adaptive immune system, might play an important role in protecting against the development of cancer. However, a number of research groups argued against the hypothesis of cancer immunosurveillance. Instead, they suggested that tumor cells would not present signals that would alert the immune system to their presence. It was proposed that tumors (which arise from the self-tissue) would likely present antigens that induce self-tolerance. Further to that, it was also suggested that chronic inflammation, which usually precedes tumor growth, is able to promote malignant transformation, thus prohibiting the protective mechanism of the immune system in controlling cancer development.

With the development of a wide array of immunodeficient mouse models on a very specific genetic background, the idea of cancer immunosurveillance re-emerged. Indeed, it has been found that mice deficient of particular components of the adaptive immune system such as T and B cells or mice that lack the production of immune-modulatory cytokines such as IFNγ are more prone or susceptible to the spontaneous development of carcinogen-induced tumors as well as spontaneous tumor growth. The idea that the immune system has the capacity to act as an extrinsic tumor suppressor mechanism is now well established. There is substantial amount of evidence to support the concept that an adaptive immune system plays an important role along with an innate immune system in protecting the host against cancer and also influencing the evolution of the disease. However, in order to develop an effective immune response that can kill cancer cells, the immune response has to go through a series of steps, which requires functional lymphatic vasculature.

The lymphatic vessels transport fluid and cells from basically all tissues in the body into lymph nodes and other lymphoid organs, making the lymphatic system ideally suited for immunosurveillance [32]. This role specifically applies to soluble antigens that are sequestered by antigen-presenting cells resident to the lymph node [33] as well as antigens that are displayed by dendritic cells moving to the lymph node after phagocytosis [34]. However, the mechanism of this action, which is fundamental for the initiation and propagation of an immune response, is not yet completely understood, but seems to require chemokines such as chemokine (C-C motif) ligand 21 (CCL21), CCL 19, and CCL 12

secreted by lymphatic endothelial cells that bind to the receptors CCR7 and CXCR4 that are present on the surface of mature dendritic cells to induce migration, vessel entry, and trafficking (reviewed in [23]).

Regulation of Tumor Immune Microenvironment by Lymphatics

In fact, a positive correlation between lymphatic density and immune cell infiltration has been revealed in a mouse model of melanoma [35]. This model utilized K14-VEGFR3-Ig mice that lack dermal lymphatics due to the constitutive expression of soluble vascular endothelial growth factor receptor (VEGFR) 3-Ig in the skin, which scavenges VEGF-C and VEGF-D. It was found that syngeneic B16F10 melanomas in these mice expressed decreased levels of proinflammatory molecules. Moreover, these melanomas had reduced infiltration of T and B cells as well as proinflammatory myeloid cells [35]. Similar observations were made using Chy mice, which harbor a heterozygous inactivating mutation of VEGFR-3 that leads to impaired dermal lymphangiogenesis. Syngeneic C3HBA breast adenocarcinomas were characterized by decreased leukocytic infiltration in Chy mice compared with wild type littermates, coinciding with reduced peritumoral lymphangiogenesis [35, 36]. Altogether, these results suggest that lymphatic vessels are indeed indispensable for the induction of an immune response in cancer.

Lymphatic vessels have also been shown to accelerate tumor antigen-loaded dendritic cell trafficking and priming of T cell immunity in a CCR7-dependent manner [37]. Additionally, decreased levels of tumor antigens and reduced number of cytotoxic CD8 T cells have been found in the tumor-draining lymph nodes of B16F10 melanoma-bearing kCYC mice, which demonstrate severe lymphatic dysfunction due to the expression of the Kaposi's sarcoma-associated herpesvirus latent-cycle gene, k-cyclin, under the control of the VEGFR-3 promoter [38].

Subsequently, a clinical study demonstrated that the assessment of the immune status in human colorectal cancer might serve as a stronger predictor of patient's survival, since distant metastases occurred more frequently in tumors with diminished immune cytotoxicity profile [39]. Additionally, tumors with decreased density of peritumoral lymphatics have been observed to metastasize more frequently, suggesting a positive correlation between lymphatic vascular density and immune cytotoxicity profile [39]. Collectively, this clinical study demonstrated that the lymphatic vasculature facilitates an antitumor immune response and shapes tumor immune microenvironment [39].

However, one of the most prominent steps in impeding the generation of an effective antitumor immune response is the development of a strong immunosuppressive tumor microenvironment. There are a number of factors that might contribute to this process, but certainly one of the most important is the recruitment of regulatory or immunosuppressive immune cells into the tumor microenvironment. Lymphatic structures are often found embedded in adipose tissue. However, the impact of lymphangiogenesis on tumor-associated adipose tissue has only recently been investigated, despite the fact though many tumors grow in close proximity to or physically interact with adipocytes or metastasize to lymph nodes that are shrouded by adipocytes [40–42]. For example, an increased number of macrophages, enriched particularly with an alternatively activated (or M2) population, have been found in tumor-associated adipose tissue from B16F10 melanoma-bearing K14-VEGFR3-Ig mice [43]. This observation suggests that the blockade of pathways regulating the formation of lymphatic vessels influences an inflammatory response within tumor-associated adipose tissue by enhancing the development of the microenvironment that facilitates tumor growth and progression.

