Pathways Targeting Tumor Lymphangiogenesis
Christoph Wissmann and Michael Detmar

Abstract
Tumor metastasis to sentinel lymph nodes represents the first step of tumor dissemination in most human cancers and serves as a major prognostic indicator for disease progression. Recent studies have revealed that tumors can actively induce the formation of lymphatic vessels, and that tumor lymphangiogenesis is correlated with lymph node metastasis in experimental cancer models and in several types of human cancers. Metastatic tumor cells may continue to promote lymphatic vessel growth even after their metastasis to sentinel lymph nodes, likely promoting further cancer spread. Vascular endothelial growth factor-C (VEGF-C) and VEGF-D were the first specific lymphangiogenesis factors identified, acting predominantly via VEGF receptor-3 (VEGFR-3) that is expressed by lymphatic endothelial cells, and a large number of recent studies have shown a correlation between tumor expression of VEGF-C or VEGF-D and lymph node metastasis. VEGFR-3 activation promotes lymphatic endothelial cell proliferation, migration, and survival via the extracellular signal-regulated kinase 1/2, the phosphatidylinositol 3-kinase/AKT, and the c-Jun NH2-terminal kinase 1/2 pathways. Additional tumor lymphangiogenesis factors have been recently identified, including VEGF-A. Importantly, blockade of the VEGF-3 pathway by specific antibodies, by soluble receptor constructs, and by small molecule kinase inhibitors efficiently inhibits experimental tumor lymphangiogenesis and metastasis and might also represent a novel therapeutic avenue for the treatment of human cancers.

Lymphangiogenesis and Cancer Metastasis
The major function of lymphatic vessels is to collect and to transport protein-rich interstitial fluid via lymph nodes, larger collecting lymphatic vessels, and the thoracic duct back to the blood vascular circulation (1, 2). The lymphatic system also contributes to the immune surveillance of the body by transporting activated immune cells from peripheral tissues to the regional lymph nodes. Tumor cells can take advantage of the lymphatic vascular system to promote their metastasis to lymph nodes and beyond. Indeed, tumor metastasis to regional (sentinel) lymph nodes often represents the first step of tumor dissemination and serves as a major prognostic indicator for the progression of human cancers (3). Recent studies have revealed that tumors can actively induce the formation of lymphatic vessels, and that tumor lymphangiogenesis promotes lymph node metastasis in experimental cancer metastasis models (4–6). Moreover, correlative clinical studies have revealed that tumor lymphangiogenesis is often associated with enhanced metastasis in several types of human cancer (3, 7, 8). As an example, human cutaneous malignant melanomas that later metastasized were characterized by increased lymphangiogenesis, and the degree of melanoma lymphangiogenesis can serve as a novel predictor of lymph node metastasis and overall patient survival (9). Moreover, the extent of lymphatic vessel growth in primary cutaneous melanomas was the most sensitive variable for predicting whether these tumors had already metastasized to the sentinel lymph node at the time of surgery (10). Recent evidence indicates that tumor cells can also induce lymph node lymphangiogenesis already before they metastasize, and that metastatic tumor cells continue to induce lymphatic vessel growth within sentinel lymph nodes, possibly promoting their further metastatic dissemination (10, 11).

Vascular Endothelial Growth Factor Receptor-3 Pathway and Tumor Lymphangiogenesis
Vascular endothelial growth factor receptor-3 (VEGFR-3), also known as Flt4, was the first lymphatic-specific growth factor receptor identified (12). VEGFR-3 is a member of the fms-like tyrosine kinase family and specifically binds VEGF-C and VEGF-D but not VEGF-A (Fig. 1A). In normal adult tissues, VEGFR-3 expression is largely restricted to the lymphatic endothelium (12). Recent studies in animal tumor models have provided direct experimental evidence that increased levels of VEGF-C or VEGF-D promote active tumor lymphangiogenesis and lymphatic tumor spread to regional lymph nodes, and that these effects are suppressed by blocking...
VEGFR-3 signaling (4–6, 13). Moreover, a large number of clinicopathologic studies have shown a direct correlation between expression of VEGF-C or VEGF-D by tumor cells and metastatic tumor spread in many human cancers, indicating an important role of this pathway also in human tumor progression (3, 7, 8).

