

Review

Role of lymphangiogenic factors in tumor metastasis

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Abstract

Nearly four centuries after the discovery of lymphatic vessels, the molecular mechanisms underlying their development are beginning to be elucidated. Vascular endothelial growth factor C (VEGF-C) and VEGF-D, via signaling through VEGFR-3, appear to be essential for lymphatic vessel growth. Observations from clinicopathological studies have suggested that lymphatic vessels serve as the primary route for the metastatic spread of tumor cells to regional lymph nodes. Recent studies in animal models have provided convincing evidence that tumor lymphangiogenesis facilitates lymphatic metastasis. However, it is not clear how tumor-associated lymphangiogenesis is regulated, and little is known about how tumor cells escape from the primary tumor and gain entry into the lymphatics. This review examines some of these issues and provides a brief summary of the recent developments in this field of research.

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1. Introduction

The major roles of the lymphatic system include the maintenance of tissue fluid homeostasis and transport of macromolecules and immune cells back into the blood circulation via the collecting lymphatic vessels and the thoracic duct. Two theories about the development of the lymphatic vessel system were proposed at the beginning of the last century; these were the venous origin of the lymphatic vessels [1] and de novo formation of primary lymph sacs in the mesenchyme [2]. Supporting evidence has been published for both views [3,4], suggesting that the development of the lymphatic system may require both mechanisms. Studies during the last few years have provided significant insights into the molecular mechanisms underlying the development of lymphatic vessels and the role of lymphangiogenesis in health and disease [5]. The achievement owes largely to the discovery of the key lymphatic growth factors (vascular endothelial growth factor C (VEGF-C) and VEGF-D) and their receptor VEGFR-3, and more recently of several specific molecular markers

to identify lymphatic vessels. Such developments have opened up a new research frontier, extending the tumor angiogenesis field to studies of tumor lymphangiogenesis and metastasis.

2. Molecular regulation of lymphatic vessel development

The identification of specific lymphatic markers has greatly facilitated studies of lymphangiogenesis. VEGFR-3 is one of the first lymphatic markers identified. During the murine development, VEGFR-3 was initially expressed in endothelial cells (ECs) of developing blood vessels, but its expression becomes restricted to lymphatic endothelial cells (LECs) after midgestation and persists in adult lymphatic vessels [6]. Expression of VEGFR-3 has also been shown in adult fenestrated blood vessel endothelia [7], and in some tumor-associated blood vessels [8,9]. Podoplanin, an integral plasma membrane protein first identified in glomerular epithelial cells, is expressed by LECs and provides a useful lymphatic endothelial marker [10–12]. However, it was also found to be expressed by some endothelial cells of skin blood vessels [13]. The lymphatic endothelial hyaluronan receptor (LYVE-1) has been used as a specific lymphatic endothelial marker to identify lymphatic vessels in normal and tumor tissues [14–16], with the exception of being

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expressed also by liver sinusoidal ECs [17]. The prospero-related homeobox protein 1 (Prox-1) is expressed by a variety of cell types but among endothelial cells it is only detected in LECs of normal and tumor tissues [3,18]. Other markers used to identify lymphatic vessels include desmoplakin [19], 5' nucleotidase [20], macrophage mannose receptor [21,22], and the β -chemokine receptor D6 [23]. It should be noted that none of the markers is entirely specific for LECs.

Two lymphangiogenic growth factors have so far been characterized, named VEGF-C and VEGF-D. Both have been shown to induce lymphangiogenesis in transgenic mice and in other *in vivo* models [24–26]. They signal primarily through VEGFR-3 [26–30]. VEGF-C null mice die before birth, and mice heterozygous for VEGF-C deficiency develop chylous fluid in the abdomen after birth, suggesting haploinsufficiency of VEGF-C for lymphatic vessel development [111]. VEGF-C and VEGF-D are first produced as pre-pro-polypeptides, and the stepwise proteolytic processing increases their affinities for VEGFR-3. The fully processed mature forms also bind to and activate VEGFR-2 [31,32]. Therefore, both factors can also exert angiogenic activity via VEGFR-2 [33–35].

