2017

Cardiac Lymphatics - A New Avenue for Therapeutics?

Vuorio Taina

Elsevier BV

Tieteelliset aikakauslehtiartikkelit
© Elsevier Ltd
CC BY-NC-ND https://creativecommons.org/licenses/by-nc-nd/4.0/
http://dx.doi.org/10.1016/j.tem.2016.12.002

https://erepo.uef.fi/handle/123456789/7914

Downloaded from University of Eastern Finland's eRepository
Cardiac lymphatics, a new avenue for therapeutics?

Taina Vuorio¹, Annakaisa Tirronen¹, Seppo Ylä-Herttuala¹²

¹Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, P.O. Box 1627, FIN-70211 Kuopio, Finland; ²Kuopio University Hospital, Science Service Center and Gene Therapy Unit, P.O. Box 1777, FIN-70211 Kuopio, Finland


Correspondence to S.Y-H. Tel. +358 403552075, seppo.ylaherttuala@uef.fi.

Keywords
atherosclerosis, cardiac lymphatic vessels, myocardial infarction, reverse cholesterol transport, therapeutics
Abstract

Progress in lymphatic vessel biology and novel imaging techniques have established the importance of lymphatic vasculature as part of the cardiovascular system. Lymphatic vessel network regulates many physiological processes important in the heart such as fluid balance, transport of extravasated proteins and trafficking of immune cells. Therefore, lymphangiogenic therapy could be beneficial in the treatment of cardiovascular diseases, for example by improving reverse cholesterol transport from atherosclerotic lesions or resolving edema and fibrosis after myocardial infarction. In this review, we first describe recent findings in the development and function of cardiac lymphatic vessels and subsequently focus on the prospects of pro- and anti-lymphangiogenic therapies in cardiovascular diseases.
Lymphatic vessels are novel players in cardiovascular health and disease

For decades the anatomy and function of cardiac lymphatic system was analyzed with dye injection techniques in large animal models [1] and the role of cardiac lymphatics in normal physiology and pathological conditions remained understudied. The identification of specific lymphatic endothelial cell (LEC) (see Glossary) regulators and markers, such as Prospero Homeobox 1 (PROX1) [2], Vascular Endothelial Growth Factor C (VEGF-C) [3], Vascular Endothelial Growth Factor Receptor 3 (VEGFR-3) [4], Podoplanin (PDPN) [5] and Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) [6] has brought several new transgenic mouse models and sophisticated confocal and multiphoton imaging methods for lymphatic research [7].

The lymphatic system is required for the maintenance of body fluid balance, chylomicron transport from the intestine and immune cell transport and surveillance. The lymphatic network is composed of blind-ended, thin-walled lymphatic capillaries that take up extra interstitial fluid, lymphatic collector vessels dedicated to the transport of the fluid, and lymph nodes that are responsible for immune cell responses. LECs are loosely connected with button-like junctions that allow the entry of fluid, proteins and leukocytes into the lymphatic capillaries. Lymph flows from capillaries into the valve-containing lymphatic collectors and through the thoracic duct and right lymphatic trunk into venous circulation [8].

As in other parts of the body, the heart has an extensive lymphatic network that regulates and maintains the correct fluid balance. The obstruction of the cardiac lymph flow in the healthy heart of an experimental animal can lead to severe cardiac edema, left ventricular dysfunction and hemorrhages (reviewed in [1]). On the other hand, the development of new lymphatics (lymphangiogenesis) may be favorable in delaying atherosclerotic plaque formation within coronary arteries [9]. In addition, therapeutically induced lymphangiogenesis has shown to be beneficial during the healing process after myocardial infarction (MI) as it reduces fluid retention and improves inflammatory cell clearance in the cardiac tissue [10, 11]. In this review, we will introduce the development and regulation of cardiac lymphatics and subsequently focus on therapeutic applications for cardiovascular pathologies

