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Review:

Lymphatic vasculature in tumor metastasis and immunobiology*

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Abstract: Lymphatic vessels are essential for tissue fluid homeostasis, immune cell trafficking, and intestinal lipid absorption. The lymphatics have long been recognized to serve as conduits for distant tumor dissemination. However, recent findings suggest that the regional lymphatic vasculature also shapes the immune microenvironment of the tumor mass and potentiates immunotherapy. This review discusses the role of lymphatic vessels in tumor metastasis and tumor immunity.

Key words: Lymphatic; Lymphatic endothelial cell (LEC); Cancer; Metastasis; Immunotherapy
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1 Introduction

Metastasis is the primary cause of morbidity and mortality in cancer patients (Guan, 2015). Although it was recognized centuries ago that patients with a higher tumor burden in sentinel lymph nodes have worse prognoses (Shayan et al., 2006), the notion that the lymphatic vasculature is fundamental to tumor metastasis was appreciated only about two decades ago (Karaman and Detmar, 2014). Pioneering murine studies demonstrated that the induction of lymphatic vascular overgrowth promotes tumor metastasis (Skobe et al., 2001; Stacker et al., 2001). A later clinical investigation revealed that tumor lymphatic vessel amplification predisposes human melanoma to metastasis (Dadras et al., 2003). However, a recent study demonstrated that enhancing lymphangiogenesis augments the efficacy of cancer vaccine- and checkpoint blockade-mediated immunotherapy (Fankhauser et al., 2017), providing the first evidence that the promotion of cancer lymphangiogenesis might be harnessed to potentiate immune modulatory treatments. Further investigation is, therefore, urgently needed to gain a better understanding of how lymphatics may affect tumor pathology. This review summarizes the role of the lymphatic vasculature in tumor metastasis as well as tumor immunobiology.

2 Lymphatic vascular system

The lymphatic system is a unidirectional circulatory network that is essential for tissue fluid homeostasis, dietary fat absorption, and the regulation of immune responses (Jiang et al., 2018). The lymphatic vascular tree begins as blind-ended capillaries comprised of a single layer of lymphatic endothelial cells (LECs) that tether to the surrounding tissue through anchoring filaments (Stacker et al., 2014); junctional proteins, such as vascular endothelial cadherin (VE-cadherin), claudin-5, occludin, zonula occludens-1 (ZO-1), and endothelial cell-selective adhesion molecule (ESAM), form discontinuous button-like structures that interconnect capillary LECs and facilitate the uptake of interstitial fluid, macromolecules, and cells (Baluk et al., 2007; Mortimer and Rockson, 2014). The capillaries converge into pre-collectors that further merge into collecting lymphatics, where continuous zipper-like junctions join LECs (Baluk

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et al., 2007). Collecting lymphatics are invested with smooth muscle cells and contain intraluminal valves that permit unidirectional lymph flow (Mäkinen et al., 2007). Lymphatic vascular deficiency or dysfunction causes lymphedema, a chronic disfiguring disorder that affects millions of patients globally (Tian et al., 2017; Jiang et al., 2018; Rockson et al., 2018). Also notable is the fact that pathological lymphatic remodeling occurs in a myriad of acute or chronic disorders, such as cardiac, pulmonary, renal, and neurodegenerative diseases, and improving the lymphatic circulation in those conditions appears to be beneficial (Aspelund et al., 2016; Henri et al., 2016; Stump et al., 2017; da Mesquita et al., 2018; Lopez Gelston et al., 2018; Vieira et al., 2018). This review focuses on lymphatics in cancer pathology.

3 Tumor lymphangiogenesis

Lymphangiogenesis is the process of new lymphatic vessel formation from preexisting vasculature, a phenomenon that is important for both embryonic development and adult tissue homeostasis (Tammela and Alitalo, 2010). It is a tightly regulated process that requires the coordination of multiple inter- and intracellular signaling cascades (Zheng et al., 2014). The vascular endothelial growth factor (VEGF)-C/D-induced activity of VEGF receptor (VEGFR)-3 elicits the central signaling that stimulates lymphangiogenesis in both physiological and pathophysiological conditions (Jeltsch et al., 1997). Other molecules, including fibroblast growth factor-2 (FGF2), angiopoietins, sphingosine-1-phosphate (S1P), neuropilin 2 (NRP2), bone morphogenetic protein 9 (BMP9), and Notch1, also participate in the generation of new lymphatic vasculature (Zheng et al., 2014). Pro-lymphangiogenic interleukin (IL)-1β and tumor necrosis factor (TNF)-α and anti-lymphangiogenic IL-4 and IL-13 also regulate lymphangiogenesis, especially during inflammation (Baluk et al., 2009, 2013; Shin et al., 2015; Sainz-Jaspeado and Claesson-Welsh, 2018). Furthermore, interstitial pressure and flow rate can guide lymphangiogenesis (Boardman and Swartz, 2003). LEC-expressed proteases, such as matrix metalloproteinase (MMP) 9 and MMP2, and integrins, such as Itga9 and α5β1, are also involved in new lymphatic vasculature formation (Vaahtomeri et al., 2017), suggesting that lymphangiogenesis requires extracellular matrix (ECM) remodeling as well as a bidirectional LEC and ECM communication. Analogous to that of blood vascular angiogenesis, the lymphangiogenic process utilizes the Notch signaling pathway to enhance lymphatic stabilization by promoting a stalk cell phenotype of proliferating LECs (Zheng et al., 2011). More recently, LEC metabolic processes, such as fatty acid oxidation (FAO) and glycolysis, have been shown to regulate lymphangiogenesis (Wong et al., 2017; Yu et al., 2017), highlighting the importance of cellular metabolism in regulating lymphatic development and regeneration.

