Lymphangiogenesis and Lymphangiogenic Growth Factors

Pauliina Hartiala, MD, PhD[1,2] Anne M. Saarikko, MD, PhD[1,3]

[1] Department of Plastic and General Surgery, Turku University Central Hospital, Turku, Finland
[2] Department of Medical Microbiology and Immunology, University of Turku, Finland
[3] Cleft Unit, Department of Plastic Surgery, Helsinki University Central Hospital, Helsinki, Finland

Address for correspondence Pauliina Hartiala, MD, PhD, Department of Plastic and General Surgery, Turku University Central Hospital, PL 52, Kiinamyllynkatu 4-8, 20251, Finland (e-mail: pauliina.hartiala@utu.fi).

J Reconstr Microsurg 2016;32:10–15.

Abstract

Lymphedema is a progressive disease caused by damage to the lymphatic network. Recent development in the fields of preclinical growth factor research and lymphedema microsurgery promise new hope for lymphedema patients. In this article, we review the latest results on basic research and highlight the role of specific growth factors in normal lymphatic development and several disease states. Lymph node transfer, a new promising method in reconstructive lymphatic microsurgery, is also dependent on the lymphatic vascular regrowth and lymphangiogenic growth factors. We discuss the scientific basis of lymph node transfer and therapeutic potential of lymphangiogenic growth factors in the treatment of lymphedema.

Keywords
- lymphangiogenesis
- lymph node transfer
- VEGF-C

Lymphatic System

The lymphatic system consists of lymph nodes, spleen, thymus, bone marrow, digestive system lymphatic tissue, and lymphatic vasculature. The main functions of the lymphatic system are production of immune cells, collection, and transportation of extravasated fluid and macromolecules from peripheral tissues, filtering lymphatic fluid, and removal of foreign material.[1] The lymphatic capillaries in the peripheral tissues merge with larger collecting lymphatic vessels, specialized for the transport of large volumes of lymph, that in turn connect with chains of lymph nodes.[1] Lymphatic vessels are typically found in all vascularized tissues, with the notable exception of bone marrow and the central nervous system.[2] From an immunological point of view, lymph nodes are strategically positioned patrol stations for antigens from peripheral tissues.[3] Naïve lymphocytes circulate through lymph nodes. When a foreign microbe enters the skin, dendritic cells (DCs) and macrophages phagocytose the invading microbes and migrate to lymph nodes via lymph vessels. To elicit an adaptive immune response, DCs must interact with antigen-specific T-cells and B-cells in the lymph node and present the foreign antigens.[3] Intruders, such as bacteria and tumor cells, have also learned to take advantage of the vast lymphatic network to facilitate their spreading and growth. Thus, lymphatic vessels and lymph nodes are involved in several human diseases, such as lymphedema, inflammation, and tumor metastasis.

Lymphedema

Malfunction of the lymphatic system leads to chronic lymphedema that is characterized by gross swelling of the affected peripheral area, commonly the limbs. As the disease progresses, edema is accompanied by fibrosis and in the late stage of the disease replaced by fibrosis and adipose tissue.[4] Lymphedema patients are susceptible to infections as a result of encouraged microbial growth due to extravasated fluid and proteins and an impaired local immune response.[5] Many patients need constant prophylactic antibiotics because of recurrent erysipelas and other soft tissue infections. Chronic neuropathic pain is also a common symptom.

received
August 25, 2014
accepted after revision
November 16, 2014
published online
February 9, 2015

DOI http://dx.doi.org/10.1055/s-0035-1544179.
ISSN 0743-684X.

Lymphedema develops after damage of the lymphatic network due to infections, surgical or radiation therapy of metastatic cancer or trauma. Rare hereditary forms of lymphedema are caused by structural defects in the lymphatic vessels.[6] A globally remarkable cause of lymphedema is filariasis, which affects more than 90 million people.[5] It has been estimated that currently several million patients suffer from acquired lymphedema in the United States alone.[7] The effective treatment of cancer often requires removal of regional lymph nodes and the associated collecting lymphatic vessels to eradicate metastases. This leads to a disruption of the lymphatic flow in the operated area, which may result in chronic lymphedema of the affected limb. Approximately 10 to 30% of breast cancer patients develop lymphedema after axillary dissection.[8–10]

The treatment of lymphedema is currently based on compression garments and physiotherapy. Occasionally surgical treatment options including liposuction,[11] lymph node transfer,[12] and lymphaticovenous or lymphaticolymphatic shunts[5,13–15] are used. These surgical treatment options are described in other review articles of this special lymphedema theme issue.