Regulators of lymphangiogenesis

Lymphatic vessels network is formed during embryogenesis and lymphangiogenesis is also activated during pathological states, such as inflammation, tumor formation and lymphedema [12]. After more than a decade of debate on the origin of LECs, in 2007 Srinivasan et al. [13] confirmed that LECs
originate primarily from embryonic veins. They used Tie2-Cre lineage tracing, a method that utilizes inducible genetical labeling of Tie2-expressing endothelial cells that can be later traced with tamoxifen induction. Lymphangio genesis starts at embryonic day 10 (E10.0) in mice [2, 14] and during weeks 6-7 in human gestation when PROX1 expression is induced in a subpopulation of endothelial cells in common cardinal vein, intersomitic veins and superficial venous plexus [15]. However, lymphatics in specific parts of the body, such as lumbar and dorsal skin regions, cannot be traced to originate from Tie2 venous endothelial source [16]. In addition, mesenteric lymphatics have shown to arise from a specific pool of naïve hematopoietic cells [17]. A study by Klotz et al. [10] described the yolk sac haemogenic endothelium as an alternative source of cardiac LECs in mice. This finding was recently challenged by Ulvmar et al. [18] proposing that Pdgfrb-Cre lineage tracing, a method used by Klotz et al. labels the yolk sac haemogenic endothelium incompletely. Thus, the most common origin of LECs is the embryonic veins but the exact identity of the cardiac LEC progenitor remains to be determined. The function of the key factors regulating lymphangiogenesis are described in the next chapters.

**PROX1**

Homeobox transcription factor PROX1 is a key molecule in the differentiation of blood and lymphatic endothelial cells as it determines the specification of LECs from endothelial precursor cells during embryogenesis [2]. Transcription factors SRY-Box 18 (SOX18) [19] and COUP transcription factor 2 (COUP-TFII) [20] are required for PROX1 activation. The expression of SOX18 is restricted to only certain cells in venous endothelium and the expression is maintained in precursor LECs migrating towards lymph sacs [19]. It has been speculated that SOX18 has an essential role in the early induction of PROX1 but it is not sufficient for maintaining PROX1 expression later [21]. In contrast to SOX18, COUP-TFII is present in all venous endothelial cells in the embryo, and it forms a heterodimer with PROX1 in the cells determined for LEC specification [22]. PROX1 and VEGFR-3 form a positive feedback loop: VEGFR-3 gene is a direct target of PROX1 and in turn, VEGFR-3 signaling is essential in PROX1 expression in LECs [23].

**VEGF-C and VEGFR-3**

VEGFR-3 expression induces the budding and migration of LECs towards the mesenchyme that expresses VEGF-C, the primary ligand for VEGFR-3 [24, 25]. Budding LECs coalesce and form lymph sacs, which represent the first primitive lymphatic structures [14]. Both in embryos and adults, VEGF-C–VEGFR-3 signaling is crucial for LEC proliferation, migration and survival [24, 25]. However, another VEGFR-3 ligand, VEGF-D, is not required for lymphatic vessel development during embryogenesis [26]. Collagen And Calcium Binding EGF Domains 1 (CCBE1) is essential for sprouting of the primitive LECs from the cardinal vein. It is highly expressed near developing lymphatic vessels and particularly in the developing heart [27]. CCBE1 has been shown to enhance
the cleavage of pro-VEGF-C to its active form by ADAM Metallopeptidase With Thrombospondin Type 1 Motif 3 (ADAMTS3) metalloproteinase [28]. In a recent study, Bui et al. [29] confirmed that a complex of CCBE1-ADAMTS3 is required for proper proteolytic activation of VEGF-C but not VEGF-D. It was speculated that VEGF-C is required for the growth of developing lymphatics whereas VEGF-D serves as a growth factor for reactive, local lymphatic growth during inflammatory reactions in adults [29]. In addition to CCBE1, neuropilin 2 (NRP2) can enhance VEGFR-3 signaling by interacting with VEGFR-3 and thereby increasing the affinity of LECs towards VEGF-C [30].

**Podoplanin**

The expression of a transmembrane $\text{O}$-glycoprotein podoplanin (PDPN) distinguishes endothelial cells inside the embryonic veins from fully budded LECs [31]. Studies in Pdpn$^{-/-}$ mice have suggested that the expression of PDPN is required for platelet activation and aggregation by binding to a platelet factor C-type lectin-like receptor 2 (CLEC-2). Platelets without PDPN function cannot aggregate and therefore PDPN knockout embryos have incomplete lymphovenous separation and blood-filled lymphatics [32, 33]. PDPN expression is sustained in mature LECs and it is widely used as a LEC marker [5].

**FOXC2**

Forkhead Box C2 (FOXC2) is required for maturation and maintenance of collecting lymphatic vessels and especially lymphatic valves [34]. In addition, FOXC2 sustains LEC quiescence and stability by maintaining the intercellular junctions and cytoskeleton organization [35]. FOXC2 has been shown to regulate Ras/ERK signaling pathway in LECs along with VEGFR-3. Therefore, it has been speculated that FOXC2 fine-tunes the functions of VEGFR-3 during lymphangiogenesis [36].