Similar to that in tumor angiogenesis, tumor lymphangiogenesis produces abnormal lymphatic vessels, which allow retrograde fluid flow and make liquid transportation inefficient (Ariffin et al., 2014). It was hypothesized that, during tumor progression, accumulating mutations enable tumor cells to produce pro-lymphangiogenic growth factors that initiate lymphangiogenesis, a process known as the lymphangiogenic switch (Cao, 2005). For example, the mutation of the tumor suppressor gene *p53* and the activation of oncogenes *Ras* and *Myc* enhance the expression of FGF2, VEGF, and platelet-derived growth factor (PDGF), which drives initial tumor lymphangiogenesis (Ueba et al., 1994; Arbiser et al., 1997; Enholm et al., 1997; Su et al., 2001; Uramoto et al., 2004). Tumor cells can produce S1P and prostaglandin E2 (PGE2) to promote lymphatic growth (Nandi et al., 2017; Yamada et al., 2018). Tumors also indirectly enhance lymphatic growth by activating fibroblasts to secrete VEGF-C and hepatocyte growth factor (HGF) (Wei et al., 2017; Gao et al., 2018), as well as by recruiting tumor-associated macrophages (TAMs) and mast cells to produce VEGF-C/D and IL-1β (Harvey and Gordon, 2012; Weichand et al., 2017; Varricchi et al., 2018). Moreover, escalated interstitial pressure, resulting primarily from increased blood vessel leakage, provides guidance cues and promotes lymphatic expansion (Kim et al., 2016). A more recent study showed that the chemotherapeutic agent docetaxel reprograms breast cancer cells to preferentially express pro-lymphangiogenic factors, including VEGF-C and TNF-α to promote lymphatic sprouting (Harris et al., 2018). Cumulatively, these findings suggest that cancer cells, tumor microenvironment, and treatment interventions enhance lymphangiogenesis and abnormal lymphatic remodeling in the tumor (Fig. 1).

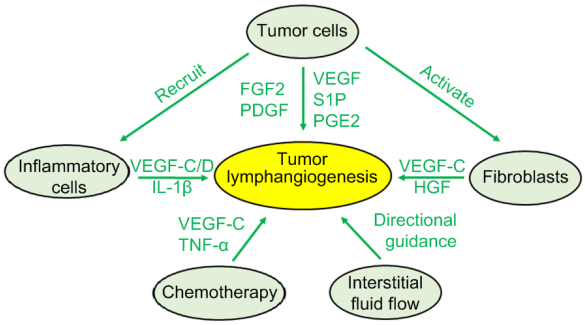

Fig. 1 Scheme of tumor lymphangiogenesis

Tumor cells produce FGF2, PDGF, VEGF, S1P, and PGE2, which directly induce lymphangiogenesis. Tumor cells also activate fibroblasts and recruit inflammatory cells, including mast cells and macrophages, which in turn produce VEGF-C/D, HGF, and IL-1β to enhance lymphangiogenesis. Chemotherapy promotes lymphangiogenesis by reprogramming cancer cells to express VEGF-C and TNF-α. Interstitial fluid flow provides directional guidance for lymphangiogenesis. FGF2, fibroblast growth factor-2; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; S1P, sphingosine-1-phosphate; PGE2, prostaglandin E2; HGF, hepatocyte growth factor; IL, interleukin; TNF, tumor necrosis factor

Tumor-induced lymphangiogenesis also occurs in the draining lymph nodes, where new lymphatic vessel formation is observed to take place before the arrival of the metastatic tumor cells, indicating that circulating pro-lymphangiogenic factors that originate from the primary tumor mass may play a central role in this process (Hirakawa et al., 2005). After arrival to the lymph nodes, tumor cells promote local lymphatic growth by continuously supplying VEGF-C (Kerjaschki et al., 2011). Lymphatic vessel expansion in draining lymph nodes appears to be an important step for distant tumor metastasis (Hirakawa et al., 2007; Karaman and Detmar, 2014).

4 Lymphatic routes for tumor metastasis

Decades of research has provided a comprehensive understanding of the cellular and molecular mechanisms involved in tumor metastasis through the blood vascular circulation (Lambert et al., 2017). Tumor cell dissemination through the bloodstream involves a sequential, multiple-step invasion-metastasis cascade. During this process, tumor cells endure hydrodynamic flow and shear stress, as well as manage to escape destruction by circulating immune cells present in the blood circulation; tumor cells also develop programs that subvert the induced dormancy during transit, which allow cancer stem cells (CSCs) within the migrating cell population to maintain their ability to re-initiate tumor growth at distant new locations (Lambert et al., 2017). Lymphatic metastasis, on the other hand, involves tumor cell transport in both lymphatic and blood vessels (Proulx and Detmar, 2013; Paduch, 2016).

Mechanisms of immune cell trafficking in lymphatics are well-studied (Randolph et al., 2016). Tumor cells highjack immune trafficking machinery to facilitate their own entry and transport within the lymphatic system. LEC-produced chemokines, including C-C motif chemokine ligand 21 (CCL21) and CCL19, are the most important factors that guide and recruit immune cells that express C-C motif chemokine receptor 7 (CCR7); mice lacking CCR7 expression have defective T cells and dendritic cells (DCs) homing to the lymph nodes (Förster et al., 2008). Increased CCR7 expression occurs in several human cancers, and a higher level of CCR7 expression correlates with increased distant metastasis (Müller et al., 2001), suggesting that tumor cell lymphatic invasion similarly employs CCR7-mediated chemotaxis. The CXCL12/CXCR4 and CX3CL1/CX3CR1 axes have been shown to play roles in directing DC trafficking (Imai et al., 1997; Kabashima et al., 2007a, 2007b; Jackson, 2014). In tumors, chemokine receptor pairs, including CXCL12/CXCR4 and CCL1/CCR8, also play a role in promoting metastasis (Hirakawa et al., 2009; Das et al., 2013) (Fig. 2). Although tumor masses often display lymph stasis, this may not significantly impede cancer cell lymphatic intravasation, because cellular uptake depends primarily on the chemotactic guidance gradient (Randolph et al., 2016). As for the site of entry, the immune cells prefer to use the regions of the lymphatic capillaries with sparse basement membrane, known as portals (Pflicke and Sixt, 2009). Tumor cells likely also enter lymphatics primarily through those locations. While the bloodstream may pose multiple obstacles to circulating tumor cells (Lambert et al., 2017), the lymphatic vasculature might provide a protective microenvironment that promotes the survival of in-transit tumor cells; LECs might also provide a niche for cells with CSC properties (Karaman and Detmar, 2014). In summary, the lymphatic

route likely has an advantage over the blood route for cancer cell dissemination, and the former may represent a preferable path for distant tumor dissemination (Mortimer and Rockson, 2014).

