Histol Histopathol (2005) 20: 283-298

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Lymphangiogenesis and its role in cancer

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Summary. In many tumour types, lymphatic vasculature serves as a major route for tumour metastasis. The dissemination of malignant cells to the regional lymph nodes is an early step in the progression of many solid tumours and is an important determinant of prognosis. Lymphangiogenesis (formation of new lymphatic vessels) is thought to be crucial for cancer cells to metastasise to the regional lymph nodes. However research in this important process has been neglected largely due to the lack of molecular markers specific to the lymphatic endothelium. Recently, several specific markers have been identified including LYVE-1, podoplanin and prox-1. Although the biology of lymphangiogeneis, particularly its regulation, is still far from clear, it is now well established that tumours are lymphangiogenic i.e. they could induce the generation of their own lymphatics and metastasise to the regional lymph nodes. It is thought that the interruption of the main signalling pathways involved in this process could help to prevent lymphatic spread of many tumours. Furthermore, understanding the molecular mechanisms in lymphangiogenesis might help to develop new therapeutic strategies against cancer lymphatic spread. Here, we reviewed the literature in regards to the biology of lymphangiogenesis, its molecular regulation, lymphatic markers and the significance in human solid tumours.

Key words: Lymphangiogenesis, VEGF-C, VEGF-D, Podoplannin, Prox-1, LYVE-1, VEGFR-3 and cancer metastasis

Introduction

The lymphatic system is involved in transport of tissue fluids, extravasated plasma proteins and cells back into the blood circulation. Lymphatics also make an important part of the body's immunological surveillance system. Formation of new lymphatics, lymphangiogenesis, occurs in both normal developing tissues and in pathological processes such as inflammation, wound healing, lymphoedema and perhaps most importantly in solid tumours. Amongst the common routes of tumour cells metastasis is the lymphatic route which occurs early and frequently with patterns of spread via afferent vessels following routes of natural drainage (Sleeman, 2000). Clinically, the extent of lymph node involvement is a key prognostic factor and constitutes an integral part of staging in many human cancers including breast, gastric, colorectal and others.

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Development of the lymphatic system

Although lymphatics were discovered by Gasper Asellius in 1627 at about the same time William Harvey described the blood circulation (Witte et al., 2001), it was over 3 decades later, when theories behind their development started to take shape. Lymphatic vessels formation occurs early during foetal development from isolated primitive lymph sacs that originate by endothelial cell budding from embryonic vein as proposed by Sabin in the early twentieth century (Jussila and Alitalo, 2002; Sabin, 1902, 1904). During early development of the blood vascular system (vasculogenesis), new capillaries arise either de novo from angioblasts or through progressive sprouting from veins (Pepper, 2001). However, recent studies have revealed that embryonic lymphangiogenesis can either develop from a local *de novo* differentiation of lymphatic endothelium from lymphangioblasts, or via sprouting from pre-existing blood capillaries i.e."veins lymphangiogenesis" (Schneider et al., 1999; Wilting et al., 1999).

Despite the importance of the lymphatic system both in normal physiological and also in pathological processes, there has been very slow progress in its studies. This is due to the fact that lymphatics are extremely similar to blood vessels even histologically. Therefore, most studies in the past involved extensive research in angiogenesis (formation of blood vessels) rather than lymphangiogeneis. With the recent detection of markers specific to the lymphatic endothelium,

studying lymphatics is now possible.

Historical aspects in lymphangiogenesis

Although it was observed that lymphatic vessels containing clusters of tumour cells do occur at the periphery of malignant tumours, lymphatic vessels have been thought to be absent from tumours themselves (Folkman, 1996). The initial concept of lymphatic spread of tumours was that tumour cells metastasise solely by the invasion of pre-existing lymphatics surrounding the tumour margin, i.e., tumours are not lymphangiogenic. Although the significance of pre-existing peritumoural lymphatics as conduits for tumour cell dissemination has been well recognised (Fisher and Fisher, 1966), it has remained unclear whether tumours can stimulate lymphangiogenesis and whether tumour metastasis stimulates molecular activation of the lymphatic system (Leu et al., 2000). In the not too distant past, several studies have failed to identify functional lymphatics within tumours leading to the suspicion that lymphangiogenesis may not play a major role in tumour metastasis (Jain, 1987; Carmeliet and Jain, 2000).

This was supported by studies involving injection of tracers into lymphatics, were they failed to show any intrinsic lymphatic vascular supply inside tumours (Tanigawa et al., 1981). However, this may simply reflect the collapse of lymphatics within tumours due to the increased pressure and mechanical stress generated by the proliferating cancer cells (Leu et al., 2000). Further studies, linked the presence of dilated and engorged lymphatics in peritumoural stroma to growth factors produced by tumour cells (Leu et al., 2000). However, these lymphatics are thought to be pre-existing and have become stimulated by the tumour cells rather than new ones formed by the tumour. These studies shed little light on whether intravasasion of tumour cells into the lymphatic system is a passive process, or indeed an active one (Hartveit, 1990; Leu et al., 2000).

With the improvements in the molecular and cellular biology studies new specific lymphatic markers have been identified. The last few years have witnessed the identification of specific markers to the lymphatic endothelium including Podoplannin, a glomerular podocyte membrane mucoprotein (Breiteneder-Geleff et al., 1999; Weninger et al., 1999); Prox-1, a homeobox gene product that is involved in regulating development of the lymphatic system (Oliver et al., 1993; Wigle and Oliver, 1999). Most recently, a novel hyaluronan receptor, LYVE-1 has been shown to be restricted to lymphatic vessels in normal tissues (Banerji et al., 1999; Mandriota et al., 2001) and associated with the tumours (Mandriota et al., 2001; Skobe et al., 2001; Stacker et al., 2001). The first real progress in studying lymphangiogenesis was the detection of the identification of the vascular endothelial growth factors (VEGFs). Among the family of these factors, VEGF-C and VEGF-D are now known to be lymphangiogenic as they are the only ligands for the tyrosine kinase, VEGF receptor 3 (Flt4). This receptor is highly expressed on the lymphatic endothelium and considered to be a lymphatic marker (see below) (Kaipainen et al., 1995; Alitalo, 1997; Karkkainen and Petrova, 2000; Karkkainen et al., 2000, 2001, 2002; Jussila and Alitalo, 2002; Karkkainen and Alitalo, 2002).