The development of antitumor immunity primarily occurs in the tumor-draining lymph nodes. The formation of new lymphatic vessels provides a route for the delivery of tumor antigens to the draining lymph nodes in order to initiate priming of T cells [44]. The lymph may deliver tumor-derived antigens directly to dendritic cells or B cells residing in the tumor-draining lymph nodes [44]. Additionally, dendritic cells that are patrolling peripheral tissues may also take up tumor antigens, invade lymphatic vasculature, and enter tumor-draining lymph nodes [44]. Antigen-loaded dendritic cells subsequently prime T cells, in order to induce antigen-specific antitumor immunity. It is also worth mentioning that the local microenvironment has the potential to influence the quality of the immune response (i.e., immune activation or induction of tolerance) during antigen presentation [44]. Additionally, the architecture and function of the tumor-draining lymph nodes may be distantly modulated by the lymphatic vasculature through the delivery of extracellular mediators (e.g., exosomes) derived from the primary tumor microenvironment [45].

To test the hypothesis that tumor-draining lymph nodes promote antitumor immunity, an adjuvant therapy specifically targeting dendritic cells in the tumor-draining lymph nodes of mice bearing B16F10 melanomas was performed via delivery of CpG oligodeoxynucleotide (CpG). In this model, treatment with CpG significantly inhibited the growth of implanted B16F10 melanomas. Additionally, alterations in immune cell repertoires such as increased frequencies of mature dendritic cells within the tumor-draining lymph nodes as well as increases in tumor antigen-specific CD8 T cells in the tumor tissue have been observed [46]. An enhanced antitumor immunity illustrated by increased cytotoxic CD8 T cell responses, has also been obtained via thera-

peutic vaccination using lymph node-targeting nanoparticle-conjugate vaccines, leading to increased tumor regression and survival [47]. Additionally, ectopic expression of CCL3, which orchestrates T cell-antigen-presenting cell encounters in the lymph nodes, in CT26 colon tumor cells resulted in reduced tumor growth, most likely by enhanced homing of dendritic cells to the tumor-draining lymph nodes and increased antitumor immunity [48]. Also of significant importance is the fact that effective checkpoint inhibition therapy requires functional tumor-draining lymph nodes [49]. Taken together, these studies suggest that tumor-draining lymph nodes are not only important for the initiation of antitumor immune response but are also critical to achieve optimal immunotherapeutic efficacy.

However, lymph nodes, apart from their function in immune activation, also appear to play an important role in the maintenance of self-tolerance [50]. The lymphatic vasculature regulates self-tolerance by generating a conduit for antigen transportation and orchestrating the structural organization of the draining lymph nodes [44]. Therefore, the lymphatic system not only triggers antitumor immune response but also promotes tolerance [51].

An increasing body of evidence suggests that lymphatic endothelial cells, under both physiological and pathological conditions, participate in the induction of peripheral tolerance through various mechanisms [52]. Lymphatic endothelial cells express peripheral tissue antigens as well as major histocompatibility complex (MHC) molecules. Accordingly, they are able to recognize and inhibit T cell activation when expression levels of inhibitory receptors are high and the expression levels of co-stimulatory molecules are low [53]. Interestingly, IFNγ has been found to strongly induce the expression level of programmed death-ligand 1 (PD-L1) in lymphatic endothelial cells [54]. Consequently, specific deletion of IFNγ receptor (IFNγR) in lymphatic endothelial cells resulted in dampened immune suppression and increased tumor immunity [55]. Thus, the induction of PD-L1 expression by IFNγ appears to constitute a feedback mechanism utilized by the immune system to achieve equilibrium. Lymphatic endothelial cells themselves also secrete immunosuppressive molecules such as nitric oxide (NO), TGF-β, and indoleamine-2,3-dioxygenase (IDO) in order to induce tolerant dendritic cells and inhibit T cell function [56, 57]. Accordingly, the lymphatics in the tumor microenvironment not only induce an active immune response characterized via immune cell infiltration, but also directly promote the formation of an immunosuppressive microenvironment through an increased expression of IDO, arginase-1, and inducible nitric oxide synthase (iNOS) by lymphatic endothelial cells [58]. The lymphatic system can also suppress the activity of CD8+ T cells by directly presenting tumor-derived antigens [59]. In summary, these findings suggest that tumor lymphatics regulate both immune activation and tolerance. Consequently, the identification of mechanisms that link lymphatics to cancer progression and/or the possibility to regulate their tumor-suppressing effects is fundamental for designing effective therapeutic strategies.

Concluding Remarks/Summary

The role of the lymphatic vascular system, in the setting of cancer, is still relatively understudied compared to the blood vascular system. Nevertheless, the research over the past few decades has established a fundamental knowledge of how the lymphatic vascular system develops, matures, and functions. Recent insights indicate that the tumor-associated lymphatic vasculature does not merely serve as a passive conduit for metastasis but also shapes the immune microenvironment in various tumors. Given the current successes in cancer immunotherapy, it is reasonable to expect that modulating the lymphatic vasculature in combination with immunotherapeutic strategies will improve treatment efficacy. Research on this topic is only in the beginning stages, and obviously much more work is needed to verify the role of lymphatics in tumor development and progression. Further confounding the issue are studies implying that the development of new lymphatic vessels might be associated with resolution of an immune response and induction of immune tolerance, which may explain why high lymphatic vessel density is often associated with poor prognosis. Therefore, it is likely that lymphatic vessels play multiple complex roles at different stages of cancer development, and that research on the impact of lymphatics on cancer will continue to increase our knowledge and understanding of the field.

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