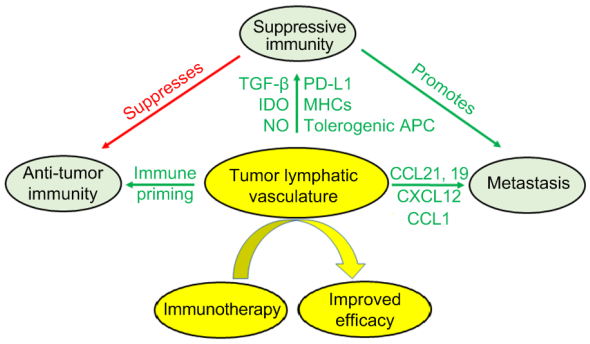

Fig. 2 Proposed scheme of tumor lymphatic vasculature in tumor metastasis, immunity, and immunotherapy
Tumor lymphatic vasculature not only primes the anti-tumor immunity but also induces suppressive immunity by producing molecules, including TGF-β, IDO, and NO. LEC expression of MHCs and PD-L1 confers them the ability to induce T cell tolerance by acting as tolerogenic APCs directly. Lymphatic vasculature provides a conduit for tumor metastasis; LEC expression of CCL21, CCL19, CXCL12, and CCL1 facilitates tumor cell lymphatic intravasation. Lymphatic-induced suppressive microenvironment not only negatively impacts anti-tumor immunity but also promotes metastasis. Promoting tumor lymphangiogenesis may augment the efficacy of immunotherapy. TGF, transforming growth factor; IDO, indoleamine-2,3-dioxygenase; NO, nitric oxide; LEC, lymphatic endothelial cell; MHC, major histocompatibility complex; PD-L1, programmed death-ligand 1; APC, antigen-presenting cell; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand

Epithelial-mesenchymal transition (EMT) is a well-described phenomenon that occurs during blood vessel metastasis (Lambert et al., 2017). Intriguingly, recent findings suggest that the EMT program may also play a role in lymphatic invasion (Karlsson et al., 2017), which may sound counterintuitive, since the unique button-like structure of the lymphatic capillary does not seem to require cellular transformation for lymphatic intravasation. However, given the notion that the transitional state between the epithelial and mesenchymal phenotypes might skew malignant cells to CSCs and facilitate tumor re-growth (Lambert et al., 2017), the EMT program in lymphatic dissemination may well be an important step.

5 Lymphatic vessels in tumor immunobiology

Cancer is a genetic disease initiated by oncogenic mutations. Once formed, tumors typically gain additional mutations or epigenetic modifications to sustain their growth. Genomic diversity provides an advantage for cancer cell survival, but also increases the likelihood that those malignant cells might be detected and killed by the host immune system (Jiang and Shapiro, 2014; Schumacher and Schreiber, 2015; Chen and Mellman, 2017). Although the lymphatic vessels are known to promote immunity (Hos and Cursiefen, 2014), a positive correlation between lymphatic density and immune response in human cancer was not revealed until recently (Lund et al., 2016). In transgenic mice (K14-VEGFR3-Ig) that lack dermal lymphatic vessels, syngeneic B16F10 melanoma displayed significantly lower infiltration of immune cells, including B cells, T cells, and proinflammatory myeloid cells, as well as diminished expression of a panel of inflammatory mediators (Lund et al., 2016). This finding suggests that induction of tumor immunity requires functional lymphatic vasculature. Consistent with this observations, lymphatic vessels were shown to facilitate tumor antigen-loaded DC trafficking and T cell priming (Roberts et al., 2016); in B16 melanoma-bearing mice with dysfunctional lymphatics, the draining lymph nodes have decreased levels of tumor antigens and lower numbers of cytotoxic $CD8^+$ T cells, and those cells possess lower anti-tumor cytotoxicity (Kimura et al., 2015). In agreement with these preclinical findings, a clinical study showed that, in colorectal cancers (CRCs), distant metastasis occurs more frequently when tumors have a lower immune cytotoxicity profile (Mlecnik et al., 2016). The same study showed that tumors with lower peritumoral lymphatic vascular density tend to be more metastatic, indicating a positive correlation between lymphatic vascular density and tumor immune cytotoxicity profile. These investigations collectively demonstrate that the lymphatics participate in promoting anti-tumor immune responses and in shaping the tumor immune microenvironment.

