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Lymphangiogenesis

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While much effort is given to the modelling of tumour angiogenesis, little attention is paid to lymphangiogenesis and its potential role in tumour progression and metastasis. This is due at least in part to the controversy that surrounds tumour lymphangiogenesis—opinion is divided as to whether tumours actively promote the development of lymph channels in the same way that they promote the development of vasculature. Given that it was decades before Folkman's theory of tumour angiogenesis became widely accepted, it is possible that the concept of tumour lymphangiogenesis will in time also become generally accepted and hence may itself become the subject of mechanistic studies and mathematical modelling. This review summarises the process of lymphangiogenesis and the potential mechanism of its induction in tumours.

Keywords: Angiogenesis; Lymphangiogenesis; Tumours; VEGF; Angiopoietins; Modelling

INTRODUCTION

Blood vessels arise through complex and highly orchestrated processes (Carmeliet, 2000). The primary capillary network develops early in embryogenesis, through the development and differentiation of endothelial cells (outlined in Fig. 1). The mature vasculature arises from remodelling processes, during which the primary network undergoes branching, splitting, sprouting and even some localised regression, giving rise to the complex system of arteries, veins and capillaries. These remodelling processes, collectively termed angiogenesis, play a predominant role during growth and development and also persist in adulthood during wound healing and in the female reproductive cycle. Many pathological states such as tumour growth and metastasis, rheumatoid arthritis and diabetic retinopathy are dependent on angiogenesis, and hence understanding the processes underlying blood vessel growth becomes fundamental to the development of new therapies.

The circulatory system delivers nutrients and oxygen to tissues, so enabling organisms to grow beyond the diffusion limit of tissues. However, blood vessels also play a number of other vital roles including the maintenance of blood fluidity (haemostasis), the recruitment of leukocytes to sites of inflammation and the maintenance of a physical barrier between blood and tissues (Jones, 2003b). Hence the contiguous layer of endothelial cells that line all blood vessels, called the endothelium, plays a number of varied and critical physiological roles. The lymphatic system develops in parallel with the vascular system but serves the opposite function in that it collects lymph and returns it to the circulatory system. The lymphatic system also removes waste products, regulates interstitial fluid balance, is involved in the transport of molecules and absorption of fat from the gut, and is an important part of the immune system (Alitalo and Carmeliet, 2002; Oliver and Detmar, 2002).

The lymphatic system consists of lymphatic capillaries, collecting vessels, lymph nodes, lymphatic trunks and lymphatic ducts (see Fig. 2). These act to drain fluid, which originated in the vascular capillaries, from the interstitial space back into the venous circulation. Lymphatic fluid ultimately drains into the thoracic duct, which enters the venous system at the junction of the left subclavian and left internal jugular veins. Lymph from the head, neck and the right side of the body drains into the venous system at the junction of the right internal jugular and right subclavian veins. Dysfunctional lymphatics can lead to problems such as lymphoedema (a swelling of an area of the body, commonly a limb) which can be congenital or acquired following clinical intervention, as well as being implicated in fibrosis, ascites and tumours such as Kaposi's sarcoma (Witte *et al.*, 1997).

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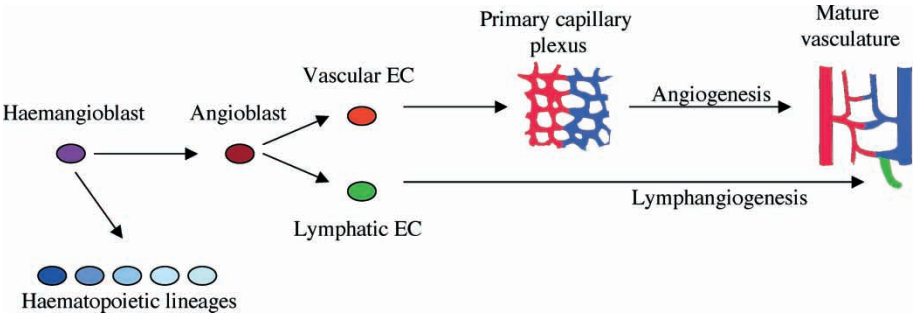

