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Mechanical forces in lymphatic vessel development: Focus on transcriptional regulation

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The lymphatic system is crucial for the maintenance of interstitial fluid and protein homeostasis. It has important roles in collecting excess plasma and interstitial fluid leaked from blood vessels, lipid absorption and transportation in the digestive system, and immune surveillance and response. The development of lymphatic vessels begins during fetal life as lymphatic endothelial progenitor cells first differentiate into lymphatic endothelial cells (LECs) by expressing the master lymphatic vascular regulator, prospero-related homeobox 1 (PROX1). The lymphatic vasculature forms a hierarchical network that consists of blind-ended and unidirectional vessels. Although much progress has been made in the elucidation of the cellular and molecular mechanisms underlying the formation of the lymphatic vascular system, the causes of lymphatic vessel abnormalities and disease are poorly understood and complicated; specifically, the mechanistic basis for transcriptional dysregulation in lymphatic vessel development remains largely unclear. In this review, we discuss the recent advances in our understanding of the molecular and cellular mechanisms of lymphatic vascular development, including LEC differentiation, lymphangiogenesis, and valve formation, and the significance of mechanical forces in lymphatic vessels, with a focus on transcriptional regulation. We also summarize the current knowledge on epigenetic mechanisms of lymphatic gene expression.

KEYWORDS

lymphatic system, lymphatic vascular development, lymphangiogenesis, valve formation, transcriptional regulation

Introduction

A well-organized lymphatic system including proper lymph fluid absorption and drainage is imperative for maintaining interstitial fluid and protein homeostasis. The lymphatic system is composed of a blind-ended, unidirectional network that contains absorptive vessels, primary lymphoid organs such as thymus and bone marrow, secondary lymphoid organs such as lymph nodes, spleen, and Peyer's patches, and lymphoid tissues as adenoids and tonsils (Ruddle and Akirav, 2009; Choi et al., 2012). Lymphatic fluid is collected from the interstitial space into lymphatic capillaries, and these lymphatic vessels merge and gradually thicken as lymphatic collecting vessels. Lymph is eventually drained at the angulus venosus, which is the junction of the subclavian vein and internal jugular

vein (Ruddle and Akirav, 2009; Choi et al., 2012; Randolph et al., 2017; Oliver et al., 2020). The lymphatic system also plays crucial roles in lipid absorption and transportation from the digestive tract to the blood circulation, as well as immune cell transport from the interstitium into the venous circulation (Alitalo et al., 2005; Tammela and Alitalo, 2010; Petrova and Koh, 2020; Landau et al., 2021). Lymphatic dysfunction causes interstitial fluid imbalance and edema, nutrient malabsorption, and inflammatory pathologies (Tammela and Alitalo, 2010; Saito et al., 2013; Abouelkheir et al., 2017). Lymphangiogenesis, the formation of new lymphatic vessels from the preexisting lymphatic vessels, relates to various diseases and pathologies such as lymphedema, tumor metastasis, and chronic inflammatory diseases including rheumatoid arthritis (Detmar and Hirakawa, 2002; Alitalo, 2011; Masood et al., 2022); however, the molecular mechanisms that regulate lymphatic endothelial cell proliferation and migration *via* transcriptional regulation remain largely unknown. In this review, we provide an update on the current knowledge regarding the development of the lymphatic vasculature and its mechanical force signals, especially focusing on transcriptional regulatory mechanisms.