Development of anti-tumor immunity takes place primarily in tumor-draining lymph nodes (TDLNs) (Chandrasekaran and King, 2014; Yeo and Angeli, 2017). Newly formed tumor lymphatic vasculature provides a conduit for the transportation of

tumor antigens to the draining lymph nodes to facilitate T cell priming (Thomas et al., 2016). Through the lymph flow, tumor-derived antigens can be directly delivered to DCs or B cells residing in the TDLNs (Roozendaal et al., 2009; Thomas et al., 2016). DCs patrolling peripheral tissues also take up tumor antigens, enter lymphatic vessels, and invade TDLNs (Thomas et al., 2016). Antigen-loaded DCs then prime naive T cells and induce antigen-specific anti-tumor immunity. It is worth noting that the local immune signaling microenvironment during antigen presentation may influence the quality of the adaptive immunity, i.e., immune activation or tolerance (Nishikawa and Sakaguchi, 2014; Thomas et al., 2016). The lymphatic vasculature within the tumor may also impact TDLN architecture and function by influencing the delivery of inflammatory modulators, exosomes, and microparticles from the tumor microenvironment (Robbins and Morelli, 2014; Rohner et al., 2015). In line with the role of TDLNs in promoting anti-tumor immunity, nanoparticle-mediated adjuvant therapy targeting TDLNs enhanced tumor immunity characterized by increased cytotoxic T cells and decreased Treg cells within TDLNs (Thomas et al., 2014). Therapeutic vaccination targeting TDLNs also enhanced anti-tumor immunity (Jeanbart et al., 2014). Elevation of CCL3 expression in the tumor microenvironment resulted in reduced tumor growth, presumably by enhancing DC homing to TDLNs and strengthening anti-tumor immunity (Allen et al., 2017). Furthermore, effective checkpoint inhibition therapy requires functional TDLNs (Fransen et al., 2018). These studies indicated that TDLNs are not only vital for the development of anti-tumor immunity but also critical for the acquisition of optimal immunotherapeutic efficacy.

In addition to their function in immune activation, lymph nodes are important sites for the maintenance of self-tolerance (Förster et al., 2008). The lymphatic vasculature also controls self-tolerance by providing a conduit for lymph-borne antigen transportation and regulating the structural organization of the draining lymph nodes (Thomas et al., 2016). The lymphatic system not only elicits anti-tumor immune responses but also promotes tolerance against tumors (Chen and Mellman, 2017). Recent studies provided evidence that LECs participate in the induction of peripheral tolerance through diverse mechanisms, under both physiological and pathological conditions (Card et al., 2014; Randolph et al., 2016; Cui et al., 2017). Because LECs express peripheral tissue antigens and major histocompatibility complex (MHC) molecules, they can directly recognize and suppress T cell activation when the expression levels of inhibitory receptor programmed death-ligand 1 (PD-L1) are high and those of co-stimulatory molecules are low (Lee et al., 2007; Nichols et al., 2007; Malhotra et al., 2012; Tewalt et al., 2012). LEC expression of PD-L1 was later shown to be strongly induced by interferon γ (IFNγ) (Dieterich et al., 2017), and lymphatic-specific deletion of IFNγ receptor (IFNγR) dampened LEC-mediated immune suppression and enhanced tumor immunity (Lane et al., 2018). Because IFNγ is a cytokine that marks immune activation and is produced predominantly by immune effector cells (Schoenborn and Wilson, 2007), PD-L1 induction by IFNγ may represent a natural feedback mechanism employed by the immune system to achieve equilibrium. LECs also produce immunosuppressive mediators, including transforming growth factor-β (TGF-β), nitric oxide (NO), and indoleamine-2,3-dioxygenase (IDO), to induce tolerant DCs and suppress T cell function (Lukacs-Kornek et al., 2011; Nörder et al., 2012; Christiansen et al., 2016). Accordingly, the lymphatics not only induce an active immune response characterized by $CD8^+$ T cell infiltration but also foster a suppressive microenvironment by increasing LEC expression of IDO, inducible nitric oxide synthase (iNOS), and arginase-1 in human tumors (Bordry et al., 2018). Lastly, the lymphatic system can also induce tolerance by directly presenting tumor-derived antigens to activated $CD8^+$ T cells to suppress their activity (Lund et al., 2012). Collectively, these recent findings indicated that tumor lymphatics control both immune activation and tolerance (Fig. 2).

6 Concluding remarks

The lymphatic circulatory system is relatively understudied in cancer compared to the blood vascular system (Folkman, 1971). Recent advances indicate that the tumor-associated lymphatic vasculature is not just a passive conduit for metastasis; it also fulfills vital functions in shaping the immune and inflammatory

responses of the tumor microenvironment. Given the current success in harnessing the immune system to improve the outcome of cancer patients (Chen and Mellman, 2017), it is conceivable that modulating the lymphatic vascular system and TDLNs in conjunction with immunotherapeutic strategies will promote treatment efficacy while ideally reducing unwanted side effects (Fig. 2).

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Compliance with ethics guidelines

Xinguo JIANG declares that he has no conflict of interest.

This article does not contain any studies with human or animal subjects performed by the author.

References

Allen F, Rauhe P, Askew D, et al., 2017. CCL3 enhances antitumor immune priming in the lymph node via IFNγ with dependency on natural killer cells. *Front Immunol*, 8:1390. https://doi.org/10.3389/fimmu.2017.01390

Arbiser JL, Moses MA, Fernandez CA, et al., 1997. Oncogenic H-ras stimulates tumor angiogenesis by two distinct pathways. *Proc Natl Acad Sci USA*, 94(3):861-866. https://doi.org/10.1073/pnas.94.3.861

Ariffin AB, Forde PF, Jahangeer S, et al., 2014. Releasing pressure in tumors: what do we know so far and where do we go from here? A review. *Cancer Res*, 74(10):2655-2662. https://doi.org/10.1158/0008-5472

Aspelund A, Robciuc MR, Karaman S, et al., 2016. Lymphatic system in cardiovascular medicine. *Circul Res*, 118(3): 515-530. https://doi.org/10.1161/CIRCRESAHA.115.306544

Baluk P, Fuxe J, Hashizume H, et al., 2007. Functionally specialized junctions between endothelial cells of lymphatic vessels. *J Exp Med*, 204(10):2349-2362. https://doi.org/10.1084/jem.20062596

Baluk P, Yao LC, Feng J, et al., 2009. TNF-α drives remodeling of blood vessels and lymphatics in sustained airway inflammation in mice. *J Clin Invest*, 119(10):2954-2964. https://doi.org/10.1172/JCI37626

