J. Biochem. 2012;152(3):213–220 doi:10.1093/jb/mvs081

JB THE JOURNAL OF BIOCHEMISTRY

JB Review

Roles of transcriptional network during the formation of lymphatic vessels

Received July 10, 2012; accepted July 16, 2012; published online July 23, 2012

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The lymphatic vascular system, also known as the second vascular system in vertebrates, plays crucial roles in various physiological and pathological processes. It participates in the maintenance of normal tissue fluid balance, trafficking of the immune cells and absorption of fatty acids in the gut. Furthermore, lymphatic system is associated with the pathogenesis of a number of diseases, including lymphedema, inflammatory diseases and tumour metastasis. Lymphatic vessels are comprised of lymphatic endothelial cells (LECs), which are differentiated from blood vascular endothelial cells. This review highlights recent advances in our understanding of the transcriptional control of LEC fate determination and reflects on efforts to understand the roles of transcriptional networks during this discrete developmental process.

Keywords: lymphangiogenesis/Prox1/Sox18/COUP-TFII/VEGFR3.

Abbreviations: BEC, blood vascular endothelial cell; COUP-TF, chicken ovalbumin upstream promoter transcription factor; LEC, lymphatic endothelial cell; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

During embryogenesis, an organism develops from a single-fertilized egg which goes through rapid cell division and differentiation into the multiple lineages that make up the diverse systems of the body plan. Various maternal and zygotic signalling cascades trigger the activation of regulatory networks of transcription factors that act as switches to turn on or off the precise expression of arrays of functional molecules that regulate differentiation of various types of cells. Combinatorial control by the hubs comprising of multiple transcription factors working in concert can also confer cell type-specific regulation of target genes to produce specialized cell types. Physical and functional interactions between multiple transcription factors at shared target genes result in complex gene regulatory networks. Unravelling of these transcriptional networks will aid in understanding the molecular mechanisms how different cell types arise. This review will focus on transcriptional regulation of embryonic lymphangiogenesis. We will explore the roles of the transcription factors during lymphatic endothelial cell (LEC) fate specification, differentiation and the maintenance of the identity of LECs.

Structures and Functions of LVs in Health and Disease

The lymphatic vasculature is an essential component of the vertebrate vascular system and plays a number of critical roles in homeostasis and disease (*1*). In mammals, the mature lymphatic system consists of the lymphatic vasculature and the lymphoid organs including the lymph nodes, Peyer's patches, tonsils, spleen and thymus. The lymphatic vasculature covers most of the body, with the exception of epidermis, cornea, retina, cartilage and central nervous system. Lymphatic vessels (LVs) function to return interstitial fluid and protein to the bloodstream, to absorb dietary fatty acids and to traffic immune cells.

LVs are involved in the pathogenesis of a number of human diseases. Reduced lymphatic vascular function often leads to lymphedema, whereas the aberrant growth of LVs has been involved in the progression of many types of cancer. Since tumour cells utilize LVs to metastasize to local lymph nodes and secondary tumour sites, the presence of lymph node metastases is typically correlated with poor prognosis (*2*). Therefore, understanding of the molecular mechanisms underlying the development of the lymphatic system will aid in designing of novel therapeutic approaches to interfere with the lymph node metastasis.

The blood and lymphatic vasculatures are lined by endothelial cells (ECs). The lymphatic vasculature is formed by LECs, which are highly similar to blood vascular endothelial cells (BECs). Although BECs and LECs represent two distinct cell populations, they can gain the characteristics of the other cell type under certain circumstances (*3*, *4*). The vascular network of the lymphatic system starts with blind-ending lymphatic capillariesin the peripheral tissues (Fig. 1). Lymphatic capillaries are composed of a single-cell layer of overlapping ECs that are interconnected by specialized discontinuous button-like junctions and that contain few intercellular tight junctions or adherens junctions (*5*). Lymphatic capillaries do not have basement membranes, or are not covered by smooth muscle cells (Fig. 1). They are tethered by anchoring filaments to collagen fibres of the extracellular matrix.