After activation by VEGFR-3 ligands, autophosphorylation of tyrosine residues Y1230/Y1231 of the intracellular VEGFR-3 domain results in binding of the signaling adaptor proteins Src homology containing protein (Shc) and growth factor receptor binding protein 2 (Grb-2), inducing the activation of the extracellular signal-related kinase 1/2 and phosphatidylinositol 3-kinase/Akt signaling, thereby promoting endothelial cell proliferation, migration, and survival (14–16). Moreover, autophosphorylation of Y1063 induces a survival signaling cascade via CRKI/II and the c-Jun NH₂-terminal kinase (γNK) 1/2 pathway, leading to induction of c-JUN expression (ref. 16; Fig. 1A). Reactive oxygen species are also able to induce tyrosine phosphorylation of VEGFR-3 and its association with Shc, Grb-2, Sos, p85, SHP-2, and phospholipase C-γ, thereby promoting endothelial cell survival under redox stress (17).

Recent studies indicate that heterodimer formation of VEGFR-3 with the related receptor VEGFR-2 might alter VEGFR-3 phosphorylation site usage in primary lymphatic endothelial cells, thereby adding an additional level of modulation of VEGFR-3 signaling (15).
Other Molecular Pathways Controlling Lymphangiogenesis

Recent studies indicate a major role of the VEGF-A/VEGFR-2 signaling pathway in lymphangiogenesis (18). VEGF-A induces proliferation of lymphatic endothelial cells in vitro (19), and injection of adenoviral murine VEGF-A resulted in pronounced in vivo lymphangiogenesis in mouse ear skin (20). Moreover, targeted overexpression of murine VEGF-A164 in the skin of transgenic mice enhanced lymphangiogenesis during tissue repair and in skin inflammation (21, 22). Importantly, VEGF-A also induced active proliferation of VEGF-2-expressing tumor-associated lymphatic vessels in a multi-step skin carcinogenesis model, leading to enhanced tumor metastasis to the sentinel and distant lymph nodes (11). Angiopoietin-1, an activating ligand for the endothelial-specific receptor tyrosine kinase Tie2, induces lymphangiogenesis in the mouse cornea and, after adenoviral gene transfer, in other adult mouse tissues (23, 24). Treatment with soluble VEGF-3 inhibited the effects of virally delivered angiopoietin-1 in mice, indicating that angiopoietin-1 also acts indirectly via the VEGF-3 pathway (24). Hepatocyte growth factor was recently identified as a potent lymphangiogenesis factor, as overexpression of hepatocyte growth factor in transgenic mice and its intradermal delivery resulted in lymphatic vessel hyperplasia (25). These effects were not inhibited by a VEGF-3 blocking antibody, indicating that hepatocyte growth factor might directly promote lymphangiogenesis. Fibroblast growth factor-2 also promotes lymphatic vessel growth in the mouse cornea assay, likely by inducing VEGF-C secretion from blood vascular endothelial and perivascular cells (26, 27). In addition, recent studies indicate that platelet derived growth factor-BB and insulin-like growth factors 1 and 2 might also induce lymphangiogenesis (28, 29). Overall, the potential effects of most of these newly identified lymphatic growth factors (Fig. 1A) on tumor-induced lymphangiogenesis and lymph node metastasis remain to be investigated.