Baluk P, Hogmalm A, Bry M, et al., 2013. Transgenic overexpression of interleukin-1β induces persistent lymphangiogenesis but not angiogenesis in mouse airways. *Am J Pathol*, 182(4):1434-1447. https://doi.org/10.1016/j.ajpath.2012.12.003

Boardman KC, Swartz MA, 2003. Interstitial flow as a guide for lymphangiogenesis. *Circul Res*, 92(7):801-808. https://doi.org/10.1161/01.RES.0000065621.69843.49

Bordry N, Broggi MAS, de Jonge K, et al., 2018. Lymphatic vessel density is associated with $CD8^+$ T cell infiltration and immunosuppressive factors in human melanoma. *Oncoimmunology*, 7(8):e1462878. https://doi.org/10.1080/2162402X.2018.1462878

Cao YH, 2005. Emerging mechanisms of tumour lymphangiogenesis and lymphatic metastasis. *Nat Rev Cancer*, 5(9): 735-743. https://doi.org/10.1038/nrc1693

Card CM, Yu SS, Swartz MA, 2014. Emerging roles of lymphatic endothelium in regulating adaptive immunity. *J Clin Invest*, 124(3):943-952. https://doi.org/10.1172/JCI73316

Chandrasekaran S, King MR, 2014. Microenvironment of tumor-draining lymph nodes: opportunities for liposome-based targeted therapy. *Int J Mol Sci*, 15(11):20209-20239. https://doi.org/10.3390/ijms151120209

Chen DS, Mellman I, 2017. Elements of cancer immunity and the cancer-immune set point. *Nature*, 541(7637):321-330. https://doi.org/10.1038/nature21349

Christiansen AJ, Dieterich LC, Ohs I, et al., 2016. Lymphatic endothelial cells attenuate inflammation via suppression of dendritic cell maturation. *Oncotarget*, 7(26):39421-39435. https://doi.org/10.18632/oncotarget.9820

Cui Y, Liu K, Lamattina AM, et al., 2017. Lymphatic vessels: the next frontier in lung transplant. *Ann Am Thorac Soc*, 14(S3):S226-S232. https://doi.org/10.1513/AnnalsATS.201606-465MG

da Mesquita S, Louveau A, Vaccari A, et al., 2018. Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. *Nature*, 560(7717):185-191. https://doi.org/10.1038/s41586-018-0368-8

Dadras SS, Paul T, Bertoncini J, et al., 2003. Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. *Am J Pathol*, 162(6):1951-1960. https://doi.org/10.1016/S0002-9440(10)64328-3

Das S, Sarrou E, Podgrabinska S, et al., 2013. Tumor cell entry into the lymph node is controlled by CCL1 chemokine expressed by lymph node lymphatic sinuses. *J Exp Med*, 210(8):1509-1528. https://doi.org/10.1084/jem.20111627

Dieterich LC, Ikenberg K, Cetintas T, et al., 2017. Tumor-associated lymphatic vessels upregulate PDL1 to inhibit T-cell activation. *Front Immunol*, 8:66. https://doi.org/10.3389/fimmu.2017.00066

Enholm B, Paavonen K, Ristimaki A, et al., 1997. Comparison of VEGF, VEGF-B, VEGF-C and Ang-1 mRNA regulation by serum, growth factors, oncoproteins and hypoxia. *Oncogene*, 14(20):2475-2483. https://doi.org/10.1038/sj.onc.1201090

Fankhauser M, Broggi MAS, Potin L, et al., 2017. Tumor lymphangiogenesis promotes T cell infiltration and potentiates immunotherapy in melanoma. *Sci Transl Med*, 9(407):eaal4712. https://doi.org/10.1126/scitranslmed.aal4712

Folkman J, 1971. Tumor angiogenesis: therapeutic implications. *N Engl J Med*, 285(21):1182-1186. https://doi.org/10.1056/NEJM197111182852108

Förster R, Davalos-Misslitz AC, Rot A, 2008. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol*, 8(5):362-371. https://doi.org/10.1038/nri2297

Fransen MF, Schoonderwoerd M, Knopf P, et al., 2018. Tumor-draining lymph nodes are pivotal in PD-1/PD-L1 checkpoint therapy. *JCI Insight*, 3(23):e124507. https://doi.org/10.1172/jci.insight.124507

Gao P, Li CJ, Chang Z, et al., 2018. Carcinoma associated fibroblasts derived from oral squamous cell carcinoma promote lymphangiogenesis via c-Met/PI3K/AKT in vitro. *Oncol Lett*, 15(1):331-337. https://doi.org/10.3892/ol.2017.7301

Guan XM, 2015. Cancer metastases: challenges and opportunities. *Acta Pharm Sin B*, 5(5):402-418. https://doi.org/10.1016/j.apsb.2015.07.005

Harris AR, Perez MJ, Munson JM, 2018. Docetaxel facilitates lymphatic-tumor crosstalk to promote lymphangiogenesis and cancer progression. *BMC Cancer*, 18:718. https://doi.org/10.1186/s12885-018-4619-8

Harvey NL, Gordon EJ, 2012. Deciphering the roles of macrophages in developmental and inflammation stimulated lymphangiogenesis. *Vascular Cell*, 4(1):15. https://doi.org/10.1186/2045-824X-4-15

Henri O, Pouehe C, Houssari M, et al., 2016. Selective stimulation of cardiac lymphangiogenesis reduces myocardial edema and fibrosis leading to improved cardiac function following myocardial infarction. *Circulation*, 133(15):1484-1497. https://doi.org/10.1161/CIRCULATIONAHA.115.020143

Hirakawa S, Kodama S, Kunstfeld R, et al., 2005. VEGF-A induces tumor and sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis. *J Exp Med*, 201(7):1089-1099. https://doi.org/10.1084/jem.20041896