Regulation of Lymphangiogenesis

Lymphatic Vessel Differentiation during Embryogenesis

The development of lymphatic vessels begins in the embryo at midgestation, at a time when the cardiovascular system is established and fully functional. The homeobox transcription factor Prox1 is essential for the establishment of the identity of the lymphatic endothelial cells.[16] Prox1 is also important for maintenance of the lymphatic vasculature during later stages of development and in adulthood as Prox± mice develop chylous ascites, abnormal lymphatic vessels and adult-onset obesity.[17] After the lymphatic vascular differentiation process, vascular endothelial growth factor C (VEGF-C) is crucial for the further growth of the lymphatic vasculature, a process called lymphangiogenesis.[18]

Importance of VEGF-C/D for Lymphatic Vascular Growth

VEGFs are the most specific and important regulators of blood and lymph vessel growth, also termed angiogenesis and lymphangiogenesis.[19,20] They stimulate cellular responses by binding to the tyrosine kinase receptors VEGFR-1, VEGFR-2, and VEGFR-3, which are specifically expressed on blood and lymphatic endothelial cells. VEGFR activation promotes cell proliferation, migration and survival.[21] VEGF-A binds to VEGFR-1 and VEGFR-2 and induces mainly angiogenesis while VEGF-C and VEGF-D bind VEGFR-3 and induce mainly lymphangiogenesis.[2,21] By targeting VEGFR-3 in the lymphatic endothelial cells VEGF-C and VEGF-D are the most important and specific lymphatic vessel growth factors known.[22] VEGF-A seems to stimulate mainly circumferential hyperplasia of lymphatic capillaries, and only indirectly lymphangiogenesis via the recruitment of inflammatory cells.[23] Several other (unspecific) growth factors such as fibroblast growth factor-2, insulin-like growth factor 1 (IGF-1), IGF-2, hepatocyte growth factor, endothelin-1, and platelet-derived growth factor subunit B have been reported to induce lymphangiogenesis in various situations. Most of these effects are associated with the induction of VEGF-C and VEGF-D in a variety of cell types including inflammatory cells and fibroblasts.[1,24] VEGFs also have coreceptors including neuropilin (Nrp)-1 and Nrp-2, certain integrins, as well as heparan sulfate proteoglycans, which modulate the signaling of VEGF receptors and provide specificity for their signal transduction.[20,25,26]

Factors Regulating Lymphatic Vessel Valve Formation

The forkhead transcription factor Foxc2 is highly expressed in the developing lymphatic vessels and also in lymphatic valves in adults.[27] The initial development of lymphatic vasculature proceeds normally in the absence of Foxc2, but at later stages of embryogenesis lymphatic vessels are severely affected.[27] Recently, transcription factors, Prox1 and Foxc2, were shown to cooperate and control connexin37 and calcineurin during lymphatic valve development.[28] Mutation in Foxc2 causes the hereditary lymphedema distichiasis syndrome.[29] Interestingly, mutations of connexin47 have been shown to be associated with increased risk of secondary lymphedema after breast cancer treatment.[30]

Tumor-Associated Lymphangiogenesis

Lymph node metastasis is an important prognostic indicator in many tumor types.[31] Tumor cells can invade either preexisting lymphatic vessels or new lymphatic vessels formed at the tumor periphery by tumor-induced lymphangiogenesis. Many types of tumors express the lymphangiogenic growth factors VEGF-C and VEGF-D, and several studies have shown that expression of these growth factors actively induces tumor-associated lymphangiogenesis, leading to lymphatic invasion, lymph node and distant metastasis, and subsequently to poor patient survival.[32] Also, tumor-associated macrophages have been shown to express VEGF-C and VEGF-D and contribute to peritumoral lymphangiogenesis.[33] In mouse models, blockage of VEGFR3 signaling inhibits lymphatic metastases (reviewed by Alitalo et al[1]).

