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Review

Lymphangiogenesis and breast cancer metastasis

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Summary. Breast cancer is one of the commonest malignancies in women in the western world. It spreads predominantly via the lymphatic system. However, the understanding of the formation of lymphatics, lymphangiogenesis, has been limited. This has been largely due to the previous lack of lymphatic specific markers. The most specific marker used in humans has been the vascular endothelial growth factor receptor 3 (VEGFR-3). However, this is also found on blood vessel endothelium. The other vascular endothelial factor receptors (VEGFR-1 and -2) are primarily blood vessel receptors. More recently, novel, specific markers for lymphatics have been discovered, such as LYVE-1, prox1 and podoplanin, enabling further research into this new field. Each of these new markers is described in detail. The article also outlines the current understanding in breast cancer metastasis, with an emphasis on the more recent research into lymphangiogenesis. Since these specific markers are now available, quantitation of lymphangiogenesis is now possible by using either immunohistochemistry or quantitative PCR approaches. In addition, to breast cancer, research into many other cancers is now possible using these methods and new markers. With this in mind, possible therapeutic strategies for the future are discussed.

Key words: Metastasis, Breast cancer, Lymphangiogenesis, LYVE-1

Introduction

Breast cancer is the most common cancer in women worldwide, with the possible exception of skin malignancies. The United Kingdom has the highest incidence, with one in ten women affected by the disease (Forbes, 1997) (approximately 26,000 new cases each year) resulting in 14,000 deaths per year. It is rare in women less than 25 years of age, but the incidence increases with advancing years. No single cause has been identified. The strongest associated risk factor is a positive family history, but only 5% of breast cancers are hereditary; the remainder occur as a result of spontaneous genetic mutations. The relative amount of oestrogen exposure to an individual is the main environmental risk factor. Other risk factors include obesity, alcohol, radiation exposure, and the failure to breast-feed children.

Breast cancer is pathologically termed invasive breast carcinoma. There are different morphological types of invasive breast carcinoma. The commonest type (>65%) is invasive ductal (also called no special type - NST) carcinoma. This is followed by lobular carcinoma (classical type and variant types) which accounts for approximately 10% of all breast cancers. The most important prognostic factor in breast cancer is lymph node status (Fisher et al., 1983; Carter et al., 1994) and the axillary nodes are routinely removed during surgery for breast cancer. The more nodes that contain cancer, the worse the prognosis. In a series of 24,740 patients with breast cancer, the overall incidence of metastatic disease in the axilla was 46% (Carter et al., 1994). The 10-year survival for node-negative patients is 60-70%, but only 20-30% for node-positive patients (Dixon and Sainsbury, 1998). The size of the tumour affects the probability of nodal metastasis. For tumours of 1 cm in diameter or less, the incidence of axillary metastases ranges from 3% to 22% (Fisher et al., 1969; Walls et al., 1993; Giuliano et al., 1996). Other prognostic factors, such as tumour grade and grade, oestrogen receptor status, are less useful.

Tumour metastasis

The biology of tumour metastasis

The term ‘metastasis’ specifically refers to the spread of cancer cells from the primary site of growth to another organ, or part of the organ, not directly connected to the primary. The metastatic spread of tumour cells is responsible for the majority of cancer deaths. When primary cancers begin to grow, malignant cells dissociate from the mass at an unknown rate and travel around the body until they settle in a distant organ.

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They then begin to replicate to increase their mass and the process may then repeat itself. This process is known as the 'metastatic cascade'. Within this process, there are a number of separate steps that the tumour cell must complete (Jiang and Mansel, 2000). In order for cancer cells to leave the primary tumour mass and disseminate there must be a reduction in their normal adhesion to other cells as well as changes in their adhesion to the surrounding extracellular matrix. The migration of cells through the matrix and across vessel walls allows dissemination via the lymphatic system and/or blood vessels. This migration is dependent upon stable adherence and traction produced by adhesion molecules and their ligands. Arrest in distant vessels may be facilitated by the adhesion of tumour cells to lymphoid cells/platelets resulting in multicellular aggregates that increase the possibility of entrapment in capillaries (Gasic et al., 1973). Development of the metastatic deposit will only occur if the local environment can provide sufficient nutrients (including oxygen and growth factors) and a suitable extracellular matrix substrate. The latter suggests that some adhesion-dependent signals may need to be initiated for optimal growth of the metastases. Cell adhesion is mediated by several families of cell-surface expressed molecules, including the cadherins (Stappert and Kemler, 1999), immunoglobulin superfamily (Simmons, 1999), selectins (Tedder et al., 1999) and the integrins (Hynes, 1992). Metastasis also relies heavily on the development of new blood vessels (angiogenesis) and lymphatics (lymphangiogenesis), so as to help provide the nutrients required for growth and also to remove cellular waste products. These processes will be described in more detail later.