The development of the lymphatic vascular system

The development of the lymphatic vascular system initiates shortly after blood circulation is established in mouse embryos (Yang and Oliver, 2014). At embryonic day (E) 9.5, a subpopulation of lymphatic endothelial progenitor cells in the anterior cardinal vein start to express the Prospero-related homeobox 1 (PROX1) transcription factor, which is a master lymphatic vascular regulator (Wigle and Oliver, 1999; Francois et al., 2008; Ducoli and Detmar, 2021), and then differentiate into lymphatic endothelial cells (LECs) (Lee et al., 2009; Yamazaki et al., 2009; Srinivasan et al., 2010; Srinivasan and Oliver, 2011; Escobedo and Oliver, 2016; Petrova and Koh, 2018). By around E10.0, *PROX1* positive lymphatic endothelial progenitor cells expressing vascular endothelial growth factor receptor (VEGFR) 3 sprout *via* stimulation with mesenchyme-derived VEGF-C ligand. These cells further migrate dorsolaterally from cardinal and intersomitic veins and establish primary lymph sacs and superficial lymphatic vessels identified as the jugular lymph sac by E11.5 (Wigle and Oliver, 1999; Karkkainen et al., 2004; Francois et al., 2012; Yang et al., 2012; Hagerling et al., 2013; Stritt et al., 2021). Another study also suggests LEC fate is decided during transition through the paraxial mesoderm (PXM) lineage. PXM-derived ECs selectively transdifferentiate from the cardinal vein to form LEC progenitors and form the lymphatic endothelium of multiple organs and tissues (Stone and Stainier, 2019). There is accumulating evidence that mesenchyme- or non-venous derived lymphatic progenitor cells contribute to the early lymphatic vasculature and the development of the lymphatic vascular network in various organs including the skin, heart, and mesentery (Bernier-Latmani et al., 2015; Klotz et al., 2015; Martinez-Corral et al., 2015; Stanczuk et al., 2015; Kazenwadel and Harvey, 2016; Ducoli and Detmar, 2021). This network spreads throughout the mouse embryo by E14.5 and subsequently goes through remodeling and maturation from E15.5-E16.0, forming the hierarchical structure of the lymphatic vascular network in which lymphatic capillaries merge to form pre-collecting and collecting lymphatic vessels (Coso et al., 2014; Norden and Kume, 2020) (Figure 1).

Lymphatic vessels are composed of lymphatic capillaries, which are also called initial lymphatics, and collecting lymphatic vessels. The basement membrane of lymphatic capillaries is discontinuous without lining of any pericytes or lymphatic smooth muscle cells (SMCs); therefore, they work for collecting excess plasma and interstitial fluid leaked from blood vessels (Norden and Kume, 2020). In contrast, collecting lymphatic vessels have lymphatic valves to prevent the backflow of lymph, and smooth muscle to transport lymph fluid by contraction (Kume, 2015). The development of the lymphatic vascular network is conducted by several critical signaling pathways including lymphangiogenic signaling such as the VEGF-C/D-VEGFR3 and Angiopoietin (Angpt)-tunica interna endothelial cell kinase (TEK, also known as Tie2) pathways (Potente and Makinen, 2017). Two transcription factors, the SRY-Box transcription factor 18 (SOX18) and the chick ovalbumin upstream promoter transcription factor 2 (COUP-TFII), also play an important role in lymphatic specification *via* the induction of *PROX1* expression, whereas different pathways such as Notch, retinoic acid, and Wnt/beta-catenin signaling are involved in this process (Nicenboim et al., 2015; Ducoli and Detmar, 2021). *VEGFR3* also regulates *PROX1* by establishing a feedback loop necessary to maintain the identity of LEC progenitor cells, and VEGF-C-mediated activation of Vegfr3 signaling is required to maintain *PROX1* expression in LEC progenitor cells (Srinivasan et al., 2014). In collecting lymphatic vessels, platelet-derived growth factor B (PDGFB) regulates lymphatic SMC recruitment, but PDGFB overexpression is insufficient to mediate recruitment to lymphatic capillaries (Wang et al., 2017).

A recent study has demonstrated the deficiency of Folliculin, a tumor suppressor, causes ectopic expression of *PROX1* in venous endothelial cells (VECs), leading to the misconnection of blood and lymphatic vessels (Tai-Nagara et al., 2020). In LEC-biased VECs deficient for Folliculin, the basic helix-loop-helix transcription factor E3 (TFE3) translocate into the nucleus, binds to a regulatory element of the *PROX1* gene, and induces its ectopic venous expression (Tai-Nagara et al., 2020). Thus, in mice, it has been shown that the transition of lymphatic specification and differentiation from venous cell fate is tightly controlled during development. Importantly, the development of the zebrafish anal fin begins along with the formation of lymphatic vessels, but not blood vessels. Following the

