Lymphangiogenesis in Development and Human Disease

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Angiogenesis and permeability of blood vessels are regulated by vascular endothelial growth factor (VEGF) via its two receptors VEGFR-1 and VEGFR-2. A decade ago we discovered VEGFR-3 that did not bind VEGF; its expression became restricted mainly to lymphatic endothelia during development (1). This marker allowed us to isolate and culture lymphatic endothelial cells and to show that the Prox1 transcription factor regulates lymphatic endothelial specific genes (2,3). We generated homozygous VEGFR-3 targeted mice which died around midgestation due to failure of cardiovascular development, suggesting a blood vascular function for VEGFR-3 (4). However, inhibition of VEGFR-3 signal transduction later during development did not affect the blood vessels, while it led to regression of the growing lymphatic vessels by endothelial cell apoptosis (5). We also purified and cloned the VEGFR-3 ligand, VEGF-C (6). Transgenic mice expressing VEGF-C or its VEGFR-3 specific mutant showed evidence of lymphangiogenesis and VEGF-C knockout mice had defective lymphatic vessels (7, 8, 9). The proteolytically processed form of VEGF-C bound also to VEGFR-2 and was angiogenic (10). VEGF-D which is closely related to VEGF-C, was similarly processed and bound to the same receptors (11). Thus VEGF-C and VEGF-D appear to be both angiogenic and lymphangiogenic growth factors (12).

Another step in the angiogenic pathway involves the angiopoietins (Ang:s). Ang-1 activates the Tie-2 receptor of endothelial cells, while the related Ang-2 is involved in the destabilization of blood vessels. We recently reported that Ang-1 stimulates also the related "orphan" Tie-1 receptor (13, 14) and that it can induce lymphatic vessel sprouting and hyperplasia (15). Lymphangiogenic factors, such as Ang-1 or fibroblast growth factor (FGF) act at least partially via VEGF-C/D induction (15, 16). Further maturation of the lymphatic vessels involves the FoxC2 transcription factor that inhibits smooth muscle cell recruitment and promotes the development of valves (17). FoxC2 mutations and heterozygous missense point mutations inactivating VEGFR-3 tyrosine kinase function (18), have been associated with human lymphedema. In a lymphedema mouse model with VEGFR-3 mutation, VEGF-C gene therapy restored functional lymphatic vessels to the treated skin (19, 20), suggesting that gene therapy could be tried also in human lymphedema. Other conditions where the VEGF-C/D/VEGFR-3 system can be targeted with clinical benefit include inflammatory diseases and cancer (21, 22).

In human tumors, VEGF-C/D expression correlates with vascular invasion, lymphatic vessel and lymph node involvement, distant metastasis, and, in some instances, poor clinical outcomes. Also studies of various tumor models have shown that VEGF-C and VEGF-D overexpression can enhance lymphatic metastasis, while a soluble VEGFR-3 fusion protein ("VEGF-C/D Trap") inhibited lymphatic metastasis (23,24). In some models lymphatic, but not lung metastases were blocked with a VEGF-C/D trap while in others the treatment inhibited both lymph node and lung metastases. Although these experiments provide strong support for the involvement of VEGF-C, VEGF-D, and their receptor VEGFR-3 in the lymphatic spread of malignancy, the underlying mechanisms have only recently been addressed.

Lymph vessel proliferation seen in tumor models overexpressing the lymphangiogenic factors may not be a prominent feature in several human cancers, and may in fact not be needed for enhanced metastasis in most solid tumors. While intratumoral lymphatic vessels have been detected in some solid tumors, such as melanomas and head and neck carcinomas, at least in experimental tumors they may not be completely functional, because of their collapse in conditions of high intratumoral pressure. On the other hand, the pressure gradient and lymph vessels at the tumor margin may be more important in spreading tumor cells through the process of vessel sprouting stimulated by tumor-secreted VEGF-C or VEGF-D. In this process the endothelial cells send long filopodia towards the growth factor producing tumor cells and then form tumor-directed vessel sprouts, where the vessel lumen opens up and may allow facilitated access of tumor cells to the lumen (25). Furthermore, the collecting lymphatic vessels draining fluid from the tumor area are stimulated to dilate by intraluminal VEGF-C via the process of endothelial proliferation in the vessel wall (25). Clumps of metastatic tumor cells could then undergo an easier transit in lymph flowing in the dilated hyperplastic vessels. The VEGF-C/D trap inhibited sprouting and vessel dilation, and seemed to restore the integrity of the vessel wall (25). Similarly, blocking monoclonal antibodies that target VEGF-C, VEGF-D or their receptor(s) and small molecules that inhibit the tyrosine kinase catalytic domain of these receptors could be used for the inhibition of experimental tumor metastasis. Further work should soon tell if these same molecules inhibit further systemic metastasis or angiogenesis in tumor models.


10. Achen, M.G., Jeltsch, M., Kukk, E., Mäkinen, T., Vitali, A., Wilks, A.F., Alitalo, K. and Stacker, S.A.: Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor-2 (Flk1) and VEGF receptor 3 (Flt3). *Proc Natl Acad Sci.* 95:548-553, 1998.