Breast cancer metastasis

Metastatic disease in breast cancer is common. 7% of women with breast cancer present with widespread metastases at initial diagnosis (Khonji et al., 2000). Of those with no evident metastases who undergo potentially curative surgery, 20-30% of patients with negative axillary nodes and 50-60% with positive axillary nodes will develop widespread metastases (Wingo et al., 1995). This implies that subclinical, micrometastatic deposits were already present when the patient initially presented to the doctor. Indeed, it has been argued by some, but not all, that breast cancer is always a systemic disease by the time of presentation (Fisher, 1997).

The rate of tumour growth decreases as the size of the tumour increases (DeWys, 1972). This is due to large tumours having retarding factors, which result in a greater number of cells being in a dormant state. This applies to both primaries and metastases. An extension of this theory is that the rate of growth of metastases may increase when the primary tumour is surgically removed. This would make metastatic foci particularly vulnerable to chemotherapy after surgery. An alternative theory is one of micrometastatic dormancy, whereby the rate of growth decreases after the initial growth spurt, only to be followed by a delayed increase in the rate of growth many months or years later (Demichelli et al., 1997). This is supported by the late presentation of both local recurrences and distant metastases that are often seen. If this were the case, the purpose of adjuvant therapy would be to help maintain this dormant state for as long as possible.

Halsted put forward the first theory as to the natural history of breast cancer. He suggested that the tumour remains localised at its site of origin until a certain time when lymphatic invasion takes place and the disease becomes loco-regional (Fig. 1). After a further interval, vascular invasion and dissemination occurs, resulting in metastatic disease (Fig. 2). This concept was the basis for radical local surgery for breast cancer, as the chance

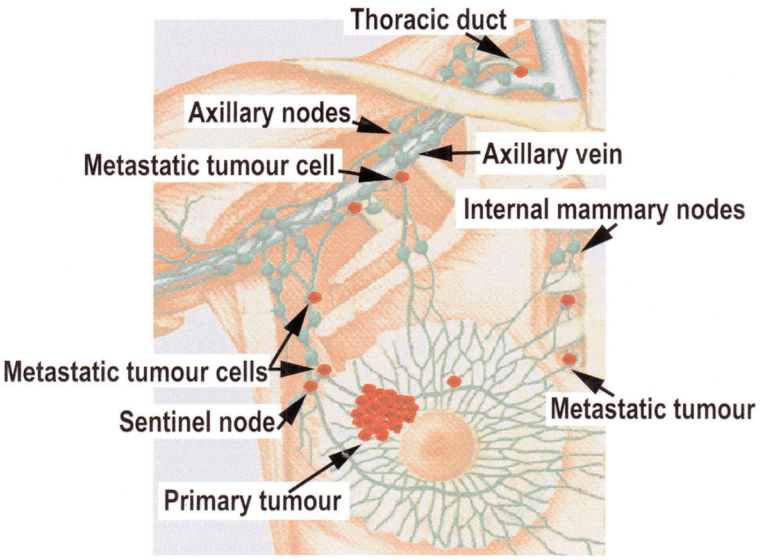

Fig. 1. Anatomy of the lymphatic drainage of the breast showing potential sites for breast cancer regional metastases.

of cure was thought to increase with the amount of tissue excised. The alternative hypothesis, subscribed to by Fisher and others, is that breast cancer is a systemic disease by the time of clinical presentation (Fisher, 1997). It has been proposed that delay in the diagnosis of breast cancer does not reduce survival (Fisher, 1988). A 1cm tumour has already undergone 30 doublings, compared to the lethal tumour burden of 40 doublings (Khonji et al., 2000). Thus it is argued that dissemination is likely to have already occurred. The failure of extensive loco-regional surgery to prevent dissemination is evidence supporting this theory. Data from the breast screening trials, however, do not support this theory, since screened patients have a 30% decrease in mortality (Tabar et al., 1985). Therefore, in such patients who often have small, impalpable cancers, surgery may remove the primary before any viable metastases have developed. However, perhaps micrometastases are present but survival rates are improved because the tumour burden is so small. This debate should be answered when long-term data from screened patients becomes available.

