The field of lymphangiogenesis has made rapid and exciting developments over the last few years more so than any other field in vascular biology. Progress in this field has been long awaited because a number of clinical disorders are associated with impaired lymphatic function. The lymphatic vasculature takes up fluid from the interstitial space, thereby maintaining interstitial fluid balance and providing lymphatic clearance of macromolecules. Abnormal development of lymphatic vessels can give rise to lymphatic malformations, large cystic structures in which lymphatic fluid accumulates. Mutations affecting the vascular endothelial growth factor receptor (VEGFR)-3, or the transcription factor forkhead box C2 (FOXCl2), have been implicated in primary congenital lymphedema. Insufficient function of lymphatic vessels and lymphedema can also result from chronic inflammation, infection, or trauma. In addition to its function in fluid drainage, the lymphatic system is also an important route for circulating immune cells that function in fluid accumulation. Mutations affecting the vascular endothelial growth factor receptor (VEGFR)-3, or the transcription factor forkhead box C2 (FOXCl2), have been implicated in primary congenital lymphedema. Insufficient function of lymphatic vessels and lymphedema can also result from chronic inflammation, infection, or trauma. In addition to its function in fluid drainage, the lymphatic system is also an important route for circulating immune cells that function in fluid accumulation. Therefore, there are a number of clinical applications for therapeutics that either inhibit or induce lymphangiogenesis.

Until recently, the molecular mechanisms of lymphangiogenesis were largely unknown, mainly because molecular markers for lymphatic endothelium were lacking. However, several molecules that are expressed preferentially in lymphatic endothelium have recently been identified, including the VEGFR-3,1 the homeobox gene Prox-1,3 the hyaluronan receptor LYVE-1,4 and the mucin-type transmembrane glycoprotein podoplanin.5 Specific antibodies allowed the isolation and characterization of lymphatic endothelial cells.6 Forced expression of Prox1 in blood vessel endothelial cells caused upregulation of lymphatic markers such as Podoplanin or VEGFR-3, indicating that Prox1 is a master control gene in the program specifying lymphatic endothelial cell fate.6,7

Florence Sabin proposed in 1902 that the lymphatic vasculature forms by budding of lymph sacs at the anterior cardinal vein, and subsequent sprouting.8 Current molecular evidence, based primarily on the analysis of genetically altered mice, supports this concept. Polarized expression of the homeodomain transcription factor Prox1 in a subpopulation of endothelial cells in the anterior cardinal vein precedes budding of the lymph sac and sprouting of lymphatic vessels.9 This process also requires the activation of VEGFR-3 by VEGF-C,9 in VEGF-C deficient mice, endothelial cells become committed to the lymphatic lineage but do not sprout to form lymph vessels. Consequently, VEGF-C null mouse embryos die prenatally because of fluid accumulation in tissues.

Various other factors have been implicated in lymphatic development. Podoplanin is first detected in mouse embryos in Prox1-positive cells. Podoplanin deficient mice die at birth because of respiratory failure and have defects in lymphatic vessel pattern formation, associated with diminished lymphatic transport, congenital lymphedema and dilation of lymphatic vessels.5 Considering the developmental relationship between lymphatic endothelium and blood vessel endothelium, it is not surprising that several growth factors that have previously been implicated in cardiovascular development are also involved in the formation of the lymphatic vasculature. Recent examples include the angiopoietins (Ang) which collaborate with VEGF during blood vessel development and adult angiogenesis. Ang-1 is associated with blood vessel stabilization and recruitment of perivascular cells, while Ang-2 destabilizes vessels during postnatal angiogenesis. Analysis of knockout mice has shown that Ang-2 is also required for lymphatic patterning, and this role can be rescued by Ang-1.10 Ang-1 stimulated lymphatic endothelial cell proliferation, vessel enlargement and the generation of new sprouts.11 Finally, even VEGF-A which has been considered specific for blood vessel endothelium, may promote lymphangiogenesis in skin wounds.12

Among the various growth factors involved in lymphangiogenesis, the functions of VEGF-C and VEGF-D in the pathology of lymphatic vessels are best studied. Both factors are produced as proforms that bind to and activate VEGFR-3. The processed forms of VEGF-C and VEGF-D also bind VEGFR-2, the primary signaling VEGF-A receptor. Several lines of evidence suggest that VEGF-C is a potent inducer of lymphangiogenesis in tumors.13 However, peritumoral lymph vessels induced by VEGF-C exhibit abnormal function.14 VEGF-C overexpression in experimental tumors promoted breast cancer metastasis.15 Similarly, VEGF-D also induced the metastatic spread of tumor cells via the lymphatics.16

