Lymphatic Function, Lymphangiogenesis, and Cancer Metastasis

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ABSTRACT The lymphatic system serves as the primary route for the metastasis of many cancers and the extent of lymph node involvement is the most important indicator of tumor aggressiveness. Despite the apparent importance of the lymphatic vessels for tumor dissemination, it has remained unclear whether activation of lymphatic endothelial cells may affect tumor progression and metastasis and the molecular mechanisms of lymphangiogenesis are just beginning to be elucidated. This overview describes the unique structural and functional characteristics of the lymphatic vessels that render them particularly suitable for invasion by tumor cells and for their efficient transport to lymph nodes. Recent evidence indicates occurrence of tumor lymphangiogenesis and its correlation with metastasis. Molecular regulation of tumor lymphangiogenesis, its significance for tumor metastasis, and implications for cancer therapy are discussed. *Microsc. Res. Tech. 55:92–99, 2001.*

INTRODUCTION

The lymphatic and blood vascular system, although structurally two distinct systems, are functionally interconnected and act in concert to maintain tissue homeostasis. The lymphatic system in many ways complements functions of the blood vascular system by regulating tissue fluid balance, facilitating interstitial protein transport, and serving immunological functions. Whereas mechanisms of angiogenesis involving blood vessels have been studied extensively over the past years, mostly due to the importance of angiogenesis in tumor growth and metastasis, little effort has been directed toward understanding regulatory mechanisms of lymphatic vessel growth and function in physiological and pathological conditions. Meanwhile, the lymphatic system is the primary route for the dissemination of many cancers and the extent of lymph node involvement is a key prognostic factor for the outcome of the disease; despite this, the major issues regarding the involvement of lymphatic vessels in tumor progression have remained unresolved.

The lymphatic vessels comprise a one-way transport system for fluid and proteins by collecting them from the interstitial space and returning to the blood circulation. As blood travels into the capillaries, plasma fluid and proteins extravasate into the interstitial space according to hydrostatic and osmotic pressure gradients. Most of this fluid gets reabsorbed into postcapillary venules, but osmotic forces resulting from the extravasated proteins cause a small net fluid flux into the tissue. The lymphatic capillaries drain this net exudate and therefore facilitate convective protein transport through the interstitium (Aukland and Reed, 1993; Schmid-Schönbein, 1990b). If the lymphatics become blocked or dysfunctional, interstitial protein accumulates, leading to continual increase of osmotic pressure and thus fluid accumulation (edema) ensues. The net fluid efflux from the blood, and therefore the net flow rate of lymph, is about two to three orders of magnitude less than the flow rate of the blood. Because of the high permeability of the lymphatic capillaries, the composition of lymph is nearly equivalent to that of interstitial fluid, which in turn is similar to, but less concentrated than, that of blood plasma. Intestinal lymph, in addition, contains a high amount of lipids resorbed directly from the intestine. The simplified relation between blood, interstitium, and lymph is depicted in Figure 1.

Lymphatic vessels and the lymph nodes are also important components of the immune system. Lymphatic vessels direct antigen-presenting cells to the lymph nodes and are thus essential for the development of cellular immunity. In the skin, for example, lymphatic vessels are an exit path for Langerhans cells. Impairment of lymphatic functioning, e.g., inadequate transport of fluid, macromolecules, or cells from the interstitium, leads to a number of diseases that are characterized by edema, impaired immunity, and fibrosis (Mortimer et al., 1990).

ORGANIZATION AND STRUCTURAL CHARACTERISTICS OF THE LYMPHATIC SYSTEM

There are five main categories of conduits in the lymphatic system: the lymphatic capillaries, collecting vessels, lymph nodes, lymphatic trunks, and ducts, whose sizes range from 10 μm to 2 mm in diameter. Lymph forms when interstitial fluid moves into the