Signaling via VEGFR-3 is also important for the remodeling of primary vascular networks into larger blood vessels, a function essential for the development of the cardiovascular system in embryos [36]. As VEGFR-3 null mice die before the emergence of lymphatic vessels, they could not be used for analysis of the significance of VEGFR-3 in lymphatic vessel development. Recent work by Makinen et al. [30] using a transgenic mouse model provided evidence for the essential role of VEGFR-3 in lymphangiogenesis. In this model, cDNA encoding a soluble VEGFR-3-Ig fusion protein, a potent antagonist of VEGFR-3 signaling that traps VEGF-C and VEGF-D, was overexpressed in mice under the human keratin 14 promoter [37]. Overexpression of this fusion protein induced apoptosis of growing LECs and resulted in the regression of already formed lymphatic vessels in embryonic skin, whereas the blood vasculature was not affected [30]. Lymphatic vessel growth was also suppressed in internal organs due to the systemic effect of this protein circulating in the blood. The absence of skin lymphatics persisted after birth and the mice developed lymphedema exemplified by the swelling of their feet. However, the lymphatics began to grow back at least partially in the internal organs when the pups were three weeks old [30]. This suggests that lymphangiogenesis is differentially regulated in the skin and in the internal organs.

The Chy mice provide an independent genetic lymphedema model where a missense mutation in the tyrosine kinase domain of VEGFR-3 inactivates the tyrosine kinase [38]. In these mice, lymphatic vessels were absent from the skin but present in the internal organs, for example around the aorta. In this regard, it is interesting that neuropilin-2 (NRP2), a possible coreceptor for VEGF-C, appeared to be

more weakly expressed in the lymphatics of the skin than in internal organs [38]. It is possible that mechanical stresses such as interstitial fluid pressure and strain of the extracellular matrix may differ between the skin and the internal organs, and may also account for the differences. A recent study using a skin regeneration model suggests that the interstitial fluid flow regulates the lymphangiogenic process to establish the lymphatic capillary network [39]. The importance of VEGFR-3 in lymphangiogenesis was also supported by evidence obtained from another transgenic mouse model [26], where a mutant form of VEGF-C (VEGF-C156S), which can only bind to VEGFR-3, was shown to induce hyperplastic lymphatic vessels similar to those seen in VEGF-C transgenic mice [24].

In addition to VEGF-C and VEGF-D, other factors crucial for lymphatic vessel development have been identified including Prox1, the integrin $\alpha 9\beta 1$, the Net Ets-domain transcription factor, NRP2 and angiopoietin-2 (Ang2). Mice deficient of Prox-1 failed to develop lymphatic vessels and heterozygous knockout mice showed accumulation of milky fluid in the intestines after birth [3]. The process of polarized endothelial cell budding from the cardinal vein became abnormal in Prox1 null embryos starting at E11.5, and the budding cells did not show markers of LECs [18]. The LEC fate-determining role of Prox1 has also been evident from studies where blood vascular endothelial cells (BECs) overexpressing Prox1 delivered by an adenoviral vector showed suppression of BEC-specific gene transcription and simultaneous induction of LEC-specific genes [40,41]. Mice null for the integrin $\alpha 9$ subunit died within 6–12 days after birth due to respiratory failure. This was shown to be associated with edema and extravascular lymphocytes surrounding the thoracic duct and other lymphatic vessels, suggesting a defect in lymphatic development [42]. In Net-targeted mutant mice, dilated lymphatic vessels were seen as early as E16.5, and mice died after birth due to respiratory failure, resulting from the accumulation of chyle in the thoracic cage (chylothorax) [43]. Loss of NRP2 function has been shown to lead selectively to hypoplasia of small lymphatic vessels and capillaries but not larger collecting lymphatic vessels or veins [44]. However, lymphatic vessels began to grow back starting from postnatal day seven onwards in different organs [44]. While Ang2 is not required for the formation of lymphatics, it plays a key role in their subsequent remodeling and maturation. Mice lacking Ang2 develop chylous ascites shortly after birth, and subcutaneous edema due to defective lymphatic vessels [45]. Further mechanistic studies are required to get a complete picture of various molecular pathways controlling the development of the lymphatic system.