FIGURE 1 Development of the vasculature. Specialist cells, the haemangioblasts, undergo differentiation into either the haematopoietic lineages (which include lymphocytes, macrophages, neutrophils and megakaryocytes) or angioblasts, which themselves can develop further into either vascular or lymphatic endothelial cells (EC). Vascular endothelial cells continue to differentiate and assemble into tubules which subsequently fuse to form the primary capillary network, characterised by the even distribution of uniformly sized vessels. This process is termed vasculogenesis. Remodelling of the primary capillary network, termed angiogenesis, includes sprouting, branching and regression, giving rise to the mature vasculature, characterised by the large and small vessels culminating in the capillary beds. Lymphatic vessels are thought to develop by sprouting from the venous side of capillary beds, incorporating lymphatic EC.

ARCHITECTURE OF LYMPHATIC CAPILLARIES

Lymphatic capillaries are small endothelial tubes which, together with lymphatic tissues (thymus, tonsils, Peyers patches, lymph nodes, spleen), make up the lymphatic system. Lymphatic capillaries lie in the interstitial space in close proximity to blood capillaries and tend to have thinner walls and be more irregular than blood vessels. There are a number of similarities between the lymphatic and vascular capillaries (see Fig. 3). Both are lined by a contiguous layer of endothelial cells (Jones, 2003a) and have vasa vasora (blood vessels which provide nutrition for the vessel wall). The wall of the lymph vessel consists of a single layer of endothelial cells, much as a vascular