Molecular mechanisms of lymphangiogenesis

Tumour cell dissemination is mediated by mechanisms including local tissue invasion, lymphatic and blood spread or direct seeding of body cavities. Regional lymph nodes are often the first sites to develop metastases (Alitalo and Carmeliet, 2002; Oliver and Detmar, 2002) either draining via pre-existing afferent lymphatic vessels and/or via newly formed lymphatic capillaries. This is indeed the basis of the sentinel lymph node biopsy and indicates the particular importance in surgical management of cancers including breast, melanoma and others. However, not all tumours metastasise to the regional lymph nodes first. Furthermore, the presence of a metastasis in a lymph node does not necessarily mean that the tumour cells have been arrived via the lymphatic vessels (Van Trappen and Pepper, 2001). Tumour cells may pass directly into the blood vascular system through veno-lymphatic communications. The mechanisms determining whether regional lymph nodes or other sites first develop metastases remain poorly understood. In fact, most disseminated tumour cells have a limited life span in blood stream. While many surviving cancer cells remain dormant in the host tissues, only a few develop into clinically detectable micrometastases. However, identification of those occult tumours cells, and prevention of their re-growth would be of great clinical significance.

Tumourigenesis in humans is a multi-step process, and these steps reflect the genetic alterations that drive the progressive transformation to cancer. Contrary to normal cells, cancer cells have defective regulatory circuits that control normal proliferation and homeostasis. While normal cells require mitogenic signals to proliferate, malignant cells are self-sufficient for the growth signals and insensitive to the growth-inhibitory signals. Therefore, tumour cells are independent in generating their own growth signals. It has been well established that a complex series of cellular interactions between several types of cells like fibroblasts, immune cells, and endothelial cells as well as malignant cells within the tumour tissues could lead to cancer cells growth and metaststasis (Hanahan and Weinberg, 2000). In addition to the ability to synthesize their own growth factors leading to an autocrine stimulation, cancer cells could indeed induce the stimulation of other cells like endothelial cells via a paracrine mechanism, thus generating neo-vascularization in the local tumour microenvironment. As tumours need neovascularization to grow and

metastasise, microvascular density has been used as a measure of tumour angiogenesis which is correlated to prognosis (Weidner, 1995). These early studies yielded little conclusive evidence as to the influence of lymphatic microvessel density on patients' survival. In ovarian cancer for example, the lymphatic vessel density has no influence on the progression of the disease and in cervical cancer an increased amount of lymphatic vessels may even be associated with a better prognosis (Birner et al., 2000, 2001).

Lymphangiogenic growth factors and receptors

The detection of the vascular endothelial growth factors (VEGFs) started with the discovery of VEGF in 1989 (Keck et al., 1989; Leung et al., 1989). Since then, other vascular growth factors were identified and the VEGF family is currently consists mainly of VEGFs -A, -B, -C, -D, -E and PlGF (placental growth factor) (Fig. 1). There are three VEGF tyrosine kinase receptors identified so far, VEGFR-1 (Flt-1), VEGFR-2 (Flk-1, KDR) and VEGFR-3 (Flt-4). VEGF-B and PlGF bind to VEGFR-1, whereas VEGF-A interacts with both VEGFR-1 and VEGFR-2. VEGF-E binds VEGFR-2 and both VEGF-C and VEGF-D bind VEGFR-3 (Fig. 1). VEGFR-1 and VEGFR-2 mediate angiogenesis, whereas VEGFR-3 is involved mainly in lymphangiogenesis.

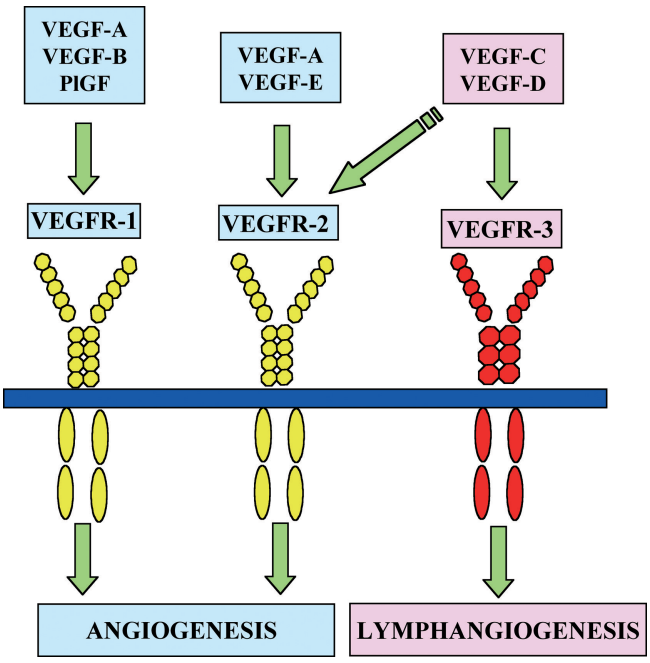

Fig. 1. The currently known VEGFs and their receptors. VEGFR-1 (Flt-1) and VEGFR-2 (KDR) have seven extracellular immunoglobulin homology domains, but in VEGFR-3 (Flt-4), the fifth immunoglobulin domain is cleaved on receptor processing into disulfide-linked subunits. VEGFR-1 and VEGFR-2 mediate angiogenesis, whereas VEGFR-3 is involved mainly in lymphangiogenesis.

In adults, VEGF-C is expressed in the heart, small intestine, placenta, ovary and the thyroid gland. VEGF-C stimulates mitosis and migration of endothelial cells and it increases vascular permeability. VEGF-C has been shown to induce lymphangiogenesis in transgenic mouse skin and in mature chick chorioallantoic membrane (Jeltsch et al., 1997; Oh et al., 1997). However, recombinant VEGF-C also promotes angiogenesis when applied to early chorioallantoic membrane of chicks, to mouse cornea or to ischaemic hindlimbs of rabbits (Cao et al., 1998). It has recently been reported that lymphatics surrounding a VEGF-C overexpressing tumour are enlarged, and it has been suggested that the increase in lymphatic diameter may be sufficient to increase metastasis. VEGF-D is structurally 48% identical to VEGF-C. VEGF-D is expressed in many adult tissues including the vascular endothelium, heart, skeletal muscle, lung, small and large bowel. VEGF-D is mitogenic for endothelial cells. The fact that VEGF-D binds also VEGFR-2 has made it to be possibly angiogenic. However, the controversy remains as it has been shown that transgenic overexpression of VEGF-D led to lymphatic hyperplasia but not angiogenesis.

