Abstract. Lymphatic vessels play a crucial role in a variety of human cancers, since invasion of lymphatic vessels by tumor cells and subsequent development of lymph node metastases significantly influences prognosis of cancer patients and therefore represent an integral part of tumor staging. Recent evidence on the important influence of lymphangiogenetic growth factors on intralymphatic cancer growth and metastasis raises hopes that lymphatic vessels and factors inducing their growth could serve as an additional target for tumor therapy. Nevertheless, in contrast to blood vessel angiogenesis, the mechanisms of new lymphatic vessel formation in human cancers, i.e. lymphangiogenesis, are still relatively unclear. In addition, only little data exist so far on the quantification and impact of this lymphangiogenesis, evident by lymphatic microvessel density (LMVD), on the prognosis of cancer patients. With expectation of possible anti-lymphangiogenic therapies, this review focuses on the mechanisms of lymphangiogenesis in general, and especially on the role of lymphatic vessels in gynecological and breast cancer, which are the so far most detailed investigated malignancies with regard to lymphangiogenesis.

1. Introduction

The majority of cancer-related deaths result from metastatic spread of tumor cells (1-3). Clinical and pathological observations show that lymph node involvement appears as one of the earliest features of metastatic disease (4). The presence of lymph node involvement is a key prognostic factor and therefore an integral part of tumor staging in a variety of human cancers. Clinical findings suggest that by providing a pathway for tumor cell dissemination, tumor-associated lymphatics are a key factor of metastatic spread. Although it has been well established that lymphatic vessels are the main avenue for dissemination of tumor cells to lymph nodes, there has been debate in the literature as to whether lymphatic vessels could exist directly within tumors because of the high interstitial pressure (5).

Tumor-induced lymphangiogenesis has traditionally been overshadowed by the greater emphasis placed on the blood vascular system (angiogenesis). This has been mostly due to the popular belief that lymphatic vessels were not recruited within tumor tissue, and to the lack of suitable markers which distinguish blood from lymphatic vascular endothelium. This scenario has changed rapidly after the identification of the first lymphangiogenic factor, vascular endothelial growth factor (VEGF)-C (6,7). However, despite remarkable progress in this field, it remains unclear whether inhibition of lymphangiogenesis might be a realistic therapeutic strategy for inhibiting tumor cell dissemination and the formation of metastasis (8). It remains to be determined whether anti-lymphangiogenetic strategies could primarily target tumor cells, a rich source of VEGF-C production (9,10), or lymphatic vessel endothelium itself (11-31).

This review presents current knowledge on the induction and biological mechanisms of lymphangiogenesis in general, the role of lymphatic vessels in gynecological and breast cancer, and discusses possible future concepts of anti-lymphangiogenic therapies.

2. Induction of lymphangiogenesis

VEGF, a highly conserved secreted glycoprotein, is considered as the main inducer of blood vessel angiogenesis (32-35). VEGF signals through two tyrosine kinase receptors, vascular endothelial growth factor receptor (VEGFR)-1 and VEGFR-2, which are expressed predominantly but not exclusively on vascular endothelial cells. VEGFR-2 appears
to be the principal signaling receptor for vascular endothelial cells, whereas VEGFR-1 most probably functions as a decoy receptor, serving to regulate availability of VEGF in a given tissue (36,37).

Since neither VEGFR-1 nor VEGFR-2 are expressed by lymphatic endothelial cells, it was not surprising when Kaipanen et al found a third VEGF receptor, VEGFR-3. This receptor is widely expressed in early embryonic vasculature and becomes restricted mainly to lymphatic endothelium at later stages of development and in postnatal life (see below) (38). The three VEGF tyrosine kinase receptors differ with respect to regulation mechanisms and patterns of expression. However, VEGF was found not to bind to VEGFR-3, appearing unlikely for VEGF to be involved in lymphangiogenesis. Instead, two novel members of the VEGF family, VEGF-C and VEGF-D, were discovered to be ligands for VEGFR-3. The critical role of VEGFR-3 and its corresponding ligands VEGF-C and VEGF-D in developmental and tumor induced lymphangiogenesis as well as in lymphatic tumor invasion has been demonstrated subsequently in a variety of studies (5,39-41). Thus, VEGF-C and VEGF-D have to be considered as main inducers of lymphangiogenesis and lymphatic spread of tumors. Therefore it is not surprising that increased expression of VEGF-C in primary tumors correlates with increased tendency of tumor cell dissemination to regional lymph nodes, as described for a variety of human carcinomas (11,39).