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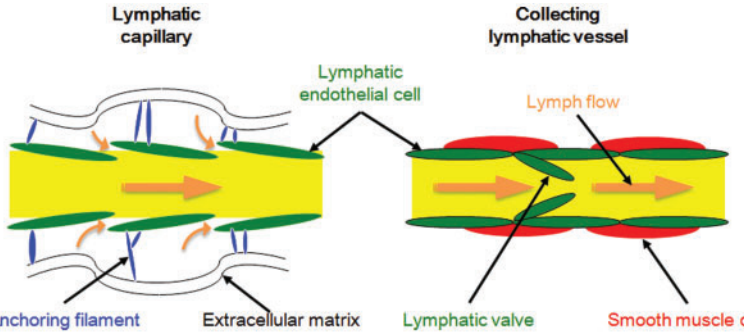

Fig. 1 Structure of the LVs. The ECs of lymphatic capillaries (green) lack tight junctions. Instead, the neighbouring ECs partly overlap, forming valve-like openings, which allow easy access for lymph (orange arrow) into the vessel lumen. Lymphatic capillaries lack vascular smooth muscle cells. Anchoring filaments (blue) connect lymphatic capillary ECs to the surrounding extracellular matrix and maintain vessel patency during increased interstitial pressure. The lymph drains from the lymphatic capillaries to collecting LVs, which are finally emptied into veins in the jugular region. The collecting LVs are surrounded by vascular smooth muscle cells (red) with intrinsic contractile activity to promote lymph flow. The collecting LVs contain valves that prevent the backflow of the lymph.

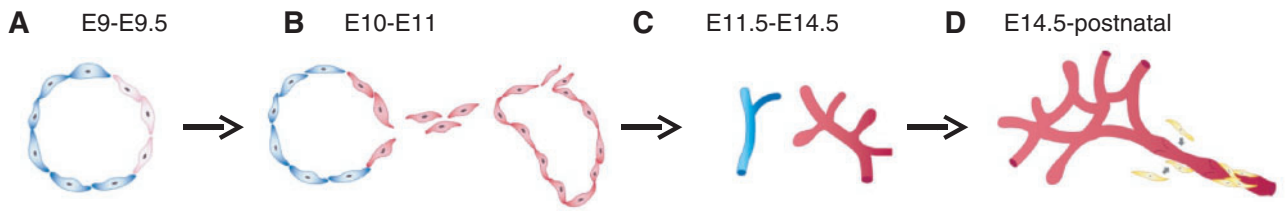

Fig. 2 Development of the LVs. (**A**) In E9.0–E9.5 mouse embryos, a subset of venous ECs (orange) express Prox1 and become specified to differentiate into the lymphatic endothelial fate. (**B**) These differentiating LECs express VEGFR3 and migrate towards VEGF-C expressing mesenchymal cells, and proliferate to form primary lymph sacs. (**C**) The primary lymphatic vascular plexus (orange) becomes separated from the blood vessels (red). (**D**) The primary lymphatic vascular plexus undergoes remodelling and maturation to create the hierarchy consisting of a lymphatic capillary and collecting LVs. Adapted from (*1*).

As the surrounding interstitial pressure changes, the anchoring filaments tighten and relax, causing the LVs to expand and fill or contract and push lymph, respectively. Under high interstitial pressure, EC junctions open, anchoring filaments extend and fluid moves into the vessel. The lymphatic capillaries descend into pre-collecting lymphatics, which eventually merge into larger secondary collecting LVs. Collecting LVs possess ECs that exhibit continuous zipper-like junctions, and are covered by smooth muscle cells that provide contractile activity to assist lymph flow (*5*). In order to ensure a unidirectional flow of the lymph from the peripheral tissues towards the blood circulation, collecting vessels contain bi-leaflet lymphatic valves. In mammals the driving forces for the generation of lymph flow are generated by contractions of the lymph vessel coat and by skeletal muscle contractions. The lymph of the body is drained into two main LVs, the thoracic duct and the right lymphatic duct. Both of them transport the lymph back into the blood circulation via connections with the left and right subclavian veins, respectively.