**Development, anatomy and function of the cardiac lymphatic system**

In mice, cardiac lymphatic vessel formation starts at E11.0-12.0, a few days after the development of coronary blood vessels. VEGFR-3 and PROX1 positive LECs migrate from extracardiac tissues towards the outflow tract on the ventral surface of the heart. At E14.5, lymphatic vessels sprout from the sinus venosus towards the ventricular surface [10]. During embryonic days E15.0-E18.0, LECs migrate from the base of the heart towards the apex, following the coronary vasculature forming the main precollector lymphatics [37]. In addition, cells that express macrophage marker F4-80 have been shown to incorporate to lymphatic vessel walls during prenatal development [38]. After birth, lymphangiogenesis continues and the lymphatic network is expanded from the subepicardium towards the myocardium [37]. In mice, the lymphatic vasculature is fully developed by post-natal day 15 [10]. The development of cardiac lymphatic vessels is described in Figure 1.
In the adult heart, the cardiac lymphatic system forms a network of capillaries and precollecting lymphatic vessels that are located most abundantly in the ventricles. The human left ventricular wall possesses approximately 30 lymphatic vessels/mm², significantly less than blood capillaries [39, 40]. There is some species-specific variation in the anatomy of cardiac lymphatics. For example, the majority of lymphatics in rabbit and mouse hearts are located in the subepicardium and outer myocardium, whereas in humans lymphatic capillaries can be visualized evenly in the myocardial, subepicardial, and subendocardial areas (reviewed by Ratajska [40]). Cardiac lymphatic flow begins from the subendocardium, runs through the myocardium and enters into the capillary lymphatic plexus in the epicardium [41]. The diameter of the capillaries varies, depending on the species and study, from 20 µm up to 400 µm [40]. Lymphatic capillaries converge into large valve-containing collecting lymphatics that run along the left conal and left cardiac veins, and finally drain into subaortic and paratracheal lymph nodes [37]. All cardiac valves also possess lymphatics, that are mainly located in the basal part but also some are scattered peripherally [39].

Many congenital lymphatic disorders cause lymphatic dysfunction and lymphedema primarily in the legs and feet of the individual but not in the heart, such as Milroy’s disease, associated with missense mutations in VEGFR-3 tyrosine kinase domain [42] and lymphedema-distichiasis syndrome, caused by mutations in the FOXC2 gene [43]. However, Noonan syndrome, affected by the upregulated RAS-MAPK signaling, is associated with several signs of lymphatic dysfunction such as lymphedema, lymphatic dysplasias, chylous reflux and intestinal lymphangiectasis, but also congenital heart defects characterized by pulmonary valve stenosis, atrial septal defects and hypertrophic cardiomyopathy [44]. Cardiac lymphatic anatomy or function has not been recorded in these patients, but lymphatic dysfunction might affect the progression of heart defects as the development of lymphatics is abnormal.

In addition to hereditable lymphatic dysfunctions, lymphatic vessels play a role in several heart-related conditions and might have a therapeutical potential. These will be discussed in the further sections of the review.

Lymphatic vessels in heart diseases

Heart diseases affect nearly half of the population in Western societies [45]. They are primarily caused by atherosclerosis, a chronic inflammatory disease of the arteries that can cause blockage of the blood flow leading to ischemia and MI. In the following sections we review the current knowledge of lymphatics in the cardiac pathologies and highlight the therapeutic potential of lymphangiogenesis (Table 1).
Atherosclerosis

Atherosclerosis is characterized by the formation of arterial fatty streaks and plaques loaded with macrophages containing cholesterol (foam cells). A continuous recruitment of monocytes and differentiation into foamy macrophages drives disease progression [46]. The so-called vulnerable plaques, are especially dangerous because they can break and cause acute occlusion in large arteries in the heart and other organs [47]. An attractive treatment option for atherosclerosis is to reduce macrophage burden and decrease cholesterol build up within artery wall by way of a reverse cholesterol transport (RCT).

RCT is a pathway responsible for cholesterol mobilization on high density lipoprotein (HDL) particles from extravascular tissues such as artery wall and muscle to the liver for excretion [48]. RCT is a key player in maintaining peripheral and total body cholesterol homeostasis and it could lead to regression of atherosclerosis by reducing the cholesterol content within the plaques. A strong inverse correlation has been established between plasma HDL cholesterol and the risk for cardiovascular disease [49]. During RCT, apolipoprotein A1 containing pre-β HDL removes cholesterol from macrophages through the ABCA1 and ABCG1 transporters, and then travels through the bloodstream to the liver for biliary excretion [48]. The exact route and mechanism for HDL RCT from peripheral tissue to the circulation still remains unclear.