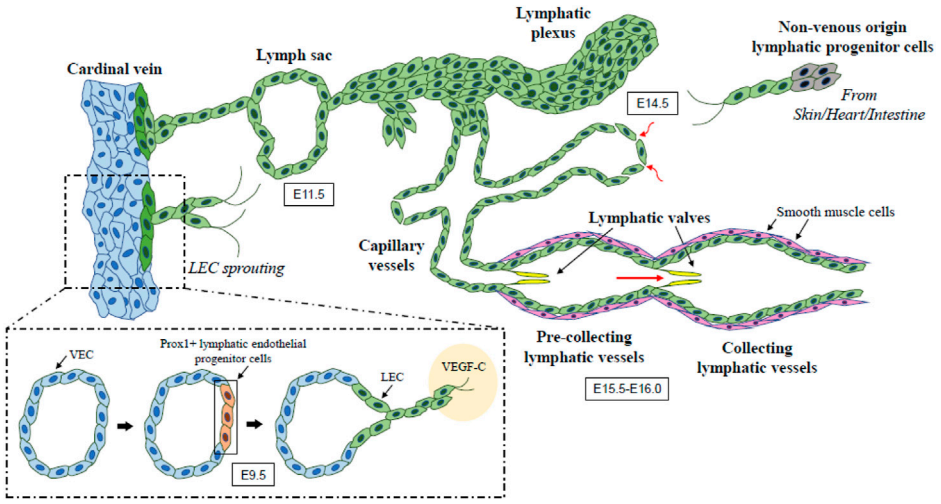

FIGURE 1
The developmental process of lymphatic vessel. Lymphatic endothelial progenitor cells in the cardinal vein begin to express *PROX1* (known as a master lymphatic vascular regulator) at E9.5 and then differentiate into LECs expressing *VEGFR3*. VEGF-C/VEGFR3 signaling promotes the sprouting and migration of *PROX1*-positive LECs, which leads to the formation of lymph sacs and initial lymphatic plexus by E11.5. Extended sprouting and migration of LECs from the initial lymphatic structures give rise to the hierarchical lymphatic vessel network. Non-venous origin lymphatic endothelial progenitor cells also contribute to the early lymphatic vasculature and the development of its network. This network undergoes remodeling and maturation during E15.5-E16.0 and forms the organized lymphatic vascular network including lymphatic capillaries and pre-collecting and collecting lymphatic vessels. Arrows show the direction of lymph flow. LEC, lymphatic endothelial cell; VEC, venous endothelial cell; VEGF, vascular endothelial growth factor.

progressive loss of lymphatic markers during the anal fin growth, these vessels subsequently acquire a blood vessel fate leading to the connection to blood circulation. Thus, this specialized blood vessel formation occurs through LEC transdifferentiation (Das et al., 2022). Single-cell RNA-sequencing analysis in this study further reveals that the loss of lymphatic fate results in the upregulation of several blood endothelial markers, such as *VEGFR1*, Delta-like (DLL) 4, and SRY-box (SOX) 17. Of note, mosaic overexpression of *SOX17* in zebrafish ECs results in reduced lymphatic gene expression in the anal fin as well as the absence or incomplete formation of the thoracic duct (Das et al., 2022), demonstrating the importance of *SOX17* function in the transition process.