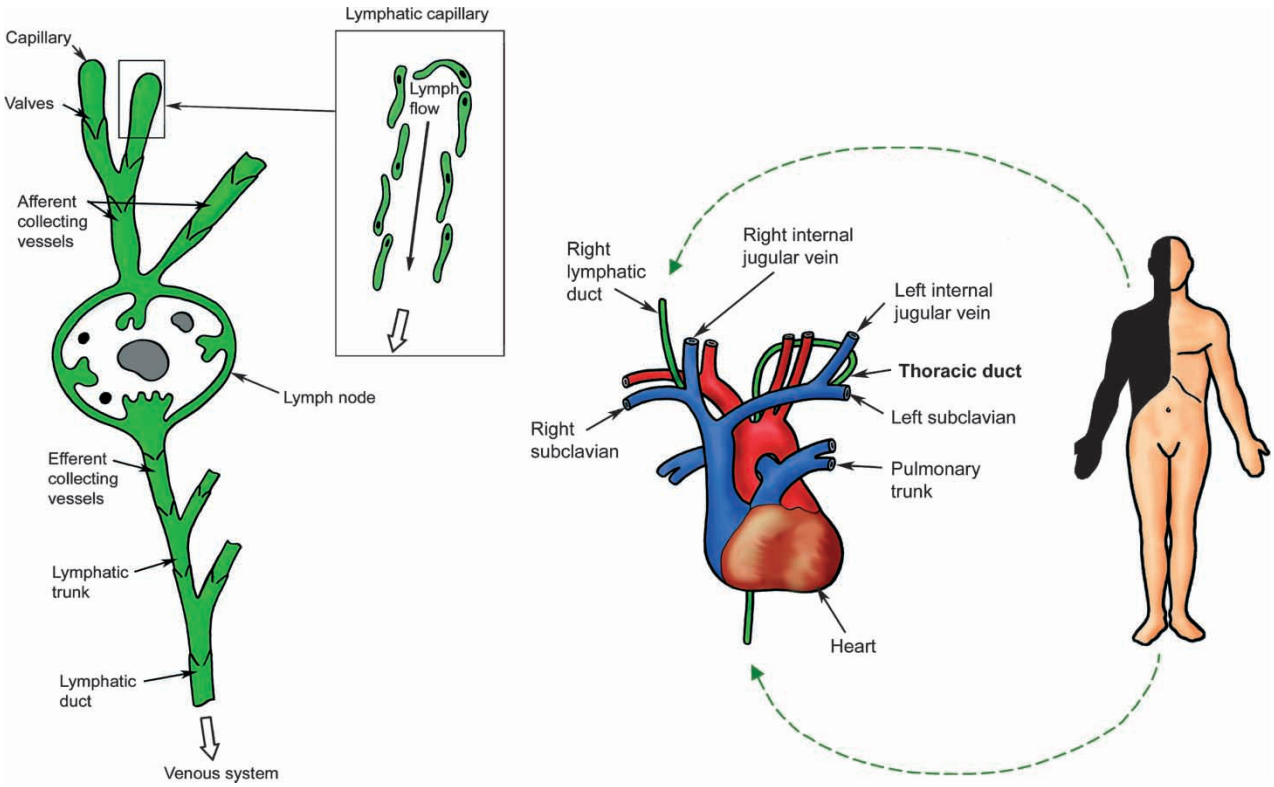

FIGURE 2 Anatomy of the lymphatic system. (A) Interstitial tissue fluid, the lymph, enters the highly permeably lymphatic capillaries (see inset). The lymphatic fluid moves down the capillary into the collecting vessel. Valves prevent fluid draining in the reverse direction and ensure negative pressure within the lymphatic capillary. Lymph nodes filter fluid through lymphocyte-rich nodules (shown in grey), which serve to remove unwanted microorganisms and also to replenish lymphocytes into the circulatory system. Lymph continues to move from the lymph nodes into the larger efferent collecting vessels and onward through the lymphatic trunk and ducts. (B) Lymph is returned to the vascular circulation at one of two points in the venous system. Lymph from the head, neck and right upper side of the body (shown in black) moves through the right lymphatic duct and enters the venous system at the junction of the right internal jugular and right subclavian veins. Lymph from the remainder of the body drains through the thoracic duct and enters the venous system at the junction of the left internal juguar and left subclavian veins. In this way, fluid that leaves the vascular system, carrying oxygen and nutrients to tissues, is collected and returned to the circulatory system. The venous and arterial compartments of the heart are shown in blue and red, respectively, lymph ducts are shown in green. Dotted arrows indicate which segment of the body drains into which lymph duct.

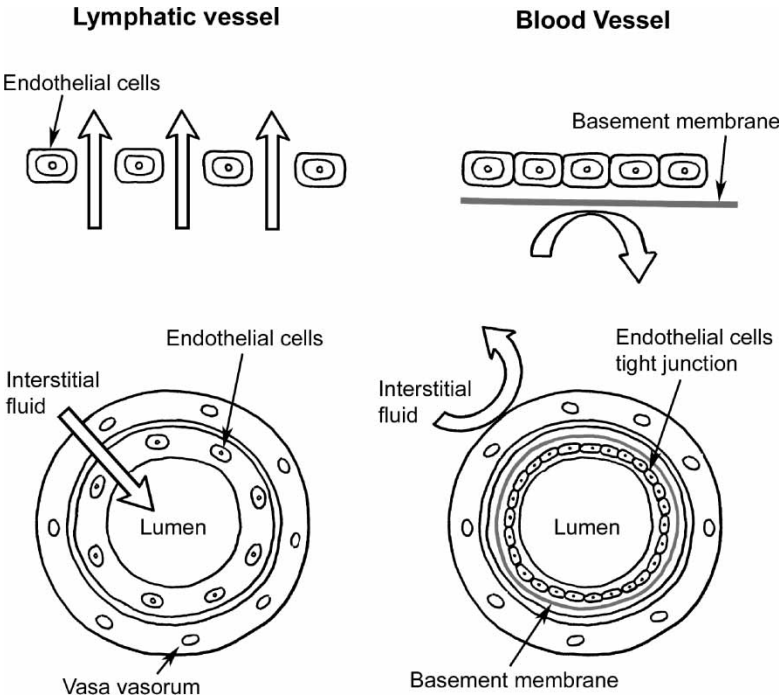

FIGURE 3 Architecture of vascular and lymphatic capillaries. Despite their similarities, vascular and lymphatic capillaries show several differences. Vascular endothelial cells form tight junctions and secrete a basement membrane which becomes part of the vessel wall. Lymphatic channels lack these features and hence are leaky, with gaps, or fenestrae, between the component lymphatic endothelial cells. The result is that fluid which is actively transported out of the vascular capillary cannot return but instead is pushed by a pressure gradient into the highly permeable lymphatic capillaries.