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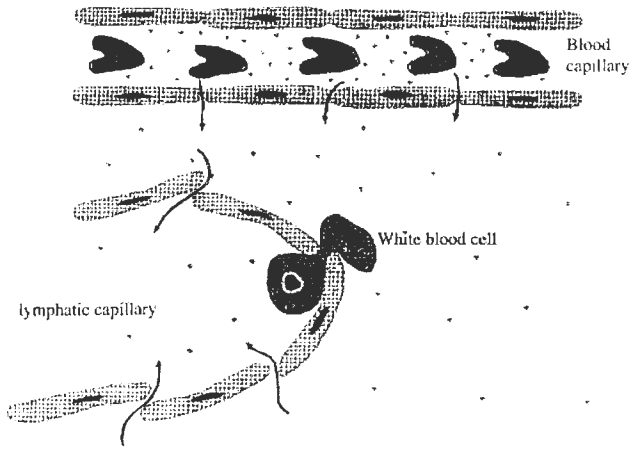

Fig. 1. Relationship between the blood and lymphatic capillaries.

lymphatic capillaries. From the capillaries it drains into the collecting vessels, which pass through at least one but usually through several clusters of lymph nodes. Collecting vessels drain into larger trunks, which lead into the lymphatic ducts. Finally, the lymphatic ducts return the lymph back into the bloodstream, completing the circuit of fluid transport.

Lymphatic capillaries (also called initial or terminal lymphatics) are blind-ended structures that are optimally suited for fluid and particle uptake. Similar to blood capillaries, lymphatics are comprised of a single nonfenestrated endothelial cell layer, but the structure of lymphatic capillaries is different from that of blood capillaries in several important aspects (Casley-Smith and Florey, 1961; Daroczy, 1988; Leak, 1970). They generally possess a more irregular and wider lumen (10–60 μm in diameter) than blood capillaries and their endothelium is typically characterized by an extremely attenuated cytoplasm, except in the perinuclear region. In contrast to blood vessels, lymphatic capillaries have absent or poorly developed basal lamina and they are not encircled by pericytes. Tight junctions and adherens junctions, the major types of intercellular junctions in blood vessels, are not as frequently seen in lymphatics. While these junctions in blood vessels are typically implicated in maintaining firm cell–cell adhesion to connect adjacent endothelial cells over entire cell boundaries, in lymphatics they represent focal points of adhesion instead. Finally, one of the most striking features of lymphatic capillaries is their intimate association with the adjacent interstitial areas. Lymphatic endothelial cells are closely connected to the surrounding tissue by fine strands of elastic fibers (Gerli et al., 1991; Pullinger and Florey, 1935). These anchoring filaments are attached to the abluminal surface of the cells and extend deeply into the connective tissue, thereby firmly attaching lymphatic endothelium to extracellular matrix fibers. Lymphatic endothelial cells are also characterized by numerous invaginations and cytoplasmic vesicles on both luminal and abluminal surfaces that are involved in transendothelial transport of molecules into the lumen (Cornford and Oldendorf, 1993; Leak, 1976; Marchetti et al., 1991).

From the lymphatic capillaries, lymph drains into the collecting lymphatics. Unlike the initial lymphatics, the collecting vessels are generally not tethered to the extracellular matrix, but instead contain smooth muscle and thus may support a circumferential hoop stress (Aukland and Reed, 1993; Schmid-Schönbein, 1990b). They also contain one-way valves that aid in lymph propulsion and prevent retrograde flow. Segments of collecting lymphatics between valves are termed lymphangions; each lymphangion serves as a contractile compartment that propels lymph into the next compartment. All collecting lymphatics pass through the lymph nodes and can be further classified as prenodal (afferent) or postnodal (efferent), to specify whether they carry lymph to or from the lymph nodes. Lymph nodes are compartmentalized into narrow fluid crevices where blood and lymphatic compartments oppose each other for fluid exchange and cell transport (Schmid-Schönbein, 1990b). From the final set of lymph nodes, lymphatic trunks drain lymph into the lymphatic ducts. The thoracic duct is the final branch of the lymphatic system that enters the lower region of the chest by passing through the aortic opening of the diaphragm; it drains into blood via the junction of the left jugular and subclavian veins.

Although lymphatic vessels are often found in proximity to blood vessels in tissues, the density of lymphatic plexus does not always match the abundance of blood supply. For example, there are no lymphatic vessels in the central nervous system and lymphatic vessels do not penetrate as far as blood vessels in several other well-vascularized tissues. In lobular organs such as the liver and mammary glands, lymphatic capillaries do not penetrate the lobules but instead surround their periphery. In skeletal muscle they are confined only to the fascial planes. Other tissues, such as the cornea of the eye and cartilage, are devoid of both blood and lymphatic vessels. Lymphatic-rich tissues include the skin, lung, and gastrointestinal tract. (Yoffey and Courtice, 1970).