The secretion of VEGF-C and VEGF-D by some tumours could induce the activation of their receptor, VEGFR-3 on the vascular endothelium and thereby inducing the formation of new lymphatic vessels. However, little is currently known about the factors that make dome tumours secret these lymphangiogenic factors. Like angiogenesis (formation of new blood vessels), factors such as hypoxia, other growth factors, cytokines and hormones have been studied. Regulation by other cytokines and growth factors seems to be promising as it has been recently found that VEGF-C and VEGF-D could indeed be regulated by IL-ß (Akagi et al., 1999) and IL-7 (Al-Rawi et al., 2003) respectively. It is well established that the downstream signalling pathways of VEGFs are similar to those activated by other cytokines such as MAPK (mutagene activated protein kinase) and PI3-K (phoshoinositol 3-kinase). Recent studies have indicated the presence of cross-talks between the MAPK and the PI3-K pathways as phosphorylation of Raf by Akt resulted in inhibition of the Raf-MEK (MAP kinase) – ERK pathway (Zimmermann and Moelling, 1999). PI3-kinase activation is known to mediate signalling transduction of many several cytokines and growth factors. The PI3-K pathway is linked to mitogenesis, but several studies subsequently have shown that this pathway has an important function in regulating cell survival by the activation of the serine-threonine kinase Akt (protein kinase B). The cross talk between MAPK and PI3-K pathways leads to increased cell survival by stimulating the transcription of the pro-survival gene(s) and by post-translational modification and inactivation of components of the cell death machinery. VEGFR-3 can also strongly activates Stat-5 (Korpelainen et al., 1999); a protein known to be activated by IL-7 suggesting that Stat-5 activation is involved in the regulation of

lymphatic endothelium.

VEGFR-3, a tyrosine kinase receptor, has been shown to control the development and growth of the lymphatic system. The importance of VEGFR-3 for the development of the lymphatic vasculature has been further strengthened by the fact that early onset primary lymphoedema is linked to the VEGFR-3 locus in distal chromosome 5q. However, in the early embryonic development, VEGFR-3 is essential in the formation of the primary cardiovascular network before the emergence of the lymphatic vessels, as VEGFR-3 knockout embryos die early in development because of cardiovascular failure.

Stimulation of VEGFR-3, using the specific ligand, induces a rapid tyrosine phosphorylation of Shc and activation of MAPK pathway results in an increased cell motility, actin reorganization and proliferation. Recently, VEGFR-3 has been found to be a strong activator of Stat-3 and Stat-5. Stat proteins were therefore identified as novel targets for the VEGFRs, suggesting that they may be involved in the regulation of endothelial function. Stat proteins are also involved in other cytokines signalling suggesting that the regulation of VEGFR-3 signalling might be controlled by other cytokines.

VEGFR-3 has been employed as a marker for lymphatic vessels in normal and pathological tissue samples and has been used to demonstrate an apparent lymphatic origin of Kaposi's sarcoma cells (Jussila et al., 1998). However, although VEGFR-3 stains PAL-E-negative capillaries, recent data show that VEGFR-3 can also be expressed in blood vessel endothelia (Partanen, 2000). It is also expressed in blood capillaries during the neovascularisation of tumours and in chronic inflammatory wounds.

IL-7 has been identified as a strong lymphangiogenic factor in endothelial cells (Al-Rawi et al., 2002). IL-7 specifically increases the expression of lymphatic markers, LYVE-1, podoplanin, and prox-1 in endothelial cells, and it induces the formation of lymphatic vessels *in vivo* (Al-Rawi et al., 2004a). Furthermore, IL-7 up-regulates the expression VEGF-D in endothelial and breast cancer cells (Al-Rawi et al., 2003, 2004a,b). Hepatocyte growth factor (HGF) has also been recently identified as a putative lymphangiogenic factor both in vitro and in vivo using a breast tumour model (Jiang et al., 2003).

The vascular endothelial growth factors-C and -D (VEGF-C and VEGF-D)

VEGF-C and VEGF-D are produced as precursor proteins with N- and C-terminal propeptides flanking the VEGF homology domain (Joukov, 1996; Joukov et al., 1996; Lee, 1996; Lee et al., 1996; Orlandini et al., 1996; Achen et al., 1998). The fully processed or mature forms of VEGF-C and VEGF-D consist of the VHD, which acts as a ligand not only for VEGFR-3, but also for VEGFR-2 (Joukov et al., 1997; Achen et al., 1998).

In midgestation embryos, VEGF-C is prominently expressed in regions where the lymphatic vessels undergo sprouting from embryonic veins, such as in the perimetanephric, axillary and jugular areas, and in the developing mesenterium (Kukk et al., 1996). In adults, VEGF-C is expressed in the heart, small intestine, placenta, ovary and the thyroid gland. VEGF-C stimulates mitosis and migration of endothelial cells and it increases vascular permeability. VEGF-C has been shown to induce lymphangiogenesis in transgenic mouse skin and in mature chick chorioallantoic membrane (Jeltsch et al., 1997; Oh et al., 1997). However, recombinant VEGF-C also promotes angiogenesis when applied to early chorioallantoic membrane of chicks, to mouse cornea or to ischaemic hindlimbs of rabbits (Cao et al., 1998; Witzenbichler et al., 1998). Therefore, VEGF-C is likely to play a dual role both as an angiogenic and a lymphangiogenic growth factor.

If VEGF-C induces lymphangiogenesis, is it sufficient enough to increase the rate of metastasis to the lymph nodes? It has recently been reported that lymphatics surrounding a VEGF-C overexpressing tumour are enlarged, and it has been suggested that the increase in lymphatic diameter may be sufficient to increase metastasis (Pepper, 2001). Furthermore, the association of VEGF-C overexpression, lymphatic vessel density and lymph node metastases has been described in a variety of carcinomas including thyroid, prostate, gastric, colorectal, and lung (Bunone et al., 1999; Ohta et al., 1999, 2000; Yonemura et al., 1999; Buju et al., 2000; Skobe et al., 2001).

VEGF-D is 48% identical to VEGF-C (Yamada et al., 1997; Achen et al., 1998). It contains the eight conserved cysteine residues characteristic of the VEGF family and has a cysteine-rich COOH terminal extension similar to VEGF-C. In midgestation mouse embryos, VEGF-D expression is particularly abundant in the developing lung. VEGF-D is expressed in many adult tissues including the vascular endothelium, heart, skeletal muscle, lung, small and large bowel.

VEGF-D is mitogenic for endothelial cells. Like VEGF-C, VEGF-D is proteolytically processed after secretion, and it binds to and activates both VEGFR-2 and -3 (Achen et al., 1998; Orlandini et al., 1996; Yamada et al., 1997). The fact that VEGF-D binds also VEGFR-2 has made it to be possibly angiogenic. However, the controversy remains as it has been shown that transgenic overexpression of VEGF-D led to lymphatic hyperplasia but not angiogenesis (Marconcini et al., 1999).