Recent studies suggest that lymphatic vessels could play an important role in RCT by transporting HDL from interstitial tissues to the bloodstream [9, 50]. It has been shown in mice that blocking lymphatic growth within the aortic wall leads to greater cholesterol retention in the aortae [9]. Furthermore, a surgical ablation of lymphatic vessels in the mouse skin blocked RCT without impairing cholesterol efflux from macrophages indicating the importance of lymphatics in HDL transport [9]. Soluble decoy VEGFR-3×Ldlr−/−/ApoB100/100 mice and Chy×Ldlr−/−/ApoB100/100 mice both display inhibited VEGF-C – VEGFR-3 signaling thus leading to impaired lymphangiogenesis. Studies with these mouse models further confirmed that lymphatic insufficiency leads to accelerated atherosclerotic lesion development highlighting the importance of lymphatic vessel function in cholesterol metabolism [51].

Additionally, it has been shown that restoration of lymphatic function in hypercholesterolemic mice improves lipid clearance. Hypercholesterolemic ApoE−/− mice carrying excess cholesterol in VLDL and chylomicron remnant fraction displayed structural alterations in lymphatics and lower expression of VEGF-C and FOXC2, which both are important factors maintaining lymphatic vasculature. However, treatment of ApoE−/− mice with a local injection of recombinant VEGF-C growth factor restored lymphatic function and reduced the accumulation of cholesterol in the skin and improved
RCT, further emphasizing the potential of lymphangiogenic therapy as a tool for cholesterol clearance [50].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) down-regulates of LDL receptor (LDLR) by binding the receptor and causing its lysosomal degradation in cells. A study employing Pcsk9<sup>−/−</sup> mice suggests that LDL receptor (LDLR) could also play a role in lymphatic dysfunction. Atherosclerosis protected Pcsk9<sup>−/−</sup> mice exhibited improved collecting lymphatic vessel function combined with enhanced expression of LDLR on LECs whereas mice deficient of LDLR (Ldlr<sup>−/−</sup>/hApoB<sup>100/100</sup>) were shown to have defects in collecting lymphatic vessels before the atherosclerotic lesion formation in LDLR-dependent manner. Treatment of Ldlr<sup>−/−</sup>/hApoB<sup>100/100</sup> mice with selective VEGFR-3 agonist, VEGF-C<sub>152S</sub> mutant, significantly increased lymphatic vasculature in the adventitia of the aortic sinus and enhanced lymphatic cellular transport. However, there is no data whether this could alter the risk for atherosclerosis [52].

Most of the studies have utilized mouse models but in 2011 Kholova et al. [39] demonstrated that lymphatics are also present in human coronary arteries as well as in atherosclerotic plaques. Lymphatics were found in intima, media and adventitia of the arterial wall, and moreover, increased medial lymphangiogenesis was present in progressive atherosclerotic lesions, which could be a natural response to resolve increased inflammation and cholesterol build up [39]. Interestingly, it has been shown that ApoA1 and HDL concentration in human artery wall is as high as in the plasma, thus emphasizing a potential role of arterial lymphatics in carrying excess cholesterol out from atherosclerotic plaques [53].

As mentioned earlier, the mechanism for HDL transport from peripheral tissue to the circulation has remained unknown. Recently, it was suggested that Scavenger receptor class B member 1 (SR-BI) is expressed in the lymphatic endothelium where it mediates the internalization and transport of HDL. Furthermore, downregulation of SR-BI by small interfering RNAs resulted in 80% inhibition of HDL uptake by LECs in vitro. It was also shown that treatment of wild type mice with an SR-BI blocking antibody inhibits the transport of HDL via lymphatics by 75%. This is the first study demonstrating that lymphatics can actively transport HDL cholesterol via SR-BI dependent mechanism [50].

All aforementioned studies show that functional lymphatic vasculature is responsible for cholesterol removal from the artery wall and therefore, influences the atherosclerotic plaque formation. Additionally, it has been proposed that HDL enters the lymphatic vasculature via SR-BI dependent mechanism before reaching the bloodstream. These results suggest that therapies aimed at reducing atherosclerosis by way of induced lymphangiogenesis and enhanced HDL transport may be beneficial. Thus far, clinical trials aiming to increase HDL levels have failed to reduce the risk of recurrent
cardiovascular events [54, 55]. This accentuates that simply increasing the HDL levels is not a sufficient treatment, thus targeting the mechanisms behind HDL transport could provide a better therapeutic outcome.