The commonest sites for distant metastasis in breast cancer are bone, liver and lungs. It is not clear why it is specifically these sites that breast cancer has a predilection for. Less common sites are the brain, adrenal gland, ovaries, peritoneal cavity and thyroid. Bony metastasis is the commonest, accounting for almost 50% of the cases of metastatic breast cancer (Khonji et al., 2000). These metastases cause bone destruction, which may often be seen on plain X-rays. This is due to an increase in osteoclastic activity caused by the tumour deposits. There are two theories to explain the latter. There is a local peptide produced by the tumour cells at the site of metastasis, which has been shown to be parathyroid hormone related peptide (PTHrP) (Vargas et al., 1992) which, in turn, is regulated by one of the growth factors TGF-ß (Yin et al., 1996). PTHrP, like parathyroid hormone itself, stimulates osteoclastic activity. The second theory is that the cancer cells themselves may destroy bone directly (Eilon and Mundy, 1978). The increased bone destruction may lead to an increase in serum calcium concentrations (hypercalcaemia) which is found in 10-15% patients with such disease (Body, 1995). Liver metastasis from breast cancer usually carries a poor prognosis – the patient is not expected to survive more than a few months. The reason that breast cancer frequently involves the liver is thought to be due to local growth factors, which induce the preferential growth of these tumour cells. Insulin-like growth factor has been shown to be present in the liver. This itself is a growth and motility factor for breast cancer (Nicolson, 1993).

Angiogenesis

Angiogenesis, which occurs in the embryo and adult, describes the formation of new blood vessels from pre-existing capillaries. The growth of many neoplastic tissues relies on angiogenesis. Primary breast carcinomas are known to produce many different angiogenic growth factors. In addition to tumour cells, macrophages, lymphocytes, natural killer cells, fibroblasts and endothelial cells are capable of releasing a number of angiogenic or anti-angiogenic growth factors (Senger, 1996). Although inhibition of a variety of angiogenic growth factors may decrease tumour growth, the vascular endothelial growth factors (VEGFs) and their corresponding receptors currently constitute a promising target for anti-angiogenic therapy.

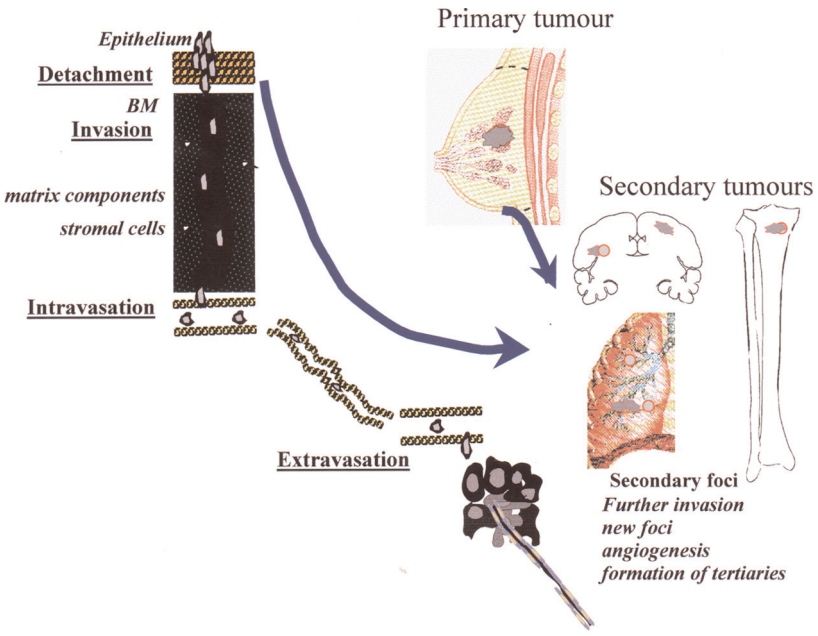

Fig. 2. Breast cancer metastasis. Schematic diagram to show malignant cells invading local tissues before reaching distant organs, such as lungs, brain and bone.