MECHANISMS OF LYMPH FORMATION

Mammalian lymphatic capillaries contain no smooth muscle and are generally observed in a partially or fully collapsed state (Aukland and Reed, 1993; Schmid-Schönbein, 1990a). To function, they are critically dependent on their connections to the extracellular matrix by anchoring filaments. These fibers, 6–10 nm in diameter, are composed of elastin similar to that found in the extracellular matrix (Gerli et al., 1990) and tether the endothelium to adjacent collagen fibers (Leak and Burke, 1966). Thus, they are highly sensitive to interstitial stresses. An increase in the interstitial fluid volume (i.e., strain or swelling of the extracellular matrix) causes the anchoring filaments to exert radial tension on the lymphatic capillary to 'pull it open' or increase its luminal volume (Aukland and Nicolaysen, 1981; Aukland and Reed, 1993; Bert et al., 1988; Hogan and Unthank, 1986) (Fig. 2). This creates a "tissue pump," or a small oscillating pressure gradient, which facilitates lymph formation (Ikomi and Schmid-Schönbein, 1996; Schmid-Schönbein, 1990a). The concept of anchoring filaments helps to explain why venules are often compressed in inflammation and other conditions associated with tissue edema, while

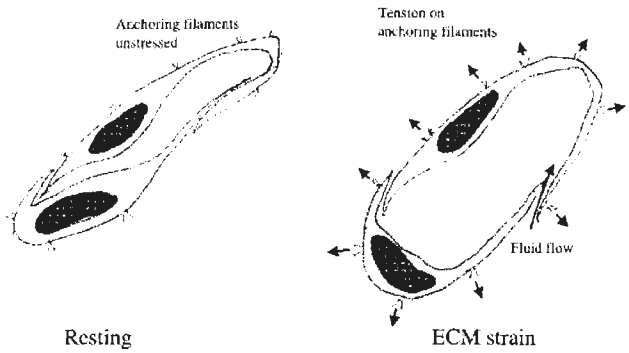

Fig. 2. The "tissue pump" that enables lymph formation: stress within the interstitium creates radial tension on the anchoring filaments, locally increasing the luminal volume of the lymphatic capillary. This creates a slight and temporary pressure difference, driving interstitial fluid into the lymphatic vessel through the passages formed by opening of overlapping endothelial cell junctions.

lymphatic capillaries are typically dilated (Pullinger and Florey, 1935). However, the dependence of lymph formation rates on local tissue pressure or volume diminishes at high interstitial fluid volumes (Guyton, 1965; Taylor et al., 1973) and is most likely limited by systemic forces that drive lymph propulsion. Overall, the functional state of lymphatic vessels cannot be necessarily determined by the vessel morphology, since an open lumen can indicate vessels both with dysfunction as well as normal function but increased load.

Lymph drainage is also accommodated by the opening of the intercellular junctions. Overlapping intercellular junctions formed by extensive superimposing of adjacent endothelial cells are a property unique to lymphatic vessels. By being loosely apposed to each other over long distances, lymphatic endothelial cells cast intercellular clefts. As the interstitium swells, anchoring filaments not only increase the vessel lumen, but also pull open the intercellular junctions to permit easy passage of fluids and particles into the vessel (Fig. 2). As fluid enters the lumen and decreases the pressure difference across the vessel wall the junctions begin to close, thereby preventing retrograde flow back into the interstitium (Ikomi and Schmid-Schönbein, 1996; Schmid-Schönbein, 1990b).

The extracellular matrix therefore plays an integral role in lymphatic function, as fluid equilibrium is controlled by the cooperation of both lymphatic function and the extracellular matrix. The elasticity and hydration of a tissue is determined by the composition and organization of the extracellular matrix; e.g., collagen provides structural framework and proteoglycans largely determine water content and resistance to fluid transport. Extensive and chronic degradation of the extracellular matrix eventually renders lymphatic vessels nonresponsive to the changes in the interstitium and therefore causes dysfunction (Negrini et al., 1996). In light of its importance in lymphatic function (i.e., the interstitial–lymphatic interface most clearly differentiates lymphatic from blood vascular capillaries), the composition and architecture of the ECM are likely to play a critical role in lymphangiogenesis and should be taken into consideration when studying the biology and pathology of the lymphatic system.