Hirakawa S, Brown LF, Kodama S, et al., 2007. VEGF-C-induced lymphangiogenesis in sentinel lymph nodes promotes tumor metastasis to distant sites. *Blood*, 109(3):1010-1017. https://doi.org/10.1182/blood-2006-05-021758

Hirakawa S, Detmar M, Kerjaschki D, et al., 2009. Nodal lymphangiogenesis and metastasis: role of tumor-induced lymphatic vessel activation in extramammary Paget's disease. *Am J Pathol*, 175(5):2235-2248. https://doi.org/10.2353/ajpath.2009.090420

Hos D, Cursiefen C, 2014. Lymphatic vessels in the development of tissue and organ rejection. *In*: Kiefer F, Schulte-Merker S (Eds.), Developmental Aspects of the Lymphatic Vascular System. Advances in Anatomy, Embryology and Cell Biology, Vol. 214. Springer, Vienna, p.119-141. https://doi.org/10.1007/978-3-7091-1646-3_10

Imai T, Hieshima K, Haskell C, et al., 1997. Identification and molecular characterization of fractalkine receptor CX_3CR1, which mediates both leukocyte migration and adhesion. *Cell*, 91(4):521-530. https://doi.org/10.1016/S0092-8674(00)80438-9

Jackson DG, 2014. Lymphatic regulation of cellular trafficking. *J Clin Cell Immunol*, 5:258. https://doi.org/10.4172/2155-9899.1000258

Jeanbart L, Ballester M, de Titta A, et al., 2014. Enhancing efficacy of anticancer vaccines by targeted delivery to tumor-draining lymph nodes. *Cancer Immunol Res*, 2(5):436-447. https://doi.org/10.1158/2326-6066

Jeltsch M, Kaipainen A, Joukov V, et al., 1997. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science*, 276(5317):1423-1425. https://doi.org/10.1126/science.276.5317.1423

Jiang XG, Shapiro DJ, 2014. The immune system and inflammation in breast cancer. *Mol Cell Endocrinol*, 382(1):673-682. https://doi.org/10.1016/j.mce.2013.06.003

Jiang XG, Nicolls MR, Tian W, et al., 2018. Lymphatic dysfunction, leukotrienes, and lymphedema. *Ann Rev Physiol*, 80:49-70. https://doi.org/10.1146/annurev-physiol-022516-034008

Kabashima K, Shiraishi N, Sugita K, et al., 2007a. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. *Am J Pathol*, 171(4):1249-1257. https://doi.org/10.2353/ajpath.2007.070225

Kabashima K, Sugita K, Shiraishi N, et al., 2007b. CXCR4 engagement promotes dendritic cell survival and maturation. *Biochem Biophys Res Commun*, 361(4):1012-1016. https://doi.org/10.1016/j.bbrc.2007.07.128

Karaman S, Detmar M, 2014. Mechanisms of lymphatic metastasis. *J Clin Invest*, 124(3):922-928. https://doi.org/10.1172/JCI71606

Karlsson MC, Gonzalez SF, Welin J, et al., 2017. Epithelial-mesenchymal transition in cancer metastasis through the lymphatic system. *Mol Oncol*, 11(7):781-791. https://doi.org/10.1002/1878-0261.12092

Kerjaschki D, Bago-Horvath Z, Rudas M, et al., 2011. Lipoxygenase mediates invasion of intrametastatic lymphatic vessels and propagates lymph node metastasis of human mammary carcinoma xenografts in mouse. *J Clin Invest*, 121(5):2000-2012. https://doi.org/10.1172/JCI44751

Kim S, Chung M, Jeon NL, 2016. Three-dimensional biomimetic model to reconstitute sprouting lymphangiogenesis in vitro. *Biomaterials*, 78:115-128. https://doi.org/10.1016/j.biomaterials.2015.11.019

Kimura T, Sugaya M, Oka T, et al., 2015. Lymphatic dysfunction attenuates tumor immunity through impaired antigen presentation. *Oncotarget*, 6(20):18081-18093. https://doi.org/10.18632/oncotarget.4018

Lambert AW, Pattabiraman DR, Weinberg RA, 2017. Emerging biological principles of metastasis. *Cell*, 168(4):670-691. https://doi.org/10.1016/j.cell.2016.11.037

Lane RS, Femel J, Breazeale AP, et al., 2018. IFNγ-activated dermal lymphatic vessels inhibit cytotoxic T cells in melanoma and inflamed skin. *J Exp Med*, 215(12):3057. https://doi.org/10.1084/jem.20180654

Lee JW, Epardaud M, Sun J, et al., 2007. Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self. *Nat Immunol*, 8(2):181-190.

https://doi.org/10.1038/ni1427

Lopez Gelston CA, Balasubbramanian D, Abouelkheir GR, et al., 2018. Enhancing renal lymphatic expansion prevents hypertension in mice. *Circul Res*, 122(8):1094-1101. https://doi.org/10.1161/CIRCRESAHA.118.312765

Lukacs-Kornek V, Malhotra D, Fletcher AL, et al., 2011. Regulated release of nitric oxide by nonhematopoietic stroma controls expansion of the activated T cell pool in lymph nodes. *Nat Immunol*, 12(11):1096-1104. https://doi.org/10.1038/ni.2112

Lund AW, Duraes FV, Hirosue S, et al., 2012. VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. *Cell Rep*, 1(3):191-199. https://doi.org/10.1016/j.celrep.2012.01.005

Lund AW, Wagner M, Fankhauser M, et al., 2016. Lymphatic vessels regulate immune microenvironments in human and murine melanoma. *J Clin Invest*, 126(9):3389-3402. https://doi.org/10.1172/JCI79434

Mäkinen T, Norrmén C, Petrova TV, 2007. Molecular mechanisms of lymphatic vascular development. *Cell Mol Life Sci*, 64(15):1915-1929. https://doi.org/10.1007/s00018-007-7040-z