At present, little is known whether factors such as hypoxia, growth factors, cytokines and hormones regulate expression of VEGF-C and VEGF-D (Bellomo et al., 2000). It has been recently shown for that IL-ßcould up-regulate VEGF-C (Akagi et al., 1999) and IL-7 could indeed up-regulate VEGFR-3 by an autocrine stimulation of endothelial cells via a VEGF-D dependent mechanism (Al-Rawi et al., 2003). Although the regulation of VEGF-C and VEGF-D by other cytokines

is still not well established, it is known that cross talks and interactions do exist between them. For example, cytokines like IL-7 induces tyrosine phosphorylation and activation of phosphoinositol-3 kinase (PI3-K) in both endothelial and cancer cells (Al-Rawi et al., 2003; Xia et al., 1996). This molecule (PI3-K) is indeed implicated in the VEGFs-induced endothelial cell survival via activation of its downstream target serine kinase Akt/PKB (Gerber et al., 1998).

Lymphatic markers

The main differences between lymphatic and blood vascular endothelium are listed in table 1. Lymphatic capillaries are identified by the fact that they are lined by a single layer of endothelial cells, which are characterized by having poorly developed junctions with frequent large gaps between cells. These loose junctions readily permit the passage of large biological macromolecules, pathogens and migrating cells. Because pressure within lymphatic capillaries is only slightly higher than the interstitium, lumen potency is maintained by anchoring filaments that connect the abluminal surfaces of endothelial cells to the perivascular extracellular matrix (Leak, 1968; Pepper, 2001). Unlike blood capillaries, lymphatic capillaries lack a continuous basement membrane, and they are devoid of pericytes (Aukland and Reed, 1993). However, it should be noted that the latter is not true for larger collecting lymphatic ducts, which are supported by a thin connective tissue coat and higher up the lymphatic drainage tree by an additional smooth muscle wall. Although the initial lymphatics have no valves, the larger collecting ducts do have (Aukland and Reed, 1993). However these anatomical differences do not provide a practical way in the differentiation between blood and lymphatic vessels, particularly in regards to studies involving lymphatics. The development of specific biological markers has made the discrimination between the two systems much accessible particularly with the development of antibodies against some of them. This has made molecular quantitation and immunohistochemical analyses readily available. The main markers for the lymphatic endothelium are listed in table 2. The ideal lymphatic endothelial marker would have some characteristics. It would be exclusively found (positive marker) on or excluded from (negative marker) lymphatic endothelial cells, rather than depending on relative differences in expression level between blood and lymphatic vessels (Sleeman et al., 2001). They should be highly stable, specific, and sensitive.

Podoplanin

Podoplanin, is a 43 kDa surface glycoprotein that was recently cloned as a cell surface protein expressed on normal rat kidney podocytes, but not on podocytes in kidneys with a puromycin aminonucleoside nephrosis (PAN), a model for human minimal change nephropathy (Breiteneder-Geleff et al., 1997). It consists of 163 amino acids and has a single membrane spanning domain, two phosphorylation sites and six O-glycosylation sites in the large ectodomain. Originally, podoplanin was first cloned as OTS-8 in TPA-treated osteoplastic cells (Nose et al., 1990) and as the antigen recognised by the E11 antibody, which binds to osteoblast and osteocytes and is a marker for cells of the late osteogenic lineage (Wetterwald et al., 1996). The identical sequence was reported by Rishi et al. (1995) as T1a, a protein expressed on alveolar epithelial type 1 cells. The lung is a major site of podoplannin expression in the adult (Rishi et al., 1995; Wetterwald et al., 1996). Intravenous injection of antibodies against podoplanin caused proteinurea and flattening of podocytes, typical of the pathology seen in PAN suggesting that podoplanin is involved in maintaining lamellar permeability and the

Table 1. Summary of the main differences between lymphatic and vascular endothelium.

	BLOOD VESSEL	LYMPHATIC VESSEL
Cell surface molecules	Von Willebrand factor (Factor VIII), VE-cadherin, ICAM, CD31, JAM1/JAM2, PAL-E (absent from arterioles and some capillaries) and others	VEGFR-3, prox-1, podoplanin, LYVE-1
Basement membrane	Present and continuous	Absent or incomplete basement membrane
Junction types	Tight junctions / Adherens / gap junctions	Overlapping Loose junctions readily permit the passage of macromolecules, pathogens and migrating cells (not for larger ducts)
Enzymes	Presence of alkaline phosphatase and lack of 5'-nucleotidase	Lack of alkaline phosphatase and presence of 5'-nucleotidase
Chemokines	SLC	IP-10, Eotaxin
Pericytes	Mostly present (unreliable)	Mostly absent

ICAM: Intracellular adhesion molecule; JAM: Junctional adhesion molecule; PAL-E: Pathologische Anatomie Leiden – Endothelium; VEGFR-3: Vascular endothelial growth factor receptor-3; Prox-1: Prospero related homeobox gene-1; LYVE-1: Lymphatic vessel endothelial receptor-1; SLC: Secondary lymphoid chemokine; IP-10: IFN-γ-inducible protein-10.

shape of podocyte foot processes (Matsui et al., 1998, 1999).

Podoplanin is also expressed on epithelial cells of the choroids plexus cells and on lymphatic endothelial cells (Wetterwald et al., 1996). Light and electron microscopic immunohistology demonstrate the specificity of podoplanin expression on lymphatic but not blood vasculature endothelia in the skin (Breiteneder-Geleff et al., 1999). Furthermore, podoplanin was found to be expressed on PAL-E-negative vessels and to co-localize with VEGFR-3 (Breiteneder-Geleff et al., 1999; Weninger et al., 1999). These data suggest that podoplanin is a very promising marker for differentiating between lymphatic and blood vascular endothelium. To-date, the exact function of podoplanin is still unknown. However podoplanin may be involved in regulating the permeability of lymphatic vessels, or perhaps in maintaining their integrity (Sleeman et al., 2001).

Prox-1 (Prospero related homeobox gene –1)

Prox-1, the homologue of the *Drosophila* homeobox gene prospero, is a marker for the sub-population of endothelial cells that bud and sprout to give rise to the lymphatic system during early development (Wigle and Oliver, 1999). Prox-1 gene spans more than 40 kb, consists of at least 5 exons and 4 introns and encodes an 83 kDa protein. Prox-1 gene is mapped to human chromosome 1q32.2 – q32.3. Chicken Prox-1 is highly expressed in the developing lens, retina, and pancreas (Tomarev et al., 1996). Mouse Prox-1 expression was detected in the young neurons of the subventricular region of the CNS as well as the developing lens and the pancreas (Oliver et al., 1993). Targeted deletion of the Prox-1 gene does not affect development of the blood vascular system, but the budding and sprouting of the developing lymphatics is ablated, suggesting that prox-1 plays a key role in lymphatic system development (Wigle and Oliver, 1999). These data point towards a possible exclusive expression of prox-1 in lymphatic endothelium

Lymphatic Vessel Endothelial receptor -1 (LYVE-1)

LYVE-1 receptor is a type I integral membrane polypeptide expressed on the cell surface as a 60 kDa protein, which is reduced to approximately 40 kDa by glycosidase treatment (Banerji et al., 1999). LYVE-1 is abundant in spleen, lymph node, heart, lung, and foetal liver, less abundant in appendix, bone marrow, placenta, muscle, and adult liver, and absent in peripheral blood lymphocytes, thymus, brain, kidney, and pancreas. Expression of LYVE-1 is largely restricted to endothelial cells lining lymphatic vessels and splenic sinusoidal endothelial cells (Banerji et al., 1999). LYVE-1 may be involved in hyaluronan metabolism in the lymphatic system (Fraser et al., 1988; Fraser and Laurent, 1989; Sleeman et al., 2001). The co-localisation of LYVE-1 and hyaluronan on the luminal surface of lymphatic vessels suggests that HA may coat the lumen of lymphatic vessels through binding to LYVE-1 allowing hyaluronan-binding cells to adhere and migrate (Banerji et al., 1999).