3. Lymphangiogenesis promotes lymphatic metastasis

Accumulating data from clinicopathological studies suggest that spread of cancer cells to regional lymph nodes is an

early event for many solid tumors, and lymphatic vessels serve as the primary route for this spread [46]. However, until recently the issue regarding the existence of lymphatic vessels in human cancers has been controversial. The availability of lymphatic markers has helped to clarify the situation. These markers have been used alone or in combination to study the presence of lymphatic vessels in tumors and their association with lymph node metastasis. Intra-tumoral lymphatic vessels have been detected in some human cancers including squamous cell carcinomas of the head and neck (HNSCC) [47], and primary melanomas [48,49]. In oropharyngeal carcinomas, it has been shown that a high density of LYVE-1-stained vascular structures correlates with the presence of regional lymph node metastases. The intra-tumoral lymphatic vessels in HNSCC are proliferating as demonstrated by positive staining for the proliferation-associated nuclear protein pKi67 in the LECs [47]. A significant correlation between lymphangiogenesis and lymph node metastasis has also been shown in human breast cancer using podoplanin as a lymphatic marker [50].

Recent studies using animal models have provided convincing evidence for the role of lymphangiogenesis in lymphatic metastasis. Lymphatic vessels were detected in a variety of tumor xenografts [51–54], and in chemically induced orthotopic squamous cell carcinomas in mice [55]. Human breast cancer cell lines MDA-MB-435 and MCF-7, genetically modified to overexpress VEGF-C, showed enhanced rates of tumor spread to regional lymph nodes by inducing peri- and/or intra-tumoral lymphatic vessel growth when implanted orthotopically into the fat pads of mammary gland [51,52,56]. In a genetic model of pancreatic islet cell carcinomas, overexpression of VEGF-C by β -cells of the endocrine pancreas increased lymphangiogenesis surrounding the primary tumor and enhanced tumor spread to the regional lymph nodes [57]. VEGF-D expression has also been shown to induce the formation of lymphatic vessels in a xenotransplant model of transformed human kidney cells. This resulted in significantly increased incidence of lymphatic metastasis to regional lymph nodes [53].

Our recent study provided evidence validating the importance of lymphangiogenesis in lymphatic metastasis (Fig. 1). Overexpression of VEGFR-3-Ig by stably transfected LNM35 cells, a human lung cancer cell line selected for a highly metastatic phenotype and expressing high levels of endogenous VEGF-C, or by systemic adenoviral delivery, inhibited tumor lymphangiogenesis and lymph node metastasis when the cells were grown as tumors in immunodeficient mice [52,54]. Subsequently, when a similar approach was employed in a syngeneic mammary tumor model in immunocompetent rats, inhibition of lymph node metastasis was also obtained [58]. However, preexisting lymphatics were not affected by the VEGFR-3-Ig treatment. This suggests that newly formed lymphatic vessels are necessary for lymphatic metastasis. Furthermore, some authors have also shown that the tumor lymphatics have unique molecular determinants that could be targeted [59].