In a study of human breast cancer, an independent correlation has been found between VEGF expression, microvessel density and clinical prognosis (Gasparini et al., 1997). In addition, tumours with a high mean vascular density are associated with a high rate of metastasis as well as a poor prognosis (Weidner, 1995). This relationship can be explained by a simple hypothetical principle: the more vessels, the greater the probability that tumour cells will invade the vascular bed and escape from the site of origin of the tumour. Proliferating tumour capillaries with fragmented, leaky basement membranes are easily penetrated by tumour cells. In fact, a considerable percentage of tumour blood flow is in direct contact with tumour cells (Hashizume et al., 2000). After overcoming this first vascular barrier, the tumour cells have to survive the circulation, attach to the microvasculature of the target organ, exit from this vasculature (usually through the postcapillary venular endothelium) and survive in the target tissue. Furthermore, in order to form macrometastases, micrometastatic cells must also be able to induce angiogenesis in their new target tissue.

Since breast cancer spreads predominantly via the lymphatic system, it is important that the mechanisms involved in lymphangiogenesis (the formation of new lymphatic vessels) are also known. Until recently, limited research has been carried out in this field. The following describes a review of the literature on this subject, including references to breast cancer.

Lymphangiogenesis and lymphatic markers

The biology of lymphangiogenesis

This term describes the formation of new lymphatic vessels. This occurs in normal development and in the growth of tumours. Clinical and pathological observations have long suggested that, for many tumours, the most common pathway of initial dissemination is via lymphatics, with patterns of spread via afferent vessels following routes of natural lymphatic drainage (Cotran et al., 1999). However, the lymphatic system has traditionally been overshadowed by the greater emphasis placed on the blood vascular system. This has been due in part to the absence of suitable markers that distinguish lymphatic from blood vascular endothelium, and to the lack of identification of lymphatic-specific growth factors.

In recent years, these limitations have been relieved by the discovery of a small number of potential lymphatic-specific markers. These include: LYVE-1, a lymphatic endothelial receptor for the extracellular matrix/lymphatic fluid mucopolysaccharide hyaluronan (Banerji et al., 1999); Prox1, a homeobox gene product involved in regulating early lymphatic development (Wigle and Oliver, 1999); podoplanin, a glomerular podocyte membrane mucoprotein (Breiteneder-Geleff et al., 1999); and the vascular endothelial growth receptor-3 (VEGF-3) which, as discussed above, is a transmembrane tyrosine kinase receptor for the vascular endothelial growth factors C and D (VEGF-C and VEGF-D). Although previous studies have used VEGFR-3 as a specific marker for lymphatic vessels, it has now been shown to be expressed in tumour blood vessels during neovascularisation (Valtola et al., 1999). It should not, therefore, be considered specific for lymphatics any longer.

Lyve-1 and hyaluronan

Hyaluronan (HA), a linear polymer of (1-,-4) D-glucuronic acid (1-,-3) N-acetyl-D-glucosamine, is a large glycosaminoglycan found in the tissue matrix and body fluids of all vertebrates which plays a fundamental role in regulating cell migration and differentiation (Banerji et al., 1999). This role is first apparent during development, when changes in the levels of matrix HA induce condensation of mesenchymal cells and lead to the onset of chondrogenesis (formation of cartilage) and myogenesis (formation of muscles). In later life, HA facilitates cell migration in processes such as wound healing and inflammation by forming a pericellular matrix surrounding fibroblasts and epithelial cells, reducing the level of intercellular adhesion (Banerji et al., 1999). The importance of HA in all these roles is underlined by the observation that deletion of HA synthases in mice results in early death of the embryo.

The highest levels of HA are found within the skin and musculo-skeletal system which together account for greater than 50% of the total body HA. Surprisingly, most degradation does not occur within these areas; rather HA is transported through the lymphatic system to distant lymph nodes where more than 90% of the HA in afferent lymph is degraded or re-enters the circulation to be rapidly endocytosed by liver endothelial HA receptors (Fraser et al., 1988). Therefore, the lymphatic vessels are a major pathway for HA transport from tissues such as skin and intestine, where flux measurements indicate they can remove 10% of the total HA content within a 24-hour period (Ostgaard and Reed, 1993). This turnover can be further increased after either tissue injury or sepsis.