The growth induction of new lymphatics in vivo can be beneficial in conditions of lymphedema. Several studies have shown that VEGF-C by itself can induce the growth of new functional lymphatic vessels in the adult organism. Adenoviral expression of VEGF-C induced lymphangiogenesis in the
in a rabbit ear model to simulate human chronic post surgical lymphatic insufficiency, therapeutic lymphangiogenesis with recombinant VEGF-C ameliorated lymphatic function, and significantly increased lymphatic vascularity.\(^{17}\) Yoon et al found that VEGF-C gene therapy both in the rabbit ear and in regenerating tail augmented postnatal lymphangiogenesis and ameliorated secondary lymphedema.\(^{18}\) In an epigastric skin flap model, adenovirus-mediated VEGF-C gene therapy restored lymphatic flow across incision wounds.\(^{20}\) The VEGF-C treated mice showed persistent lymphatic function even 2 months postoperatively although a number of the lymphatic vessels regressed after the cessation of adenoviral gene expression. Taken together, the results of these studies suggest that VEGF-C has potential for therapeutic lymphangiogenesis in diseases lacking adequate lymphatic drainage.

In this issue of Circulation Research, however, Goldman et al report that the application of VEGF-C did not increase lymphangiogenesis in a mouse model of skin regeneration, although it induced transient lymphatic vessel hyperplasia.\(^{21}\) The authors implanted a suspension of VEGF-C overexpressing breast tumor cells, embedded in a collagen scaffold, as source for the continuous supply of the growth factor. These cells only transiently increased the density of regenerating lymphatic vessels after 10-17 days, whereas at later time points, when VEGF-C levels diminished, hyperplasia disappeared and the number of lymphatic endothelial cells, their spatial distribution, and the density of lymphatic vessels were similar to control groups. This result is unexpected because the same VEGF-C overexpressing cells, when grown as tumors in mice, potently increased intra- and peri-tumoral lymphangiogenesis, resulting in significantly enhanced metastasis to regional lymph nodes and to lungs.\(^{15}\) However, the discrepancy might be explained by differences between the microenvironment of a solid tumor (which would otherwise remain devoid of lymphatic vessels) and that of regenerating skin (in which physiological lymphangiogenesis occurs also in the absence of exogenous factors).\(^{21}\)

The observations by Goldman et al\(^{21}\) seem, at first sight, to contradict the study by Yoon et al who reported that local VEGF-C gene transfer augmented lymphangiogenesis in a similar model up to 3 weeks.\(^{19}\) However, Goldman et al, too, observed lymphatic endothelial cell hyperplasia at similar time points (10-25 days). By studying later time points, they made the important observation that lymphatic hyperplasia in their model was only transient.

Interestingly, Goldman et al found that control transfected tumor cells and VEGF-C overexpressing tumor cells enhanced lymphatic fluid channel formation in regenerating skin to a similar extent.\(^{21}\) In line with this observation, excess VEGF-C did not increase lymphatic endothelial cell migration through fibrin (or chemoindvasion) in vitro. Thus, the control transfected tumor cells used in their study produced all growth factors and proteases necessary for lymphatic endothelial cell migration and fluid channel formation, and increased VEGF-C levels did not enhance these processes. Which factors are involved? There is no doubt that VEGF-C which is expressed at low levels in regenerating tissue in the control groups, is a key player in this process, because VEGFR-3 inhibition prevented lymphatic regeneration in the same model.\(^{22}\) However, the results of Goldman et al suggest that additional factors would be necessary to sustain lasting effects on lymphangiogenesis in regenerating adult skin.

The observation that VEGF-C by itself can induce functional lymphatics in several, but not all, experimental models might simply reflect the fact that VEGF-C acts in concert with other factors required for physiological lymphangiogenesis, which, depending on the experimental setting, may or may not be present in sufficient amounts to complement VEGF-C activity. Thus, it might turn out that certain aspects of the lesson that we learned from therapeutic blood vessel angiogenesis will apply also to therapeutic lymphangiogenesis. Initial studies involving VEGF-A delivery were encouraging but soon followed by the insight that VEGF-A application alone is either not sufficient or may have side effects, such as vascular hyperpermeability.\(^{22}\) Although VEGF-A can induce angiogenesis in adult tissue, its needs to acts in concert with several other factors, including angiopoietins and certain ephrins, to generate functional new blood vessels, a problem that has not yet been solved in therapy. Whether VEGF-C can induce the growth of new functional lymphatic vessels in vivo by itself, or whether additional factors such as Ang-1 are also required,\(^{11}\) will most likely depend on the specific condition, the microenvironment, and the mode of growth factor application. As always, the answer to the question, “Which treatment is effective in patients?” will eventually come from clinical trials.

References


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