Lymphatic vessels in cancer metastasis: bridging the gaps

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Distant organ metastasis is the most important factor in determining patient survival in cancer. This is thought to occur via the body’s own systems for transporting fluid and cells, the blood vascular and lymphatic systems. Cancer cells may exploit these vascular systems by expressing growth factors, which alter the normal pattern of angiogenesis and lymphatic vessel growth (lymphangiogenesis), thus creating conduits for tumour metastasis. With respect to lymphatic metastasis, techniques which allow the mapping of a tumour’s lymphatic drainage and sampling of the ‘sentinel node’ from the regional lymph node group provide crucial prognostic information, determine further treatment and offer a window into tumour-host immune interactions. Aberrant drainage patterns so identified are both clinically significant, and highlight important anatomical and molecular complexities not explained by existing models of lymphatic development or anatomy. The molecular controls of tumour lymphangiogenesis and factors determining which lymphatic vessel subtypes are induced may be targets for novel therapeutics designed to restrict cancer metastasis. Furthermore, analyses of these control mechanisms will enhance our understanding of the interactions between the tumour cells and the lymphatic vasculature. For many years, disparate groups of clinical researchers and basic scientists have been working to unravel the mysteries of the lymphatic system. This review aims to summarize these contributions, in terms of the history, identification, structure and function of lymphatic vessels in cancer and the role they play in tumour metastasis. Current ideas about the roles of lymphangiogenic growth factors, their signalling pathways in lymphatic metastasis and therapeutic opportunities to restrict this spread will also be explored.

Brief history of the lymphatic system in cancer

The French surgeon Le Dran (1) first noted in the sixteenth century that cancers of the breast which spread to axillary lymph nodes had significantly worse survival outcomes than those that were localized only to the primary tumour. Some 200 years later, Halsted (2) set about performing sometimes disfiguring radical excisions of both the primary breast cancer and the metastatic lesions in the axillary lymph nodes (3). The next major clinical development regarding the lymphatics in cancer occurred in the 1950s when clinicians began to use radioisotope injections to better understand which regional lymph node groups drain different parts of the body (4), essential information for identifying potential routes of cancer metastasis via the lymphatic vasculature.

The 1990s saw the adaptation of lymphatic mapping for the prediction of each patient’s lymphatic drainage from a specific tumour, and for identification of the ‘sentinel lymph node(s)’ (i.e. the lymph node(s) most likely to contain spreading cancer cells) within the lymphatic drainage basin (5). The same decade also yielded the discovery of lymphatic-specific molecular markers to identify lymphatic vessels, which were hitherto histologically indistinguishable from blood vessels (6). The advent of these techniques led to the realization that lymphatics are fundamental to cancer metastasis and many other pathological processes, and has generated intense clinical and scientific interest in the lymphatic vasculature as a potentially valuable therapeutic target (7).

Structure, function and development of the lymphatic system

The lymphatic vessels are integral for interstitial fluid volume regulation and absorption of dietary fat. In addition, the lymphatics are important for immune function and in the metastatic spread of cancer (8). The intricate network of thin-walled vessels which constitutes the lymphatic vasculature commences within the superficial dermis of the skin as highly permeable blind-ending sacs, and are referred to as ‘lymphatic capillaries’ or ‘initial lymphatics’ (Figure 1) (8,9). Fibrillin filament anchors by lymphatic endothelial cells (LECs) and the extracellular matrix (ECM) translate interstitial fluid volume expansion into lateral displacement, to create temporary intercellular fenestrations (10). The lack of a continuous basement membrane surrounding the initial lymphatics facilitates entry of fluid into these vessels and restoration of normal interstitial volume, resulting in a slackening of the fibrillin filaments, and eventual return of the LECs to their overlapping resting position. Studies by Weber et al. (11) suggest that ECM signalling stimulates additional cytoskeletal structural alterations, which further aid fluid influx.