Malhotra D, Fletcher AL, Astarita J, et al., 2012. Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat Immunol*, 13(5):499-510. https://doi.org/10.1038/ni.2262

Mlecnik B, Bindea G, Kirilovsky A, et al., 2016. The tumor microenvironment and immunoscore are critical determinants of dissemination to distant metastasis. *Sci Transl Med*, 8(327):327ra26. https://doi.org/10.1126/scitranslmed.aad6352

Mortimer PS, Rockson SG, 2014. New developments in clinical aspects of lymphatic disease. *J Clin Invest*, 124(3):915-921. https://doi.org/10.1172/JCI71608

Müller A, Homey B, Soto H, et al., 2001. Involvement of chemokine receptors in breast cancer metastasis. *Nature*, 410(6824):50-56. https://doi.org/10.1038/35065016

Nandi P, Girish GV, Majumder M, et al., 2017. PGE2 promotes breast cancer-associated lymphangiogenesis by activation of EP4 receptor on lymphatic endothelial cells. *BMC Cancer*, 17:11. https://doi.org/10.1186/s12885-016-3018-2

Nichols LA, Chen YM, Colella TA, et al., 2007. Deletional self-tolerance to a melanocyte/melanoma antigen derived from tyrosinase is mediated by a radio-resistant cell in peripheral and mesenteric lymph nodes. *J Immunol*, 179(2):993-1003. https://doi.org/10.4049/jimmunol.179.2.993

Nishikawa H, Sakaguchi S, 2014. Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol*, 27:1-7. https://doi.org/10.1016/j.coi.2013.12.005

Nörder M, Gutierrez MG, Zicari S, et al., 2012. Lymph node-derived lymphatic endothelial cells express functional costimulatory molecules and impair dendritic cell-induced allogenic T-cell proliferation. *FASEB J*, 26(7):2835-2846. https://doi.org/10.1096/fj.12-205278

Paduch R, 2016. The role of lymphangiogenesis and angiogenesis in tumor metastasis. *Cell Oncol*, 39(5):397-410. https://doi.org/10.1007/s13402-016-0281-9

Pflicke H, Sixt M, 2009. Preformed portals facilitate dendritic cell entry into afferent lymphatic vessels. *J Exp Med*, 206(13):2925-2935. https://doi.org/10.1084/jem.20091739

Proulx ST, Detmar M, 2013. Molecular mechanisms and imaging of lymphatic metastasis. *Exp Cell Res*, 319(11):1611-1617. https://doi.org/10.1016/j.yexcr.2013.03.009

Randolph GJ, Ivanov S, Zinselmeyer BH, et al., 2016. The lymphatic system: integral roles in immunity. *Annu Rev Immunol*, 35:31-52. https://doi.org/10.1146/annurev-immunol-041015-055354

Robbins PD, Morelli AE, 2014. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol*, 14(3):195-208. https://doi.org/10.1038/nri3622

Roberts EW, Broz ML, Binnewies M, et al., 2016. Critical role for $CD103^+/CD141^+$ dendritic cells bearing CCR7 for tumor antigen trafficking and priming of T cell immunity in melanoma. *Cancer Cell*, 30(2):324-336. https://doi.org/10.1016/j.ccell.2016.06.003

Rockson SG, Tian W, Jiang XG, et al., 2018. Pilot studies demonstrate the potential benefits of antiinflammatory therapy in human lymphedema. *JCI Insight*, 3(20):e123775. https://doi.org/10.1172/jci.insight.123775

Rohner NA, McClain J, Tuell SL, et al., 2015. Lymph node biophysical remodeling is associated with melanoma lymphatic drainage. *FASEB J*, 29(11):4512-4522. https://doi.org/10.1096/fj.15-274761

Roozendaal R, Mempel TR, Pitcher LA, et al., 2009. Conduits mediate transport of low-molecular-weight antigen to lymph node follicles. *Immunity*, 30(2):264-276. https://doi.org/10.1016/j.immuni.2008.12.014

Sainz-Jaspeado M, Claesson-Welsh L, 2018. Cytokines regulating lymphangiogenesis. *Curr Opin Immunol*, 53:58-63. https://doi.org/10.1016/j.coi.2018.04.003

Schoenborn JR, Wilson CB, 2007. Regulation of interferon-γ during innate and adaptive immune responses. *Adv Immunol*, 96:41-101. https://doi.org/10.1016/S0065-2776(07)96002-2

Schumacher TN, Schreiber RD, 2015. Neoantigens in cancer immunotherapy. *Science*, 348(6230):69-74. https://doi.org/10.1126/science.aaa4971

Shayan R, Achen MG, Stacker SA, 2006. Lymphatic vessels in cancer metastasis: bridging the gaps. *Carcinogenesis*, 27(9):1729-1738. https://doi.org/10.1093/carcin/bgl031

Shin K, Kataru RP, Park HJ, et al., 2015. T_H2 cells and their cytokines regulate formation and function of lymphatic vessels. *Nat Commun*, 6:6196. https://doi.org/10.1038/ncomms7196

Skobe M, Hawighorst T, Jackson DG, et al., 2001. Induction of

tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. *Nat Med*, 7(2):192-198. https://doi.org/10.1038/84643

Stacker SA, Caesar C, Baldwin ME, et al., 2001. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat Med*, 7(2):186-191. https://doi.org/10.1038/84635

Stacker SA, Williams SP, Karnezis T, et al., 2014. Lymphangiogenesis and lymphatic vessel remodelling in cancer. *Nat Rev Cancer*, 14(3):159-172. https://doi.org/10.1038/nrc3677

Stump B, Cui Y, Kidambi P, et al., 2017. Lymphatic changes in respiratory diseases: more than just remodeling of the lung? *Am J Respir Cell Mol Biol*, 57(3):272-279. https://doi.org/10.1165/rcmb.2016-0290TR

Su WC, Shiesh SC, Liu HS, et al., 2001. Expression of oncogene products HER2/Neu and Ras and fibrosis-related growth factors bFGF, TGF-β, and PDGF in bile from biliary malignancies and inflammatory disorders. *Dig Dis Sci*, 46(7):1387-1392.