Lymphatic Vessel and Inflammation

Lymphatic vessels proliferate during inflammation. Macrophages have been shown to produce VEGF-C and contribute to lymphatic regeneration after tissue transfer.[34] In a mouse diabetes (db/db) model, injection of IL1-β stimulated db/db macrophages to diabetic wounds induced lymphatic vessel formation and accelerated wound healing.[35] Furthermore, newly formed lymphatic vessels expressed both macrophage marker F4/80 and lymphatic marker LYVE-1 suggesting that new lymphatic vessels were formed from F4/80 cells, namely, macrophages.[35] IL-1α, IL-1β, and TNF-α have been shown to induce VEGF protein and VEGF-C mRNAs in human lung fibroblasts.[36] IL-1β induces expression of VEGF-C at the transcriptional level.[37] High levels of lymphangiogenic factors are also produced by macrophages and granulocytes in inflamed tissue, and blocking these factors suppresses

lymphangiogenesis and reactive inflammation of the lymph node.[38] On the other hand, soft tissue infections and inflammation also play a role in the development of lymphedema, as nonsteroidal anti-inflammatory drugs have been shown to affect lymphedema development.[39] However, the exact molecular mechanisms of this process are not fully understood. Also, lymphedema patients are susceptible to infections as a result of encouraged microbial growth due to extravasated fluid, proteins, and an impaired local immune response.[5]

Lymphatic Vascular Growth after Surgical Lymph Node Transfer

Lymph node transfer, introduced by Becker, is a fairly new promising technique that aims to rebuild the lymphatic vascular anatomy after surgical treatment of metastatic cancer (see separate review by Becker in this JRM issue). However, lymphatic anastomoses in this technique are expected to form spontaneously. Thus, the outcome of this treatment relies on the spontaneous lymphatic regrowth (lymphangiogenesis) in the axillary area. Patel et al documented the integration of distally placed lymph node flaps using indocyanine green (ICG) lymphography and showed ICG uptake in all the flaps as a sign of integration.[40] Interestingly, we have recently shown, that human lymph nodes express VEGF-C, which provides a scientific explanation for this technique.[41] The therapeutic effect of this surgical method has been tested in different experimental lymphedema animal models and recently also in human lymphedema patients.[12,41–43] In patients, lymphatic tissue can be harvested as a vascularized free flap from the lower abdominal wall and transferred into the axilla (see ►**Fig. 1A**). All clinical studies have also shown an improvement of skin infections (erysipelas, lymphangitis, and cellulitis) after lymph node transfer.

As we are operating on patients that have previously developed lymphedema symptoms in their upper extremities we need to be extremely careful with the donor site morbidity. Harvesting lymph nodes from the lower abdominal wall seems to induce seroma formation[12,42] but so far there are no reports on lymphedema symptoms of the lower limb after the lymph node transfer surgery. In our patient material postoperative lymphoscintigraphy revealed minor subclinical changes in the lymphatic flow of the donor site limbs in 6 out of 10 patients. However, none of the patients had changes in their lower limb circumferences during the 8- to 56-month follow-up.[44]

Lymphangiogenic Therapy

In the lymph node transfer technique the lymphatic vascular anastomoses are expected to form spontaneously. Clinical and experimental data however suggests that even though spontaneous lymphangiogenesis after lymph node transfer does occur, the incorporation of the transferred lymph nodes into the existing lymphatic network may fail.[12,42,45,46] This poor incorporation efficiency may compromise the outcome of the operation, because connection with lymphatic vessels is required for maintenance and function of the lymph nodes.[46,47]

Results from the preclinical lymphedema models employing VEGF-C or VEGF-D gene transfer[48–52] or application of recombinant protein[53,54] have demonstrated the potential and specificity of these factors for inducing growth of new lymphatic vessels. The short half-life of the VEGF proteins favors the use of transient gene transfer as a mode of treatment.[55] First experimental lymphedema animal models focused on regrowth of new lymphatic capillary vessels in response to lymphatic growth factor therapy.[50–52] Together with other groups we have more recently shown that also the collecting lymphatic vessels can be rebuilt with the VEGF-C/D therapy.[45,46,54] Adenoviral VEGF-C or VEGF-D gene transfer results into transient lymphatic vessel growth factor overexpression in the targeted area.[45,46,50] During the first 2 weeks robust growth of lymphatic vessels can be detected but after 2 weeks, as the adenoviral growth factor expression is downregulated, the lymphatic vessel network regresses.[50] However, the newly formed vessels that have lymphatic flow seem to stabilize and maturate into collecting lymphatic vessels spontaneously over the course of 6 months.[46] Thus, the growth factor stimulated vessels seem to engage an intrinsic differentiation and maturation program into collecting lymphatic vessels.