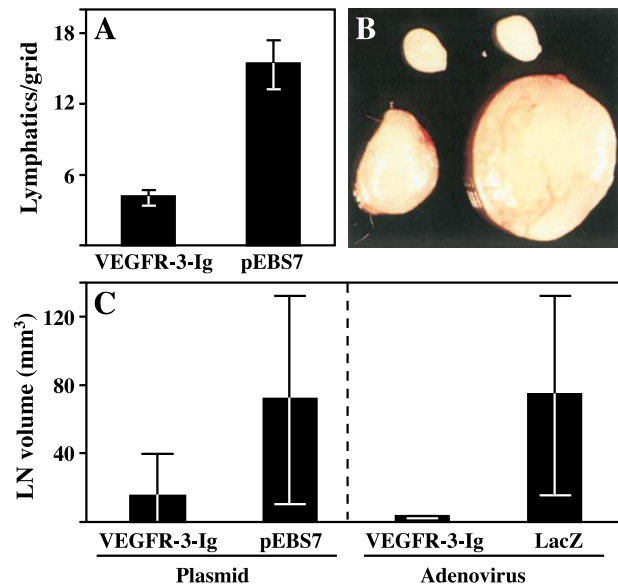


Fig. 1. Blockade of lymph node metastasis by the inhibition of tumor-associated lymphatic vessel growth. (A) The human lung cancer cell line NCI-H460-LNM35 (LNM35) is a subline of NCI-H460, a human large cell carcinoma of the lung. LNM35 tumor cells were selected for a highly metastatic phenotype and express high levels of endogenous VEGF-C. Overexpression of VEGFR-3-Ig by stably transfected LNM35 cells, or by systemic adenoviral delivery, inhibited tumor lymphangiogenesis and lymph node metastasis when the cells were grown as tumors in immunodeficient mice. LYVE-1-stained vessels in three microscopic fields of the highest vessel density were counted, and the results were compared by using the unpaired *t* test. There is a statistically significant decrease of LYVE-1-stained vessels ($P < 0.001$) in VEGFR-3-Ig expressing LNM35 tumors. Error bars = 95% confidence intervals. (B) Typical lymph nodes in mice bearing VEGFR-3-Ig tumors (upper pair) and control LNM35 tumors (lower pair). (C) Lymph node (LN) volume and 95% confidence intervals (plasmids: $n = 12$, $P = 0.070$; adenovirus: $n = 14$ for AdR3-Ig; 7 for control; $P < 0.001$). The figure was modified from He et al. [54], by permission of Oxford University Press.

However, it should be emphasized that although newly formed lymphatic vessels are necessary components for lymphatic metastasis, lymphangiogenesis alone cannot cause lymph node metastasis. Overexpression of VEGF-C by the poorly metastatic N15 tumor cells was shown to induce intra- and peri-tumoral lymphatic vessels, but no increase of lymph node metastasis was detected [54]. This suggests that the switch from poorly to highly metastatic phenotype of tumor cells is a complex process and may involve changes in the expression of many genes [60]. Studies using high-throughput approaches such as microarray analysis should provide significant insights into this phenomenon.

Intensive research during the last few years has provided a better understanding of tumor spread via lymphatic vessels. However, there are still many questions waiting to be addressed. One prominent one is how tumor-associated lymphangiogenesis is regulated. Tumor-associated lymphatics could be formed by direct vessel co-option, by sprouting and/or splitting from preexisting lymphatic vessels in surrounding tissues, or by recruitment of LEC progenitors from bone marrow. The existence of LEC

progenitors has been suggested in avian embryogenesis [4]. VEGFR-3⁺/CD34⁺ endothelial precursors have also recently been identified from human fetal liver and blood, and upon culture they were shown to express both vascular and lymphatic EC markers [61]. It remains to be investigated which is the main mechanism contributing to tumor lymphangiogenesis.

So far only some types of cancer have been shown to contain lymphatic vessels although expression of VEGF-C mRNA and/or protein occurs in a variety of human cancers examined (Table 1) [46,62]. Mechanisms underlying the regulation of VEGF-C expression in tumors are not fully understood. Very recently, Heregulin- β 1 has been demonstrated to potently up-regulate VEGF-C expression in human breast cancer cells via a signaling pathway including the HER2 receptor, p38 serine kinase and NF- κ B transcription factor [63]. Proinflammatory cytokines such as IL-1 α or TNF could also be important regulators of VEGF-C expression in tumors [60,64]. However, lymphatics are not homogeneously distributed within tumors [47,52,54], which suggests that the growth of lymphatic vessels may also be restrained by other microenvironmental factors such as hydrostatic pressure or mechanical stress exerted by the proliferating tumor cells [65,66]. Furthermore, it will be interesting to find out whether intra-tumoral lymphatic vessels are functional for tumor dissemination into the lymph nodes. Lymphatic vessels at the tumor periphery but not intra-tumoral lymphatics were shown to be functional by dye uptake test, and it was proposed that lymphatic vessels at the tumor margin might play a major role in the lymphatic spread of tumor cells [67]. However, there is no convincing evidence so far to exclude the possibility that tumor cells spread through the intra-tumoral lymphatics. Although fluid drainage via lymphatics is mainly controlled by interstitial mechanical stresses [68,69], tumor cell spread via lymphatics may be governed by additional mechanisms.