Myocardial infarction

As in other parts of the body, cardiac lymphatic vessels are required for the maintenance of fluid balance and immune surveillance, both important factors during and after MI. Briefly, MI is primarily a manifestation of coronary artery disease where a part of the heart muscle is damaged by a blockage in blood flow. In most cases, the rupture of the atherosclerotic plaques in the coronary artery wall lead to a clotting cascade and complete blockage of the arterial lumen. If this ischemic condition is prolonged, cardiac cells within the region die leading to the formation of an infarction scar [56]. Recruited inflammatory cells clear the affected area from dead cells and matrix debris and stimulate regenerative processes. However, inflammatory reaction can also cause deleterious cardiac remodeling [57].

Only a few studies have addressed the role of lymphatic vessels in MI. Recently, it was shown that adverse remodeling of epicardial collector lymphatics in the infarcted area causes reduced lymphatic flow and persistent edema [11]. Cardiac edema has been shown to induce myocardial stiffness, fibrosis and LV dysfunction by increasing the amount of collagens in the ventricle walls [58]. As a response to the fluid accumulation into the myocardium, lymphangiogenesis has been observed after experimental LAD ligation in mice [10] and rats [11] and also in post-mortem human MI samples [59].

Therapeutical lymphangiogenesis appears to be beneficial for post-MI healing. Klotz et al. showed that left ventricular ejection fraction was significantly improved 14 and 21 days after MI, when VEGFR-3 specific recombinant protein VEGF-C_{156S} therapy was utilized [10]. Henri et al. [11] studied the effect of microparticles carrying VEGF-C_{C152S} protein, another selective VEGFR-3 ligand, in an MI rat model. While, no effect was seen in infarct scar size, cardiac hypertrophy was decreased. In addition, precollector remodeling was attenuated and fluid balance was improved. Both VEGF-C and VEGF-D therapy has been shown to promote blood flow and healing in the rabbit and mouse hind limb after ischemia, but the role of lymphangiogenesis in these studies remains questionable [60-63]. In addition, gene therapy trial in humans showed beneficial effects of VEGF-C therapy on angina score [64, 65]. Unfortunately, lymphangiogenesis was not analyzed in these studies.

Diseases of heart valves

Lymphatic vessels have been shown to be present both in normal and diseased human heart valves. As compared to blood vessels, lymphatic vasculature is proportionately more prominent in valves.
than in the other areas of the heart [39]. A few publications have reported that increased lymphangiogenesis is observed during valve pathologies [39, 66, 67]. Human heart valve endocarditis is characterized by inflammation and abnormal growth, as well as inflammation-associated lymphangiogenesis. Lymphatic vasculature within the diseased heart valves was shown to be increased, both in vessel size and density [67]. However, it is not clear whether lymphangiogenesis within the valves is enough to resolve the inflammation and would lymphangiogenic therapy be a sufficient treatment.

Valvular stenosis involves narrowing of the valve caused by calcification and accumulation of lipids and inflammatory cells [66]. Syvaranta et al. [66] showed that the lymphatic vasculature is present in human stenotic aortic valves and lymphatic capillaries are associated with areas rich in inflammatory cells and neovascularization. Furthermore, VEGF-D and VEGFR-3 mRNA levels in the stenotic aortic valves were upregulated and conceivably are responsible for the increased lymphangiogenesis. Lymphangiogenesis may provide a pathway for lipid and inflammatory cell clearance leading to reduced valvular thickening.

Heart transplantation

The lymphangiogenesis observed after MI appears to be beneficial in resolving inflammation by increasing the clearance of fluid and immune cells as well as inflammatory mediators from the injured heart. However, sustained lymphangiogenesis may also provoke unwanted adverse effects by supporting the transport of immune cells and antigens. Lymphatic vasculature is an important bridge between the innate and adaptive immunity, and in the case of heart transplantation, it may evoke adaptive immunity with serious consequences [68]. Activated antigen-presenting cells such as dendritic cells migrate to the recipient’s draining lymph nodes to trigger adaptive immune responses by presenting foreign antigens to T cells, thus aggravating inflammation. Because increased lymphangiogenesis enables antigen-presenting cell migration, lymphangiogenesis can serve as a increased exposure of lymph nodes target to modulate adverse immune reactions [68].