3. Markers of lymphatic endothelium

Until recently, research on lymphatic vessels was seriously hampered by the fact that standard pathological processing of cancer specimens did not allow accurate distinction between blood and lymphatic vessels and their endothelium. Immunohistochemistry was not suitable, since both lymphatic and blood endothelial cells express a similar set of endothelial markers, such as CD31, CD34, podocalyxin, or von Willebrand factor (vWF) (42). The identification of VEGFR-3 restricted to lymphatics was the first step to allow a specific discrimination between the two types of endothelia. Immunostaining with specific antibodies to VEGFR-3 was considered a useful tool to distinguish between lymphatic and blood vessels and capillaries in formalin-fixed, paraffin-embedded tissue sections (43). Three years later a new study by Jussila et al (43) demonstrated VEGFR-3 also to be widely expressed in embryonic blood vessels and to be re-expressed in tumor blood vessels (44). Thus, antibodies against VEGFR-3 are no longer considered as specific for lymphatic endothelium. To date three specific markers exist for lymphatic endothelium: a) podoplanin, a 43 kDa glomerular podocyte membrane mucoprotein (45-47); b) Prox-1 (48), a homeobox gene product involved in regulating early lymphatic development; and c) LYVE-1, a lymphatic endothelial receptor for the extracellular matrix/lymphatic fluid glycosaminoglycan, hyaluron (49,50).

From these three identified markers, podoplanin has to be considered as the most specific and most widely used one in the field of cancer research. Recently podoplanin has even been shown to serve as a tool for distinguishing and isolating endothelial cells of blood vessels and lymphatic channels, showing their specialization and adaptation to distinct functions (51).

4. Lymphangiogenesis and breast cancer

Metastasis of breast cancer occurs primarily through the lymphatic system, and the extent of lymph node involvement is a key prognostic factor for the disease, determining further therapy. The development of the sentinel node technique has further raised interest in lymphatic spread of tumor cells (52-55).

Increased number of lymphatic vessels has been described in ductal carcinoma in situ and in invasive breast cancer (56,57). The first study in which two different antibodies were used to distinguish lymphatic vessels from small blood vessels in formalin-fixed, paraffin-embedded tissue sections, and their respective densities were correlated with other clinical parameters was performed by Nathanson et al (58). Using immunohistochemical staining for factor VIII related antigen, type IV collagen, and VEGFR-3, Nathanson et al (58) showed that the odds of a patient with breast cancer having axillary lymph node metastasis increased substantially as the proportion of putative lymphatic microvessels increased and the relative proportion of blood microvessels in angiogenic hot spots decreased. Patients with the highest relative number of VEGFR-3-immunostained microvessels and the lowest relative number of blood vessels were shown to have the highest probability of lymph node metastases. Even though the association proposed by these results appeared causal, there was still the obvious fact that antibodies against VEGFR-3, used by Nathanson et al (58) were not a useful tool to discriminate between blood and lymphatic vessels (56). A major contribution concerning lymphangiogenesis in breast cancer was done with the recent data published by Skobe et al (39) and Mandriota et al (41). They demonstrated that VEGF-C overexpressing breast cancer cells increased intratumoral lymphangiogenesis after orthotopic transplantation into nude mice, leading to significant enhanced metastasis to regional lymph nodes. For staining of lymphatic endothelia the novel marker LYVE-1 was used. These data established VEGF-C as a molecular link between tumor lymphangiogenesis and metastasis in an animal model.