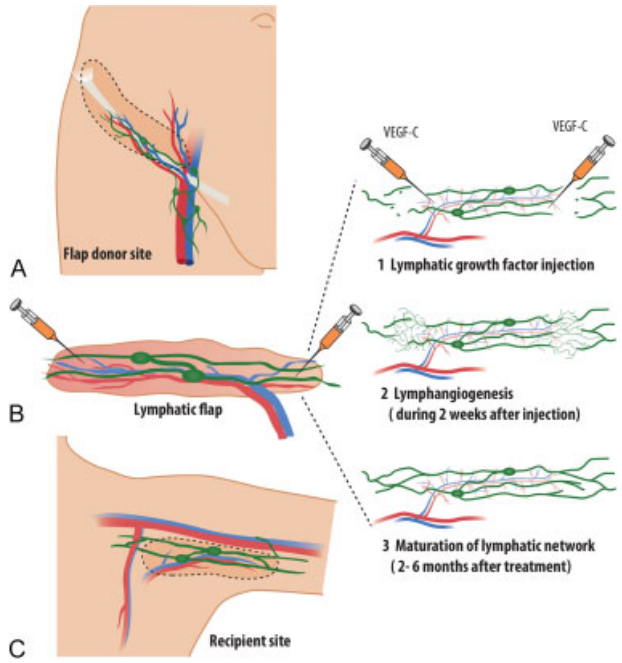

Fig. 1 Future vision on how to reconstruct the lymphatic vascular anatomy of the axilla after axillary lymph node dissection and oncologic cancer treatment. (A) Lymphatic tissue from the lower abdominal wall can be harvested as a vascularized free flap. (B) Adenoviral vector encoding vascular endothelial growth factor C (VEGF-C) is injected into the distal edges of the lymphatic flap to enhance lymphangiogenesis and further lymphatic network maturation. (C) VEGF-C treated lymphatic flap is placed into the axilla to replace the removed scar tissue.

Combining Lymphangiogenic Growth Factor Treatment with Lymph Node Transfer

Lymph nodes need lymphatic flow to remain functional.[45–47] Without connections with the afferent and efferent lymphatic vessels lymph nodes will regress.[47] The idea of combining growth factor treatment with the lymph node transfer as a

lymphedema treatment was first presented by Tammela et al.[46] The results of this study showed that the sentinel node function of transferred lymph nodes was regained and intracutaneously injected lung carcinoma cells were trapped in the transferred lymph node.[46] Previously it has been shown that transplanted lymph nodes retain the ability to mount a cytotoxic immune response against tumor cells and that they recover a normal architecture.[46,47,56,57]

More recently the efficacy of the VEGF-C and -D growth factor therapies and lymph node transfer was also tested in a lymphedema large animal model.[45,54,58] Postoperative lymphatic drainage was significantly improved in the VEGF-C/D–treated animals compared with controls. Importantly, the structure of the transferred lymph nodes was best preserved in the VEGF-C–treated animals.[45,58] Control-treated lymph nodes regressed and their follicular structure was replaced by adipose and fibrotic tissue. This is in concordance with previous data, which indicate that the lymph nodes need lymphatic vasculature and exposure to lymph flow to remain intact.[46,47] Perinodal VEGF-C injection induces less inflammatory macrophage deposition and lymph node lymphangiogenesis compared with intranodal VEGF-C.[58]