Abbreviations: E, embryonic day; ECM, extracellular matrix; LEC, lymphatic endothelial cell; MAPK, mitogen activated protein kinase; PC, proprotein convertase; PTK, protein tyrosine kinase; RTK, receptor tyrosine kinase; SMC, smooth muscle cell; VEGF vascular endothelial growth factor; VEGFR vascular endothelial cell growth factor receptor.
Pre-collecting lymphatic vessels, located in the deep dermis, drain fluid from the initial lymphatics (Figure 1). They have segments which contain valves and are surrounded by a basement membrane and smooth muscle cells (SMCs) (12). These segments alternate with other regions that are morphologically akin to the initial lymphatic capillaries. The pre-collecting lymphatics, in turn, drain into the ‘collecting lymphatics’, which are located in the subcutaneous tissue. These vessels have circumferential SMCs and regular intraluminal valves (13). Those collecting lymphatics which are >200 μm in diameter also have arterial-type mural intima, media and adventitia (13); however, do not normally possess pericytes (14). They propel lymph at an average of 10 μm/s by a combination of intrinsic wall motion (generated by specialized pacemaker-SMCs), and compression by adjacent arterial pulsation and skeletal muscle contraction (15–17), eventually returning lymph to the central veins. The collecting lymphatics coalesce into lymphatic trunks, then intrathoracic ducts. Unaided by ‘lymphatic hearts’, found in animals such as toads (18), the larger mammalian lymphatic vessels generate surges of up to 100 μm/s several times each minute to drain fluid from most tissues to a lymph node within 20 min (19–21).

Alterations to flow may be mediated by nitric oxide synthase in LECs, acting via nitric oxide to produce SMC dilatation (22,23) and may be under autonomic control, as illustrated by splanchnic stimulation influencing thoracic duct flow (24). Recent work by He et al. (25) indicates that these lymphatic vessels may also dilate in response to tumour-derived growth factors, hence increasing the lymph flow and capacity to transport tumour cells to lymph nodes.

Lymph fluid passes through a series of lymph nodes or other secondary lymphoid organs (gastrointestinal submucosal lymphoid aggregations such as Peyer’s patches and tonsils) (26). Afferent lymph node ducts divide before passing beneath the capsule of the node into cortical sinuses then pass through a reticuloendothelial cell filter (26). Antigen-presenting cells display antigen epitopes to lymphocytes within these organs, triggering them to clonally expand and tailor an individualized ‘antigen-specific’ immune response (27). Whilst lymph continues through the medullary sinus to the hilar region of the lymph node and into efferent ducts, tumour cells may become trapped and proliferate here or spread further to distal organs (21).

The first lymphatic vessels emerge in the mouse at about embryonic day (E) 10.5, when endothelial cells sprout from the
anterior cardinal vein to form the primary jugular lymph sacs (28). These endothelial cells express the lymphatic transcription factor Prox-1, which is essential for lymphatic development and is thought to be a major determinant of LEC fate (28). These cells also express the receptor tyrosine kinase (RTK) vascular endothelial growth factor receptor-3 (VEGFR-3), the cell surface receptor that binds the lymphangiogenic growth factors VEGF-C and VEGF-D. It is the nearly expression of VEGF-C which initiates the first sprouting of lymphatics from the anterior cardinal vein, via VEGFR-3 stimulation (29). The primary jugular lymph sacs subsequently also sprout to form a primitive lymphatic plexus, which spreads throughout the head and neck, thorax and forelimbs (8). The lymphatics continue to evolve from E12.5 onwards, via a series of posterior lymphatic sacs which are derived from local veins, and progress to penetrate interstitial tissues nearby (28). They form a primitive dermal plexus by E15.5 (28), and continue to develop after birth into a deep dermal pre-collecting layer, from which sprout the beginnings of a subdermal lymphatic capillary plexus (30). The LECs of the latter lymphatic vessels differ from those of the deep dermal lymphatics both morphologically and in terms of the cell surface molecular markers expressed (Figure 1) (30). The timing of the formation of the initial lymphatic capillary layer also varies depending on the location of the skin, but occurs after the second postnatal day in the mouse (30). The endothelium of mature lymphatic vessels expresses the cell surface markers podoplanin and LYVE-1, although LYVE-1 appears to be more abundant in lymphatic capillaries than in larger vessels (Figure 1) (30). In contrast, EphrinB2, a cell surface tyrosine kinase, is expressed on LECs in pre-collecting lymphatic vessels, where it is essential for the maturation of these vessels after birth, and on collecting lymphatic vessels, but is not expressed on LECs in lymphatic capillaries (30).