Overview of Lymphatic Development

Florence Sabin postulated that LVs develop by sprouting from blood vessels more than 100 years ago. This 'centrifugal' theory has been verified during the last 10 years (*1*). The LVs form after the blood vascular system is established during embryogenesis. Shortly after the separation of arteries and veins, around embryonic day (E) 9.0 in mice, a distinct population of ECs of the anterior cardinal vein starts to express LYVE-1, a hyaluronan receptor. In E9.75 mouse embryos, Prox1 transcription factor starts to be expressed in a subset of LYVE-1 expressing cells of the cardinal vein (Fig. 2) (*6*, *7*). Around E10.5, when the Prox1 expressing cells become committed to their LEC fate, they sprout and bud off the cardinal vein, and migrate in a polarized manner towards the surrounding tissue, where they form primary lymphatic vasculature. Expansion of the lymphatic sacs by sprouting and proliferation leads to the formation of the primitive lymphatic sacs (Fig. 2). Several lines of evidence have suggested that Prox1 expressing lymphatic endothelial progenitors are attracted by Vascular Endothelial Growth Factor (VEGF)-C, which is expressed by the mesenchymal cells adjacent to the growing LVs.

VEGF-C and VEGF-D signal through their cognate receptor VEGF receptor (VEGFR) 3 (*8*), and promote the migration and proliferation of LECs *in vitro* (*9*). Furthermore, adenoviral or transgenic expression of VEGF-C induces lymphangiogenesis *in vivo* (*10*, *11*). Studies using knockout mice revealed that allelic loss of VEGF-C is sufficient to cause severe lymphedema, and homozygous VEGF-C deletion results in the complete loss of the lymphatic vasculature. In *Vegfc*-null mouse embryos, while specification of LEC takes place, the embryos lack a lymphatic vasculature because the committed LECs fail to sprout from the cardinal vein (*12*). In contrast to VEGF-C, genetic deletion of VEGF-D exhibits no phenotypes on the

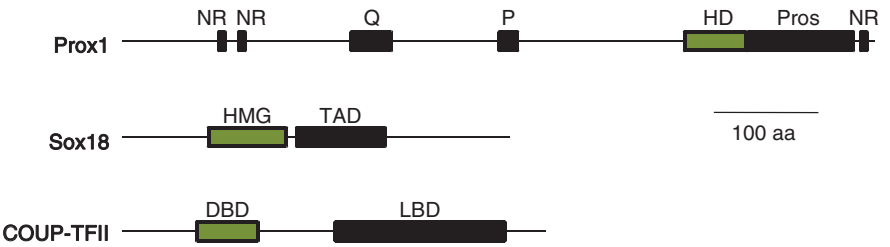

Fig. 3 Schematic illustration of protein structures of key transcription factors of lymphatic development. DNA binding motifs (green boxes) and other protein structural motifs (black boxes) of Prox1, Sox18 and COUP-TFII are shown. NR: nuclear receptor domain, Q: Glutamine-rich domain, P: Proline-rich domain, HD: homeodomain, Pros: *prospero* domain, HMG: high mobility group domain, TAD: transcription activation domain, DBD: DNA binding domain, LBD: ligand binding domain, aa: amino acids.

formation of lymphatic vasculature (*13*). Since VEGFR3 is expressed in all BECs during the early stages of development, loss of VEGFR3 signalling in this period results in embryonic lethality due to vascular failure before the lymphatic vascular development begins (*14*). VEGFR3 expression becomes restricted to LECs later in development (~E12.5) (*15*), when defective VEFGR3 signalling interferes with the development of a proper lymphatic vasculature. Intriguingly, mutations in the tyrosine kinase domain of VEGFR3 result in a loss of signalling activity and lead to lymphatic hypoplasia and lymphedema in human patients (Milroy Disease) (*16*) and in mice (Chy mutant mice) (*17*).