Transcriptional and epigenetic regulation in lymphangiogenesis

Transcriptional regulation during lymphangiogenesis is strictly controlled, and recent evidence suggests the specific functions of several key transcription factors in lymphangiogenesis. The transcription factor V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB), which is involved in the differentiation of various cell types, regulates the transcriptional changes invoked by VEGF-C in LECs (Dieterich et al., 2015; Dieterich et al., 2020; Rondon-Galeano et al., 2020; Arnold et al., 2022). *MAFB* induces the expression of *PROX1*, other transcription factors and markers of differentiated LECs, indicating the role of *MAFB* in the maintenance of the mature LEC phenotype (Dieterich et al., 2015). LEC-specific *MAFB* deficiency in mice causes increased lymphatic branching in the diaphragm at P7, enhanced tumor-induced lymphangiogenesis, increased perinatal lethality associated with cyanosis, and excessive smooth muscle cell coverage indicating a defect in the maturation of lymphatic networks. This suggests *MAFB* could be a potential target for therapeutic modulation of lymphangiogenesis (Dieterich et al., 2020; Rondon-Galeano et al., 2020). The transcription factor hematopoietically expressed homeobox (HHEX), an upstream regulator of VEGF-C/VEGFR3/PROX1 signaling during angiogenic sprouting and lymphatic formation, is required cell-autonomously in endothelial cells to promote venous and lymphatic sprouting. Mice deficient for *HHEX* exhibit severe vascular defects in blood and lymphatic vessel development (Gauvrit et al., 2018).

Forkhead box (Fox) O1, a member of the Fox transcription factor family, acts an essential role in developmental lymphangiogenesis by promoting LEC migration toward the chemokine (C-X-C motif) ligand (CXCL) 12 and regulating their proliferative activity (Niimi et al., 2020). The LEC-specific deletion of *FOX O1* in mice decreases LEC migration toward CXCL12 by downregulating C-X-C chemokine receptor

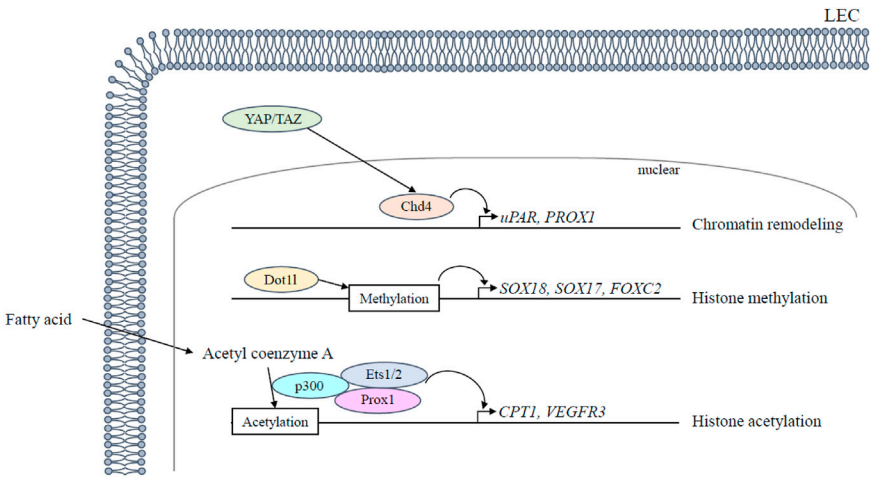

FIGURE 2
Epigenetic regulation in lymphangiogenesis. Epigenetic regulation regarding lymphatic development is mediated in LECs as follows: 1) *Chd4* is functionally associated with the Hippo signaling pathway and downregulates *Prox1* expression together with Hippo pathway final effectors *YAP/TAZ*; 2) *Dot1l* promotes transcription by histone methylation of chromatin; 3) Acetyl coenzyme A is used by the histone acetyltransferase p300 that interacts with *Prox1* to acetylate histones. ETS 1/2 participates in *VEGFR3* gene expression by recruiting the histone acetyltransferase p300 to the *VEGFR3* locus and leading to histone acetylation. LEC, lymphatic endothelial cell.

(CXCR) 4, induces excess LEC proliferation, and decreases LEC apoptosis, which leads to the disconnected and dilated structure of the lymphatic vessels (Niimi et al., 2020).

Brahma-related gene 1 (BRG1), a chromatin-remodeling enzyme, epigenetically regulates COUP-TFII expression, by remodeling chromatin within the *COUP-TFII* promoter and impacting the ability of transcriptional machinery to access the promoter (Davis et al., 2013). The EC-specific deletion of *Brg1* in mice results in downregulation of COUP-TFII expression in developing veins (Davis et al., 2013).