Using an antibody against podoplanin in immunohistochemistry, we investigated the peritumoral lymphatic microvessel density and lymphovascular tumor cell invasion in patients with invasive breast cancer. We found no lymphatic vessels directly within tumor formations, but in the peritumoral stroma. High peritumoral lymphatic microvessel density (LMVD) and the presence of lymphovascular invasion were strongly associated with the presence of lymph node metastases (59). Although VEGF-C is commonly expressed by human cancers, several other studies have also failed to identify functional lymphatic vessels within tumors, but found them increased within the peritumoral stroma (58-61). This is in contrast to the results of Skobe et al (39) and Mandriota et al (41), who observed intratumoral lymphatic vessels in animal models, with VEGF-C overexpressing tumors. An explanation for this phenomenon might be that neoplastic cells grown in a confined space generate mechanic stress which may compress or inhibit the development of lymphatic channels inside the tumor. In contrast, (newly formed) blood vessels are numerous, probably due to their better ‘resistance’ to increased pressure (62). Alternatively there may be a true difference in the
results indicate that mechanisms of lymphangiogenesis is and the degree of inflammatory stroma reaction (31). These interestingly, we observed a good correlation between LMVD and lymph node status (63). Most of more lymphatic vessel invasions, no association was metastasis, and high LMVD was associated with the presence metastases appears to be lymphovascular invasion (59). We are currently investigating the prognostic relevance of lymphatic vessels and lymphovascular invasion in breast cancer.

5. Lymphangiogenesis and cervical cancer

Currently, early-stage cervical cancer is one of the best studied human malignancies with regard to lymphatic vessels (31,60,63,64).

Cervical cancer is one of the most common cancers in females worldwide (65). Pelvic lymph node status, inflammatory stroma reaction, depth of invasion and vascular space involvement are established prognostic factors in these patients (66,67). Using immunohistochemical staining for factor VIII related antigen, which is considered a marker for vascular endothelium, and for podoplanin (lymphatic endothelium) we were able for the first time to distinguish between lymphatic and blood microvessels in cervical cancer specimens.

Lymphatic vessel invasion by tumor cells was highly associated with the presence of lymph node metastases (60). In contrast, increased lymphatic microvessel density was associated with favorable prognosis in patients with early-stage invasive cervical cancer. This finding was particularly interesting as, in comparison, increased blood microvessel density has been shown to be associated with shorter survival in our collective (63). This controversy could not be easily explained. It had originally been expected that increased lymphatic microvessel density would be associated with a dismal prognosis as it would provide an increase of the ‘lymphatic window’ and therefore easier access of cancer cells to the lymphatic system, and, hence, raises the opportunity of lymphatic spread. However, this appears not to be the case. Although invasion of lymphatic vessels by tumor cells was highly associated with the presence of lymph node metastasis, and high LMVD was associated with the presence of more lymphatic vessel invasions, no association was found between LMVD and lymph node status (63). Most interestingly, we observed a good correlation between LMVD and the degree of inflammatory stroma reaction (31). These results indicate that mechanisms of lymphangiogenesis is highly complex in early-stage cervical cancer. Although it is well known that pro- and anti-angiogenic molecules can emanate from cancer as well as from endothelial and stroma cells, the secretion of various proangiogenic factors by tumor cells is considered as the main factor inducing blood and lymph vessel neoangiogenesis in human cancers.

Recently, a high VEGF-C mRNA level in cervical cancers has been reported of being associated with an increased rate of lymph node metastases (16). Our findings provide first indirect evidence that in early-stage cervical cancer, inflammatory stroma reaction seems to play a crucial role in lymphangiogenesis (31). This might also explain the almost complete absence of lymphatic vessels within tumors, and their increased number in the surrounding tumor stroma. Although the presence of a strong inflammatory stroma reaction increases the possibility for lymphovascular invasion, this strong immunological response might by itself inhibit the formation of lymph node metastases out of these lymphovascular invasions.

It is well known that human cancer cells can be successfully destroyed by immunological response, which is currently exploited by the development of immunotherapies against cancer (68,69). A pre-requisite for generation of a primary immune responses is that tumor antigens move into lymphoid organs via blood, lymph fluid or mobile antigen-presenting cells (70).

