The lymphatic system regulates tissue fluid homeostasis and acts as a conduit for leukocytes. Under high hydrostatic pressure, the blood system delivers oxygen, nutrients, and hormones to distant cells, while acellular fluid is forced out of blood capillaries into interstitial space. Most of this fluid drains back into lymphatic capillaries that are under low pressure and leaky due to their unique endothelial lining with microvalves between endothelial cells (ECs). Lymphatic ECs (LECs) are distinguished from blood ECs (BECs) by expression of the transcription factor Prox1, the cell surface proteins LYVE-1 and podoplanin, and the growth factor receptors VEGFR3 and neuropilin 2, whereas BECs express PECAM-1, endoglin, VEGFR2, and neuropilin 1 (1, 2). The lymphatic system develops from an endothelial outgrowth of the anterior cardinal vein, with several proteins required to induce LEC differentiation, most notably transcription factors Sox18 and Prox1 (1, 3, 4). Others are required for maturation of the lymphatic system, including some that function in arterial EC specification (1, 2) (Fig. 1). Yoshimatsu et al. (5) highlight the role of a ligand–receptor system that represses LEC differentiation, proliferation, and lymphangiogenesis. The receptor ALK-1, previously implicated in arteriovenous specification (6), is activated by the TGF-β-related ligand bone morphogenetic protein-9 (BMP-9) and inhibits the switch from BEC to LEC differentiation by repressing the latter and promoting the former. Their study adds another dimension to the molecular control of EC specification.

Gene targeting and lineage tracing analyses in model organisms identified several molecular players that orchestrate budding of LECs from venous endothelium (1, 2). In early development, VEGFR3 and LYVE-1 are not only expressed in LECs but also in venous BECs, together with the transcription factor COUP-TFII. Sox18 is expressed with the appearance of the lymphatic rudiment and cooperates with COUP-TFII to activate Prox1 (Fig. 1). Prox1 and COUP-TFII then interact to instigate a LEC transcription program that enables continued expression of VEGFR3, Neuropilin 2, LYVE-1, and podoplanin (7). As development ensues, VEGFR3 expression is repressed in BECs but, due to persistent Prox1 expression, remains active in LECs. Prox1 is a master regulator of lymphatic development required to maintain LEC identity (1, 2, 7), because repression of Prox1 reprograms LECs into BECs (4).

A number of signaling pathways regulate LEC generation and lymphatic vessel development. The tyrosine kinase receptor VEGFR3 is activated by the ligands VEGF-C and VEGF-D, secreted from mesenchyme at sites of lymphangiogenesis, and is required for expansion of the LEC population and lymphangiogenesis (1, 2). EphrinB2 controls internalization of VEGFR3, and its loss also results in lymphatic defects (8). Angiopoietins, acting through the Tie-2 tyrosine kinase receptor, also control lymphangiogenesis. Whereas Tie-2 is expressed in both BECs and LECs, and angiopoietins control both hem- and lymphangiogenesis, only angiopoietin-2 expression is essential for development of the lymphatic system (1, 2). Notch signaling is also required for lymphatic vessel development, by regulating EphrinB2 expression and, consequently, VEGFR3 signaling (9). However, none of these signaling pathways directly controls Prox1 expression and LEC lineage commitment, although Erk MAPK signaling was shown to activate Sox18 and Prox1 expression and LEC specification (10).

Yoshimatsu et al. (5) define the role of the BMP/activin receptor-like kinase 1 (ALK-1) ligand–receptor pathway as an inhibitor of LEC differentiation and lymphangiogenesis. Unlike receptor tyrosine kinases that mediate VEGF or angiopoietin signaling, TGF-β family proteins act through complexes of two type II and two type I receptors that primarily phosphorylate Ser and Thr, enabling the type I receptors to activate Smads through C-terminal phosphorylation on Ser. Using mouse models, the authors show that in LECs, the type I receptor ALK-1 relays signals in response to BMP-9 and that BMP-9–ALK-1 signaling represses LEC differentiation and lymphangiogenesis in normal development and tumors. This repression is accompanied by increased BEC-marker expression, positioning BMP-9–ALK-1 signaling as a switch that regulates the reciprocal balance of BEC and LEC differentiation (5), resulting from repression of the Prox1 gene by ALK-1–activated Smads (4, 7).

These observations extend previous findings on the roles of ALK-1 and BMP-9 in hem- and lymphangiogenesis. ALK-1 was shown to antagonize the activities of the canonical TGF-β type I receptor, TjRI/ALK-5, in the control of endothelial function by TGF-β (11). With the identification of BMP-9 as a ligand for ALK-1 in ECs, BMP-9–ALK-1 signaling was shown to inhibit EC proliferation and angiogenesis (12), yet was also seen to promote EC proliferation and capillary sprouting (13). In lymphatic development, blockade of ALK-1 signaling in mice results in impaired lymphangiogenesis (14), whereas BMP-9 deficiency causes abnormal lymphatic development and impaired lymphatic draining (15).