Relatively little is known about development of lymph nodes or other lymphoid tissues. These structures arise from connective tissue protruding into embryonic lymph sacs, which later forms the first lymph node anlagen tissue at the predetermined site of future nodes (26,27). This involves differentiation of mesenchymal cells into stromal organizer cells and aggregates of CD45⁺CD4⁺CD3⁺ lymphoid ‘tissue inducer cells’ (27). The two cell types interact within the anlagen to induce expression of adhesion molecules on stromal organizer cells and release homeostatic chemokines such as CCL21, CCL19 and CXCL13. These in turn attract further lymphoid ‘tissue inducer cells’ and other hemopoietic cells (27,31). Interestingly, studies in mutant mice have demonstrated that lymph node development is genetically distinct from that of the lymphatic vasculature (32).

The involvement of the lymphatics in cancer
The initial spread of cancer cells is seen histologically as tumour escape beyond a defined boundary and may include the invasion of local lymphatic vessels (33). Micrometastasis to lymph nodes, thought to occur before most primary tumours are clinically detectable, often heralds distant organ metastasis at the time of diagnosis or after some period of delay, and is therefore the most significant prognostic indicator in many human cancers (34). Hence, lymph node staging may alter the type and timing of treatment offered to the patient, and it is critical that all nodes potentially containing metastatic tumour cells are sampled surgically for histological analysis (34). Failure to detect occult metastasis may be responsible for around one-third of early stage colorectal cancer patients who were originally classed as lymph node-negative on histopathology, later developing systemic or recurrent disease (35,36).

‘Sentinel lymph node biopsy’ is one method of reliably sampling lymph nodes to which tumour cells have metastasized. Originally developed for melanoma (37), it has since been adapted for application to several other malignancies (38,39), in particular, breast carcinoma (40). It is a procedure in which a pre-operative map of lymph node(s) draining a primary cancer is obtained by injection of radiolabelled colloid (41). The sentinel node(s) is then located intraoperatively with a hand held gamma probe (42), in conjunction with visualization of blue dye injected at the commencement of the procedure (43). This enables accurate identification and surgical excision of the sentinel lymph node(s), deemed to be those node(s) most likely to harbour metastatic cancer cells (5,37). If histological analysis of the sentinel lymph node(s) demonstrates the presence of metastatic cells, surgical clearance of the remaining lymph nodes within the same anatomical area is required for further histological staging and prognostication. Furthermore, histologically positive sentinel lymph nodes usually pre-date distant metastasis and are, therefore, an indicator of poor prognosis (5,37), even if they occur outside the regional lymph node group (44,45). Detection of positive sentinel lymph node(s) results in reclassification of a patient to a group with higher risk of recurrence, which may make them eligible for systemic adjuvant anti-metastasis treatment and thus potentially result in a survival benefit (36,46–48). In contrast, the absence of metastatic cancer cells within the sentinel node is taken to indicate disease-free status in the remaining lymph nodes in the nodal group (49), and the patient is consequently spared the morbidity of further lymphablation surgery, which could lead to undesirable side effects such as lymphoedema (50). Ablation of metastatic lymph node(s) and focussed external beam radiation therapy also aid local disease control, as misdiagnosis or inadequate clearance of metastatic nodes may result in bulky, unresectable disease (51).