capillary and the larger lymphatic vessels have a connective tissue coat lying outside the endothelium. The largest lymphatic vessels have layers of tunica intima, media and adventitia much as is seen in blood vessels. The tunica media contains smooth muscle cells, and the tunica adventitia is composed of a mix of fibrous tissue and smooth muscle which can create a pumping force. Both veins and the larger lymphatics have valves to ensure that fluid flows in only one direction.

Despite the similarities between vascular and lymphatic channels there are also many differences. Blood vessels have a continuous basement membrane and tight endothelial junctions which make them semi-permeable but the lymphatic vessels often lack a basement membrane so that interstitial fluid can readily enter. Also contributing to the increased permeability are the larger spaces in between lymphatic capillaries than those in between blood capillaries. Unlike veins, lymphatic vessels have special anchoring filaments to ensure they remain patent as surrounding tissue pressure rises. There are fewer attachments between endothelial cells in lymphatic vessels, although fenestrae do exist in the sub-serous lymphatics compared to tight junctions which exist in vascular channels (Jones, 2003a). Overall, the lymphatic system is of a lower flow rate and lower pressure than the vascular system.

LYMPHANGIOGENESIS

Lymphangiogenesis is the formation of new lymphatics. It is seen physiologically during embryogenesis and in adult life during wound healing. It is thought that the physiological driving force for lymphangiogenesis is the need for organised interstitial fluid flow (Swartz and Boardman, 2002), but the mechanism behind the formation of new lymph vessels is unclear. It was originally thought that primitive lymph sacs develop from endothelial budding from embryonic veins, and then the lymphatic system develops by sprouting into the surrounding tissues and organs (Oliver and Detmar, 2002). An alternative theory is that lymph sacs arise from ‘lymphangioblasts’ (mesenchymal pre-cursor cells), and these then form vascular connections. This theory has been supported by the identification of lymphangioblasts contributing to the lymphatic system development in the avian wing bud (Schneider *et al.*, 1999). It is likely that the formation of new lymphatic vessels in the embryo arises from a combination of these two mechanisms.

LYMPHANGIOGENESIS IN TUMOURS

The lymphatic system is commonly the first place to which tumours metastasise (Stacker *et al.*, 2002) and evidence of lymphatic spread of a tumour correlates with increased tumour aggressiveness (Swartz and Skobe, 2001). Hence many tumours utilise the lymphatic system as a method of dissemination and are capable of migration through lymph channels—not dissimilar to the movement of immune cells or the spread of infection. However, while there is firm evidence for angiogenesis in tumour development and metastasis the question as to whether

lymphangiogenesis occurs in a malignant tumour has been debated at length and remains controversial.

Until recently, tumour-driven lymphangiogenesis had not been demonstrated. Indeed, some studies had failed to identify any functional lymphatics within tumours (Jain, 1987; Leu *et al.*, 2000), and it was proposed that this was because the vessels were lost or collapsed, or that they could not penetrate into the tumour. The presence of intra- and peritumoural lymphatics has been reported (de Waal *et al.*, 1997); however, it remains unclear whether these vessels were pre-existing or newly developed. Pre-existing lymphatic vessels may expand, and vessels may penetrate into the tumour or become trapped between expanding tumour foci (Stacker *et al.*, 2002), or new vessels may grow. Tumours may grow towards and utilise pre-existing lymphatics, or lymphangiogenesis may be required for tumour dissemination (Mandriota *et al.*, 2001; Pepper, 2001).

One of the main problems in studying lymohangiogenesis has been the difficulty in distinguishing between vascular and lymphatic endothelial cells and channels (Clarijs *et al.*, 2001; Swartz and Skobe, 2001). However, as more molecular analyses are undertaken, the number of identifying markers is increasing, and a role in embryonic lymphangiogenesis has been identified for some previously characterised angiogenic factors such as bFGF and VEGF. More recently, roles in tumour lymphangiogenesis have also been identified for some of the known angiogenic regulators.