The lymphatic system becomes completely separated from the blood vascular system except for the two connections with the veins in the neck region, the right lymphatic and thoracic duct (Fig. 2). Maturation of the lymphatic vasculature begins at ~E14.5 and lasts until birth. During this time period, the primitive lymphatic plexus develops into a hierarchical network of lymphatic capillaries and collecting LVs. The structural remodelling of collecting vessels involves the formation of intraluminal valves and the attainment of smooth muscle coverage (Fig. 2).The separation of lymphatic vascular system from the blood vasculature and maturation of the lymphatic system depend on multiple signalling pathways and transcription factors, whose roles have been reviewed elsewhere (*1*, *18*).

Transcription Factors Involved in Lymphatic Development

Identification of lymphatic endothelial-specific markers and functional studies of regulators by developing genetically modified animals and *in vitro* studies have identified Prox1, Sox18 and COUP-TFII as the key transcription factors essential for LEC fate specification.

Prox1

Prox1 is a homeobox-containing transcription factor and is related to *Drosophila prospero* (*6*). Prox1 has a homeobox DNA binding domain and Prospero domain in its carboxyl-terminal region (Fig. 3). Previous studies have revealed that three nuclear receptor boxes in Prox1 play important roles in its interaction with nuclear receptors including liver receptor homologue-1.

In E9.75 mouse embryos, Prox1 is expressed in a subset of BEC of the cardinal vein, from which they sprout to form primary lymph sacs (*6*, *7*). Several lines of evidence have suggested that Prox1 expression is necessary and sufficient to specify the LEC phenotype in venous BECs. In *Prox1*-null mice, sprouting of lymphatic endothelial progenitors from the veins appears unaffected at E10.5, but their migration is arrested at around E11.5-E12.0, leading to a complete absence of the lymphatic vasculature, suggesting that Prox1 is necessary to specify LEC phenotypes in a subset of venous ECs. Furthermore, as a homeobox transcription factor, Prox1 has been shown to up-regulate the expression of LEC markers, and to down-regulate BEC markers in mature ECs (*3*, *19*). These *in vivo* and *in vitro* findings suggest that Prox1 regulates the program of differentiation of embryonic BECs to LECs by functioning as a binary transcriptional switch, turning the BEC program off and the LEC program on.

Prox1 homologues have been shown to be necessary for the formation of the lymphatic system in other vertebrate models including *Xenopu laevis* (*20*) and zebrafish (*21*), suggesting its conserved roles in lymphatic development throughout evolution. However, to date, there have been no lymphatic disorders reported to be associated with mutations in human *PROX1* gene.

Sox18

SRY-related HMG-box (Sox) 18 is a member of the F-group of Sox transcription factors, a subfamily that also contains the closely related Sox7 and Sox17 proteins (*22*). Sox genes are characterized by a homologous sequence named the high mobility group (HMG) box (Fig. 3). The HMG box is a DNA binding domain that is highly conserved throughout eukaryotic species (*22*). Previous structure-function studies have revealed that Sox18 has a transactivation domain in the central region (Fig. 3). Sox transcription factors are known to play a number of essential roles during vascular development and often act redundantly in these processes (*23*, *24*). For example, Sox18 is necessary for specification of arteries and veins, but functions redundantly with Sox7 in this process (*25*).

The physiological significance of Sox18 in the development of lymphatic vasculature was confirmed in *Ragged Opposum* (Ra^{Op}) mutant mice, which carry a naturally occurring dominant negative mutation of *Sox18* gene (*26*). In the Ra^{Op} mice, Prox1 expression in venous ECs is not observed, which results in arrested lymphatic vascular development (*27*). *Sox18* null mice also display a complete loss of venous Prox1 expression.