A recent study demonstrated that cardiac allograft ischemia-reperfusion injury in the rat heart increased graft VEGF-C expression and lymphatic vessel activation. Treatment with a VEGFR-3 inhibitor led to reduced lymphatic vessel activation and dendritic cell maturation, subsequently reducing chronic inflammation and resulting in attenuated acute and chronic rejection. Furthermore, mouse studies using transplanted hearts carrying a LEC specific VEGFR-3 deletion confirmed the previous results that VEGFR-3 inhibition leads to prolonged cardiac allograft survival [69].

Another method to prolong allograft heart transplant survival is to destroy the bridge between innate and adaptive immunity. Azzi et al. (2016) injected microparticles containing T cell proliferation
inhibitors directly to the transplanted mouse heart. These microparticles mimic lymphocyte migration
to the lymph nodes and therefore target the activation of the adaptive immunity. Microparticles
delivered to draining lymph nodes successfully prolonged heart allograft survival [70].

From mice to humans?

Most of the successful cardiac studies within the lymphatic field have utilized rodent models (see
Table 1), which leads us to question whether the methods and results from the small animal studies
can be translated to complex and chronic human diseases. To resolve this question, the next important
step is large animal studies. There are only a few publications utilizing pig models, which demonstrate
that lymphedema can be treated with local injections of adenoviruses encoding lymphangiogenic
VEGF-C [71-73], VEGF-C_{156S} [72] or VEGF-D [73]. The same treatment strategies could be
employed to treat cardiac pathologies in large animal models. There is an ongoing clinical trial for
enhancing cardiac angiogenesis, which emphasizes that the methods to treat MI patients are already
available [74]. The NOGA electroanatomical mapping and injection catheter provides a tool for
lymphangiogenic therapy by enabling to detect and target the borderline areas of the infarction scar
and to deliver the intramyocardial treatment simultaneously [74, 75]. Clearly, the tools to move on to
large animal studies and even to clinical trials in cardiac lymphatic field in the near future are readily
available.

The activation of lymphangiogenesis may result in increased exposure of lymph nodes to
inflammatory mediators and promote the development of metastasis (reviewed in [68]). Therefore, the
safety and efficacy of the lymphangiogenic therapy need to be carefully evaluated within the context
of each disorders and the desired therapeutical goals. Most current studies have used recombinant
proteins or plasmids for treatments but these generally suffer from short duration and low efficacy.
Another option for gene delivery would be viral vectors which efficiently carry and transduce the
therapeutic factor into the target cells and tissues [76]. Also, biodegradable microparticles have been
utilized as carriers of therapeutic factors into lymph nodes [70] and myocardial wall [11, 70]. Most
commonly used pro-lymphangiogenic factors are VEGFR-3 ligands VEGF-C and VEGF-D and their
splice variants. Other options, such as angiopoietins and fibroblast growth factors and their
combinations require further evaluation in this context [12, 77].

Concluding Remarks and Future Perspectives

After years of ignorance cardiac lymphatic system has become an active target for research and novel
findings are published at increasing pace. Recent advances in the lymphatic field have provided new
insights in the treatment of cardiovascular diseases (See Outstanding Questions box and Figure 2, Key
It has been shown that the lymphatic vessels are present around atherosclerotic lesions and provide an important pathway for cholesterol transportation. Therefore, novel lymphangiogenic therapies could accelerate RCT leading to inhibition or regression of atherosclerosis. Lymphangiogenic therapy has also been successfully utilized to resolve edema formation, inflammatory cell accumulation and fibrosis during MI in mice [10, 11]. On the other hand, anti-lymphangiogenic therapy could be beneficial in preventing undesired inflammation in heart transplants.

In addition, modulation of lymphatic function could be beneficial in treating lipid-related diseases, such as weight-gain and hypercholesterolemia. One of the first reports describing the connection between lymphatics and lipids was in 2005, when Harvey et al. [78] showed that lymphatic dysfunction in Prox1+/- mice caused leakage of lymph into surrounding tissues and resulted in adult-onset obesity. Fat accumulation has also been observed in humans with surgical ablation of lymph nodes [79] but not in other mouse models of lymphatic deficiency [8]. Evidence from the RCT studies indicate that lymphatics are also required for lipoprotein transport and metabolism. It is well established that lymphatics mediate chylomicron transport from intestine into bloodstream, but the function of lymphatics in regulating lipid and lipoprotein uptake into the lacteals has not yet been characterized. Further, the question still remains if lymphatics participate in the trafficking and regulation of endogenous lipoproteins, namely VLDL and atherogenic LDL. There are many open questions in the relationship of lymphatics and lipid metabolism and when clarified, new avenues might open for the treatment of lipid-related diseases, primary causes of many heart pathologies.