The precise mechanisms by which tumour cells move preferentially towards particular lymph node(s) remain poorly defined. It was originally envisaged that lymphatic invasion took place passively as an advancing tumour front eroded the walls of any vessels in its path and that metastasis occurred by passive drainage (52); however, recent evidence indicates that a more complex interaction between tumour cells and the lymphatic endothelium may take place (53). The availability of markers with specificity for LECs has enabled the identification and quantification of previously undiscernible lymphatic vessels in human cancer (54), and other pathological states (55,56).

The main ‘workhorse’ reagent for identification of lymphatic vessels, since the late 1990s, has been anti-LYVE-1 antibody (6,57,58). LYVE-1 is a CD44 homologue protein which is involved in hyaluronan and immune cell transport (6,57), and may itself be implicated in tumour cell trafficking to lymph nodes (59). Heterogeneous LYVE-1 expression on cultured and tumour-induced LECs (59), and differential expression between normal lymphatic vessel subtypes (Figure 1) (30), means that a combination of LEC molecular markers are required to reliably identify different lymphatic vessels (9). Podoplanin is a mucin-type transmembrane glycoprotein, which despite being expressed on kidney podocytes,
osteoblasts and Type I alveolar cells is expressed uniformly across the lymphatic vessel subtypes and has thus proven to be another useful LEC marker (60–62). Whilst its specific function remains elusive, mutant mice lacking podoplanin display lymphatic defects which contribute to respiratory compromise and death, but do not interfere with normal blood vasculature (63). Podoplanin expression is regulated by the homeobox transcription factor Prox-1, which, whilst not widely used to identify cancer lymphatics, has been important for understanding lymphatic development during embryogenesis (64,65).

VEGFR-3 was amongst the earliest markers used to characterize lymphatic vessels (66); however, the observation of VEGFR-3 expression on blood vessels in tumours and wound granulation tissue has meant that it is less appropriate for specific identification of lymphatics in these conditions (67,68). Finally, molecules recently identified on lymphatic vessels, such as EphrinB2 and EphB4, which are responsible for blood vessel territorial demarcation, are differentially expressed between lymphatic vessel subtypes (Figure 1) (30), and may therefore become useful in distinguishing different lymphatic vessels in cancer.

Whilst immunohistological studies using lymphatic-specific markers have demonstrated the existence of proliferating intratumoral lymphatic vessels in several types of human tumour, the functional significance of intratumoral lymphatic vessels remains controversial (69). It has been proposed that intratumoral lymphatics are not able to transport tumour cells because the elevated hydrostatic pressure within a tumour may compress these vessels (69,70). Certain studies have reported intratumoral lymphatic density as an accurate independent predictor of poor disease-free survival, and of increased metastatic propensity in such important human tumours as melanoma, and carcinoma of the breast, endometrium, colon, lung, prostate, ovary, pancreas and in the head and neck (71–79).

Peritumoral lymphatics are the lymphatic vessels immediately surrounding a tumour. It has been suggested that these may represent pre-existing vessels compressed into a peritumoral rim by the expanding tumour mass (80). However, LECs in peritumoral lymphatics have been observed to be proliferating around some human cancers, suggesting that these vessels can also arise due to lymphangiogenesis (71–74). Additionally, peritumoral lymphangiogenesis was significantly associated with regional metastasis and poor disease-free and overall survival in malignant melanoma (81), and detection of lymphangiogenic growth factors was also shown to be of prognostic significance in human carcinoma of the cervix, ovary, breast and gastrointestinal tract (74,75,77,78,82–85). Although the relative importance of intratumoral versus peritumoral lymphatics for metastatic spread remains a subject of debate, it is clear that the lymphatic vessels associated with a tumour can be important for metastasis and patient outcome (86). Further, experimental and clinopathological studies indicate that lymphangiogenesis can contribute to the formation of these lymphatic vessels (86), therefore, an understanding of the molecular control of this process may identify novel therapeutic targets for restricting the spread of cancer (7).