Chromodomain helicase DNA-binding 4 (CHD4), an ATPase subunit of the nucleosome remodeling deacetylase (NuRD) chromatin-remodeling complex, regulates vascular integrity in the mid-gestation (Ingram et al., 2013). Specifically, *CHD4* controls the development of lymphovenous valves, which regulates the return of lymph to the blood circulation by forming fibrin-rich thrombi that prevent blood from entering the lymphatic system (Crosswhite et al., 2016). The LEC-specific deletion of *CHD4* in mice leads to increased transcription of the urokinase plasminogen activator receptor (uPAR), thereby facilitating activation of the fibrin-degrading protease plasmin and then degrading the fibrin near the lymphovenous valves (Crosswhite et al., 2016). In addition, CDH4 is functionally associated with the Hippo signaling pathway in lymphatic endothelial cells (Figure 2). The Hippo pathway final effectors Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) promote remodeling of lymphatic plexus patterning and postnatal lymphatic valve maintenance by negatively regulating Prox1 expression (Cho et al., 2019). LEC-specific deletion of *YAP/TAZ* in mice suppresses both lymphatic plexus patterning and valve initiation *via* upregulation of *PROX1*, whereas LEC-specific *YAP/TAZ* overexpression downregulates *PROX1*, disrupts lymphatic specification, and restricts lymphatic sprouting (Cho et al., 2019).

Disruptor of telomeric silencing 1-like (DOT1L), a histone H3 lysine (H3K) 79 methyltransferase, promotes transcription by histone methylation of chromatin and is a crucial factor in the homeostasis of various organs such as the heart and hematopoiesis (Jo et al., 2011; Nguyen et al., 2011). Vascular endothelial cell (VEC)-specific, but not LEC-specific, deletion of *DOT1L* causes fully penetrant lymphatic aplasia by altering the lymphatic transcription program and reducing H3K79me2 enrichment at lymphatic genes, including the transcription factors *SOX18*, *SOX17*, and *FOXC2*, which are critical for LEC differentiation and valve formation, as well as Vegfr3, which is critical for LEC proliferation and migration (Yoo et al., 2020) (Figure 2).

LECs use fatty acid β-oxidation for proliferation and epigenetic regulation of *PROX1*, which mediates epigenetic changes that promote lymphangiogenesis during LEC differentiation (Figure 2): 1) *PROX1* upregulates carnitine palmitoyltransferase (CPT) 1A expression, which increases fatty acid β-oxidation-dependent acetyl coenzyme A production; 2) Acetyl coenzyme A is used by the histone acetyltransferase p300 to acetylate histones at lymphangiogenic genes; 3) histone acetyltransferase p300 interacts with Prox1 to facilitate preferential histone