Therapeutic Targeting of the VEGFR-3 Pathway

Several studies have investigated whether specific inhibition of the VEGF-3 pathway (Fig. 1B) might inhibit cancer metastasis to lymph nodes and beyond. Inhibition of VEGF-3 activity by a blocking anti-VEGFR-3 antibody reduced the incidence of lymph node and organ metastasis in a mouse breast carcinoma model (30). Moreover, a specific blocking antibody to VEGF-D inhibited tumor lymphangiogenesis and lymphatic metastasis of VEGF-D-dependent mouse tumors (6). Furthermore, inhibition of tumor cell VEGF-C expression by stably transfected small interfering RNA reduced lymphangiogenesis, lymph node, and lung metastasis of murine mammary cancers (31). As an alternative approach, soluble VEGF-3 fusion protein ("VEGF-C/-D-trap") has been shown to inhibit VEGF-C-induced tumor lymphangiogenesis and metastasis spread in a breast cancer xenotransplant model (32). Overexpression of soluble VEGF-3 by lung cancer cells also reduced the number of intratumoral lymphatic vessels and the incidence of metastasis to the draining lymph nodes (13), and ectopic overexpression of soluble VEGF-3 in an immunocompetent rat mammary tumor model suppressed metastasis formation in lymph nodes and lungs (33). A number of small molecule kinase inhibitors of VEGFR-2 have been found to also inhibit VEGFR-3 signal transduction, including SU11248, AZD2171, BAY 43-9006, PTK/ZK, MAZ51, and CEP-7055. These small molecular compounds also affect, however, a number of other tyrosine kinases, and their distinct effects on tumor lymphangiogenesis in vivo remain to be investigated. More recently, a gene therapy approach using recombinant adenovirus expressing soluble VEGF-3 resulted in blockade of lymph node metastasis in a melanoma model in mice (34). Together, these findings indicate that blockade of the VEGF-3 pathway efficiently inhibits lymph node metastasis and likely also reduces the incidence of distant organ metastases.

Clinical-Translational Perspectives

Tumor-associated lymphangiogenesis has now been firmly established as a novel mechanism for cancer progression, and an impressive amount of preclinical data indicate that blockade of the VEGF-3 pathway inhibits tumor spread to lymph nodes and likely beyond. Moreover, therapeutic tools have been developed to inhibit lymphatic vessel growth also in humans (35). Several questions remain unanswered, however, regarding the potential clinical use of lymphangiogenesis inhibitors in human cancer patients. It remains unknown whether blockade of lymphatic vessel growth will be as efficient in human cancers as it has been found to be in mouse tumor models, and whether chronic blockade of the VEGF-3 pathway might result in unwanted adverse effects, in particular regarding hematopoiesis. Which patients might benefit from anti-lymphangiogenic therapies, considering that primary cancers are usually completely removed by surgery? It is conceivable that treatment of patients with tumors that are not suited for complete surgical removal, such as some types of head and neck cancers, might reduce the incidence of lymph node and organ metastases. Moreover, blockade of tumor-induced lymphatic vessel growth within metastatic lymph nodes might prevent further cancer spread to distant organs. Because VEGF-C and VEGF-D are also able to activate VEGFR-2, and because VEGF-A has been shown to promote tumor lymphangiogenesis, it is tempting to speculate that combined inhibition of VEGFR-2 and VEGFR-3, or of VEGF-A and VEGF-C, might result in an even more potent blockade of tumor-induced lymphatic vessel growth. Indeed, a combination of both anti-VEGFR-2 and anti-VEGFR-3 blocking antibodies was shown to be more efficient in reducing experimental lymph node and distant breast cancer metastasis than each antibody alone (30) and will be of interest to see whether a recently developed bispecific antibody against VEGFR-2 and VEGFR-3 will also show enhanced activity in vivo (36). Because several antiangiogenic drugs have been approved (or are in late-stage clinical tests) for cancer therapy, such as the VEGF-blocking antibody bevacizumab (37) and several small molecule kinase inhibitors, it will be important to determine whether some of these drugs might also be able to reduce tumor lymphangiogenesis. Finally, because recent evidence indicates that VEGFR-3 might also be expressed by some types of cancer cells (38), therapies designed to block lymphatic vessel growth might also directly interfere with the autocrine stimulation of tumor growth.
References


Pathways Targeting Tumor Lymphangiogenesis

Christoph Wissmann and Michael Detmar


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/23/6865

Cited articles
This article cites 37 articles, 25 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/23/6865.full#ref-list-1

Citing articles
This article has been cited by 16 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/12/23/6865.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.