Molecular regulation of lymphangiogenesis in cancer

VEG-C and VEG-D constitute the lymphangiogenic ‘sub-family’ of the VEGF growth factors, and have become important predictors of tumour growth, lymphangiogenesis and metastatic behaviour (85). All of the members of the VEGF family (the others being VEGF-A, placenta-derived growth factor and VEGF-B) are related by a common VEGF-homology domain (VHD), which contains a 30% conserved amino acid sequence and a characteristic cystine-knot motif (87). Unique to the two-member lymphangiogenic ‘sub-family’, N-terminal (N-pro) and C-terminal (C-pro) propeptides (87,88) are proteolytically cleaved from the VHD by enzymes including plasmin (89) and ‘proprotein convertases’ (PCs) (88,90). This process generates mature, bioactive forms of VEGF-C and VEGF-D, which consist of VHD dimers and display enhanced affinity for VEGFR-3 (88,91), and thus make this group of enzymes important to the tumorigenicity of cancers which secrete these growth factors (92,93). Developmentally, VEGF-C is vital for normal lymphatic embryogenesis (87), a finding supported by transgenic and gene deletion animal models (55,94). Additionally, exogenous administration of both VEGF-C and VEGF-D were sufficient to rescue lymphatic vessel sprouting (29), and transgenic expression of either ligand alone induced lymphangiogenesis independent of angiogenesis (95,96).

VEGFR-3 is a major transducer of lymphangiogenic signaling, acting predominantly through phosphorylation of Akt, a PI-3 kinase-dependent serine-threonine kinase, and a PKC-dependent p42/p44 mitogen activating protein kinase (MAPK) (94,96,97). VEGF-3 activation not only prevents LEC apoptosis in culture but also stimulates proliferation, migration and cell survival under serum deprivation conditions via this pathway (98).

Experimental models of lymphangiogenesis in cancer

The VEGF-C expressing MCF-7 human breast cancer cell line demonstrates increased rates of lymph node metastasis in the presence of increased intratumoral, and particularly peritumoral lymphatics (99). Furthermore, soluble antibody to VEGFR-3 was shown to inhibit lymphangiogenesis and subsequent metastasis (99). A similar phenomenon of increased intratumoral and peritumoral lymphatic vessels was seen in an alternative VEGF-C expressing breast cancer line, MBA-MD-435 (100). The enhanced access to lymphatics was also associated with an increase in regional lymph node spread, and particularly pulmonary visceral metastasis (100). Consistent with Kukl et al.’s work (101), however, which showed the unprocessed predominantly VEGFR-3-specific 31 kD form of VEGF-C (as produced by these breast cancer models) to be insufficiently processed to stimulate VEGFR-2, significant angiogenesis did not occur in either setting (99,100). In contrast, the VEGF-C overexpressing MeWo human melanoma-derived cell line caused increased proliferation of both blood and lymphatic vessels, both intratumorally and peritumorally, due to increased proteolytic processing of VEGF-C (102). Enlarged neolymphatics in and around the VEG-F-C expressing AZ521 human gastric cancer model resulted in lymph node metastasis in 95% of AZ521-VEGF-C mice, compared with 29% of controls (103), and similar findings were demonstrated in the normally benign pancreatic β-cell tumours, when implanted in VEGF-C overexpressing transgenic mice (104).

In the case of VEGF-D, growth factor expressing, stably transfected 293 EBNA tumours xenografted into SCID-NOD mice demonstrated active roles in both tumour growth and lymphangiogenesis, with a concomitant increase in regional lymph node metastasis, compared with their VEGF-A expressing counterparts (105). Whilst VEGF-A promoted growth and
angiogenesis, neither lymphangiogenesis nor nodal dissemination were enhanced, and empty vector controls produced well contained, indolent tumours (105). Lymph node metastasis rates occurred in a VEGF-D dose-related fashion, and monoclonal antibodies raised to the bioactive region of VEGF-D competitively antagonised mature VEGF-D binding of VEGFR-2 and VEGFR-3, and inhibited tumour metastasis (106).