4. Lymphatic metastasis: a passive or an active event?

Tumor cells or emboli have to overcome a series of barriers to establish metastases in distant organs. Multiple molecular and cellular responses initiated by a combination of various stimuli may be required for the metastatic event. These sequential processes are thought to include induction of angiogenesis and/or lymphangiogenesis, detachment from surrounding tumor cell mass and access to blood or lymphatic vessels, survival in the circulation, random or specific arrest in the microvasculature of target organs, exit from the vessels, and growth and invasion into the organs to form a metastatic focus. When compared with the blood vessels, the lymphatic vessels have a discontinuous basement membrane and lack tight interendothelial junctions [70]. It is therefore believed that it would be easier for tumor cells to enter the lymphatic vessels rather than the blood circulation (see Fig. 2 for a schematic illustration).

Table 1

Correlation between VEGF-C or VEGF-D expression and lymphatic metastasis in the primary human tumors (modified from Stacker et al. [62])

Tumor	Primary findings	Reference
<i>VEGF-C</i>		
Breast cancer	VEGF-C expression correlated with lymphatic vessel invasion. Five-year disease-free survival of the VEGF-C positive group was significantly poorer ^{a,b}	[78]
Cervical cancer	VEGF-C expression was an independent factor influencing pelvic lymph node metastasis. Patients with VEGF-C expression had significantly poorer prognosis ^{a,b}	[79–81]
Colorectal cancer	VEGF-C mRNA correlated with lymph node metastasis, lymphatic involvement and invasion depth ^{a,b}	[82–84]
	VEGF-C expression correlated significantly with poorer histologic grade, depth of invasion, lymphatic invasion, lymph node metastasis, venous invasion, liver metastasis and Duke's stage ^b	[85]
	Tumors with lymph node metastasis had higher levels of VEGF, but there was no association between VEGF-C expression and lymphatic spread ^{a,b}	[86]
Endometrial carcinoma	VEGF-C expression correlated with vascular invasion, depth of invasion, lymphatic vessel invasion and lymph node metastasis ^b	[87]
Esophageal cancer	VEGF-C correlated with depth of invasion, tumor stage, venous invasion, lymphatic invasion and lymph node metastasis ^b	[88]
Gastric cancer	Grade of VEGF-C expression correlated with lymph node status ^a	[89]
	VEGF-C expression correlated with lymphatic and venous invasion. VEGF-C expression had a significant negative impact on the prognosis of patients who did not express VEGF ^b	[90]
	There was a significant correlation between the expression of VEGF-C and lymphatic invasion in adenocarcinomas of undifferentiated type, but not in those of differentiated tumors ^b	[91]
	VEGF-C immunoreactivity was associated with tumour invasion, lymphatic invasion and lymph node metastases ^b	[92]
	VEGF-C protein expression was significantly higher in lymphatic invasion-positive early gastric cancer ^b	[93]

Table 1 (continued)

Tumor	Primary findings	Reference
<i>VEGF-C</i>		
Oral cancer	The VEGF-C positivity was significantly higher in squamous carcinomas than in normal tissues and benign lesions. Expression in squamous carcinomas correlated with pathological grade and lymph node metastasis ^{a,b}	[94,95]
Head and neck squamous cell carcinoma	Increased expression of VEGF-C was detected in tumors. VEGF-C had predictive value for the presence of cervical nodal metastases ^a	[96]
Lung adenocarcinoma	Lymph node metastasis was associated with high VEGF-C expression. A low VEGF-D/VEGF-C ratio correlated with the presence of lymphatic invasion ^a	[97]
Neuroblastoma	No correlation was observed between VEGF-C expression and lymph node metastasis ^a	[98]
Non-small cell lung cancer (NSCLC)	VEGF and VEGF-C were associated with nodal microdissemination ^a VEGF-C expression was significantly associated with lymph node metastasis, lymphatic vessel invasion and poor outcome ^b	[99] [100,101]
Pancreatic cancer	VEGF-C expression was associated with increased lymphatic vessel invasion and lymph node metastasis, but not with decreased patient survival ^{b,c}	[102]
Prostate cancer	VEGF-C mRNA was more abundant in lymph node positive patients ^d	[103]
Gallbladder cancer	VEGF-C expression correlated significantly with lymphatic vessel involvement, lymph node metastasis, and poor outcome ^b	[104]
Thyroid tumors	VEGF-C correlated with lymph node invasive tumors ^a Significantly higher levels of VEGF-C mRNA were detected in patients with papillary carcinoma and nodal involvement than in those without nodal involvement ^a	[105] [106]
Ovarian carcinoma	Increased expression of VEGF-C was significantly associated with lymph node metastasis and poor survival ^b	[107]
Melanoma	Tumor-associated lymphatic vessel density (LVD) did not correlate with VEGF-C but with bFGF expression. Increased intra-tumoral and peri-tumoral LVD correlated significantly with improved survival in multivariate analysis ^b	[49]