The role of BMP-9–ALK-1 signaling as a differentiation switch in LECs is reminiscent of similar key roles of TGF-β family proteins in differentiation. TGF-β induces...
epithelial–mesenchymal transition (EMT), thus repressing the epithelial state and activating mesenchymal differentiation (16). TGF-β also represses osteoblastic and myogenic differentiation, whereas some BMPs activate osteoblast differentiation (17), and BMP7 induces brown adipocyte differentiation in mesenchymal progenitors (18). The underlying mechanisms reveal a potent ability of Smads to either activate or repress the expression and/or activities of master transcription factors that drive differentiation programs (19). In EMT induction, TGF-β–activated Smad3 induces expression of Snail transcription factors (20), whereas, in inhibiting osteoblast differentiation, Smad3 cooperates with Runx2 to repress Runx2 target genes (21), and, in myogenesis inhibition, Smad3 associates with myogenic bHLH transcription factors to prevent activation of their target genes (22). By analogy, we surmise that ALK-1–activated Smad1 or Smad5 repress Sox18–mediated transcription (Fig. 1), consistent with the cooperation of Smads with other Sox transcription factors such as Sox2 and Sox9 (19, 23). Thus, functional silencing of Sox18 by Smad1 or Smad5 may repress Prox1, preventing LEC differentiation and expanding the BEC population. When during embryogenesis ALK-1 expression first limits LEC expansion remains to be tested, because Alk1−/− mice die before lymphangiogenesis begins, and BMP10 can substitute for loss of BMP9 in BMP9−/− embryos (24). Nevertheless, Yoshimatsu et al. (5) observed defective lymphatic development in BMP9−/− embryos five days after first budding of the lymphatic rudiment.

Mutations in controlling lymphatic development, including SOX18 and FOXC2, were found to be causative in human lymphedema syndromes (1). In contrast, hemizygous loss-of-function ALK1 mutations cause a vascular malformation syndrome named hereditary hemorrhagic telangiectasia (HHT), rather than affecting lymphatics. HHT patients present with recurrent nose bleeds and develop cutaneous and mucosal blood vessel dilations. Some patients also develop life-threatening arteriovenous malformations (25). HHT can be caused by mutations in other ALK-1 pathway components, including the ALK-1 coreceptor endoglin that aids ligand binding, and Smad4, which partners with TGF-β– and BMP-activated Smads (25). Recently, causative BMPs missense mutations have been implicated in an HHT–related syndrome (26). Thus, HHT-causing mutations appear to define a BMP–9–endoglin–ALK–1–Smad4 pathway. Despite the identification of causative genes, the basis for HHT pathology is still uncertain. Mouse models of HHT show an arterial to venous EC transition within vascular lesions, evident by decreased EphrinB2 and increased COUP-TFI expression (6). It will be of interest to study transitions from BEC– to LEC–like properties within human HHT lesions.

The control of LEC differentiation by BMP–9–ALK–1 signaling raises interesting questions. Why do ALK-1 and BMP-9 mutations primarily affect the vascular system in humans, whereas mutations in other key regulators of lymphangiogenesis cause lymphedema syndromes? The answer to this question lies in the fact that regulatory molecules are often repurposed for varied functions in different tissues and at different stages of development. For example, EphrinB2 not only acts in axon guidance but also regulates angiogenesis, lymphangiogenesis, and arterial EC fate (8, 27). Similarly, VEGF3, an LEC marker, is essential for hemangiogenesis, where it can act with or independently of VEGF or VEGFR-2 to activate angiogenesis (9, 28). In this respect, it is intriguing that polymorphisms in PTEN14, a gene mutated in a lymphedema syndrome (29), show genetic association with the incidence of arteriovenous malformations in HHT patients (30), suggesting an interaction with endoglin/ALK-1 signaling in vascular development. PTPN14 interacts with VEGFR3 (29) and regulates EphrinB2 expression (30), suggesting further functional interaction between ALK-1 and VEGFR3. Direct functional cross-talk between ALK-1–activated Smads and VEGFR3 signaling, possibly complementing ALK–1–Notch signaling cross-talk as seen in hemangiogenesis (31), may additionally coordinate LEC and BEC proliferation and differentiation. Finally, a lingering question is what other ligands might control LEC differentiation and lymphangiogenesis through ALK-1. TGF-β can activate ALK-1 in ECs (11), and BMP-10 acts through ALK-1 in early vascular development (24). Defining the context-dependent roles of ALK-1 and its ligands in determining EC differentiation status during vascular and lymphatic development continues to be challenging but is of great biomedical interest.