

Lymphatics in the broken heart

Ebba Bråkenhielm,¹ Yuguo Chen,² and Yihai Cao³

¹Normandy University, UniRouen, Inserm (Institut National de la Santé et de la Recherche Médicale) UMR1096 (EnVI Laboratory), FHU CARNAVAL, Rouen, France. ²Department of Emergency Medicine, Shandong Provincial Clinical Research Center for Emergency and Critical Care Medicine, Institute of Emergency and Critical Care Medicine of Shandong University, Qilu Hospital of Shandong University, Jinan, Shandong, China. ³Department of Microbiology, Tumor and Cell Biology, Biomedicum, Karolinska Institutet, Stockholm, Sweden.

Cardiac lymphatics have emerged as a therapeutic target in cardiovascular diseases to limit myocardial edema and inflammation, notably after myocardial infarction (MI). While most experimental therapeutic approaches have focused on vascular endothelial growth factor C (VEGF-C) delivery, it remains uncertain to what degree the beneficial cardiac effects are related to lymphatic expansion in the heart. In this issue of the *JCI*, Keller, Lim, et al. reexamined the acute functional impact of endogenous cardiac lymphangiogenesis in the infarct zone after MI in mice. Their data, obtained by elegant comparisons of several complementary genetic mouse models, indicate that infarct expansion and left ventricular dilation and function after MI are unaffected by infarct lymphangiogenesis. This Commentary places the results into the context of previous findings. We believe these data will help further advance the research field of cardiac lymphatics to guide better clinical translation and benefit patients with ischemic heart disease.

Lymphangiogenesis in the infarcted heart

Earlier studies in large mammals have demonstrated that lymphatic drainage of the heart is essential for maintenance of cardiac tissue homeostasis (1). Recently, there has been renewed interest in cardiac lymphatics, as the cardiac lymphatic network was found to be remodeled through the process of lymphangiogenesis following myocardial infarction (MI) (1–4). As with other organs, cardiac lymphatic expansion and remodeling in health and disease are mainly controlled by vascular endothelial growth factor receptor 3 (VEGFR-3) and its two ligands, VEGF-C and VEGF-D (ref. 5 and Figure 1). Activation of VEGFR3, together with its coreceptor neuropilin (Nrp), induces migration, proliferation, and differentiation of lymphatic endothelial cells (LECs) to expand the lymphatic vascular network (6). The pathophysiological trig-

gers for lymphangiogenesis include both tissue inflammation and edema, and emerging studies indicate that mechanotransduction may contribute to the regulation of VEGFR3 activity in the heart (7). Concerning the origin of endogenous VEGF-C and -D ligands in the heart, both cardiomyocytes and cardiac-infiltrating immune cells are rich sources of these lymphatic growth factors (8, 9). In the setting of myocardial ischemia, hypoxia-inducible growth factors, including VEGF-A and PDGF-B, may also stimulate lymphangiogenesis (10, 11). In addition, other factors, such as adrenomedullin (12), contribute to cardiac lymphatic expansion after MI.

Impact of genetic loss of function of VEGF-C, VEGF-D, and VEGFR3

Studies in transgenic animals reveal that although *Vegfd*-null mice lack any clear

phenotypes, *Vegfc* homozygous deletion is embryonic lethal due to lymphatic deficiency (13). *Vegfc*-haploinsufficient embryos survive to adulthood, but display hypomorphic lymphatic vasculature. Similarly, neutralization of endogenous VEGF-C and -D ligands, through transgenic constitutive expression of a soluble VEGFR3-Ig construct (sVEGFR3), severely delays lymphatic development, including in the heart where the lymphatics are present but dilated in adult mice (14, 15). An even more severe phenotype occurs in adult Chy mice, which harbor an inactivating mutation in VEGFR3 (16) and whose cardiac lymphatics are profoundly abnormal (15). In contrast, constitutive *Vegfr3* deletion is embryonic lethal, owing to early effects on blood vessel formation. Indeed, during embryonic development VEGFR3 is not yet restricted to LECs, and VEGF-C and -D contribute to embryonic angiogenesis via VEGFR3 expressed on blood endothelial cells (BECs). In adult mice, VEGF-C and -D also stimulate angiogenesis by binding to VEGFR2 expressed in BECs (17, 18). Together, these data uncover the impact of the VEGF-C/VEGF-D/VEGFR3 axis on the development and postnatal maturation of lymphatics, including those of the heart. However, in healthy adult mice, inhibition of VEGF-C/VEGF-D/VEGFR3 has limited, if any, functional impact, as quiescent lymphatic vessels in most organs are insensitive to reduced growth factor signaling (19).