ANGIOGENIC FACTORS WITH A ROLE IN LYMPHANGIOGENESIS

Angiogenic processes occur through a complex series of events involving extracellular signals, intracellular signalling pathways, gene activation, protein synthesis and activation. Many factors are involved in the regulation of these processes, and much attention has focused on extracellular growth factors. Some of these factors, such as vascular endothelial growth factor (VEGF), act in an endothelial-specific manner, whereas others, such as basic fibroblast growth factor (bFGF), act in a far wider spectrum of cell types. The contribution of known angiogenic regulatory factors to lympangiogenic processes is discussed below.

VEGFs

The vascular endothelial growth factors are a group of polypeptide growth factors which have a well-established role in the regulation of blood vessel formation (reviewed in Ferrara *et al.*, 2003). VEGF is an established angiogenic factor; angiogenesis is important for the growth of solid tumours (Kim *et al.*, 1993). The biological effects of VEGF are mostly specific for endothelial cells and include stimulation of proliferation and migration and the regulation of vascular permeability (Joukov *et al.*, 1997). The VEGF family of proteins consists of VEGF-A (often referred to as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor (PlGF) (Ferrara, 1999). VEGF exists as one of four different isoforms, termed $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$, named by the length of the amino acid chain, having 121, 165, 189 and 206 amino acids, respectively with $VEGF_{165}$ being the major isoform (Tischer *et al.*, 1991). These isoforms are formed by alternative splicing from the same gene and are all thought to be involved in angiogenesis with distinct but overlapping functions (reviewed in Ferrara *et al.*, 2003). $VEGF_{121}$ is a freely soluble protein; $VEGF_{165}$ is also secreted, although some remains bound to the cell surface and the extracellular matrix. $VEGF_{189}$ and $VEGF_{206}$ are mostly sequestered in the extracellular matrix (reviewed in Ferrara and Davis-Smyth, 1997). The different distributions of the different isoforms suggest discrete although possibly overlapping functions.

VEGF-A (which is the homologue normally referred to as VEGF) has recently been implicated in the control of lymphangiogenesis. VEGF-A has been shown to cause the proliferation of lymphatic endothelium in adult mouse tissues (Nagy *et al.*, 2002). The endothelium is abnormal, with enlarged lymphatics containing incompetent valves, resulting in sluggish flow and hence delayed lymph clearance. Thus, VEGF-A may be implicated in diseases characterised by abnormal lymphatics (Nagy *et al.*, 2002).

The role of VEGF-C in lymphatic capillaries has been far better characterised. VEGF-C (a ligand for VEGFR-3) is known to regulate both physiological and pathological blood vessel growth *in vivo* (Jussila and Alitalo, 2002). It has also been shown to induce a lymphangiogenic response in the avian chorioallantoic membrane (CAM; Oh *et al.*, 1997) and overexpression leads to the induction of hyperplastic lymphatic vessels (Jeltsch, 1997). VEGF-C has been shown to induce lymphangiogenesis in the ears of mice injected with a recombinant adenovirus (Jeltsch, 1997). Expression of VEGF-C under the control of rat insulin promoter II resulted in the formation of an extensive lymphatic vasculature around the islets of Langerhans (Pepper, 2001) leading to the hypothesis that a specific lymphangiogenic response is mediated by VEGF-C (Karkkainen and Petrova, 2000). Furthermore, increased expression of VEGF-C correlates with increased dissemination of tumour cells to regional lymph nodes, although there is no evidence of a direct role for VEGF-C in tumour metastasis (Pepper, 2001). Hence it has been postulated that VEGF-C may increase tumour metastasis by increasing the number and size of lymphatic vessels, or it may alter the structure of the existing lymphatics to facilitate tumour cell intravasation (Pepper, 2001; Swartz and Skobe, 2001).