The central core of the LYVE-1 Link module (C2-C3) is 57% identical to that of the human CD44 HA receptor, the only other Link superfamily HA receptor described to date with the closest homologue to LYVE-1. Nevertheless, there are distinct differences between LYVE-1 and CD44 suggesting that the two homologues

Table 2. Specific markers for the lymphatic endothelium, their expression sites and main biological function.

LYMPHATIC MARKER	MOLECULAR TYPE	SITES OF EXPRESSION	BIOLOGICAL ACTIVITY	REFERENCE
Podoplannin	Glomerular podocyte mucoprotein	Co-expressed with VEGFR-3 in lymphatic capillaries, vascular tumours, osteoblasts, renal podocytes and lung alveolar type-1 cells	Involved in maintaining lamellar permeability and the shape of podocyte foot processes in the kidneys	(Breiteneder-Geleff et al., 1997)
Prox-1	Transcription factor	Lens, heart, liver, pancreas and nervous system	Homeobox gene involved in the development and differentiation of lymphatic vessels. Prox-1 deficient neonates have failure of lymphatic sprouting and differentiation	(Wigle and Oliver, 1999b)
LYVE-1	HA receptor-1	Kidney, pancreas, adrenal glands and thyroid gland	Transport of hyaluronan from extracellular matrix to lymph nodes	(Banerji et al., 1999)
VEGFR-3	Receptor tyrosine kinase	Mainly on lymphatic vessels, but also reactivated in blood vessels in pathological conditions	Receptor for VEGF-C and VEGF-D	(Kaipainen et al., 1993)
Desmoplakin	Endothelial adhering junction (complexus adhaerentes)	Small lymphatic endothelium, but absent from large lymphatic vessels such as the thoracic duct	Provide gaps through which macromolecules and circulating cells pass	(Schmelz et al., 1994b)

differ either in the mode of HA binding or in its regulation. LYVE-1 receptor is almost exclusively restricted to lymph vessel endothelial cells, while CD44 is almost completely absent (Banerji et al., 1999). While the highest concentration of LYVE-1 expression was found in submucosal lymph vessels underlying smooth muscle in the colon, and the lacteal vessels of intestinal villi that transport dietary lipid absorbed from the small intestine. CD44 is expressed abundantly in blood vessels and largely absent from lymphatic vessels (Picker et al., 1989). However, LYVE-1 is also expressed on sinusoidal endothelial cells of the spleen and placental syncytiotrophoblasts (Sleeman et al., 2001).

The development of antibodies against LYVE-1 has made detection of lymphatics within tumours possible. For example, proliferating intratumoural lymph vessels have been identified in head and neck cancer (Beasley et al., 2002). Studies on LYVE-1 as a lymphatic marker was also helped in detecting lymphatics in primary malignant melanoma (Oliver and Detmar, 2002). Furthermore, the presence of LYVE-1 in tumours can indeed promote lymph node metastasis. Overexpression of VEGF-C in orthotopically transplanted MDA-435 or MCF-7 breast carcinoma (Mattila et al., 2002; Skobe et al., 2001) or RIP1/Tag2-RIP1/VEGF-C transgenic mice (Mandriota et al., 2001) promoted proliferation of LYVE-1-positive lymph vessels and increased subsequent metastasis of tumour to lymph nodes.

Vascular endothelial growth factor receptpr-3 (VEGFR-3)

While, VEGFR-1 and -2 are expressed almost exclusively on vascular endothelial cells, VEGFR-3 is the restricted to lymphatic endothelium (Eriksson and Alitalo, 1999; Olofsson et al., 1999; Veikkola et al., 2000). However, VEGFR-3 can also be up-regulated on tumour blood vessels (Partanen et al., 2000; Valtola et al., 1999). VEGFR-3, a tyrosine kinase receptor, has been shown to control the development and growth of the lymphatic system. The importance of VEGFR-3 for the development of the lymphatic vasculature has been further strengthened by the fact that early onset primary lymphoedema is linked to the VEGFR-3 locus in distal chromosome 5q (Ferrell et al., 1998; Witte et al., 1998; Evans et al., 1999). However, in the early embryonic development, VEGFR-3 is essential in the formation of the primary cardiovascular network before the emergence of the lymphatic vessels, as VEGFR-3 knockout embryos die early in development because of cardiovascular failure (Dumont et al., 1998).

In humans, two isoforms of the VEGFR-3 protein occur, VEGFR-3S (short) and VEGFR-3L (long). The difference between the two lies in their carboxyl termini as a result of alternative mRNA splicing (Galland et al., 1993; Pajusola et al., 1993). VEGFR-3L is the predominant isoform in the tissues. It contains three additional tyrosyl residues, of which Tyr1337 serves as an important autophosphorylation site in the receptor (Pajusola et al., 1993; Fournier et al., 1995). The long isoform was able to mediate anchorage independent growth in soft agar and tumourigenicity in nude mice (Pajusola et al., 1994; Borg et al., 1995; Fournier et al., 1995).

Stimulation of VEGFR-3, using the specific ligand, induces a rapid tyrosine phosphorylation of Shc and activation of MAPK pathway results in an increased cell motility, actin reorganization and proliferation (Cao et al., 1998; Joukov et al., 1998). In a human erythroleukemia cell line which expresses high levels of the VEGFR-3, VEGF-C stimulation induced activation of the signalling molecules Shc, Grb2 and SOS which lead to cell growth response (Wang et al., 1997). In these cells VEGF-C also induced tyrosine phosphorylation of the cytoskeletal protein paxillin by RAFTK, a member of the focal adhesion kinase family. The binding of VEGFR-3 to Grb2 is mediated by the Grb2 SH2 domain. The PTB domain of Shc is required for Shc tyrosine phosphorylation by VEGFR-3 (Pajusola et al., 1994; Fournier et al., 1995, 1999). Mutations in Shc phosphorylation sites increased VEGFR-3 transforming activity in the soft agar assay, suggesting that Shc has an inhibitory role in VEGFR-3 mediated growth response. Recently, VEGFR-3 has been found to be a strong activator of Stat-3 and Stat-5. Stat proteins were therefore identified as novel targets for the VEGFRs, suggesting that they may be involved in the regulation of endothelial function. Stat proteins are also involved in other cytokines signalling suggesting that the regulation of VEGFR-3 signalling might be controlled by other cytokines.