The dendritic cell family compromises a unique system of antigen-presenting cells present in every human organ except the brain and the central cornea. Dendritic cells exhibit the most potent ability to initiate T-lymphocyte mediated immunological response by migration from the site of primary antigen exposure to T cell-rich areas of lymphoid organs and presenting of antigen to specific T cells. Dendritic cells appear to migrate through the lymphatic system to sites of T cell activation (lymph nodes and spleen) (71,72). Local presence of dendritic cells was shown to lead to significantly prolonged survival of patients with early-stage invasive cervical cancer (73). It is possible that high lymphatic microvessel density promotes migration of dendritic cells to lymphatic organs, in turn resulting in improved T cell mediated immune response against cancer cells. The lymphangiogenic properties of cells within the inflammatory tumor stroma reaction and the mechanisms of interaction between tumor cells and surrounding stroma with regard to lymphangiogenesis are under investigation by our group.

6. Lymphangiogenesis and ovarian cancer

Of all gynecologic cancers, ovarian malignancies represent the greatest clinical challenge, since two thirds of patients present with already advanced disease, requiring major surgery, with intensive and often complex additional therapies (74). Therefore ovarian cancer also has the highest mortality rate of all gynecologic malignancies: out of 24,000 new cases annually in the United States, 13,600 patients are expected to succumb to their disease (75). Only one study exists that investigates the role of lymphatic microvessels in ovarian cancer (76). In contrast to cervical and breast cancer, lymphatic vessels seem to play no important role in this type of cancer. Again, no intratumoral lymphatic vessels are present, but
there is an accumulation of compressed-looking lymphatic capillaries in the capsule of the tumors. In addition, we only observed few cases with lymphatic vessel invasion by tumor cells, showing no correlation of this event with the corresponding lymph node status.

Survival analysis of ovarian cancer patients shows no significant influence of lymphatic microvessel density on the prognosis of patients. The fact that we observed de facto no lymphangiogenesis in ovarian cancer might be explained by the generally only scarce inflammatory stroma reaction in this type of cancer.

7. Summary and perspectives

Many questions concerning lymphangiogenesis and tumor metastasis still remain unanswered: e.g. what are the exact molecular mechanisms and the effectors of lymphangiogenesis and could inhibition of lymphangiogenesis be a realistic therapeutic strategy for preventing tumor cell dissemination and formation of metastases? Due to the possible role of the immune system in lymphangiogenesis, and the possible role of lymphatic vessels in the anti-tumor immune response, the development of concepts for anti-lymphangiogenic therapy regimens seems problematic. In addition, the role of lymphatic vessel appears different among various types of cancers.

In cervical cancer, for example, future therapies should selectively aim at inhibition of lymphatic vessel invasion of tumor cells, but should not hamper the mechanisms of the local immune response, of which lymphangiogenesis seem to be an integral part of. In contrast, in ovarian cancer, the application of anti-lymphangiogenic therapies seems not to be a promising approach. Another problem of possible lymph-angiogenic therapies is the fact that at time of diagnosis and primary surgery, in a considerable number of cancer patients lymphatic invasion and dissemination of tumor cells has already occurred. Currently there is no information on the possible impact of lymphangiogenesis on the formation of metastases out of already disseminated cells (77). So the actual importance of future anti-lymphangiogenic therapies will only become evident if lymphangiogenesis proves to be important for the development of metastasis out of ‘minimal residual disease’, similar to blood vessel angiogenesis (78). A further problem could be the danger of elevating the tumor interstitial pressure by destroying a main drainage for interstitial fluid and to hamper delivered drugs to reach their designation (79,80). An attractive possibility for lymphatic vessel-associated therapy could be to target anti-cancer drugs into tumor lymphatics and to selectively destroy peritumoral lymphatic vessels, which in consequence could inhibit lymphatic metastasis.

Further studies will have to investigate these problems in detail, and upcoming future results of these studies could lead to concepts of possible anti-lymphangiogenic therapies for the benefit of cancer patients.

References