Detmar et al. (107) compared a VEGF-A producing, GFP-expressing, K14 promoter-driven transgenic mouse model with a non-VEGF-A expressing control, in which squamous cell carcinoma was generated by multistep, topical chemical induction. Interestingly, not only did VEGF-A appear to play a role in tumour growth but also stimulated both angiogenesis and lymphangiogenesis, apparently signalling via an upregulated VEGFR-2 on LECs (107).

Overall, these animal models correlate with findings in several human tumours. Significant tumour-induced lymphangiogenesis has been noted in human melanoma samples, and importantly, were directly related to the risk of lymph node metastasis and patient survival (46,71). Of note, several authors have found that human breast carcinoma samples seem to demonstrate metastasis in the absence of intratumoral lymphangiogenesis (69,86,108). Human breast cancer specimens in which intratumoral vessels were often collapsed and poorly staining with proliferation markers, but in which peritumoral lymphatics were increased and contained tumour emboli (86), may indicate particular significance of the peritumoral microenvironment in tumour lymphatic proliferation and metastatic activity. They may also illustrate the technical limitations of using single lymphatic markers, such as LYVE-1, alone (69), as they may fail to stain relevant vessels if downregulated in the particular lymphatics subtypes formed (30). Consistent with the regression to the primitive embryological state often seen in pathological contexts, blood vessel VEGFR-3 expression is observed on immunohistochemical studies of both benign and malignant vascular tumours (Kaposi sarcoma, 80% of spindle cell hemangiomas, 80% of angiosarcomas), non-vascular tumours, (including melanomas, carcinomas, other sarcomas) and perivascular tumours (109). These findings may implicate VEGFR-3 in maintaining the endothelial integrity during tumour angiogenesis, which generates vessels more akin to primitive, rather than mature blood vessels (68).

**Targeting lymphatics vessels for clinical benefit**

**Therapeutics**

The VEGF-C/VEGF-D–VEGFR-3 pathway. Therapeutic approaches for the inhibition of RTKs such as VEGFR-3, include monoclonal antibodies, small molecule inhibitors, peptide drugs and antisense techniques (7,110,111). Folkman’s vision of anti-angiogenesis as a cancer treatment has been realized with the release of an anti-VEGF agent for the treatment of metastatic colorectal carcinoma (112,113). Analogous to VEGF-A blockade to reduce tumour angiogenesis (114,115), an approach to block VEGFR-3 ligands VEGF-C/VEGF-D is promising for the inhibition of tumour lymphangiogenesis and lymphogenous metastasis (105,106,116). Hence, Achen et al. (106) raised monoclonal antibodies to the VHD of human VEGF-D which bind both unprocessed and fully processed VHD with high affinity, and inhibit their binding to both VEGFR-2 and VEGFR-3.

Overall, experimental models demonstrating suppression of VEGFR-3 signalling have shown inhibition of both peritumoral and intratumoral lymphangiogenesis, angiogenesis and metastatic spread (116,117). Administration of soluble VEGFR-3-immunoglobulin fusion protein, which binds VEGF-C and blocks VEGFR-3 signalling, and intravenous recombinant adenoviruses expressing VEGFR-3-immunoglobulin, both induce regression of tumour-induced lymphatic vessels (116). In addition, the interference with ligand–receptor interactions and the resulting inhibitory effect on lymphangiogenesis and metastasis produced by adeno-associated virus-delivered soluble VEGFR-3 decoy receptor was dose-related in VEGF-C secreting PC-3 and A375 tumour models (117). Further evidence of the therapeutic potential of soluble VEGFR-3 was provided by transgenic expression of soluble VEGFR-3 in mouse skin, which inhibited fetal lymphangiogenesis, and induced regression of already formed lymphatic vessels, though the blood vasculature was unaffected (93). These transgenic mice develop a lymphoedematous phenotype, characterized by foot oedema and dermal fibrosis. They survive the neonatal period despite almost complete absence of lymphatic vessels in several tissues, which later regenerate (94). Similarly, missense mutations interfere with VEGFR-3 signal transduction to cause primary (congenital) lymphoedema (56).