Sox18 expression during development of lymphatic vasculature is first detected in a subpopulation of ECs in the anterior cardinal vein of E9.0 embryos (*27*). This expression precedes approximately half a day before the initiation of Prox1 expression. During later stages of embryonic lymphangiogenesis, Sox18 expression in the lymphatic vasculature decreases at approximately E14.5. Molecular mechanisms by which Sox18 expression is turned on and off in BECs and LECs, respectively, remains to be elucidated.Sox18 has also been implicated in tumour-induced lymphangiogenesis by the findings that suppressing SOX18 function is sufficient to inhibit the tumour lymphangiogenesis in a B16-F10 mouse melanoma model, and impede tumour metastasis (*28*).Importantly, mutations inhuman *SOX18* gene have been characterized as causative for the pathogenesis of hypotriochosis-lymphdedema-telangiectasia (HLT) (*16*).

COUP-TFII

Chicken ovalbumin upstream promoter transcription factors (COUP-TFs) are orphan members of the steroid/thyroid hormone receptor superfamily that modulates the activity of transcriptional binding partners (*29*). Two genes termed COUP-TFI (also known as EAR3/NR2F1) and COUP-TFII (also known as ARP-1/NR2F2) are closely related members, and contain the amino-terminal highly conserved DNA binding domain and a carboxyl-terminal ligand binding domain (Fig. 3).

COUP-TFII plays important roles in the regulation of organogenesis, neurogenesis and cellular differentiation during embryonic development. In blood vessels, COUP-TFII is expressed in the venous ECs from E8.5 but not in arterial endothelium. In LECs, which are differentiated from venous ECs, COUP-TFII expression is observed throughout embryogenesis and adulthood (*30*).

Endothelial-specific knockout of COUP-TFII gene results in a loss of venous cell identity, leading to the conversion of embryonic venous ECs to those with arterial characteristics (*31*). Since the formation of venous ECs is defective, the specification of lymphatic endothelial precursor cells is also arrested (*32*). In order to examine the roles of COUP-TFII in the specification and maintenance of LECs, Srinivasan and colleagues deleted the COUP-TFII gene in the differentiating and mature LECs (*33*). They reported that COUP-TFII directly activates the *Prox1* expression in venous LEC progenitors, and maintains the *Prox1* expression via direct binding to Prox1 promoter (*33*). In addition to the *in vivo* data, multiple groups reported that COUP-TFII physically and functionally interact with Prox1 to regulated the expression of LEC markers including VEGFR3 in cultured LECs (*34*, *35*).

Although there have been no reports on the mutations in the human COUP-TFII gene associated with lymphatic disorders, COUP-TFII has been shown to be indispensable for lymphatic development in zebrafish and *X. laevis* (*36*), suggesting an evolutionally conserved role in the development of lymphatic vasculature.

Transcriptional Networks in Lymphatic Development

Based on previous reports, it has been suggested that the formation of LVs is comprised of multiple steps (*37*). In the first step, a subset of venous ECs acquires the competence to become lymphatic endothelial progenitors (Fig. 4). Next, such cells are specified to become LECs. Finally, the identity of LECs is maintained by intrinsic mechanisms of LECs. These processes are regulated by the hubs comprised of multiple transcription factors including Prox1, COUP-TFII, Sox18 and other transcription factors as described below.

Acquisition of Competence to Become Lymphatic Endothelial Progenitor Cells by BEC in the Cardinal Veins

During mouse embryogenesis, the earliest differentiation of LECs is detected in the anterior cardinal vein (*32*). This process starts when Prox1 starts to be expressed in a subset of venous ECs at ~E9.75. Since Prox1 expression is restricted to a polarized subpopulation of BECs in cardinal vein, molecular profile of venous ECs is not enough to specify the expression pattern of Prox1. Francois and colleagues found that Sox18 is such a candidate molecule to specify the Prox1 expression (*27*). Sox18 is expressed in a subset of ECs located in the anterior cardinal vein starting at ~E9.0, approximately half a day before Prox1 expression starts. In Sox18-deficient embryos, Prox1 expression is not induced in venous ECs, resulting in the defective LEC specification and the arrested formation of the lymphatic vasculature (*27*). Furthermore, it was shown that the Prox1 promoter contains two conserved Sox consensus binding sites (*27*). These sites are bound by Sox18 and are essential for transactivation of the Prox1 promoter both *in vitro* and *in vivo* (Fig. 5). These results suggest that Sox18 is an *in vivo* direct activator of Prox1 expression.