More recently, VEGF-D, with 61% sequence identity with VEGF-C, has been associated with a role in lymphangiogenesis (Achen *et al.*, 2001). Murine studies

of tumours overexpressing VEGF-D had intratumoural lymphatic vessels and promoted lymph node metastases, whereas expression of VEGF did not. Lymphatic spread induced by VEGF-D could be blocked with an antibody specific for VEGF-D (Stacker *et al.*, 2001). VEGF-D has also been shown to be lymphangiogenic in several other models (reviewed in Baldwin *et al.*, 2002). It is thought that VEGF-D is secreted by tumour cells; studies have shown that tumour vessels positive for VEGF-D were also positive for VEGFR-2 but were negative for VEGF mRNA (Achen *et al.*, 2002). What does this mean?

A study of tumour cell lines expressing VEGF-C and VEGF-D found that expression of these lymphangiogenic factors did not correlate with metastatic potential *in vivo*. It is also reported that tumours derived from cell lines which did not express VEGF-C or VEGF-D in culture may express either or both of these factors. This suggests that tumour cell–host interactions determine tumour expression of VEGF-C and VEGF-D (Krishnan *et al.*, 2003).

VEGF Receptors

The VEGFs bind to a family of endothelial cell-specific tyrosine kinase receptors, termed VEGFR-1, VEGFR-2 and VEGFR-3 (also known as Flt-1, Flk-1/KDR and Flt-4, respectively). The binding of the different VEGFs is selective—not all ligands bind to each receptor. VEGF-A binds to VEGFR-1 and VEGFR-2 (Ferrara, 2001) and VEGF-B binds to VEGFR-1 (Olofsson *et al.*, 1998). VEGF-C and -D bind to VEGFR-2 and -3 and VEGF-E (the viral homologue) binds to only VEGFR-2 (for reviews, see Karkkainen and Petrova, 2000; Jussila and Alitalo, 2002; Stacker *et al.*, 2002).

Expression of VEGFR-1 and VEGFR-2 are largely restricted to the vascular endothelial cells and haematopoietic cells. VEGFR-2 has been shown to be the major mediator of the mitogenic, angiogenic and pemeability-inducing responses of endothelial cells to VEGF (Ferrara *et al.*, 2003). VEGFR-1 plays a more subtle signalling role, "fine tuning" the endothelial response to VEGF through modulation of expression of other genes involved in the angiogenic response such as plasminogen activators and metalloproteinases and release of other growth factors.

In the late 1990s, VEGFR-3 was identified as the first receptor found to be expressed mainly in lymphatic endothelium (reviewed in Stacker *et al.*, 2002) and was subsequently shown to be expressed in tumour blood vessels during neovascularization (Dumont *et al.*, 1998). Since VEGF-C binds to VEGFR-2 and VEGFR-3 and as VEGF binds to VEGFR-1 and VEGFR-2, it follows that the lymphangiogenic response is mediated by the activation of VEGFR-3 (Enholm *et al.*, 2001). In the adult, VEGFR-3 appears to be restricted to lymphatic endothelium yet VEGFR-2 is expressed by both vascular and lymphatic endothelium (Jussila and Alitalo, 2002).

Angiopoietins

The angiopoietin family comprises three structurally related ligands, termed Angiopoietin-1 (Ang-1), Ang-2 and Ang-4 (represented by Ang-1, Ang-2 and Ang-3, respectively, in mouse; Davis *et al.*, 1996; Maisonpierre *et al.*, 1997; Valenzuela *et al.*, 1999). To date, Ang-1 and Ang-2 are the better-characterised members of the family, with little known about Ang-3/4. All three ligands bind with similar affinity to their common receptor, Tie2, an RTK expressed predominantly on the endothelial cell surface. Despite their structural similarity, Ang-1 and Ang-2 elicit opposing effects on Tie2, with Ang-2 specifically inhibiting the Ang-1 induced Tie2 phosphorylation seen in endothelial cells (Maisonpierre *et al.*, 1997). This phenomenon suggests that intracellular signalling *via* Tie2 requires precise regulation. Both Ang-1 and Ang-2 have been shown to be critically involved in angiogenesis, where their involvement is thought to involve regulation of endothelial cell interactions with supporting perivascular cells. Ang-1 serves as both a maturation factor for developing vessels as well as a stabilising signal to maintain the integrity of the adult vasculature (Suri *et al.*, 1996; Jones, 2003b). Conversely, Ang-2 acts as a highly localised destabilising signal, necessary to either prime the vasculature for sprouting, or to allow vascular regression.