LYMPH TRANSPORT THROUGH THE LYMPHATICS

Transport of lymph through the lymphatic system (lymph propulsion) is coupled to lymph formation and both components contribute to the net flow rate in the lymphatics. The term "formation" describes fluid transport from the interstitium into the initial lymphatics and "propulsion" refers to the systemic forces that drive lymph from the initial capillaries to the larger vessels and eventually back to the blood. If there is blockage in the systemic route (e.g., removal of a lymph node), interstitial fluid may enter the initial lymphatics but will eventually "back up" as fluid is not drained from them, causing edema. Likewise, if the interstitial–lymphatic interface is destroyed and lymphatic capillaries cannot function, no lymph will be drained from that local region despite the baseline systemic drainage forces.

The driving forces for lymph formation are local: namely, interstitial fluid pressure and strain of the extracellular matrix and can be affected by skeletal motion and massage as well as the slight strains associated with pressure oscillations caused by arterial pressure pulsations and vasomotion of neighboring arterioles. The forces that drive lymph propulsion through the lymphatics, on the other hand, include systemic forces such as respiration (Negrini et al., 1994; Schad et al., 1978; Schmid-Schönbein, 1990b; Swartz et al., 1996), blood pressure (Parsons and McMaster, 1938), exercise (Olszewski et al., 1977), and massage (Ikomi and Schmid-Schönbein, 1996; McGeown et al., 1988; Mortimer et al., 1990), and are largely independent of the lymph formation rate.

The measurements of lymph flow velocity that have been reported in the literature are limited to superficial vessels in organs that can be visualized by in vivo microscopy. In the human skin lymph flow velocity averages 10 μm/s (Fischer et al., 1994); in the tail skin of anesthetized mice 3 μm/s (Berk et al., 1996; Swartz et al., 1996). However, lymph flow seems to fluctuate and oscillate, with a broad range of velocities of up to ±20 times the mean (Berk et al., 1996). Anesthesia decreases overall lymph flow since it reduces the systemic driving forces for lymph propulsion such as the respiration rate, blood flow and pressure, and skeletal movements (Colantuoni et al., 1984; McHale and Thornbury, 1989; Schad et al., 1976).

To transport lymph through the lymphatic system, collecting vessels possess smooth muscle and valves (Lauweryns et al., 1976; Leak and Burke, 1966). The smooth muscle exhibits spontaneous contractions in the form of peristaltic waves between lymphangions at approximately 5 mm/s (Hall et al., 1965; Hargens and Zweifach, 1977; Ohhashi et al., 1980; Olszewski and Engeset, 1980; Zawieja et al., 1993). The valves facilitate this peristaltic propulsion of lymph by allowing emptying and filling of each lymphangion—two neighboring valves are never open at the same time (Ohhashi et al., 1980)—which results in stepwise pressure changes from one lymphangion to the next. Since the spontaneous contractions can be evoked by distension (Mislin, 1976; Reddy and Staub, 1981), the presence of the valves is essential to contraction because they allow a lymphangion to distend before emptying

into the next segment. This results in a net pressure drop along the length of the collecting vessels and lymph flow ceases when rhythmic contractions stop (Ohhashi et al., 1980).

Lymphatic function is often characterized by a tissue clearance rate, which describes the removal of injected molecules or particles in terms of amount per unit time per unit tissue volume. Lymph formation can be observed in skin and mesentery by injecting an optical contrast agent such as mercury, radiolabeled particles, or fluorescently labeled macromolecules (Bollinger et al., 1981; McNeill et al., 1989; Mortimer et al., 1990; Swartz et al., 1996). This procedure is commonly termed 'microlymphangiography' and can be used to diagnose lymphatic dysfunction. Other methods for evaluating lymphatic function include measurements of solute concentration ratios between plasma and lymph (Renkin and Wiig, 1994) as well as local measurements of lymphatic capillary pressures (Bates et al., 1994; Wen et al., 1994; Zaugg-Vesti et al., 1993).