Future Perspectives and Conclusions

Approximately, 200,000 new cases of breast cancer are diagnosed annually in the United States alone. Besides breast cancer, many other cancer types spread via the lymphatic vessels necessitating regional surgery of the lymph nodes and making a large patient population susceptible to the development of lymphedema. Recent data suggests that some of the patients on whom we operate may be genetically predisposed to develop lymphedema symptoms after any surgical operation—cancer operation or microvascular flap operation.[30]

Lymph node transfer is a new technique in lymphedema surgery. In our patient material, 10 to 20% of all postmastectomy patients who ask for breast reconstruction also have lymphedema symptoms of the upper arm. The fact that lymph node transfer can easily be performed in combination with routine microvascular breast reconstruction has made this method attractive.[12,42,59] However, the lack of randomized clinical trials and the methodological variance in the published studies still leave open questions about the efficacy of the lymph node transfer surgery.[60] To truly show the efficacy of this new technique we need results from large clinical studies and different units. According to our own experience, not all patients seem to benefit from the lymph node transfer surgery. This might be partly explained by the differences in lymph node VEGF-C expression. Recent findings from the experimental animal models clearly favor the use of growth factors in conjunction with lymph node transfer to augment incorporation of the grafted lymph node into the resident lymphatic vascular tree.[45,46,58] Vectors inducing short-term overexpression of the patients' own endogenous lymphatic growth factors in the distal edge of the lymphatic tissue flap could be used to enhance the therapeutic effect of lymph node transfer technique (►**Fig. 1**).

VEGF-C and -D are also involved in lymphatic metastasis of several human tumors and therefore patient safety is an important issue. However, human lymph nodes also produce endogenous VEGF-C and lymph node transfer is already used in cancer patients.[42] In experimental models newly formed lymphatic vessels seem to stabilize after the short-term (1 week) VEGF-C overexpression.[45,46,58] Thus, long lasting lymphatic growth factor therapy is not needed.

Disclosures
Anne Saarikko is a member of the board of directors of OY Lx Therapies Ltd.

Funding Sources
This study was supported by the Academy of Finland, the Turku University Foundation, Special Governmental Funding (EVO) allocated to Turku University Central Hospital, the Paavo and Eila Salonen Foundation, the Ida Montini foundation, the Aarne and Aili Turunen foundation, the Emil Aaltonen foundation and the TYKS Foundation.

References

1 Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. Nature 2005;438(7070):946–953
2 Tammela T, Alitalo K. Lymphangiogenesis: Molecular mechanisms and future promise. Cell 2010;140(4):460–476
3 Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767–811
4 Warren AG, Brorson H, Borud LJ, Slavin SA. Lymphedema: a comprehensive review. Ann Plast Surg 2007;59(4):464–472
5 Rockson SG. Lymphedema. Am J Med 2001;110(4):288–295
6 Ferrell RE, Finegold DN. Research perspectives in inherited lymphatic disease: an update. Ann N Y Acad Sci 2008; 1131:134–139
7 Tabibiazar R, Cheung L, Han J, et al. Inflammatory manifestations of experimental lymphatic insufficiency. PLoS Med 2006;3(7): e254
8 Gross DM, Forsthuber T, Tary-Lehmann M, et al. Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. Science 1998;281(5377):703–706
9 Clas F, Loos M. Antibody-independent binding of the first component of complement (C1) and its subcomponent C1q to the S and R forms of Salmonella minnesota. Infect Immun 1981;31(3): 1138–1144
10 Barthold SW, de Souza M. Exacerbation of Lyme arthritis in beige mice. J Infect Dis 1995;172(3):778–784
11 Suami H, Chang DW. Overview of surgical treatments for breast cancer-related lymphedema. Plast Reconstr Surg 2010;126(6): 1853–1863
12 Becker C, Assouad J, Riquet M, Hidden G. Postmastectomy lymphedema: long-term results following microsurgical lymph node transplantation. Ann Surg 2006;243(3):313–315
13 Baumeister RG, Seifert J, Hahn D. Autotransplantation of lymphatic vessels. Lancet 1981;1(8212):147
14 Chang DW. Lymphaticovenular bypass for lymphedema management in breast cancer patients: a prospective study. Plast Reconstr Surg 2010;126(3):752–758
15 Nagase T, Gonda K, Inoue K, et al. Treatment of lymphedema with lymphaticovenular anastomoses. Int J Clin Oncol 2005;10(5): 304–310

