Lymphatics thrive on stress: mechanical force in lymphatic development

Martin A Schwartz* and Michael Simons*

Departments of Medicine and Cell Biology, Yale Cardiovascular Research Center, Yale University, New Haven, CT, USA

*Correspondence to: martin.schwartz@yale.edu or michael.simons@yale.edu

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The arteries, veins, capillaries and lymphatic vessels that comprise the vertebrate vascular system function to transport blood and lymphatic fluid to and from the tissues. It is therefore unsurprising that development, maintenance and physiology of these structures are strongly influenced by mechanical forces such as pressure and fluid shear stress associated with fluid flow (Hahn and Schwartz, 2008; Culver and Dickinson, 2010). Both embryonic morphogenesis and adult homeostasis of blood vessels appear to be organized so that vessel dimensions are matched to tissue demand. The accompanying paper from Planas-Paz *et al* (2012) elegantly demonstrates that a similar principle operates for lymphatic development as well.

The lymphatic system is composed of a network of blind-ended, thin-walled capillaries that coalesce into larger vessels, which in turn drain into the venous circulation via the thoracic duct (Schulte-Merker *et al*, 2011). The primary role of lymphatics is to remove the protein-rich interstitial fluid from the extracellular spaces, thereby reducing interstitial pressure and maintaining the overall fluid balance in the body. The development and maintenance of the lymphatic vasculature is orchestrated by several key players including homeobox transcription factors of the SOX F family, lymphatic-specific transcription factor Prox1, VEGF-C and its receptor VEGFR3.

Lymphatic development in mice proceeds in two distinct steps (Schulte-Merker *et al*, 2011). The first is an induction of polarized expression of Sox18 transcription factor in cardinal vein endothelial cells at around E9.5. This cell population then acquires expression of Prox1, a prospero-related homeodomain transcription factor that, together with COUP-TFII, converts them to lymphatic fate. The newly specified lymphatic endothelial cells then sprout out of the cardinal vein to form jugular lymphatic sacs. The second step that takes place around E13.5 is the sprouting of lymphatic sacs leading to formation of the lymphatic vasculature. Little is known about signals responsible for initiation of these events, except that lymphatic sprouting clearly requires VEGF-C/VEGFR3 signalling.

The study by Planas-Paz *et al* (2012) shows that an increase in tissue hydrostatic pressure promotes lymphangiogenesis. The data suggest that increasing tissue turgor is due to increasing filtration of plasma from the arterial circulation into the interstitial space as the vascular system grows and expands. This pressure is suggested to stretch the nascent lymphatics, triggering proliferation of lymphatic endothelial

cells and hence enlargement of lymphatic vessels (Figure 1). The authors propose that pressure-induced activation of VEGFR3 mediated by $\beta 1$ integrin, perhaps with the involvement of Src, is the key step. VEGF-C also is required for this

Figure 1 Force-dependent lymphatic growth. Fluid leaking out of newly formed arteries swells the tissue and stretches nascent lymphatic vessels (A, B). Lymphatic endothelial cells subsequently proliferate and enlarge the lymphatics, which can drain the excess fluid from the tissues (C).

process. While an intriguing concept, the role of VEGF-C and VEGFR3 are not fully developed in the current study.

The concept that tissue hydrostatic pressure promotes lymphangiogenesis neatly couples the growth of arterial blood circulation, which is accompanied by increasing interstitial fluid, to the growth of lymphatics driven by the very same interstitial fluid pressure. It furthermore illustrates an attractive mechanism by which lymphatic growth is coupled to levels of interstitial fluid, thereby ensuring that lymphatic dimensions match demand. The current challenge is to link this mechanistic insight to the known need for VEGFR3 signalling and to molecular mechanisms.

Pressure-driven activation of VEGFR3 during lymphangiogenesis bears an interesting similarity to shear stress-driven activation of VEGFR2 during arteriogenesis (Tzima *et al.*, 2005). In that system, force from shear acts upon PECAM1 (CD31), leading to activation of a src family kinase (SFK), which subsequent studies suggested to be fyn (Chiu *et al.*, 2007). Active SFK then induces ligand-independent transactivation of VEGFR2, which mediates several flow-dependent signalling events (Tzima *et al.*, 2005). Interestingly, binding of integrins to their ligands can affect receptor tyrosine kinase (RTK) signalling in at least two different ways (Somanath *et al.*, 2009). First, integrins can initiate ligand-independent, SFK-dependent transactivation of several RTKs, including VEGFR2 and VEGFR3. Second, integrins can synergize with

growth factors to enhance RTK activation and signalling downstream of growth factor binding.

The current observations therefore have a number of interesting connections. They add to the growing list of similarities between arterial and lymphatic systems that include key events modified by the same players such as SOX18, ephrin B2 and neuropilins (Nrp1 in the arterial versus Nrp2 in lymphatic endothelium) (Larrivee *et al.*, 2009). They also fit into a broader set of phenomena in which integrins and RTKs cooperate to integrate information from multiple sources within the cellular environment, including growth factor concentration, extracellular matrix composition and mechanical forces. A number of other issues remain unanswered. What is the molecular mechanism of cooperation (ligand-independent transactivation versus ligand-dependent synergy)? What is the mechanism by which interstitial fluid pressure applies forces to lymphatic endothelial integrins? Does force from interstitial fluid provide directional information to guide lymphatic growth? The study from Planas-Paz therefore opens a new window on vascular development; a good deal of further investigation will be required to answer these novel questions.

Conflict of interest

The authors declare that they have no conflict of interest.

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