LYMPHATIC SYSTEM AND CANCER

The lymphatic system serves as the primary route for the metastasis of most cancers and the spread of tumor cells via lymphatic vessels to the regional lymph nodes is one of the most important indicators of tumor aggressiveness for the majority of human malignancies. Whereas lymphatic vessels containing clusters of tumor cells are frequently observed at the periphery of malignant tumors, it has been generally accepted that lymphatic vessels are absent from tumors themselves (Carmeliet and Jain, 2000; Folkman, 1996; Gilchrist, 1950; Lee and Tilghmanm, 1933; Leu et al., 2000; Tanigawa et al., 1981; Zeidman et al., 1955). Some early studies reported intratumoral lymphatic vessels in certain types of cancer (Evans, 1908; Reichert, 1926), but this has been interpreted mainly as co-option of preexisting lymphatic vessels by invading tumor cells. Hence, although the significance of preexisting peritumoral lymphatics as conduits for tumor cell dissemination has been well recognized (Fisher and Fisher, 1968), it has remained unclear whether tumors can stimulate lymphangiogenesis and whether tumor metastasis necessitates molecular activation of the lymphatic system (Folkman, 1996; Leu et al., 2000; Witte et al., 1997).

Several studies have failed to identify functional lymphatics within tumors (Jain, 1987; Leu et al., 2000), leading to the concept that lymphangiogenesis may not play a major role in tumor metastasis (Carmeliet and Jain, 2000). However, the absence of detectable perfusion of lymphatic vessels does not necessarily imply the absence of anatomically distinguishable lymphatic vessels from tumors. The formation of an intratumoral lymphatic network, whether fully functional in fluid transport or not, may promote metastatic tumor spread by creating increased opportunities for metastatic tumor cells to leave the primary tumor site. The presence and potential function of lymphatic vessels in tumors have remained controversial mostly due to the lack of molecular markers to reliably distinguish the lymphatic vasculature from blood vessels (Skobe and Detmar, 2000). Recently, several novel molecules have been identified that allow a more precise distinction between lymphatic and blood vascular endothelium. These include VEGFR-3 (FLT-4), the receptor for the vascular endothelial growth factors VEGF-C and VEGF-D (Veikkola et al., 2000); podoplanin, a glomerular podocyte membrane mucoprotein (Breiteneder-Geleff et al., 1999; Weninger et al., 1999); and the homeobox gene product Prox-1 that is involved in regulating development of the lymphatic system (Wigle and Oliver, 1999). Most recently, a novel hyaluronan receptor termed LYVE-1 has been shown to be restricted to lymphatic vessels in normal tissues (Banerji et al., 1999) and associated with tumors (Mandriota et al., 2001; Skobe et al., 2001a; Stacker et al., 2001).

MOLECULAR REGULATION OF TUMOR LYMPHANGIOGENESIS AND LYMPHATIC METASTASIS

Vascular endothelial growth factor-C (VEGF-C), a novel member of the VEGF family of growth factors (Joukov et al., 1996; Lee et al., 1996), was the first growth factor that was demonstrated to stimulate lymphangiogenesis in addition to angiogenesis (Jeltsch et al., 1997; Oh et al., 1997; Witzenbichler et al., 1998). The specific effects of VEGF-C on lymphangiogenesis depend on its proteolytic processing. The mature form of human VEGF-C stimulates both VEGFR-2 and VEGFR-3 and can therefore stimulate both angiogenesis and lymphangiogenesis, whereas the partially processed form preferentially binds and activates VEGFR-3 (Joukov et al., 1997) and specifically stimulates lymphangiogenesis (Skobe et al., 2001a). Structurally, VEGF-C is closely related to vascular endothelial growth factor-D (VEGF-D), which also binds to and activates VEGFR-2 and VEGFR-3 in a similar manner (Achen et al., 1998) and stimulates angiogenesis and lymphangiogenesis (Stacker et al., 2001).