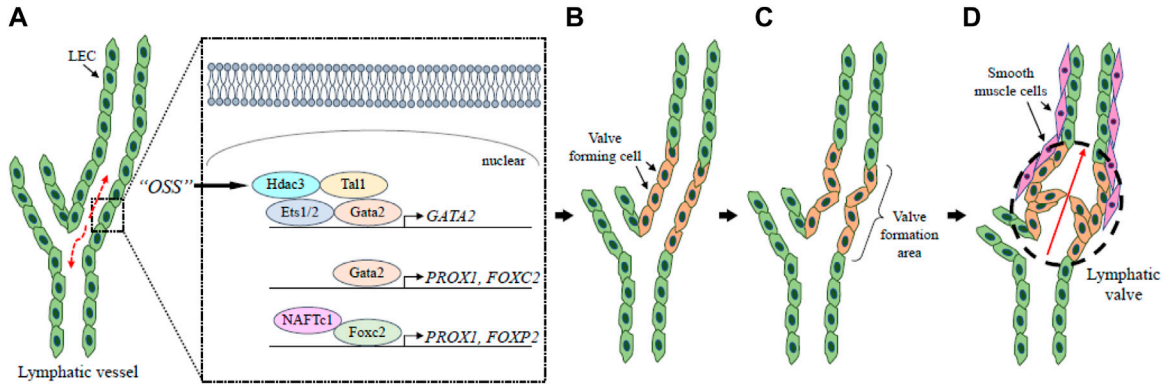

FIGURE 3
Lymphatic valve formation. The lymphatic valves are formed through lymph flow-dependent lymphatic vessel remodeling. At the bifurcation of lymphatic vessels, the flow of lymph generates OSS (broken arrows) that leads to the upregulation of *GATA2* in LECs *via* Hdac3 **(A)**. Gata2 regulates *PROX1* and *FOXC2* expression to establish valve-forming cells **(B)**. Following these processes, the valve formation area is determined **(C)**, and the lymphatic valve (broken circle) has a role in keeping the lymph flow unidirectional **(D)**. Red arrow shows the direction of lymph flow. LEC, lymphatic endothelial cell; OSS, oscillatory share stress.

acetylation at the loci of *PROX1*-targeted genes (Wong et al., 2017). LEC-specific deletion of *CPT1A* in mice impairs lymphatic vessel development by exhibiting severe impairment of dermal lymphatic vessel outgrowth and branching at E16.5. (Wong et al., 2017). Other transcription factors expressed in LEC, E26 avian leukemia oncogene (ETS) 1 and 2, act as downstream effectors of the Ras/MAPK pathway and participate in *VEGFR3* gene expression in LECs by recruiting the histone acetyltransferase p300 to the *VEGFR3* locus and leading to histone acetylation and transcriptional activation of the *VEGFR3* promoter (Ichise et al., 2012). In addition, *ETS2* enhances inflammatory lymphangiogenesis and endothelial migration towards VEGF-C through the induction of *VEGFR3* expression by binding to the *VEGFR3* promoter in concert with *PROX1* (Yoshimatsu et al., 2011). Additionally, mitochondrial complex III also regulates the critical *PROX1-VEGFR3* feedback loop. The functional inactivation of mitochondrial complex III impairs lymphatic vessel development by disrupting the maintenance of the *PROX1-VEGFR3* feedback loop through the reduction in H3K4me3 and H3K27ac histone modifications at the *VEGFR3* and *PROX1* promoters (Ma et al., 2021).

Transcriptional and epigenetic regulation in lymphatic valve formation

Lymph flow is essential for the development and maturation of lymphatic valves (Kume, 2015), which play a critical role in preventing the backflow of lymph fluid. The lymphatic valves are formed from lymphatic endothelial cells, a process that is occurred by flow-dependent lymphatic vessel remodeling caused by oscillatory share stress (OSS) at branching points in the lymphatic plexus during the early stage of lymphatic development (Sabine et al., 2012; Shin and Lawson, 2021). The OSS response leads to an increase in the expression of GATA-binding protein 2 (GATA2), Prox1, and Foxc2, which induce valve forming cells to the site of valve formation (Kazenwadel et al., 2015; Sweet et al., 2015; Shin and Lawson, 2021). In valve forming cells, Gata2 directly regulates *PROX1* and *FOXC2* expression, whereas Foxc2 regulates valve maturation in cooperation with Prox1 to control intraluminal invagination of LECs and reorganization into valve forming leaflets by postnatal day (P)1 (Kazenwadel et al., 2015). As an upstream epigenetic factor, the histone-modifying enzyme histone deacetylase 3 (HDAC3) regulates lymphatic valve formation. In response to OSS, Hdac3 is recruited to the Gata2 enhancer element and physically interacts with the transcription factors T-cell acute lymphocytic leukemia protein 1 (TAL1), Gata2, and Ets1/2 to promote Gata2 expression (Janardhan et al., 2017) (Figure 3).

Human *FOXC2* is a causative gene whose mutations are dominantly associated with lymphedema-distichiasis syndrome characterized by failure of lymph drainage in limbs, venous valve failure, and the growth of an extra set of eyelashes (Fang et al., 2000; Traboulsi et al., 2002; Mellor et al., 2007; Tavian et al., 2016). Foxc2 regulates connexin 37 expression and activation of calcineurin/nuclear factor of activated T-cells (NFAT) signaling during lymphatic collecting vessel maturation and valve formation (Petrova et al., 2004; Norrmen et al., 2009; Sabine et al., 2012). Foxc2 is also identified as a crucial factor for lymphatic valve maintenance by regulating LEC junctional integrity and cellular quiescence under reversing flow conditions *via* restriction of TAZ-mediated proliferation (Sabine et al., 2015). Foxc1 is a closely related member of the Fox transcription factor family, and LEC-specific deletion of

TABLE 1 Factors involved in transcriptional regulation regarding lymphatic vascular development.