Whereas Ang-2 appears redundant in embryonic vascular development, it is involved in postnatal remodelling shown by severely compromised retinal vascularisation, which normally occurs after birth (Hackett *et al.*, 2002). Overexpression of Ang-1 does not correct this defect, suggesting a requirement for both angiopoietins (Gale *et al.*, 2002). Furthermore, shortly after birth, animals defective for Angiopoietin-2 develop characteristics of abnormal lymphatic function, with chylous ascites and subcutaneous oedema. More detailed examination of the lymph system shows disorganisation in dermal and intestinal lymphatic patterning with reduced recruitment of smooth muscle cells. This suggests that in the lymphatic system, Ang-2 drives recruitment of supporting cells, a function of Angiopoietin-1 in vascular channels (Gale *et al.*, 2002). Ang-1 has been implicated in the recruitment of perivascular supporting cells (Suri *et al.*, 1996) as well as in formation of endothelial junctions (Gamble *et al.*, 2000) and cell–cell contacts (Kim *et al.*, 2001) in vascular endothelium. Hence the recruitment of fewer supporting cells as well as the lack of tight junctions between endothelial cells may be due to the action of Ang2 rather than Ang1 in the lymphatic channels. Furthermore, Ang-1 has been shown to act as an anti-permeability factor, capable of negating the permeabilising action of VEGF (Thurston *et al.*, 1999). Since Ang-2 is a naturally occurring antagonist of Ang-1, its role in the lymphatic system may contribute to their permeability.

MARKERS OF LYMPHATIC VASCULATURE

One factor contributing to the limited information available regarding lymphangiogenesis is the lack of specific endothelial markers. Commonly used endothelial markers such as CD31 and vWF are expressed in both vascular and lymphatic channels and hence cannot be used to distinguish between the two. However, suitable markers are being identified, and their number is steadily increasing (Sleeman *et al.*, 2001). Some markers appear to be more useful, although again their expression is not necessarily restricted to the lymphatic endothelium. For example, VEGFR-3 was the first specific growth factor receptor identified in lymphatic vessels identified; however, although it is mainly expressed in lymphatic endothelium it is also found in blood vessels (Jussila and Alitalo, 2002).

Prox1 is a transcription factor involved in the embryonic development of lymphatic vessels. It is thought that Prox1 expression in venous endothelial cells switches the cell to the lymphatic lineage. Prox1, as a protein involved in regulation of gene expression, then plays a major role in the control of the growth and elongation of lymphatic vessels (Swartz and Skobe, 2001). Embryonic mouse studies have shown that Prox1 promotes the development of the lymphatic vasculature (Oliver and Harvey, 2002) and inactivation of Prox1 in mice leads to embryonic lethality due to multiple developmental defects (Chang *et al.*, 2002). Prox1 undoubtedly plays a major role in the development of the lymphatic system, but it is also expressed in non-endothelial cells in the eye, pancreas, liver, heart and nervous system (Jussila and Alitalo, 2002) and hence as a marker must be used judiciously.

Podoplanin is a plasma membrane protein found in glomerular epithelial cells but is also expressed in endothelial cells of the small lymphatics. However, in larger lymphatic channels which have acquired a covering of smooth muscle cells, expression of podoplanin is lost (Stacker *et al.*, 2002). Vascular endothelial cells do not express podoplanin, and so it is a suitable marker to distinguish between small lymphatics and vascular channels, but negative staining in larger channels is not diagnostic.