Table 1 (continued)

Tumor	Primary findings	Reference
<i>VEGF-D</i>		
Colorectal cancer	Tumors with lymph node metastasis had higher levels of VEGF, but there was no association of VEGF-D expression with lymphatic spread ^{a,b} The expression of VEGF-D correlated with lymphatic involvement and was found to be an independent prognostic marker for disease-free and overall survival in colorectal carcinoma ^b	[86] [108]
Gastric cancer	VEGF-D expression correlated with lymphatic invasion in undifferentiated early gastric carcinoma ^b	[91]
Lung adenocarcinoma	Lymph node metastasis was associated with low VEGF-D expression ^a	[97]
Head and neck squamous cell carcinoma	Decreased expression of VEGF-D was associated with cervical node metastases ^a	[96]
Ovarian carcinoma	Increased expression of VEGF-D was significantly associated with lymph node metastasis and was found to be an independent predictor of poor outcome ^b	[107]
Breast cancer	The expression of VEGF-D was significantly associated with lymph node metastasis in invasive breast carcinomas ^b	[109]

^a mRNA expression detected by reverse transcription polymerase chain reaction.

^b Protein detected by immunohistochemistry.

^c mRNA expression detected by Northern blot analysis.

^d mRNA expression detected by in situ hybridization.

Necrosis, a phenotype frequently observed in solid tumors, could be a major determinant of tumor cell detachment [71,72]. Intra-tumoral vessels, which usually display a tiny lumen or are completely compressed within the expanding tumor mass, are often enlarged and bleed around necrotic areas. High interstitial fluid pressure inside the tumor due to vascular leakage may greatly increase the chance of tumor cell seeding into the circulation. Lymphatic vessels with an enlarged lumen can be observed in the interstitial space between expanding tumor masses and sometimes close to necrotic areas. One can speculate that proliferating endothelial cells may actively invest on the walls of interstitial spaces inside tumors as suggested by a recent study [39]. They would then envelope detached tumor cells or tumor emboli which can then be transported away with lymph or with blood into the circulation and arrested in regional lymph nodes or internal organs. Such a passive tumor cell metastasis was observed in a naturally occurring mouse mammary carcinoma model where intra-