16 Wigle JT, Harvey N, Detmar M, et al. An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype. EMBO J 2002;21(7):1505–1513
17 Harvey NL, Srinivasan RS, Dillard ME, et al. Lymphatic vascular defects promoted by Prox1 haploinsufficiency cause adult-onset obesity. Nat Genet 2005;37(10):1072–1081
18 Karkkainen MJ, Haiko P, Sainio K, et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol 2004;5(1):74–80
19 Oliver G. Lymphatic vasculature development. Nat Rev Immunol 2004;4(1):35–45
20 Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol 2009;21(2):154–165
21 Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. Nat Rev Mol Cell Biol 2006;7(5):359–371
22 Tervala T, Suominen E, Saaristo A. Targeted treatment for lymphedema and lymphatic metastasis. Ann N Y Acad Sci 2008; 1131:215–224
23 Wirzenius M, Tammela T, Uutela M, et al. Distinct vascular endothelial growth factor signals for lymphatic vessel enlargement and sprouting. J Exp Med 2007;204(6):1431–1440
24 Karpanen T, Bry M, Ollila HM, et al. Overexpression of vascular endothelial growth factor-B in mouse heart alters cardiac lipid metabolism and induces myocardial hypertrophy. Circ Res 2008; 103(9):1018–1026
25 Caunt M, Mak J, Liang WC, et al. Blocking neuropilin-2 function inhibits tumor cell metastasis. Cancer Cell 2008;13(4):331–342
26 Iozzo RV, San Antonio JD. Heparan sulfate proteoglycans: heavy hitters in the angiogenesis arena. J Clin Invest 2001;108(3): 349–355
27 Petrova TV, Karpanen T, Norrmén C, et al. Defective valves and abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis. Nat Med 2004;10(9): 974–981
28 Sabine A, Agalarov Y, Maby-El Hajjami H, et al. Mechanotransduction, PROX1, and FOXC2 cooperate to control connexin37 and calcineurin during lymphatic-valve formation. Dev Cell 2012; 22(2):430–445
29 Fang J, Dagenais SL, Erickson RP, et al. Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. Am J Hum Genet 2000;67(6):1382–1388
30 Finegold DN, Baty CJ, Knickelbein KZ, et al. Connexin 47 mutations increase risk for secondary lymphedema following breast cancer treatment. Clin Cancer Res 2012;18(8):2382–2390
31 Sleeman JP, Thiele W. Tumor metastasis and the lymphatic vasculature. Int J Cancer 2009;125(12):2747–2756
32 Holopainen T, Bry M, Alitalo K, Saaristo A. Perspectives on lymphangiogenesis and angiogenesis in cancer. J Surg Oncol 2011; 103(6):484–488
33 Schoppmann SF, Birner P, Stöckl J, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. Am J Pathol 2002; 161(3):947–956
34 Yan A, Avraham T, Zampell JC, Aschen SZ, Mehrara BJ. Mechanisms of lymphatic regeneration after tissue transfer. PLoS ONE 2011; 6(2):e17201
35 Maruyama K, Asai J, Ii M, Thorne T, Losordo DW, D'Amore PA. Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. Am J Pathol 2007;170(4):1178–1191
36 Ristimäki A, Narko K, Enholm B, Joukov V, Alitalo K. Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-C. J Biol Chem 1998; 273(14):8413–8418
37 Li J, Perrella MA, Tsai JC, et al. Induction of vascular endothelial growth factor gene expression by interleukin-1 beta in rat aortic smooth muscle cells. J Biol Chem 1995;270(1):308–312
38 Baluk P, Tammela T, Ator E, et al. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. J Clin Invest 2005;115(2):247–257
39 Nakamura K, Radhakrishnan K, Wong YM, Rockson SG. Anti-inflammatory pharmacotherapy with ketoprofen ameliorates experimental lymphatic vascular insufficiency in mice. PLoS ONE 2009;4(12):e8380
40 Patel KM, Lin CY, Cheng MH. From Theory to Evidence: Long-Term Evaluation of the Mechanism of Action and Flap Integration of Distal Vascularized Lymph Node Transfers. J Reconstr Microsurg 2014