A number of studies have recently reported VEGF-C expression in human tumors and its correlation to metastasis to regional lymph nodes. VEGF-C has been shown to be expressed in breast (Kurebayashi et al., 1999; Salven et al., 1998), colon (Akagi et al., 2000; Andre et al., 2000), lung (Niki et al., 2000; Ohta et al., 2000; Salven et al., 1998), thyroid (Bunone et al., 1999; Fellmer et al., 1999; Shushanov et al., 2000), gastric (Yonemura et al., 1999), and squamous cell cancers (Salven et al., 1998), mesotheliomas (Ohta et al., 1999), as well as neuroblastomas (Eggert et al., 2000), sarcomas (Salven et al., 1998), and melanomas (Salven et al., 1998). Increased expression of VEGFR-3 has been detected in lymphatic endothelium adjacent to cancer cells and in lymph nodes containing carcinoma metastases (Jussila et al., 1998; Kaipainen et al., 1995). Moreover, correlation between the VEGF-C expression and the rate of metastasis to lymph nodes has been found in breast (Kurebayashi et al., 1999), colorectal (Akagi et al., 2000), gastric (Yonemura et al., 1999), thyroid (Bunone et al., 1999; Fellmer et al., 1999), lung (Ohta et al., 2000), and prostate (Tsurusaki et al., 1999) cancers.

In addition to the abundant correlative clinical data, very recently a functional role of VEGF-C in tumor lymphangiogenesis and metastasis has been demonstrated (Mandriota et al., 2001; Skobe et al., 2001a). Overexpression of VEGF-C in genetically fluorescent human breast cancer cells transplanted onto nude mice resulted in enlargement of peritumoral lymphatic ves-

sels and in strikingly increased intratumoral lymphangiogenesis, without any obvious effects on tumor angiogenesis. Increased intratumoral lymphatic vessel density was associated with a significantly increased incidence of metastases in regional lymph nodes as well as with increased lung metastases. In fact, the extent of intratumoral lymphatic vessel density was highly correlated with the extent of lung metastases, implying an important role of the lymphatic system for the metastatic tumor spread to distant sites (Skobe et al., 2001a).

VEGF-C-induced lymphangiogenesis has also been shown to promote metastases to lymph nodes in a model of pancreatic cancer. Transgenic mice in which VEGF-C expression is driven by the rat insulin promoter (Rip) were crossed with Rip1Tag2 mice which spontaneously develop pancreatic β-cell tumors as a consequence of SV40 large T-antigen expression under the same promoter (Mandriota et al., 2001). The tumors of the Rip1Tag2 mice are locally invasive, but are neither lymphangiogenic nor metastatic (Hanahan, 1985). In the double transgenic model, VEGF-C induced lymphangiogenesis at the periphery, although not within the pancreatic β-cell tumors, which promoted the metastatic spread to regional lymph nodes (Mandriota et al., 2001). Taken together, these results provide a mechanistic explanation for the previously reported correlation of VEGF-C expression in the primary tumors with high incidence of lymph node metastases.

Another recent study demonstrated the important role of VEGF-D in tumor lymphangiogenesis and metastasis. Similar to VEGF-C, VEGF-D overexpressing epitheloid tumors induced the formation of intratumoral lymphatic vessels and promoted lymph node metastases in mice. Importantly, lymphatic spread induced by VEGF-D could be blocked with a neutralizing anti-VEGF-D antibody, suggesting inhibition of lymphangiogenesis as a useful strategy to inhibit metastatic spread of cancer (Stacker et al., 2001). While VEGF-D promoted tumor dissemination to lymph nodes, VEGF overexpression in the same experimental model did not, implying differential roles of VEGF family members in determining the route of metastases. In analogy with these findings, VEGF-C expression was detected only in node-positive human breast cancers, whereas expression of VEGF was detected in both node-positive and node-negative tumors (Kurebayashi et al., 1999).

Evidence for the existence of intratumoral lymphangiogenesis using molecular markers of the lymphatic vessels has so far been obtained only in experimental tumor models. In addition to VEGF-C overexpressing breast cancer (Skobe et al., 2001a) and VEGF-D overexpressing epitheloid tumors (Stacker et al., 2001), intratumoral lymphatic vessels were also frequently detectable within experimental cutaneous squamous cell carcinomas and VEGF-C overexpressing malignant melanomas (Skobe et al., 2001b). Furthermore, intratumoral lymphangiogenesis has been observed within human melanomas with high endogenous expression of VEGF-C transplanted onto avian chorioallantoic membrane (CAM) (Papoutsi et al., 2000). Moreover, in a breast cancer model lymphangiogenesis was induced not only within VEGF-C expressing tumors but also within nontransfected, control tumors, suggesting the production of lymphangiogenic factors other then VEGF-C in these tumors (Skobe et al., 2001a). The presence of intratumoral lymphangiogenesis in spontaneously developing human tumors and its potential prognostic significance remains to be determined.