**Proteolytic processing of VEGF-C/VEGF-D**

VEGF-C expressing tumours which induced angiogenesis and lymphangiogenesis, but were inhibited by mutating the pro-VEGF-C cleavage site, demonstrated that processing of pro-VEGF-C is crucial to receptor activation, and thus generation of its biological effects (93,118). The enzymes responsible include the PCs and the serine protease, plasmin, which has been shown to cleave both C-propeptides and N-propeptides from the human VEGF-C and VEGF-D VHD, to promote lymphangiogenesis and cancer metastasis (89). As a potent proteolytic enzyme which is active at cleaving both propeptides of the lymphangiogenic growth factors, the parallel role that plasmin plays in fibrinolysis, a process which is crucial in regulating blood clotting, may help coordinate lymphangiogenesis in the later stages of tissue repair. Promisingly, alterations to specific PC growth factor binding sites have been shown to inhibit PDGF-A precursor processing by furin, the phosphorylation of its cognate RTK, and the resulting cellular proliferation (119). Furthermore, specific PC blockade in several stably transfected, growth factor secreting tumour models has been found to reduce tumour growth, malignant phenotype and migration (93,118). Thus, inhibitors of the PCs and plasmin, which restrict the cleavage of VEGF-C and/or VEGF-D, may also constitute novel agents for the treatment of human tumours and/or in adjuvant therapy to prevent tumour growth, metastasis or recurrence (93,118,119).

**Intracellular signalling cascades**

The majority of work in this area has focused on inhibiting tumour angiogenesis (110,120,121); however, blockade of intracellular signalling cascades may also prove to be useful for suppression of downstream VEGFR-3 signalling, as many of the VEGF family of RTKs employ common signalling mechanisms (97,98,120,122,123). In particular, PI-3 kinase-Akt and PKC-p42/p44 MAPK signalling cascades are the pathways activated by growth factor stimulation of VEGFR-3 (94,96–98), and may represent potential therapeutic targets for inhibiting lymphangiogenesis induced by tumour-secreted...
growth factors (110,121). Several small molecular agents targeting cytoplasmic protein tyrosine kinase (PTK) in preclinical models inhibit angiogenesis and tumour progression, and have reached clinical trials (124). One example is SU11248 (sunitinib or Sutent), which has reached Phase III clinical trials, and offers targeted treatment in select tumours such as carcinoma of the lung, renal cell carcinoma, and gastrointestinal tumours (125–127). Other PTK inhibitors which target VEGFR-2 and VEGFR-3 pathways, also currently involved in clinical cancer treatment trials include CEP-7055 (128), PTK 787/ZK 222584 (129,130) and BAY 43-9006 (131). The latter is a Raf-1 kinase inhibitor, which demonstrates anti-angiogenesis and anti-tumour activity via MAPK signalling and VEGFR tyrosine kinase blockade in preclinical tumour xenograft models, and is being trialed for use in a range of human tumour settings, including such common cancers as carcinomas of the breast, prostate, lung, pancreas, kidney, thyroid, pancreas and ovary (131,132).

Chemokines and adhesion molecules

Certain receptor–ligand relationships between tumour and host tissues, and soluble factors, known as chemokines, which regulate haemopoietic cell migration, may be ‘hi-jacked’ by cancer cells to facilitate the location of and entry into lymphatic vessels, and their movement along them towards lymph nodes (133). Furthermore, tumour–endothelial interactions may be responsible for particular cancers displaying metastatic affinity towards specific tissues, and chemokine–ligand guided interactions may create a chemical gradient which predisposes circulating metastatic cells to home toward, and settle within a certain distant organ tissue (133).