The majority of HA-binding proteins within the tissues belong to the Link protein superfamily. These structures contain a disulphide-linked domain of approximately 100 amino acid residues termed the Link module, which binds the minimal recognition unit HA_{6-8}. There are other members of this superfamily apart from the Link protein. One of these is a single cell surface HA receptor, called the CD44 molecule (Aruffo et al., 1990). CD44 is expressed on epithelial, mesenchymal, and lymphoid cells, where it is thought to constitute the main receptor for HA (Aruffo et al., 1990). HA and CD44 interactions occur in several diverse functions, including the maintenance of tissue structure within the epithelia, the extravasation of lymphocytes through inflamed vascular endothelium, and the haematogenous dissemination of tumour cells (Banerji et

al., 1999). Four other HA receptors have been described: RHAMM (receptor for HA-mediated motility), LEC (liver endothelial cell receptor), the nuclear protein cdc37 and LYVE-1. There is no evidence that the first three of these receptors are involved in the transport, or degradation, of HA by lymphatic vessels. In addition, deletion of the *CD44* gene in mice produced no significant disruption of lymphatic function (Schmits et al., 1997).

LYVE-1 was first described in 1999 (Banerji et al., 1999). This is a very important HA receptor, found on the lymph vessel wall. The deduced amino acid sequence of LYVE-1 predicts a 322-residue integral membrane polypeptide, 41% similar to the CD44 receptor, with a 212-residue extracellular domain containing a single Link module, the prototypic HA binding domain of the Link protein superfamily. Like CD44, the LYVE-1 molecule binds both soluble and immobilised HA. However, unlike CD44, the LYVE-1 molecule co-localises with HA on the luminal surface of the lymph vessel wall and is completely absent from blood vessels. Hence, LYVE-1 is the first HA receptor which is specific for lymphatics. This enables it to be used as a uniquely powerful marker for lymph vessels themselves. In fact, recent studies observing the relationship between VEGFs and VEGFRs and lymphangiogenesis have now exploited this unique characteristic of LYVE-1 (Skobe et al., 2001; Stacker et al., 2001). LYVE-1 has recently been quantified in breast cancer tissue (Cunnick et al., 2001a).

Proxi 1

The homeobox gene *Prox1* was originally cloned by homology to the *Drosophila melanogaster* gene *prospero* (Oliver et al., 1993). Analysis of the expression pattern suggested that it has a functional role in a variety of tissues, including lens, heart, liver, pancreas and central nervous system (Oliver et al., 1993). It has also been shown that Prox1 is expressed in a subpopulation of endothelial cells that by budding and sprouting give rise to the lymphatic system in mice (Wigle and Oliver, 1999). These authors showed that in Prox1 null mice, budding and sprouting of the lymphatic system was arrested, although vasculogenesis and angiogenesis of the vascular system were unaffected. This suggests that, in mice, Prox1 is a specific and required regulator for the development of the lymphatic system.

Podoplanin

This protein was first described in 1997 in a rat model of human minimal change nephropathy, called puromycin aminonucleoside nephrosis (PAN) (Breiteneder-Geleff et al., 1997). These two conditions are characterised by extensive flattening of the glomerular epithelial cells (called podocytes). Podoplanin was the name given to a 43 kD membrane glycoprotein that was present on normal rat podocytes, but significantly reduced (less than 30%) on PAN rat podocytes, suggesting down regulation in the latter. It consists of 163 amino acids and has a single membrane spanning domain, two phosphorylation sites and six potential O-glycosylation sites in the large ectodomain. It is thought that podoplanin could be associated with transformation of the normal arborised podocyte foot processes to flattened processes, hence its name (Latin: pes planus, flat feet).

Since the discovery of this novel protein, podoplanin has been discovered in human podocytes, where it is a slightly smaller, 38 kD membrane glycoprotein (Breiteneder-Geleff et al., 1999). By using immunohistochemistry, expression of podoplanin was found in the endothelium of lymphatic capillaries, but not in the blood vasculature (Breiteneder-Geleff et al., 1999). In addition, it was found to co-localise with VEGFR-3 in normal skin and kidney. In purely lymphatic tumours (lymphangiomas and hygromas), high levels of staining for podoplanin were found. In purely vascular tumours (e.g. angiosarcomas), much lower levels of podoplanin staining were found when compared to other known vascular endothelial markers (such as CD31, CD34 and factor VIII-related antigen). The authors concluded that podoplanin can therefore be used as a specific marker for lymphatic endothelium. However the expression of podoplanin in the vascular tumours may suggest that it is also present in low levels on blood vessel endothelium. The fact that podoplanin co-localises with VEGFR-3, which is also partially expressed on blood vessels, strengthens this argument.