In cardiovascular pathology, where lymphangiogenesis is reactivated in the inflamed and edematous heart, sVEGFR3 strikingly reduces post-MI cardiac lymphangiogenesis, again indicating the key role of VEGF-C and -D ligands (8, 15). However, the functional impact was found to differ depending on whether attenuation of the VEGFR3 signaling was chronic or acute; whereas transgenic constitutive sVEGFR3 expression increased post-MI mortality due to enhanced cardiac rupture (15), adeno-associated virus (AAV) delivery

► **Related Article:** <https://doi.org/10.1172/JCI147070>

Conflict of interest: The authors have declared that no conflict of interest exists.

Copyright: © 2021, American Society for Clinical Investigation.

Reference information: *J Clin Invest.* 2021;131(20):e153448. <https://doi.org/10.1172/JCI153448>.

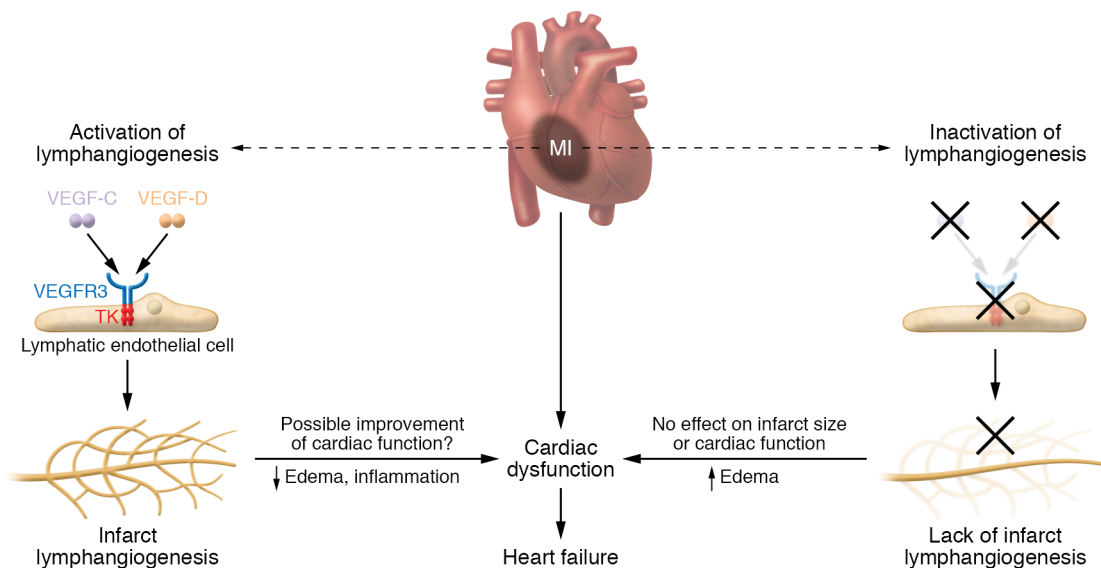


Figure 1. Impact of the VEGF-C/VEGF-D/VEGFR3 axis on cardiac lymphangiogenesis. Cardiac lymphangiogenesis is reactivated, notably in the infarct zone, after myocardial infarction (MI). It is mainly regulated by binding of the growth factors VEGF-C and VEGF-D to the plasma membrane-spanning (PM-spanning) VEGFR3 tyrosine kinase (TK) receptor selectively expressed on lymphatic endothelial cells. This signaling leads to expansion of the preexisting lymphatic network in the heart, which, by modulating infarct scar maturation through accelerated resolution of edema and inflammation, may reduce cardiac dysfunction and development of heart failure. However, Keller, Lim, et al. reveal that inactivation of VEGF-C/VEGF-D/VEGFR3-induced infarct lymphangiogenesis had no functional effect on either infarct size or cardiac function 14 days after MI in mice. These data call into question the functionality of infarct lymphatics, because although myocardial edema tended to increase, there was no change in infarct macrophage density following deletion of VEGFR3.

of the gene encoding sVEGFR3 at the time of coronary ligation in mice surprisingly limited post-MI cardiac dysfunction (8). In the latter case, the mechanism included a reduction in infarct remodeling, with less infarct scar thinning due to decreased cardiac T cell density. This reduction in infarct remodeling was proposed to reflect nonlymphatic effects of VEGF-C, with immune modulation through alteration of dendritic cell maturation, as previously described (20).