LYVE-1 (lymphatic vessel endothelial hyaluron receptor-1) is a surface receptor molecule located on both sides of the lymphatic vessel wall. Measuring LYVE-1 mRNA in tissues has been used to provide an estimation of the rate of lymphangiogenesis (Swartz and Skobe, 2001; Pepper, 2001; Chang *et al.*, 2002). Again interpretation of expression patterns needs care as LYVE-1 has also been shown to be expressed in some vascular endothelial cell types in the normal liver.

Several other markers are reported to contribute to the identification of the lymphatic phenotype (reviewed in Stacker *et al.*, 2002; Karkkainen and Alitalo, 2002; Jussila and Alitalo, 2002). These include β-chemokine receptor D6 (restricted to subset of lymphatics), desmoplakin (involved in cell–cell interactions), macrophage mannose receptor, CCL21 (chemokine receptor), 5′-nucleotidase (enzyme involved in nucleotide metabolism), Neuropilin-2 (involved in VEGF isoform signalling), integrin α9β1, the transcription factor Net, Tie1, Tie2 and Angiopoietin-2 The diversity of function of these proteins is striking and suggests that expression is not necessarily restricted to the lymphatic system. Hence it would seem prudent to undertake multiple staining protocols, to confirm the endothelial as well as the lymphatic identity.

CONCLUSION

Since the development of markers specific to lymphatic endothelium there has been a significant progression in the field of lymphangiogenesis. There is no dispute that lymphangiogenesis exists during embryogenesis but its role in tumour growth and metastasis remains controversial. The lack of lymphatics in some tumours may be due to mechanical compression or may be secondary to the tumour generating a lymphangiogenesis inhibitor. It is not yet known if lymphatic vessel density in cancers is related to prognosis and/or metastatic spread.

Lymphangiogenesis is regulated by VEGF-C and VEGF-D *via* VEGFR-3 and there is mounting evidence to suggest that VEGF-A (Nagy *et al.*, 2002) and bFGF (Chang *et al.*, 2002) may also be involved. In addition, angiopoietin-2 has been shown to be required for correct lymphatic patterning. These data suggest that both Tie and VEGFR signalling are required during lymphangiogenesis. Both the VEGFs (Ferrara *et al.*, 2003; Ferrara and Davis-Smyth, 1997) and the angiopoietins (Jones, 2003b) are well documented to be expressed in many tumour types suggesting that tumours themselves are generating the necessary factors to allow not only angiogenesis but also lymphangiogenesis to occur. However, it is not yet clear whether tumour expression of lymphatic-specific factors is sufficient to induce lymphangiogenesis or whether other mechanisms are involved. As the distinction between lymphatic and vascular channels becomes easier to define, our understanding of the induction of lymphangiogenesis in tumours, as well as the role of the lymphatic system in the metastatic spread of tumours, will become clearer.

GLOSSARY OF TERMS

Vasculogenesis	The *de novo* synthesis of vasculature, normally seen only in the early embryo, resulting from the differentiation and specialisation of endothelial precursors. Gives rise to the primary capillary network.
Angiogenesis	The remodelling of existing vasculature through a combination of

	vessel sprouting, branching, splitting and regression. Gives rise to the mature vasculature.
Lymphangiogenesis	The growth of new lymphatic channels.
VEGF	Vascular endothelial growth factor—the "gold" standard of factors responsible for the regulation of angiogenesis. Results in the proliferation, migration and differentiation of endothelial cells.
Angiopoietin	A family of endothelial cell-specific angiogenic factors, involved in the maturation and maintenance of the adult vasculature.
RTK	Receptor tyrosine kinase—single transmembrane domain receptor for extracellular ligands, often involved in mitogenic and other growth pathways.
VEGF R	Family of RTKs that bind the VEGFs.
Tie	Endothelial cell-specific RTKs. Tie2 binds all the angiopoietins with similar affinity, but shows differential activation or inactivation depending on context and ligand. There is no known ligand for Tie1.
Transcription factor	Proteins involved in regulation of gene expression.
FGF	Fibroblast growth factor—a more widespread growth factor resulting in mitogenesis in a wide range of cell types. Plays a role in angiogenesis, but its action is not restricted to endothelial cells

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