**Acknowledgements:** This work was supported by Finnish Academy Center of Excellence in Cardiovascular and Metabolic Diseases, Erkko Foundation and Urho Käänänen Foundation.
Table 1. Modulation of lymphangiogenesis in cardiovascular diseases

<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>Treatment</th>
<th>Delivery</th>
<th>Outcome</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein transport</td>
<td>mouse</td>
<td>rVEGF-C</td>
<td>skin</td>
<td>improved RCT</td>
<td>[50]</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>human</td>
<td>pVEGF-2 (i.e pVEGF-C)</td>
<td>endocardium</td>
<td>improved symptoms of angina</td>
<td>[64]</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>mouse</td>
<td>rVEGF-C</td>
<td>systemic (ip)</td>
<td>improved cardiac function</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>microparticles carrying VEGF-C&lt;sub&gt;C152S&lt;/sub&gt;</td>
<td>myocardium</td>
<td>reduced edema, reduced fibrosis</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td>pig</td>
<td>AdVEGF-C</td>
<td>myocardium</td>
<td>prevention of MI progression</td>
<td>[80]</td>
</tr>
<tr>
<td>Peripheral ischemia</td>
<td>rabbit</td>
<td>pVEGF-C and rVEGF-C</td>
<td>angioblasty balloon and intra-arterial</td>
<td>improved flow</td>
<td>[60]</td>
</tr>
<tr>
<td>Intact muscle</td>
<td>mouse</td>
<td>AdVEGF-D</td>
<td>hind limb</td>
<td>angiogenesis, lymphangiogenesis</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>rabbit</td>
<td>AdVEGF-D, AdVEGF-C</td>
<td>hind limb</td>
<td>lymphangiogenesis</td>
<td>[62]</td>
</tr>
<tr>
<td>Cardiac allograft</td>
<td>rat</td>
<td>VEGF-C/D trap, VEGFR-3 antibody</td>
<td>intra-arterial</td>
<td>improved allograft survival</td>
<td>[69]</td>
</tr>
</tbody>
</table>

r: recombinant, p: plasmid, Ad: adenoviral, ip: intraperitoneal
Figure Legends

Figure 1. Development of cardiac lymphatic vessels

(A) Cardiac lymphatic endothelial cells (LECs) originate from common cardinal vein (CCV) and the yolk sac hemogenic endothelium. (B) PROX1 expression is activated by COUP-TFII and SOX18 in a subset of endothelial cells that are then designated for LEC specification. (C) PROX1 activates VEGFR-3 expression and initiates budding of LECs from CCV. (D) LECs migrate towards the systemic venous sinus along the VEGF-C gradient. NRP2 and a complex of CCBE1 and ADAMTS3 enhance VEGF-C signaling through VEGFR-3. (E) LECs enter the heart by E12.5 and (F) begin to form the first primitive lymphatic capillaries. (G) Lymphatic network expands from base towards the apex following the coronary veins. (H) By P15, cardiac lymphatic network is fully developed. Panels E-H adapted from Klotz et al. [10].

Figure 2. Key figure. Potential lymphangiogenic therapies in cardiac diseases

A schematic diagram illustrating the role of lymphangiogenesis in atherosclerosis and MI. (A) Atherosclerosis is characterized by lipid and inflammatory cell build up within arterial walls. (B) Lymphangiogenic therapy could enhance RCT leading to greater lipid clearance from atherosclerotic plaques and regression of atherosclerosis through accelerated HDL turnover. HDL removes cholesterol from macrophages through ABCA1 and ABCG1 and exits via SR-BI to the adventitial lymphatic vessels to the blood stream. (C) Atherosclerotic plaque rupture leads to coronary artery occlusion and MI. MI is followed by adverse remodeling of epicardial collector lymphatics and subsequently edema. Additionally, severe inflammation and fibrosis is developed. (D) Therapeutic lymphangiogenesis after MI increases the lymph flow and resolves inflammation leading to improved cardiac function and healing of MI.
**Glossary box**

**Atherosclerosis:** A progressive disease characterized by accumulation of cholesterol, extracellular matrix, fibrosis, calcium deposits and inflammatory cells within the arterial wall.