VEGFS and VEGFRS in lymphangiogenesis

There are three known vascular endothelial growth factor receptors (named VEGFR-1, VEGFR-2 and VEGFR-3) (Saaristo et al., 2000). VEGFR-1 and VEGFR-2 are not significantly expressed in lymphatic endothelium. However, VEGFR-3 is predominantly expressed on lymphatic vessels (Plate, 2001). There are four known vascular endothelial growth factors (VEGF, VEGF-B, VEGF-C and VEGF-D). VEGF, which is associated with angiogenesis, does not bind to VEGFR-3. Instead, two novel members of the VEGF family which are not involved in angiogenesis, VEGF-C and VEGF-D, were found to bind to this lymphatic receptor (Joukov et al., 1996; Achen et al., 1998). VEGF-C, discovered in 1996, is a polypeptide containing 419 amino acid residues (Joukov et al., 1996). This growth factor is proteolytically processed, binds to the extracellular domain of VEGFR-3 and induces tyrosine phosphorylation of both VEGFR-2 and VEGFR-3; it also stimulates the migration of endothelial cells in collagen gels. Transgenic mice expressing VEGF-C under a basal keratin promoter developed a hyperplastic lymphatic vessel network in the skin (Jeltsch et al., 1997). In addition, however, VEGF-C has angiogenic effects if artificially applied to vessels (Cao et al., 1998). VEGF-C mRNA transcription is upregulated by PDGF,

EGF, TGF-α, TNF-α and IL-1 (Enholm et al., 1997), but not hypoxia which induces VEGF mRNA transcription. VEGF-C mRNA expression is increased in pathological conditions, such as inflammation and cancer (Salven et al., 1998). It has been shown to be present in human breast carcinoma cells and its receptors are expressed in the vasculature of breast carcinomas (Valtola et al., 1999). More specifically, VEGF-C was found to be located in the cytoplasm of the cancer cells.

VEGF-D, discovered in 1998, is a polypeptide containing 426 amino acid residues (Achen et al., 1998). It is most closely related to VEGF-C by virtue of the presence of N- and C-terminal extensions that are not found in other VEGF family members. It is also proteolytically modified before binding to VEGFR-2 and VEGFR-3 to induce tyrosine phosphorylation and is a mitogen for endothelial cells (Achen et al., 1998). In view of the biochemical similarities, VEGF-D is thought to have similar physiological characteristics to VEGF-C. However, some differences may exist, since recent studies have shown that VEGF-D promoted tumour angiogenesis whereas VEGF-C did not (Stacker et al., 2001; Skobe et al., 2001; Mandriota et al., 2001). This effect is surprising since both factors activate VEGFR-2 (in addition to VEGFR-3), which is a regulator of tumour angiogenesis. The discrepancy may be partly explained by the fact that the experimental system of Skobe et al. 2001 involved the 31-kD form of VEGF-C, which preferentially activates VEGFR-3, whereas the fully processed form (which also activates VEGFR-2) was barely detectable.

In humans, two isoforms of the VEGFR-3 receptor occur, designated VEGFR-3s (short) and VEGFR-3l (long), differing in their carboxyl terminals as a result of alternative mRNA splicing (Karkkainen and Petrova, 2000). The long form is the predominant one in the tissues, and thought to be the main one involved in lymphangiogenesis. It contains three additional tyrosyl residues, of which Tyr1337 serves as an important phosphorylation site in the receptor (Karkkainen and Petrova, 2000). Mutations of the VEGFR-3 receptor are associated with human hereditary lymphoedema, which emphasises the importance of this receptor in the development of lymphatic vessels. More specifically, early onset primary lymphoedema has been linked to the VEGFR-3 locus in chromosome 5q (Karkkainen and Petrova, 2000). A soluble form of VEGFR-3 has been shown to act as a potent inhibitor of VEGF-C and VEGF-D signalling of the VEGFR-3 receptor in transgenic mice (Makinen et al., 2001). The mice subsequently developed abnormalities in dermal lymphatic vessels, but not in their blood vessels. This suggests that in vivo angiogenesis and lymphangiogenesis are separately controlled by different members of the VEGF family.