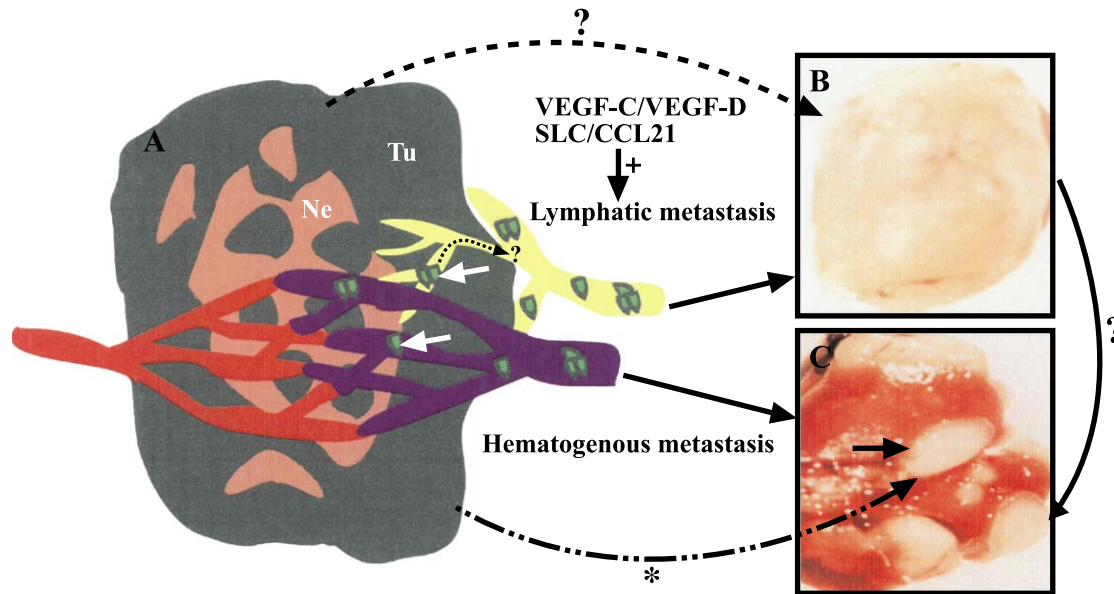


Fig. 2. Schematic illustration of lymphatic tumor metastasis as a combination of passive and active events. (A) BECs induced to proliferate by factors such as VEGF and LECs stimulated by VEGF-C or VEGF-D may envelope detached tumor cells or emboli. Necrosis, which is frequently observed in solid tumors, might facilitate the tumor cell detachment. Lymphatic vessels would then provide the channels for tumor spread to the lymph nodes (B), and blood vessels would conduct tumor dissemination to distant organs such as the lung to form metastases (C). However, lymphatic endothelial cells can also secrete chemokines such as SLC/CCL21, which can attract tumor cells that happen to express its receptor CCR7. It remains to be investigated whether intra-tumoral lymphatics can be conduits for tumor cell spread into the regional lymph nodes, and how often the tumor cells which enter the lymphatic system can pass through the lymph nodes and spread further via access into the blood circulation (question mark). Interesting recent results from Dr. Masabumi Shibuya [110] also suggest that the primary tumor can send signals that induce conditioning of the metastatic site for tumor growth before the metastatic cells can be detected (asterisk). The existence of similar signals for the lymphatic metastasis has not yet been demonstrated (question mark). Tu, tumor; Ne, necrosis; white arrow in A, intravascular tumor cells; black arrow in C, metastatic nodules in the lung.

vasating tumor cells and tumor emboli retained their nested architecture within a continuous basement membrane and were surrounded by an endothelial cell layer [73].

However, different tumor types show distinct patterns of spread to distant organs, suggesting that metastasis formation in a specific organ might be a guided process. Experimental evidence has been obtained suggesting that LECs could attract tumor cells by secreting chemokines, and therefore actively promote lymphatic metastasis. One of the chemokines, named secondary lymphoid chemokine (SLC/CCL21), is highly expressed in lymph nodes, specifically in endothelial cells of high endothelial venules and T cell-rich areas, and also in the lymphatic endothelium of multiple organs [74]. SLC/CCL21 has been shown to be chemotactic for naive T cells [74], and it is implicated in T lymphocyte homing and in the migration of antigen-stimulated dendritic cells into secondary lymphoid organs [75]. It has been shown recently that CCR7 and CXCR4, receptors for SLC/CCL21 and CXCL12, respectively, are highly expressed in human breast cancer cells. Their ligands exhibit peak levels of expression in regional lymph nodes, bone marrow, lung and liver, which represent the first destinations of breast cancer metastasis [76]. Furthermore, overexpression of CCR7 by B16 murine melanoma cells enhanced the incidence of lymph node but not lung metastasis when the tumor cells were implanted into the footpads of mice. CCR7-mediated increase of lymphatic metastasis was also shown

to be completely suppressed by treatment with neutralizing anti-SLC antibodies [77]. These data suggest active interactions between tumor cells and endothelial cells. Therefore, it is likely that tumor metastasis may be a combination of both active and passive events.

5. Concluding remarks

Recent studies have demonstrated that tumor lymphangiogenesis does occur in some human cancers, and tumor-associated lymphatics are necessary for lymph node metastasis. In animal models, inhibition of lymphangiogenesis by blocking VEGFR-3 signaling or by neutralization of chemokines such as SLC/CCL21 has been shown to suppress lymph node metastasis. This implies the therapeutic potential of targeting proliferating LECs or their paracrine interactions with the tumor cells. However, it remains to be seen whether these deductions can be applied to human patients.

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