Controversies of lymphangiogenic therapy for MI

Several experimental studies have reported that therapeutic lymphangiogenesis, achieved by VEGFR3-selective VEGF-C gene or protein therapy, suffices to improve post-MI cardiac function in rodents. In parallel, clinical trials based on *Vegfd* delivery in patients with coronary artery disease are currently ongoing (21). However, there are some controversies regarding the cellular mechanisms of these treatments. While one study indicated that intraperitoneal delivery of VEGF-C protein in mice reduced the infarct size and strikingly improved post-MI cardiac function (3), other studies based on either intramyocardial protein delivery or AAV delivery of *Vegfc* did not report any effects on the

infarct size, with more moderate cardiac functional benefits linked to lymph-mediated improvement of tissue homeostasis in noninfarcted, viable myocardium (4, 8). Given that infarct maturation is heavily regulated by immune cells, it is conceivable that stimulation of infarct lymphangiogenesis could accelerate resolution of edema and/or inflammation by enhancing clearance of extracellular fluid, debris, and immune cells from the developing scar tissue. However, whether the kinetics of lymphatic vessel regrowth is sufficiently rapid to influence this process of infarct maturation is debatable. Moreover, given the fact that cardiac lymphatics rely on contracting cardiomyocytes to propel the lymph, the functionality of lymphatic vessels in the infarct scar, which is nearly devoid of contractile elements, remains uncertain.

In this issue of the *JCI*, Keller, Lim, et al. address this debatable issue (22). Using an elegant setup of several complementary genetic models, including inducible LEC-selective deletion of the gene that encodes VEGFR3 (*Vegfr3*) or inducible global deletion of *Vegfc* in *Vegfd*-null mice, the authors investigated the functional impact of cardiac lymphangiogenesis on the infarct zone after MI. They found that inactivation of the VEGF-C/

VEGF-D/VEGFR3 axis potently inhibited infarct lymphangiogenesis. However, reduced lymphangiogenesis had no effect on the infarct size, nor on left ventricular dilation or function 7 or 14 days after MI (Figure 1). While myocardial edema during post-MI stages tended to increase in VEGFR3-deleted mice, there was no difference in infarct macrophage density, indicating limited functionality of infarct lymphatic vessels. These unbiased, experimental data from multiple genetic models convincingly demonstrate that although endogenous lymphangiogenesis is potently induced in the post-MI infarct zone, it has no clear pathophysiological relevance, at least in the short term following MI.

Outlook and concluding remarks

The study by Keller, Lim, et al. (22) raises several important questions, one of which is whether inactivation of the VEGF-C/VEGF-D/VEGFR3 axis similarly impacted lymphangiogenesis in the noninfarcted viable myocardium. Previous studies have shown that the endogenous lymphangiogenesis in the viable left ventricular wall in rodents after MI is both weak and slow (4, 8), hence the need for therapeutic lymphangiogenesis. In the Keller, Lim,

et al. experiments, it is possible that other factors partially compensated for loss of VEGF-C/VEGF-D signaling, given that cardiac lymphatic vessels eventually form in sVEGFR3-transgenic mice (14, 15). Furthermore, VEGF-C and -D also impact cardiac angiogenesis; although reduction in VEGFR3 signaling may upregulate VEGFR2 expression (15), it remains to be determined whether inactivation of the VEGF-C/VEGF-D/VEGFR3 axis, as performed by Keller, Lim, et al., influenced myocardial angiogenesis. Last, but not least, given that VEGF-C, notably by activating VEGFR2, regulates many other cell types, including BECs, immune cells, and neurons, the cardiovascular impact linked to nonlymphatic effects of the VEGF-C/VEGF-D/VEGFR3 axis modulation needs further investigation. For the clinical outlook, deepening our understanding of where and how to stimulate lymphangiogenesis is essential for improving lymphatic clearance in the heart to limit edema and inflammation in cardiovascular diseases.

Acknowledgments

EB is funded by the Agence Nationale de la Recherche of France (ANR-18-CE17-0021) and YC's laboratory is supported through research grants from the Swedish Research Council (project nos. 2011-04091, 2016-02215, and 2019-01502), the Swedish Cancer Foundation (project no. 200734PjF), the Swedish Children's Cancer Foundation (project nos. PR2015-0159 and PR2018-0107), the Strategic Research Areas (SFO) – Stem Cell and Regenerative Medicine Foundation, the Karolinska Institute Foundation (project no. 2020-02080), the Karolinska Institute distinguished professor award,

the NOVO Nordisk Foundation, and the Knut and Alice Wallenberg's Foundation (project no. KAW2014.0112).