Production of lymphangiogenic factors in tumors may promote the incidence of lymphatic metastases by increasing the number of lymphatic vessels in the vicinity of tumor cells and therefore creating increased opportunities for tumor cells to leave the primary tumor site. It is also possible, however, that the activation of lymphatics by VEGF-C, VEGF-D, or related growth factors could promote molecular interactions of tumor cells with lymphatic endothelial cells, thereby facilitating tumor cell entry into the lymphatics. Therefore, even when the tumor itself lacks lymphatic vessels, as in the VEGF-C-expressing pancreatic cancer (Mandriota et al., 2001), an increase and/or activation of peritumoral lymphatics might promote tumor metastasis. Finally, the physiology of the lymphatic system is optimally suited for the entry and transport of cells (i.e., immune cells) (Witte et al., 1997) and therefore has many advantages over the blood circulation as a transport route for a metastasizing tumor cell or embolism. The smallest lymphatic vessels are still much larger than blood capillaries and flow velocities are orders of magnitude slower. Lymph fluid is nearly identical to interstitial fluid and promotes cell viability. In contrast, tumor cells in the bloodstream experience serum toxicity, high shear stresses, and mechanical deformation leading to an extremely low success rate for metastasis (Liotta et al., 1991; Weiss and Schmid-Schönbein, 1989). The preferential metastasis via lymphatics, due to expression of lymphangiogenic factors in tumors, might therefore promote survival of disseminating tumor cells and consequently increase their metastatic efficiency. Nearly all investigations of the details of metastatic process, such as intravasation, survival, and extravasation, have focused on tumor cell behavior in the bloodstream (Liotta et al., 1991; Zetter, 1993) and there is currently a great need for clarifying the interactions between tumor cells and lymphatics and to develop a paradigm for lymphatic metastasis similar to that of hematogenous metastasis.

PERSPECTIVES

Recent findings that demonstrate the occurrence of peri- and intratumoral lymphangiogenesis in cancer and its relationship to cancer metastasis (Mandriota et al., 2001; Skobe et al., 2001a; Stacker et al., 2001) have created a basis for exploring new strategies in cancer diagnosis and therapy. However, a large amount of work is still required to evaluate the significance of tumor lymphangiogenesis in spontaneously arising human tumors and its relevance for distinct tumor types. Although correlations between expression of the lymphangiogenic factor (VEGF-C) and lymph node metastases in human tumors have been reported (Akagi et al., 2000; Bunone et al., 1999; Fellmer et al., 1999; Kurebayashi et al., 1999; Ohta et al., 2000; Tsurusaki et al., 1999; Yonemura et al., 1999), the relationship between lymphangiogenesis and metastasis in these tumors remains to be established. Preliminary evi-

dence from experimental models suggests that lymphangiogenesis might be of particular importance in tumor types that preferentially metastasize through the lymphatic system, such as breast carcinoma (Skobe et al., 2001a), melanoma (Skobe et al., 2001b), and squamous cell carcinoma (Skobe et al., unpublished data); therefore, targeting lymphangiogenesis may be therapeutically significant, in particular for certain tumor types. Clearly, targeting VEGF-C, VEGF-D, and VEGFR-3 requires further evaluation as a strategy to inhibit tumor metastases. In addition to being potential targets for inhibiting tumor metastasis, factors implicated in tumor lymphangiogenesis and specific molecules found on the activated lymphatic endothelium may prove valuable in diagnosis of particularly aggressive, metastatic cancers.

Finally, common treatments of many cancers, such as lymph node resection and radiation therapy, are frequently associated with lymphedema, a chronic condition that is a major clinical problem. Edema is characterized both by changes in the extracellular matrix and alterations of lymphatic vessels and the interplay between these two factors remains to be elucidated. It is possible that therapy aimed at promoting lymphatic regeneration may lead to an overall increase in lymphatic function in edematous tissue; however, it remains to be determined whether this can be achieved by application of lymphangiogenic growth factors alone. The continued discovery and characterization of factors that regulate lymphangiogenesis, as well as understanding the role of the extracellular matrix in lymphangiogenesis, will be essential for creating rational therapies for secondary edema associated with cancer and for the development of new therapeutic strategies for limiting cancer spread.

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