Tammela T, Alitalo K, 2010. Lymphangiogenesis: molecular mechanisms and future promise. *Cell*, 140(4):460-476. https://doi.org/10.1016/j.cell.2010.01.045

Tewalt EF, Cohen JN, Rouhani SJ, et al., 2012. Lymphatic endothelial cells induce tolerance via PD-L1 and lack of costimulation leading to high-level PD-1 expression on CD8 T cells. *Blood*, 120(24):4772-4782. https://doi.org/10.1182/blood-2012-04-427013

Thomas SN, Vokali E, Lund AW, et al., 2014. Targeting the tumor-draining lymph node with adjuvanted nanoparticles reshapes the anti-tumor immune response. *Biomaterials*, 35(2):814-824. https://doi.org/10.1016/j.biomaterials.2013.10.003

Thomas SN, Rohner NA, Edwards EE, 2016. Implications of lymphatic transport to lymph nodes in immunity and immunotherapy. *Annu Rev Biomed Eng*, 18:207-233. https://doi.org/10.1146/annurev-bioeng-101515-014413

Tian W, Rockson SG, Jiang XG, et al., 2017. Leukotriene B_4 antagonism ameliorates experimental lymphedema. *Sci Transl Med*, 9(389):eaal3920. https://doi.org/10.1126/scitranslmed.aal3920

Ueba T, Nosaka T, Takahashi JA, et al., 1994. Transcriptional regulation of basic fibroblast growth factor gene by p53 in human glioblastoma and hepatocellular carcinoma cells. *Proc Natl Acad Sci USA*, 91(19):9009-9013. https://doi.org/10.1073/pnas.91.19.9009

Uramoto H, Hackzell A, Wetterskog D, et al., 2004. pRb, Myc and p53 are critically involved in SV40 large T antigen repression of PDGF β-receptor transcription. *J Cell Sci*, 117(17):3855-3865. https://doi.org/10.1242/jcs.01228

Vaahtomeri K, Karaman S, Makinen T, et al., 2017. Lymphangiogenesis guidance by paracrine and pericellular factors. *Genes Dev*, 31(16):1615-1634. https://doi.org/10.1101/gad.303776.117

Varricchi G, Loffredo S, Galdiero MR, et al., 2018. Innate effector cells in angiogenesis and lymphangiogenesis. *Curr Opin Immunol*, 53:152-160. https://doi.org/10.1016/j.coi.2018.05.002

Vieira JM, Norman S, del Campo CV, et al., 2018. The cardiac lymphatic system stimulates resolution of inflammation following myocardial infarction. *J Clin Invest*, 128(8):3402-3412. https://doi.org/10.1172/JCI97192

Wei R, Lv MQ, Li F, et al., 2017. Human CAFs promote lymphangiogenesis in ovarian cancer via the Hh-VEGF-C signaling axis. *Oncotarget*, 8(40):67315-67328. https://doi.org/10.18632/oncotarget.18621

Weichand B, Popp R, Dziumbla S, et al., 2017. S1PR1 on tumor-associated macrophages promotes lymphangiogenesis and metastasis via NLRP3/IL-1β. *J Exp Med*, 214(9):2695-2713. https://doi.org/10.1084/jem.20160392

Wong BW, Wang XW, Zecchin A, et al., 2017. The role of fatty acid β-oxidation in lymphangiogenesis. *Nature*, 542(7639):49-54. https://doi.org/10.1038/nature21028

Yamada A, Nagahashi M, Aoyagi T, et al., 2018. ABCC1-exported sphingosine-1-phosphate, produced by Sphingosine kinase 1, shortens survival of mice and patients with breast cancer. *Mol Cancer Res*, 16(6):1059-1070. https://doi.org/10.1158/1541-7786

Yeo KP, Angeli V, 2017. Bidirectional crosstalk between lymphatic endothelial cell and T cell and its implications in tumor immunity. *Front Immunol*, 8:83. https://doi.org/10.3389/fimmu.2017.00083

Yu PC, Wilhelm K, Dubrac A, et al., 2017. FGF-dependent metabolic control of vascular development. *Nature*, 545(7653):224-228. https://doi.org/10.1038/nature22322

Zheng W, Tammela T, Yamamoto M, et al., 2011. Notch restricts lymphatic vessel sprouting induced by vascular endothelial growth factor. *Blood*, 118(4):1154-1162. https://doi.org/10.1182/blood-2010-11-317800

Zheng W, Aspelund A, Alitalo K, 2014. Lymphangiogenic factors, mechanisms, and applications. *J Clin Invest*, 124(3):878-887. https://doi.org/10.1172/JCI71603

中文概要

题　目：淋巴血管在肿瘤转移和免疫生物学中的作用

概　要：淋巴系统被认为是肿瘤转移的重要途径之一，所以通常情况下肿瘤引起的淋巴血管增生会降低肿瘤预后，治疗上也建议淋巴清扫。但是最新的研究显示，淋巴系统可能对肿瘤免疫治疗有促进作用。这篇综述的主要目的是对相关领域做一个简短总结，以期待将来有更多的研究来关注淋巴系统对肿瘤治疗的影响。文章首先介绍肿瘤淋巴血管增生和淋巴转移的分子机制，然后介绍淋巴系统在肿瘤免疫中的作用，最后利用最新研究来证明淋巴系统有增强肿瘤免疫治疗的作用。

关键词：淋巴系统；淋巴内皮细胞；肿瘤；转移；免疫治疗