VEGFR-3 has been employed as a marker for lymphatic vessels in normal and pathological tissue samples (Lymboussaki et al., 1998) and has been used to demonstrate an apparent lymphatic origin of Kaposi's sarcoma cells (Jussila et al., 1998). However, although VEGFR-3 stains PAL-E-negative capillaries (Lymboussaki et al., 1998; Paavonen et al., 2000), recent data show that VEGFR-3 can also be expressed in blood vessel endothelia (Partanen et al., 2000). It is also expressed in blood capillaries during the neovascularisation of tumours and in chronic inflammatory wounds (Partanen et al., 1999; Valtola et al., 1999; Kubo et al., 2000; Paavonen et al., 2000). A mutation in VEGFR-3 has recently been linked to hereditary lymphoedema (Ferrell et al., 1998). The mutation, which converts proline 1114 to leucine, occurs in the VEGFR-3 tyrosine kinase domain, indicating that a disturbance in VEGFR-3 signalling may play a part in the development of this disease.

Other less specific markers

5'-Nucleotidase

5'-nucleotidase is an enzyme that acts on nucleoside-5'-phosphates, such as AMP and adenylic acid, releasing inorganic phosphate. It has been shown that 5'-nucleotidase activity is stronger in lymphatic than

in blood vessels (Werner et al., 1987; Weber et al., 1994). Conversely, the activity of another enzyme called 5'-Nase alkaline phosphatase (ALPase) is higher in blood vessels than that in the lymphatics (Werner et al., 1987; Kato et al., 1991). ALPase catalyses the hydrolysis of monophosphate esters at alkaline pH and vascular endothelial cells express on particular iso-enzyme (Zoellner and Hunter, 1989). Methods have been developed to differentiate between lymphatic and blood vessels, by using different enzyme activities to produce different coloured histochemical products (Werner et al., 1987; Kato et al., 1991). Vessels that are ALPase negative but 5'-nucleotidase positive are classified as lymphatic vessels. However, these methods rely on quantative rather than on qualitative measurements and therefore they are considered to be subjective and non-specific.

Weibel-Palade bodies and their contents

Weibel-Palade bodies are electron-dense rod-like inclusions that are present in the cytoplasm of blood vascular endothelial cells. Although some investigators reported that lymphatics do not contain Weibel-Palade bodies (Erhard et al., 1996; Sauter et al., 1998), i.e. considered as negative marker, other groups claim that these bodies are present in both lymphatic and blood vessel endothelial cells (Harrison et al., 1986; Nagle et al., 1987; Magari and Ito, 1988; Marchetti et al., 1992; Otsuki et al., 1990). These and other studies indicate that Weibel-Palade bodies cannot differentiate reliably between blood and lymphatic endothelia.

Basement membrane components

As stated above, peripheral lymphatic capillaries are characterised by the absence of basement membrane (Ryan, 1989). Antibodies against basement membrane components such as collagen type IV, fibronectin, vitronectin and laminin have therefore been suggested to be useful in distinguishing blood from lymphatic microcapillaries (Yoshizawa et al., 1994; Erhard et al., 1996; Sauter et al., 1998). However, in tumour angiogenesis, the basement membrane of blood capillaries that are newly developed may be also absent or incomplete (Paku and Paweletz, 1991; Madri et al., 1996).

Pericytes

Pericytes are, as stated above, absent from the peripheral lymphatic capillaries (Aukland and Reed, 1993). Thus, lack of pericytes around vessels in histological sections has been considered a sign to differentiate between lymphatic and blood capillaries. However, during angiogenesis the immature endothelial network is also lacking pericytes (Benjamin et al., 1998). Therefore, pericytes cannot be considered to be lymphatic endothelial specific marker.

Gap junctions

The intercellular junctions between lymphatic endothelial cells have distinguishing features, including overlapping, interdigitated and attenuated interconnections which are open to provide large gaps through which macromolecules and circulating cells can pass (Ryan, 1989). This junction contains a protein called desmoplakin, that is absent from blood vessels gap junctions. Thus, desmoplakin has been suggested as a possible marker for small lymphatic capillaries (Sawa et al., 1999). However, it is not expressed in larger lymphatic collecting ducts such as the thoracic duct (Schmelz et al., 1994). Furthermore, desmoplakin can also be detected in the junctions between cultivated blood vessel endothelial cells (Valiron et al., 1996; Kowalczyk et al., 1998). Therefore, desmoplakin cannot be considered as a specific lymphatic marker.

PAL-E (Pathologische Anatomie Leiden – Endothelium)

PAL-E has been widely reported to be absent from lymphatics, i.e. a negative marker (Ruiter et al., 1993; Erhard et al., 1996; Sauter et al., 1998; Lymboussaki et al., 1999). Thus, a lack of PAL-E staining on a capillary in a histological section is a good indication of lymphatic origin. However, when interpreting negative PAL-E staining factors, it should be remembered that PAL-E is also absent from arterioles (Ruiter et al., 1993) and from blood capillaries located in anatomical sites with a patent blood-brain barrier (Schlingemann et al., 1997).

Monoclonal antibodies

Monoclonal antibodies have been raised against thoracic endothelial cells with the aim to be used as a lymphatic-specific marker (Ezaki et al., 1990; Sawa et al., 1999). Some antibodies have been shown to bind blood vessels as well. However, a double stain with collagen type-IV antibodies could be used to differentiate lymphatic from blood vessels (Ezaki et al., 1990).

Lymphangiogenesis and malignancies

Breast cancer

Breast cancer is one of the most common cancers in women worldwide. The United Kingdom has the highest incidence, with one in ten women affected by the disease (Forbes, 1997). Early metastasis to lymph nodes is a frequent complication in human breast cancer. However, the extent to which this depends on lymphangiogenesis or on invasion of existing lymph vessels remains ill-defined. It has been suggested that breast carcinomas invade and destroy lymph vessels rather than promoting their proliferation and nodal metastasis can proceed via

pre-existing lymphatics (Williams et al., 2003). In another study, it was postulated that lymphangiogenesis does not appear to be a feature of invasive breast carcinomas (Williams et al., 2003). However, the same study revealed that a proportion of the peritumoural lymphatics contained tumour emboli associated with hyaluronan, indicating a possible role for LYVE-1/ hyaluronan interactions in lymphatic invasion or metastasis (Williams et al., 2003). Intra-tumoural lymphatic vessels have been demonstrated immunohistochemically in breast cancer (Witte et al., 1997) and lymphangiogenesis has indeed been recently quantified using real-time quantitative PCR (Cunnick et al., 2001). LYVE-1 level of expression was found to be higher in tumours that had spread to the regional lymph nodes. Other studies interpreted this merely as the presence of pre-existing lymphatic vessels by invading tumour cells, and has been proposed that lymphatic vessels are absent from most tumours (Tanigawa et al., 1981; Folkman, 1996; Carmeliet and Jain, 2000; Leu et al., 2000).