A prime example in lymphatic metastasis is CCL21/SLC, a ligand expressed on LECs for lymphocyte and dendritic cell signalling, and guidance towards lymph nodes via the CCR7 receptor (134–136). Human melanoma and breast cancer cell lines expressing CCR7 have demonstrated increased affinity for lymphatic endothelium and resulted in increased lymph node metastasis in animal models, a process which was inhibited by the administration of neutralizing anti-CCL21 antibodies (137). The CXCR4–CXCL12 pathway is also an strikingly well-characterized T-lymphocyte signalling mechanism, and CXCL12 is differentially expressed between individual colorectal carcinomas, providing a prognostic factor for local recurrence, liver metastases and poor overall survival (133). Interference with CXCR4 signalling may present a further target in disease-directed therapy for colorectal carcinoma (133).

Expression of adhesion molecules, which normally participate in immune cell traffic between interstitial and vascular compartments, may also be important in tumour entry to, or exit from the lymphovascular space during metastasis (138). Bevacqua et al. (138) demonstrated an in vitro correlation between attachment potential and metastatic propensity in malignant melanoma and breast carcinoma cell lines, using adhesion assays with human tumour cells. One particular adhesion molecule, L-selectin, forms a ligand–receptor pair with mannose receptor, which is expressed on lymphatic endothelium (139). It orchestrates the intravasation of circulating lymphocytes from interstitial tissues into lymphatic vessels, and subsequent homing to lymph node high endothelial venule addressins, a process which may be exploited by L-selectin expressing tumour cells, in order to promote lymph node metastasis, and which has been inhibited in animal studies by administration of anti-L-selectin monoclonal antibody (53). Both mannose receptor and common lymphatic endothelial and vascular endothelial receptor (CLEVER)-1 have been implicated in tumour metastasis via adhesion of malignant cells to lymphatic endothelium (140), in some human cancers, and represent further potential anti-metastatic therapeutic targets (141). Proinflammatory cytokines and the resulting immune cell aggregations may also stimulate additional lymphangiogenic growth factors, and so provide an indirect method of inhibiting lymphatic vessel proliferation in the region of a tumour (142,143).

Diagnostics

Imaging localization of lymphatic drainage pathways

The cutaneous lymphatic drainage pathways from the site of a primary tumour may be highly variable between patients, even within the same areas of the body (144). Up to 30% of these tumours therefore defy clinical predictability of which lymph nodes, in which regional node groups, may contain cancer cells (145), and contradict long-held anatomical ideas of drainage patterns (146).

Significant examples detected using lymphatic mapping technology include drainage (and potential tumour spread) from superficial back skin to deep lymph nodes in paraaortic and retroperitoneal areas; across the midline; or from periumbilical skin through the chest wall (147,148). These routes bypass the classical drainage pathways through regional lymph nodes and may involve multiple nodal fields (149). Such cancers would be erroneously classified as lymph node-negative using conventional methods (147,150), in ~14–37% of patients with melanomas on their limbs, trunk, or head and neck (147–152).

Future directions for lymphatic imaging will include the ongoing development of lymphatic mapping for cancers in deeper viscera such as the gastrointestinal and respiratory tracts (48). The next challenge is to develop methods of sentinel lymph node assessment that are non-invasive, yet as accurate as present methods. Adaptations using labelled antibodies or other tracers with specificity for lymphatic vessels or lymph nodes, could assist in analysing the levels at which abnormalities of lymphatic drainage occur, whether they involve unrecognized pattern variations in ‘collecting lymphatics’, or ‘lymphaticovenous’ or ‘interlymphatic subtype’ shunts, and whether aberrant lymphatic channels may be influenced by tumour-derived growth factors.

Conclusion

Understanding lymphogenous tumour metastasis represents a crucial step in the treatment of human cancer. Since the development of lymphatic markers, several diverse disciplines may now converge in an attempt to define the respective roles played by lymphatic vessels and tumour cells and products in facilitating metastasis to lymph nodes and beyond. Understanding the complexities of lymphatic development, anatomy and pathophysiology in terms of the influences of lymphangiogenic growth factors, receptor signalling and tumour immunomodulation may yield an array of new therapeutic targets for application in the treatment of cancer.

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