Address correspondence to: Ebba Bråkenhielm, Inserm UMR1096, Normandy University, UniRouen, Faculty of Pharmacy and Medicine, 22 Boulevard Gambetta, 76183 Rouen, France. Phone: 33.235.148370; Email: ebba.brakenhielm@inserm.fr. Or to: Yihai Cao, Department of Microbiology, Tumor and Cell Biology (C1 MTC), Biomedicum Karolinska Institutet, Solnavägen 9, 17177 Stockholm, Sweden. Phone: 46.8.524.875.96; Email: yihai.cao@ki.se.

1. Laine GA, Allen SJ. Left ventricular myocardial edema. Lymph flow, interstitial fibrosis, and cardiac function. *Circ Res*. 1991;68(6):1713–1721.
2. Ishikawa Y, et al. Lymphangiogenesis in myocardial remodelling after infarction. *Histopathology*. 2007;51(3):345–353.
3. Klotz L, et al. Cardiac lymphatics are heterogeneous in origin and respond to injury. *Nature*. 2015;522(7554):62–67.
4. Henri O, et al. Selective stimulation of cardiac lymphangiogenesis reduces myocardial edema and fibrosis leading to improved cardiac function following myocardial infarction. *Circulation*. 2016;133(15):1484–1497.
5. Brakenhielm E, Alitalo K. Cardiac lymphatics in health and disease. *Nat Rev Cardiol*. 2019;16(1):56–68.
6. Cao Y. Opinion: emerging mechanisms of tumour lymphangiogenesis and lymphatic metastasis. *Nat Rev Cancer*. 2005;5(9):735–743.
7. Urner S, et al. Identification of ILK as a critical regulator of VEGFR3 signalling and lymphatic vascular growth. *EMBO J*. 2019;38(2):e99322.
8. Houssari M, et al. Lymphatic and immune cell cross-talk regulates cardiac recovery after experimental myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2020;40(7):1722–1737.
9. Vieira JM, et al. The cardiac lymphatic system stimulates resolution of inflammation following myocardial infarction. *J Clin Invest*. 2018;128(8):3402–3412.
10. Cao R, et al. Comparative evaluation of FGF-2-, VEGF-A-, and VEGF-C-induced angiogenesis, lymphangiogenesis, vascular fenestrations, and permeability. *Circ Res*. 2004;94(5):664–670.
11. Cao R, et al. PDGF-BB induces intratumoral lymphangiogenesis and promotes lymphatic metastasis. *Cancer Cell*. 2004;6(4):333–345.
12. Trincot CE, et al. Adrenomedullin induces cardiac lymphangiogenesis after myocardial infarction and regulates cardiac edema via connexin 43. *Circ Res*. 2019;124(1):101–113.
13. Haiko P, et al. Deletion of vascular endothelial growth factor C (VEGF-C) and VEGF-D is not equivalent to VEGF receptor 3 deletion in mouse embryos. *Mol Cell Biol*. 2008;28(15):4843–4850.
14. Mäkinen T, et al. Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. *Nat Med*. 2001;7(2):199–205.
15. Vuorio T, et al. Downregulation of VEGFR3 signaling alters cardiac lymphatic vessel organization and leads to a higher mortality after acute myocardial infarction. *Sci Rep*. 2018;8(1):16709.
16. Karkkainen MJ, et al. A model for gene therapy of human hereditary lymphedema. *Proc Natl Acad Sci U S A*. 2001;98(22):12677–12682.
17. Cao Y, et al. Vascular endothelial growth factor C induces angiogenesis in vivo. *Proc Natl Acad Sci U S A*. 1998;95(24):14389–14394.
18. Anisimov A, et al. Activated forms of VEGF-C and VEGF-D provide improved vascular function in skeletal muscle. *Circ Res*. 2009;104(11):1302–1312.
19. Nurmi H, et al. VEGF-C is required for intestinal lymphatic vessel maintenance and lipid absorption. *EMBO Mol Med*. 2015;7(11):1418–1425.
20. Hajrasouliha AR, et al. Vascular endothelial growth factor-C promotes alloimmunity by amplifying antigen-presenting cell maturation and lymphangiogenesis. *Invest Ophthalmol Vis Sci*. 2012;53(3):1244–1250.
21. Hartikainen J, et al. Adenoviral intramyocardial VEGF-DANAC gene transfer increases myocardial perfusion reserve in refractory angina patients: a phase I/IIa study with 1-year follow-up. *Eur Heart J*. 2017;38(33):2547–2555.
22. Keller IV TC, et al. Genetic blockade of lymphangiogenesis does not impair cardiac function after myocardial infarction. *J Clin Invest*. 2021;131(20):e147070.