Increased lymphangiogenesis was correlated to VEGF-C over-expression in metastatic breast cancer (Skobe et al., 2001). This was associated with profound lung metastasis and enlargement of the peritumoural lymphatics (Makinen et al., 2001; Skobe et al., 2001). The rate of lung metastases was directly correlated with the extent of lymphatic microvascular density inside the tumour mass (Skobe et al., 2001). A recent study found that VEGF-C expression was only detectable in node positive breast cancers, whereas expression of VEGF-A detected in both node positive and node negative tumours (Kurebayashi et al., 1999). However, other studies claim that although VEGF-C is present, it is not always sufficient to induce the formation of functional lymphatic vessels (Leu et al., 2000).

Pancreatic carcinoma

Pancreatic cancer is a common, highly lethal disease each year affecting 8–12 per 100000 of the population in Europe, North America and Australia (Parkin and Muir, 1992; Fernandez et al., 1994). Regional lymph nodes metastasis renders the disease not suitable for surgical resection. It has been recently found that transgenic mice overexpressing VEGF-C in ß-cells of the endocrine pancreas (Rip-VEGF-C with a rat insulin promoter) developed extensive lymphangiogenesis around the endocrine islets of Langerhans (Mandriota et al., 2001). Furthermore, when tumours were induced in these VEGF-C overexpressing islets, by mating the mice with transgenic mice expressing the simian virus 40 T-antigen oncogene in the ß-cells (Rip1-Tag2), metastatic tumour cell aggregates of ß-cell origin were observed in the surrounding lymphatic vessels. These mice also frequently developed metastases in the lymph nodes, which drain the pancreas, whereas tumours in mice lacking the VEGF-C transgene never metastasised, nor were tumour cells observed inside the lymphatic vessels (Mandriota et al., 2001).

Oesophageal cancer

Oesophageal cancer has a poor prognosis, which is again dependent on the presence of lymph node metastases. VEGF-C expression is associated with neoplastic progression in the oesophageal mucosa (Auvinen et al., 2002). There is an increase in VEGF-C expression in Barrett's epithelium as it progresses through dysplasia to adenocarcinoma. This is consistent with a similar increase in VEGFR-3 expression on lymphatic vessels (Auvinen et al., 2002). Furthermore, VEGF-C expression was correlated with depth of tumour invasion, tumour stage, lymphatic and venous invasion and lymph node metastasis in oesophageal cancer (Kitadai et al., 2001). However, a similar study did not find a significant correlation between VEGF-C expression and lymphatic invasion or lymph node metastases, although the expression was related to histopathological grade and hence prognosis (Noguchi et al., 2002).

Gastric cancer

Gastric cancer is one of the leading cause of cancers deaths worldwide (Stanley et al., 1988). Lymph node status is important in determining patients' prognosis and the extent of surgical resection. It has been recently demonstrated that VEGF-C expression in gastric cancer cells was significantly related to depth of invasion, lymphatic invasion and lymph node metastases (Yonemura et al., 1999; Ichikura et al., 2001; Kabashima et al., 2001; Takahashi et al., 2002). However, it seems that there is no correlation between VEGF-C expression and the degree of differentiation in gastric adeno-carcinoma (Amioka et al., 2002). The clinical impact of the association between VEGF-C expression and prognosis is not fully understood. Nevertheless, there exists a relationship between the expression of VEGF-C in tumour tissues and poor prognosis as well as reduced survival in gastric cancers (Yonemura et al., 1999; Ichikura et al., 2001; Takahashi et al., 2002). The role of VEGF-D in oesophageal carcinomas is yet to be explored. A positive correlation between VEGFR-3 and VEGF-C mRNA expression was found in gastric cancer tissues and the majority of VEGFR-3 positive vessels are indeed considered as lymphatics (Yonemura et al., 2001). Furthermore, there is higher number of VEGFR-3 positive vessels in gastric cancers that are lymph node positive, with lymphatic invasion or are poorly differentiated (Yonemura et al., 2001). The expression of VEGFR-3 was significantly higher in the poorly differentiated gastric adenocarcinomas and in cancers with higher lymph node metastasis rate (Yonemura et al., 2001).

Colorectal cancer

There are several reports suggesting a correlation between VEGF-C expression and poor clinico-pathological outcome in colorectal cancer (Akagi et al.,

2000; Furudoi et al., 2002; Parr and Jiang, 2003). VEGF-C expression was correlated with lymphatic and venous invasion, lymph node status, Dukes'stage, liver metastasis, depth of invasion, poorer histological grade and microvessel density (Akagi et al., 2000; Furudoi et al., 2002). However, VEGF-C expression and lymph node metastasis were independent prognostic factors for 5-year survival (Furudoi et al., 2002). It has been recently revealed that a positive correlation exists between the levels of VEGF-C expression and lymphatic spread metastasis in colorectal cancer (Furudoi et al., 2002). However, other studies have not demonstrated such a relationship between VEGF-C levels of expression and lymph node status in colorectal cancer (George et al., 2001; Parr and Jiang, 2003).

VEGF-D expression was found to be higher in colorectal cancer tissues and is associated with lymph node involvement and reduced overall and disease-free survival (White et al., 2002; Parr and Jiang, 2003). However, in another study, colorectal tumour expression of VEGF-D mRNA was less than in normal tissue (George et al., 2001). In the latter study, it was suggested that a reduction in VEGF-D levels in the adenoma–carcinoma sequence allowed the more potent angiogenic cytokines VEGF-A and VEGF-C to bind more readily to the signalling receptors VEGFR-2 and VEGFR-3. Furthermore up-regulation of VEGFR-3 protein expression in colorectal cancer tissues and increased expression was associated with poorer overall survival (White et al., 2002). This demonstrates the potent paracrine nature of the interaction between VEGFR-3 on the vascular endothelium and its ligands, VEGFs -C and –D in the tumour microenvironment. Additionally, levels of lymphatic markers (Prox-1 and 5'-nucleotidase) were found to be significantly higher in colonic cancer tissues compared to normal tissues and levels of podoplannin mRNA was also higher in colonic cancer tissues although was not statistically significant (Parr and Jiang, 2003).