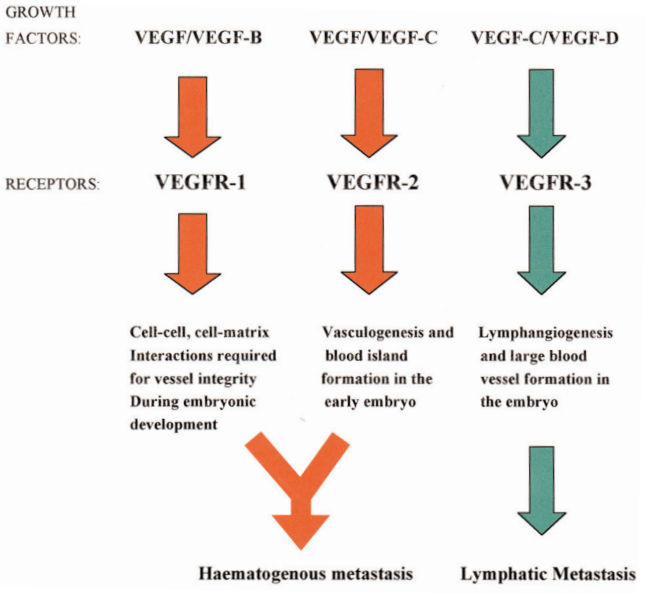

Fig. 3. Biological functions of the vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs).

The formation of lymphatic metastases

Like lymphangiogenesis, this field has been poorly studied. However, recent work has shown that the VEGF family are important in this process. In the year 2001, it was shown that both VEGF-C and VEGF-D promote lymphangiogenesis and lymphatic metastasis in tumours (Makinen et al., 2001; Skobe et al., 2001; Stacker et al., 2001). Using an anti-LYVE-1 antibody, these three research groups demonstrated an increase of tumour lymph vessels when they compared VEGF-C or VEGF-D over-expressing tumours with control tumours. These findings are supported by the observation that neutralising antibodies to VEGF-D decreased the number of lymphatic metastases in the VEGF-D-producing tumours (Stacker et al., 2001). This is the first direct experimental evidence that tumours are able to activate tumour lymphangiogenesis. There was also a correlation between expression of VEGF-C and VEGF-D with lymphatic metastasis in these mouse tumour models. It thus seems likely that tumour angiogenesis and lymphangiogenesis are driven by different pathways; angiogenesis being driven by VEGF and lymphangiogenesis driven by VEGF-C and VEGF-D (Fig. 3).

In a recent human study on lymph node metastasis in breast cancer, VEGFR-3 (short and long isoforms), VEGFR-2 and VEGF-C mRNA levels were compared between 61 breast cancers and 11 normal breast tissue controls (Gunningham et al., 2000). No significant difference in VEGF-C expression was observed between normal and neoplastic breast tissues. No association was found between lymph node metastasis and either VEGF-C or VEGFR-3. However, the workers did find a significant loss of VEGFR-3l in tumours compared with normal breast tissue, but not with VEGFR-3s.

Therapeutic implications of lymphangiogenesis

If human tumours that express high concentrations of VEGF-C and VEGF-D show a higher incidence of lymphatic metastasis, as do mouse tumours, inhibition of VEGFR-3 function may be a new approach to inhibit lymphatic metastasis in patients. The inhibition of VEGFR-3 could be achieved by using the soluble extracellular domain of VEGFR-3, as used by Makinen et al (Makinen et al., 2001), neutralising antibodies against VEGFR-3, or neutralising antibodies against VEGF-C or VEGF-D. Inhibition of angiogenesis is currently considered to be a promising therapeutic strategy to inhibit cancer growth because it can act on any tumour type, does not induce resistance of tumour cells (and can therefore be repeated) and has little effect on normal tissues (Plate, 2001). This may also hold true for lymphangiogenesis, although little human experimental work has been carried out thus far.

Now that lymphangiogenesis can be quantified using these newer lymphatic-specific markers (Cunnick et al., 2001b), it needs be shown whether there is any correlation between lymphangiogenesis and lymph node status and/or prognosis in breast cancer. If there is such a relationship, then these factors may me measured in the primary cancer after surgical resection, and indeed in the axillary lymph node or sentinel lymph node, so as to aid prognosis and subsequent treatment.

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