However, during differentiation of LECs, Sox18 expression in the vasculature is not restricted to venous BECs. Sox18 is also expressed in arterial BECs, which do not express Prox1. This observation predicts the presence of either arterial-specific repressor(s) or venous-specific co-activator(s) that modulate the functions of Sox18 to induce Prox1 expression. Srinivasan and colleagues showed that COUP-TFII, which is expressed in all venous BECs, directly binds to an evolutionally conserved region of Prox1 promoter in lymphatic endothelial progenitors of cardinal veins (*33*) (Fig. 5). Although the physical interaction

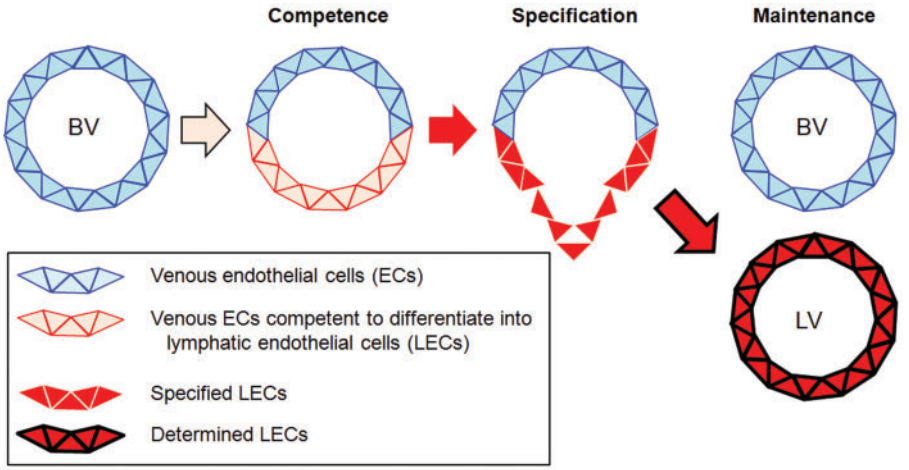

Fig. 4 Stepwise development of LVs. In mice, venous ECs of embryonic blood vascular vessels (BV) start to develop under the control of COUP-TFII. In E9.0 embryo, a subset of venous ECs expressing Sox18 in addition to COUP-TFII becomes competent to differentiate into LECs and start expressing *Prox1*. *Prox1*-expressing venous ECs are specified to differentiate to LECs, and migrate towards VEGF-C expressing cells, at which LECs form primary LVs. Later, during the differentiation and maturation steps, LECs are determined to maintain the LEC identity.

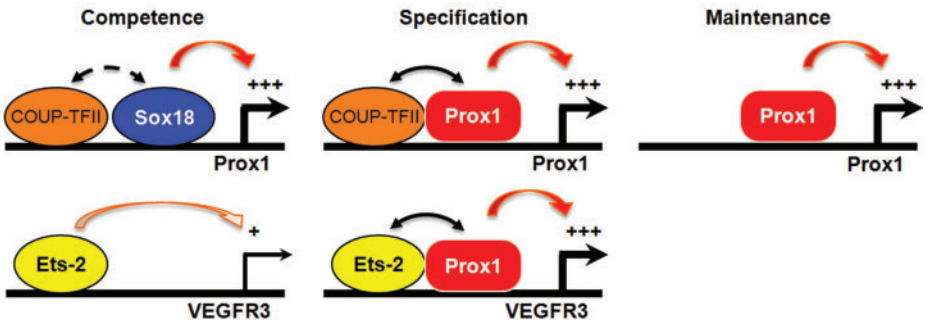

Fig. 5 Transcriptional network that controls lymphatic vascular development. In E9.5 mouse embryo, venous ECs express Ets-2, which induces VEGFR3 expression. Furthermore, a subset of ECs located in the dorsolateral side of the cardinal vein express Prox1, which is directly activated by COUP-TFII and Sox18. From E10.5 until E11.5, Prox1 collaborates with Ets-2 to induce the VEGFR3 expression at a higher level, resulting in the polarized migration of LECs towards to VEGF-C. Prox1 and COUP-TFII also synergistically induce the expression of Prox1 via direct interaction. Prox1 expression in later stages of lymphatic development is maintained by Prox1 itself and/or Prox1 targets including HoxD8.

between COUP-TFII and Sox18 has not been reported, they speculate that COUP-TFII cooperates with Sox18 to initiate Prox1 expression in lymphatic endothelial progenitor cells.