Factor	Function	References
PROX1	A master lymphatic vascular regulator	
	Promotes differentiation of lymphatic endothelial progenitor cells in the cardinal vein into LECs	Wigle and Oliver, (1999)
		Francois et al. (2008)
		Lee et al. (2009)
		Yamazaki et al. (2009)
		Srinivasan et al. (2010)
		Srinivasan and Oliver, (2011)
		Escobedo and Oliver, (2016)
		Petrova and Koh, (2018)
		Ducoli and Detmar, (2021)
MAFB	Induction of Prox1 expression in differentiated LECs	Dieterich et al. (2015)
	Maintenance of mature LEC phenotype	
FOXO1	Acts an essential role in normal developmental lymphangiogenesis by promoting LEC migration toward CXCL12 and regulating their proliferative activity	
	Control the expression of valve forming genes including *FOXC2*, *PROX1*, and *GATA2*	
	Repressor for lymphatic valve formation and maintenance	Niimi et al. (2020)
		Scallan et al. (2021)
HHEX	Promotes venous and lymphatic sprouting	Gauvrit et al. (2018)
CHD4	Acts normal lymphovenous valve development	
	Regulates the return of lymph to the blood circulation by forming fibrin-rich thrombi that prevent blood from entering the lymphatic system	Crosswhite et al. (2016)
YAP/TAZ	Promotes remodeling lymphatic plexus patterning and postnatal lymphatic valve maintenance	Cho et al. (2019)
DOT1L	Promotes transcription by histone methylation of chromatin and promotes the expression of important transcription factors such as Sox18, Foxc2, and VEGFR3 in lymphatic endothelium	Yoo et al. (2020)
GATA2	Induces valve forming cells to the site of valve formation	Kazenwadel et al. (2015)
	Directly regulates *PROX1* and *FOXC2* expression in valve forming cells	
FOXC1/ FOXC2	Required for LEC junction integrity in lymphatic valves, collecting vessels, and dermal lymphatics	Petrova et al. (2004)
		Norrmen et al. (2009)
		Sabine et al. (2015)
		Fatima et al. (2016)
		Norden et al. (2020)
FOXP2	Maintenance of collecting lymphatic vessel and valve formation	Hernandez Vasquez et al. (2021)
PIEZO1	Maintenance of the lymphatic valve protrusion such as collective cell migration, actin polymerization, and remodeling of cell-cell junctions	
	Upregulates *FOXC2* and *GATA2* under the absence of OSS in valve forming cells	Nonomura et al. (2018)
		Choi et al. (2019)

LEC, lymphatic endothelial cell.

FOXC1, *FOXC2*, or both in mice leads to increased LEC proliferation, enlarged lymphatic vessels, and abnormal lymphatic vessel morphogenesis, accompanied by increased Ras/ERK signaling during embryonic lymphangiogenesis (Fatima et al., 2016). Unlike *FOXC2*, LEC-specific *FOXC1* mutant mice normally develop initial mesenteric lymphatic valves; however, the formation of matured lymphatic vessels (v-shaped or semilunar bi-leaflet structures) is significantly impaired (Norden et al., 2020). Importantly, the number of mesenteric lymphatic valves is remarkably reduced in the LEC-specific deletion of *FOXC1* and *FOXC2* compared to LEC-specific *FOXC2* deletion alone, suggesting that *FOXC1* and *FOXC2* function cooperatively in the maturation and maintenance of lymphatic valves (Norden et al., 2020).