Carcinoma of the prostate

Prostatic carcinoma is one of the major concerns in men. It is well known that the progression of the disease is highly associated with metastasis to the bone and the lymph nodes (Slack et al., 1986). It has been demonstrated that the expression of VEGF-C in human prostatic carcinoma cells was significantly associated with the presence of lymph nodes metastasis (Tsurusaki et al., 1999). Furthermore, there was a positive correlation between the expression of VEGFR-3 and VEGF-C (Tsurusaki et al., 1999), suggesting the presence of a paracrine loop between prostatic cancer cell and the lymphatic endothelium within the tumour stroma.

Malignant melanoma

Metastatic melanomas had significantly more and larger tumour-associated lymphatic vessels and a relative lymphatic vessel area of >1.5% was significantly associated with poor disease-free and overall survival (Dadras et al., 2003). VEGF-D expression was shown to be up-regulated in human melanomas compared with melanocytes (Achen et al., 2001). VEGF-D was detected in melanoma cells and in vessels adjacent to immunopositive tumour cells, but not in vessels distant from the tumours. This suggests that VEGF-D binds to the endothelial cells of nearby vessels and contributes in a paracrine manner to the regulation of tumour lymphangiogenesis. The incidence of intratumoural lymphatics (assessed using LYVE-1 as a marker) was significantly higher in metastatic melanomas and correlated with poor disease-free survival (Dadras et al., 2003).

Lung carcinoma

There is paucity of lymphangiogenesis studies in relation to lung cancer. In one study, it has been indicated that a low ratio of VEGF-D:VEGF-C (low VEGF-D and high VEGF-C) is associated with lymph node metastasis and lymphatic invasion in lung Adenocarcinoma (Niki et al., 2000).

Head and neck carcinomas

Head and neck squamous cell carcinomas frequently spread to the neck lymph nodes. Proliferating intratumoural lymph vessels have been identified in these carcinomas (Beasley et al., 2002). Quantification of VEGF-C by real-time PCR and immunohistochemistry in Head and Neck carcinomas revealed higher levels of mRNA in tumour tissue than in normal samples (Beasley et al., 2002). Furthermore, intra-tumoural LYVE-1 positive lymphatic vessels were found to be associated with a higher risk for local relapse as well as with poor disease-specific prognosis in Head and Neck squamous cell carcinomas (Maula et al., 2003). However, the same study had found that a high density of peritumoural LYVE-1 positive vessels was a sign of favourable survival (Maula et al., 2003).

Lymphatic vessels as targets of anti-cancer therapy

The current targeting technologies make it possible to develop drugs into a targeted compound, thereby increasing the potency of the drug at the intended target tissue while reducing side effects elsewhere in the body. Inhibition of angiogenesis for example, is already considered a promising area in cancer therapy. As stated above, tumours with a higher incidence of lymph node positivity express high levels of VEGF-C and VEGF-D, inhibition of VEGFR-3 signalling might be an attractive approach to inhibit cancer lymphatic metastasis. In transgenic mice with targeted expression of a soluble form of VEGFR-3 in the skin, lymphatic vessels initially formed normally, but the onset of the transgene

expression led to regression of lymphatic vessels in embryos (Makinen et al., 2001b). Furthermore, a soluble VEGFR-3 protein produced via an adenovirus vector could inhibit lymphangiogenesis in a transplantable human breast carcinoma model using MCF-7 cell line in SCID mice (Karpanen and Alitalo, 2001). In another study, microhaemorrahge and the subsequent collapse of large tumour vessels was also reported in mice injected with blocking monoclonal antibodies against VEGFR-3 (Kubo et al., 2000). Primary lymphoedema, a rare autosomal dominant disorder of the lymphatic system, was recently linked to mutations in the VEGFR-3 tyrosine kinase domain (Karkkainen et al., 2000). Interruption of VEGFR-3 signalling results in lymphatic hypoplasia, underlining the importance of VEGFR-3 in the maintenance of lymphatic function during embryonic development (Karkkainen et al., 2000; Makinen et al., 2001).

Neutralising antibodies against VEGF-C and VEGF-D might also be an area of interest. It was recently revealed that the use of neutralizing antibodies against VEGF-D decreases the number of lymphatic metastases of the VEGF-D-293 tumours in the mammary fat pads of SCID/NOD mice (Stacker et al., 2001). Therefore, the association of lymphangiogenic factors with increased lymphatic growth and metastasis of cancers (Karpanen et al., 2001; Mandriota et al., 2001; Skobe et al., 2001; Stacker et al., 2001) has made them an attractive target for an additional therapeutic modality against cancer.

Perspectives and conclusions

The past few years have witnessed a rapid progress in identifying specific markers for lymphatic endothelial cells and lymphatic vessels. This, together with the discovery of the first few lymphangiogenic factors has already inspired a rapid expansion in the knowledge in both the biology of lymphangiogenesis and in clinical cancer and cancer spread. However, a number of challenges are facing us. The lack of an established lymphatic specific endothelial cell line that is available to research into lymphangiogenesis, has been the main constraint in studying lymphangiogenesis. The complex issue of autocrine and paracrine pathways and signalling in tumour lymphangiogenesis needs to be further explored. Understanding lymphangiogenesis and intra-tumour lymphatics and how are they connected to lymphatic invasion and lymphatic spread is fundamentally important in understanding the biology of cancer metastasis.

It has been recognised that lymphangiogenesis occurs inside tumours and is associated with nodal and distal metastasis. There is now evidence to suggest that there is significant correlation between the expression of these molecules and several clinicopathologiocal parameters in several human cancers. This might be of particular importance in determining patients' prognosis and survival.

Although tumours can secrete lymphangiogenic growth factors like VEGF-C and VEGF-D and can induce the growth of new lymphatic vessels, several questions remain unanswered. For example, why different tumours have heterogeneity in regards to the expression and secretion of these growth factors? What are the intrinsic or extrinsic factors that regulate VEGFR-3 signalling? Further work is required to clarify whether these growth factors could also induce pre-existing lymphatic vessels formation? Does interrupting VEGFR-3 signalling have any impact on lymphatic spread and cancer metastasis? The elucidation of molecular components of VEGFR-3 signalling could be beneficial both in terms of diagnosis and therapy by selective targeting of this pathway. Angiogenesis that occurs only in tumour, also known as tumour-specific angiogenesis, has been recently described (St Croix et al., 2000; Carson-Walter et al., 2001; Davies et al., 2004). Does tumour-specific lymphangiogenesis exists? Are there potential markers to distinguish the existing lymphactic vessels from the newly derived ones? Finally, the early research results have tentatively suggested that the degree of lymphangiogenesis have prognostic importance in solid tumours. They also pointed a strong possibility that targeting tumour-associated lymphatics may have potential therapeutic value.

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Accepted September 20, 2004