**Edema:** The abnormal accumulation of fluid in the interstitium leading to tissue swelling and pain.

**High density lipoprotein (HDL):** A small, ApoA-rich lipoprotein responsible for reverse cholesterol transport of cholesterol from extrahepatic tissues to liver.

**Lymphangiogenesis:** The formation of new lymphatic vessels from pre-existing lymphatic vessels.

**Lymphatic endothelial cells (LECs):** specialized form of epithelium that lines the lymphatic vessels.

**Lymph node:** An organ linked to lymphatic vessels. It is full of immune cells that recognize foreign particles and remove bacteria, viruses, toxins as well as cancer cells from the body.

**Macrophage:** A white blood cell that engulfs and digests microbes, cellular debris and any foreign substances. Also acts as an antigen-presenting cell to activate immune responses.

**Myocardial infarction (MI):** Cardiac muscle damage and/or necrosis due to an occlusion of a coronary artery caused by atherosclerotic plaques.

**Reverse cholesterol transport (RCT):** Movement of cholesterol from peripheral tissues back to the liver via plasma lipoproteins.

**Vascular endothelial growth factor 3 (VEGFR-3):** A tyrosine kinase receptor that binds vascular endothelial growth factors C and D. It is essential for the development and maintenance of lymphatic vessels.

**Trends Box**

- Cardiac lymphatic endothelial cells originate from multiple sources. In the heart, they form a network of capillary lymphatic vessels and larger collector lymphatics that drain fluid, macromolecules and inflammatory cells.
- The role of lymphatic vessels in chylomicron metabolism is well-established and lymphatics are also required for reverse cholesterol transport. Therefore, lymphatics may play even a more significant role in lipid and lipoprotein metabolism than previously thought.
- Lymphangiogenic therapy may become a useful option for the treatment of cardiovascular diseases, such as atherosclerosis and myocardial ischemia. In addition, anti-lymphangiogenic therapy could be utilized to resolve inflammatory reaction in cardiac allografts.
Outstanding Questions Box

- What is the role of lymphatics in lipoprotein metabolism?
- How could arterial wall lymphangiogenesis be enhanced? Would it increase reverse cholesterol transport and could it be used as a treatment for atherosclerosis?
- What is the most applicable delivery route and method for lymphangiogenic therapy for myocardial ischemia?
- Could attenuation of lymphatic vessel activation through VEGFR-3 inhibition therapy be employed in the clinics to treat heart transplantation patients to prolong cardiac allograft survival?
- What are the side effects of lymphangiogenic therapy and how could they be avoided?
References


3 Joukov, V. et al. (1996) A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. Embo J. 15, 290-298

4 Kukk, E. et al. (1996) VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. Development 122, 3829-3837

5 Breiteneder-Geleff, S. et al. (1999) Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am. J. Pathol. 154, 385-394


11 Henri, O. et al. (2016) Selective Stimulation of Cardiac Lymphangiogenesis Reduces Myocardial Edema and Fibrosis Leading to Improved Cardiac Function Following Myocardial Infarction. 
*Circulation* 133, 1484-1497


14 Yang, Y. et al. (2012) Lymphatic endothelial progenitors bud from the cardinal vein and intersomitic vessels in mammalian embryos. *Blood* 120, 2340-2348


18 Ulvmar, M.H. et al. (2016) Pdgfrb-Cre targets lymphatic endothelial cells of both venous and non-venous origins. *Genesis* 54, 350-358


20 Srinivasan, R.S. et al. (2010) The nuclear hormone receptor Coup-TFII is required for the initiation and early maintenance of Prox1 expression in lymphatic endothelial cells. *Genes Dev.* 24, 696-707


50 Lim, H.Y. *et al.* (2013) Lymphatic vessels are essential for the removal of cholesterol from peripheral tissues by SR-BI-mediated transport of HDL. *Cell. Metab.* 17, 671-684


60 Witzenbichler, B. et al. (1998) Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia. Am. J. Pathol. 153, 381-394


72 Visuri, M.T. et al. (2015) VEGF-C and VEGF-C156S in the pro-lymphangiogenic growth factor therapy of lymphedema: a large animal study. *Angiogenesis* 18, 313-326


75 Nurro, J. et al. (2016) AdVEGF-B186 and AdVEGF-DDeltaNDeltaC induce angiogenesis and increase perfusion in porcine myocardium. *Heart*


79 Brorson, H. et al. (2009) Breast cancer-related chronic arm lymphedema is associated with excess adipose and muscle tissue. *Lymphat Res. Biol.* 7, 3-10