Foxo1 is crucial for controlling the expression of valve forming genes including *FOXC2*, *PROX1*, and *GATA2* as a key downstream effector of shear stress by regulating lymphatic valve maintenance, and LEC-specific deletion of *FOXO1* in mice leads to the formation of additional lymphatic valves compared to control mice (Scallan et al., 2021). Another study also reveals the role of Foxo1 as a repressor for lymphatic valve formation and maintenance *via* the inhibition of OSS-induced upregulation of lymphatic valve-specific genes such as *PROX1* and *FOXC2* (Niimi et al., 2021).

A recent study has shown that Foxp2, another Fox transcription factor previously implicated in speech development, is expressed in lymphatic endothelial cells of collecting vessels and their valve-forming cells, and that Foxp2 is induced after initiation of lymph flow and upon OSS on LECs (Hernandez Vasquez et al., 2021). LEC-specific *FOXP2* mutant mice exhibit enlarged collecting vessels and defective lymphatic valves characterized by loss of NFATc1 activity (Hernandez Vasquez et al., 2021).

Piezo type mechanosensitive ion channel component 1 (PIEZO1), a cation channel activated by mechanical forces such as fluid shear stress or membrane stretch, is a causative gene associated with congenital lymphedema with pleural effusion (Nonomura et al., 2018). The LEC-specific deletion of *PIEZO1* in mice leads to a reduction in the number of lymphatic valves and impairments in lymphatic valve protrusion such as collective cell migration, actin polymerization, and remodeling of cell-cell junctions, whereas the expression patterns of Foxc2 and NFATc1, both of which are crucial factors for lymphatic valve development, are normally detected in these mutant mice (Nonomura et al., 2018). Another study demonstrated that PIEZO1 is the force sensor in the mechanotransduction pathway controlling lymphatic valve development and maintenance, although *PIEZO1* knockdown in cultured LECs largely abrogated the OSS-induced upregulation of the lymphatic valve signature genes including *FOXC2* and *GATA2* (Choi et al., 2019). Moreover, overexpressing *PIEZO1* in cultured LECs upregulates *FOXC2* and *GATA2* in the absence of OSS, demonstrating that ectopic expression of *PIEZO1* can recapitulate the lymphatic valve gene profile (Choi et al., 2019). Treatment with Yoda1, a chemical agonist of PIEZO1, leads to changes in LEC morphology by inducing the remodeling of actomyosin and/or VE-cadherin^{+} cell–cell adhesion and activates the expression of some lymphatic valve genes such as *FOXC2* and *GATA2* in a PIEZO1-dependent manner (Nonomura et al., 2018; Choi et al., 2019). Together, these results suggest that the activation of mechanosensitive PIEZO1 can control, at least in part, transcriptional regulation of lymphatic valve forming cells under the absence of mechanical forces.

Concluding remarks

Many studies have been conducted on the formation, maintenance, and function of lymphatic vessels, which are essential to maintain homeostasis. This review focuses on the mechanisms of transcriptional regulation in LECs during lymphatic vessel development (Table 1), but the precise control of lymphatic gene expression in lymphangiogenesis under physiological and pathological conditions remains unexplored. Recent single-cell RNA-sequencing studies using LEC markers such as *PROX1* and *VEGFR3* have started to clarify LEC heterogeneity in various organs including functional multiformity. For example, single-cell RNA-sequencing analysis using the zebrafish anal fin was the key to characterizing the different endothelial cell populations and transition states involved in the LEC transdifferentiation process (Das et al., 2022). Moreover, single-cell transcriptomic analysis of normal and glaucomatous Angpt1 deficient eyes has recently identified distinct trabecular meshwork (TM) and Schlemm's canal (SC) cell populations and revealed additional TM-SC signaling pathways (Thomson et al., 2021). Yet, additional comprehensive studies are needed to fully elucidate the mechanisms of transcriptional regulation of LECs with which the signaling pathways are associated. In particular, uncovering the transcriptional mechanisms underlying lymphangiogenesis will likely lead to the development of new therapeutic strategies for various diseases regarding lymphatic vessels.

Author contributions

NU contributed to the writing of the manuscript and making the figures. TK contributed to the concepts, editing, and final formatting of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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