41 Lin CH, Ali R, Chen SC, et al. Vascularized groin lymph node transfer using the wrist as a recipient site for management of postmastectomy upper extremity lymphedema. Plast Reconstr Surg 2009; 123(4):1265–1275
42 Saaristo AM, Niemi TS, Viitanen TP, Tervala TV, Hartiala P, Suominen EA. Microvascular breast reconstruction and lymph node transfer for postmastectomy lymphedema patients. Ann Surg 2012;255(3):468–473
43 Hartiala P, Saaristo AM. Growth factor therapy and autologous lymph node transfer in lymphedema. Trends Cardiovasc Med 2010;20(8):249–253
44 Viitanen TP, Mäki MT, Seppänen MP, Suominen EA, Saaristo AM. Donor-site lymphatic function after microvascular lymph node transfer. Plast Reconstr Surg 2012;130(6):1246–1253
45 Lähteenvuo M, Honkonen K, Tervala T, et al. Growth factor therapy and autologous lymph node transfer in lymphedema. Circulation 2011;123(6):613–620
46 Tammela T, Saaristo A, Holopainen T, et al. Therapeutic differentiation and maturation of lymphatic vessels after lymph node dissection and transplantation. Nat Med 2007;13(12):1458–1466
47 Mebius RE, Streeter PR, Brevé J, Duijvestijn AM, Kraal G. The influence of afferent lymphatic vessel interruption on vascular addressin expression. J Cell Biol 1991;115(1):85–95
48 Enholm B, Paavonen K, Ristimäki A, et al. Comparison of VEGF, VEGF-B, VEGF-C and Ang-1 mRNA regulation by serum, growth factors, oncoproteins and hypoxia. Oncogene 1997;14(20): 2475–2483
49 Rissanen TT, Markkanen JE, Gruchala M, et al. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. Circ Res 2003; 92(10):1098–1106
50 Saaristo A, Veikkola T, Tammela T, et al. Lymphangiogenic gene therapy with minimal blood vascular side effects. J Exp Med 2002; 196(6):719–730
51 Yoon YS, Murayama T, Gravereaux E, et al. VEGF-C gene therapy augments postnatal lymphangiogenesis and ameliorates secondary lymphedema. J Clin Invest 2003;111(5):717–725
52 Karkkainen MJ, Saaristo A, Jussila L, et al. A model for gene therapy of human hereditary lymphedema. Proc Natl Acad Sci U S A 2001; 98(22):12677–12682
53 Szuba A, Cooke JP, Yousuf S, Rockson SG. Decongestive lymphatic therapy for patients with cancer-related or primary lymphedema. Am J Med 2000;109(4):296–300
54 Baker A, Kim H, Semple JL, et al. Experimental assessment of prolymphangiogenic growth factors in the treatment of post-surgical lymphedema following lymphadenectomy. Breast Cancer Res 2010;12(5):R70
55 Veikkola T, Jussila L, Makinen T, et al. Signalling via vascular endothelial growth factor receptor-3 is sufficient for lymphangiogenesis in transgenic mice. EMBO J 2001;20(6):1223–1231
56 Rabson JA, Geyer SJ, Levine G, Swartz WM, Futrell JW. Tumor immunity in rat lymph nodes following transplantation. Ann Surg 1982;196(1):92–99

57 Fu K, Izquierdo R, Vandevender D, Warpeha RL, Fareed J. Transplantation of lymph node fragments in a rabbit ear lymphedema model: a new method for restoring the lymphatic pathway. Plast Reconstr Surg 1998;101(1):134–141
58 Honkonen KM, Visuri MT, Tervala TV, et al. Lymph node transfer and perinodal lymphatic growth factor treatment for lymphedema. Ann Surg 2013;257(5):961–967
59 Viitanen TP, Visuri MT, Hartiala P, et al. Lymphatic vessel function and lymphatic growth factor secretion after microvascular lymph node transfer in lymphedema patients. Plast Reconstr Surg Glob Open 2013;1(2):1–9
60 Penha TR, Ijsbrandy C, Hendrix NA, et al. Microsurgical techniques for the treatment of breast cancer-related lymphedema: a systematic review. J Reconstr Microsurg 2013;29(2):99–106