It is clear that significant questions remain to be answered: how is Sox18 expression activated in the ECs of dorsolateral region of cardinal vein, or repressed in the ventrolateral region? Since Sox18 is expressed in a polarized manner, diffusible factor(s) that influence either dorsolateral or ventrolateral region of cardinal vein need to be identified in the future.

Specification of LECs

Once venous BECs express Prox1 by cooperative action between COUP-TFII and Sox18, they start to become committed to a LEC fate. The initial phase of this process is the specification of the LEC phenotype (Fig. 4). During this specification stage, Prox1 plays central roles in the differentiation of venous BECs into LECs by down-regulating the BEC markers and up-regulating the LEC markers.

A detailed analysis of *Prox1*-knockout embryos suggested that the Prox1 is essential in the polarized budding and guided migration of lymphatic endothelial progenitors towards the VEGF-C expressed by the neighbouring mesenchymal cells, thereby ensuring the appropriate formation of the primary lymph sacs (*6*, *7*) (Fig. 2). Therefore, the Prox1-induced expression of VEGFR3, receptor for VEGF-C, can be considered as a reliable indicator of the progression of LEC specification. However, it remains to be determined how Prox1 induces the expression of LEC markers specifically in LECs. Although Prox1 is expressed not only in LECs but also in multiple organs including liver (*38*) and lens (*6*), Prox1 induces VEGFR3 expression only in LECs. Regulation of the activities of transcription factors often critically depends on their interaction with other transcription factors on composite DNA elements. Tissue-specific transcriptional activities of Prox1 can thus be directed by additional transcription factors.

Although Flister and colleagues reported that VEGFR3 expression in mature LECs is collaboratively

regulated by Prox1 and NFκB, which is activated by inflammatory signals (*39*), the roles of inflammatory signals during embryonic lymphatic differentiation remain to be determined. Yoshimatsu and colleagues reported that Ets-2 transcription factor is expressed in embryonic BECs and LECs and positively regulates Prox1-induced expression of VEGFR3 (*40*). Consistent with the effects on VEGFR3 expression, Ets-2 induces the migration of LECs towards VEGF-C. They found that endogenous Ets-2 interacts with Prox1 in LECs and that both Prox1 and Ets-2 bind to the VEGFR3 promoter in intact chromatin (Fig. 5). These findings suggest that Ets-2 may function as a transcriptional co-factor that enhances Prox1-induced lymphangiogenesis.

Ets-2 is one of prototypical members of the Ets family. Nineteen Ets transcription factors are expressed in BECs (*41*), and several members have been shown to play essential roles in vascular development by studies using knockout mice. Although mice that are single-mutant for either Ets-2 or Ets-1, another prototypical member of the Ets family, exhibit no phenotypic changes in vascular development, double-mutant mice for Ets-1 and Ets-2 exhibited defective blood vessel branching (*42*), suggesting that Ets-1 and Ets-2 play redundant roles during embryonic vascular development. Inhibition of the transcriptional activities of Ets family members by introducing the dominant negative mutant of Ets-1 (TM-Ets-1) decreased the expression of VEGFR3 in BECs, suggesting that Ets family members play critical roles in the VEGFR3 expression in BECs (Fig. 5). In order to elucidate the *in vivo* roles of Ets-1 and Ets-2 during lymphatic development, genes encoding for Ets-1 and Ets-2 need to be conditionally deleted in LECs in the future.

A series of experiments using *in vitro*-cultured cells suggested that Prox1 and COU-TFII synergistically induce the expression of the Prox1 target gene *FGFR3* in cultured BECs (*35*). Furthermore, siRNA-mediated knockdown of COUP-TFII in LECs reduced the expression of various LEC markers (*34*, *35*). Taken together with the finding that endogenous Prox1 and COUP-TFII bind in LECs, these results suggest that COUP-TFII and Prox1 physically and functionally interact to control the identity of LECs *in vitro*.

Maintenance of LEC Identity

Several lines of *in vitro* and *in vivo* evidence have suggested that endogenous expression of Prox1 in LECs is necessary for the maintenance of LEC identity. Interestingly, siRNA-mediated knockdown of Prox1 in cultured LECs not only decreased the expression of LEC markers such as VEGFR3 (*43*), podoplanin and SLC (*44*), but also increased the expression of BEC markers including endoglin and CD34, suggesting that mature LEC phenotype is a plastic and reprogrammable condition that depends on constant Prox1 activity for its maintenance. This notion is confirmed by multiple *in vivo* experiments (*44–47*). Intriguingly, when LECs are exposed to similar levels of shear stress as that in blood vessels, the Prox1 expression is decreased, which leads to the reprogramming of LECs to BECs (*47*). These results suggest that the molecular mechanisms by which the endogenous Prox1 expression is sustained to maintain the identity of LECs.

Although Sox18 plays essential roles in the induction of Prox1 in venous BECs, Sox18 is not involved in the maintenance of the Prox1 expression in mature LECs because Sox18 expression is not detectable in mature LECs (from E14.5) (*27*). Interestingly, the *in vivo* Prox1 promoter is initially active in *Prox1*-null embryos, but becomes turned-off a few days later, suggesting that the *Prox1*-expressing LECs provide a positive auto-regulatory mechanisms to maintain *Prox1* expression (*7*). Two mechanisms have been proposed to maintain *Prox1* expression in differentiating LECs. Prox1 could be recruited to its own promoter due to its interaction with COUP-TFII and subsequently regulate and maintain its own expression (Fig. 5). This model is supported by the finding that tamoxifen-inducible deletion of COUP-TFII gene in mature LECs resulted in dramatic loss of LEC identity (*33*). Alternatively, the COUP-TFII–Prox1 complex might activate transcription factor(s), which in turn maintain *Prox1* expression. Harada and colleagues reported that Prox1 induces the expression of HoxD8 transcription factor in LECs and that siRNA-mediated decrease in HoxD8 expression in LECs resulted in the decreased level of endogenous Prox1 expression (*48*). These results suggest that HoxD8 may be a candidate Prox1 target molecule that maintains endogenous Prox1 expression in mature LECs. Physiological relevance of these *in vitro* findings need to be verified by the genetic studies using LEC-specific deletion of HoxD8 gene in the future.

Conclusion

The previous reports described above have vastly expanded our understanding of how the networks of multiple transcription factors regulate the multiple steps during the development of lymphatic vasculature. Nonetheless, recent progress in high-throughput sequencing technologies has made it possible to unravel the genome-wide network of interaction between transcription factors and DNA. Genome-wide mapping of the targets of key transcription factors such as Prox1, Sox18 and COUP-TFII will provide a more comprehensive model of transcriptional pathways during the differentiation of LECs.

Better understanding of the molecular mechanisms how lymphangiogenesis is regulated by the transcription factor networks will aid in developing novel therapeutic strategies. Growing evidence has suggested that tumour-associated LVs play critical roles in tumour metastasis to sentinel lymph nodes. Furthermore, defective formation of LVs causes the pathogenesis of lymphedema. It is thus of critical importance to develop strategies to control lymphangiogenesis in order to prevent the metastasis of tumours and to cure lymphedema.

Acknowledgements

We thank the members of the Department of Molecular Pathology of the University of Tokyo for discussion and Keiko Yuki for technical assistance.

Funding

This research was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Science, Sports and Technology of Japan.

Conflict of interest

None declared.

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