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The New Era of the Lymphatic System: No Longer Secondary to the Blood Vascular System

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The blood and lymphatic systems are the two major circulatory systems in our body. Although the blood system has been studied extensively, the lymphatic system has received much less scientific and medical attention because of its elusive morphology and mysterious pathophysiology. However, a series of landmark discoveries made in the past decade has begun to change the previous misconception of the lymphatic system to be secondary to the more essential blood vascular system. In this article, we review the current understanding of the development and pathology of the lymphatic system. We hope to convince readers that the lymphatic system is no less essential than the blood circulatory system for human health and well-being.

The human body has two major circulatory systems: the blood and lymphatic systems. Although both systems were initially described by Hippocrates and share so many functional, structural, and anatomical similarities, the two vascular systems have had very different fates in science and medicine: Although the blood vascular system has been intensively and extensively studied for a long time, the lymphatic system, in contrast, has been considered less important, invisible, secondary to the blood vascular system, and thus largely neglected by scientists and clinicians until recent years. However, a series of landmark discoveries in past decades has unraveled much of the mystery of the lymphatic system and yielded a burst of new knowledge in the field of vascular biology and medicine. Modern molecular, cellular, and genetic approaches as well as the state-of-the-art imaging technologies have allowed true appreciation of the value of the lymphatic system as the other vascular system, no longer secondary to the blood vascular system. In this review, we discuss the current understanding of the development and function of the lymphatic system and human diseases related to the lymphatic system.

STRUCTURE AND FUNCTION OF THE LYMPHATIC SYSTEM

The lymphatic system is a linear network of lymphatic vessels and secondary lymphoid organs. Macroscopically, the blood vascular system is literally a circular system in which the fluid (blood) leaves the heart; runs through the arteries, arterioles, capillary plexus, venules, and veins; and returns to the heart (Fig. 1A)

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(Alitalo 2002; Alitalo and Carmeliet 2002; Karkkainen et al. 2002). In contrast, the lymphatic system is a blunt-ended linear system, in which tissue fluids, cells, and large extracellular molecules, collectively called lymph, are drained into the initial lymphatic capillary vessels that begin at the interstitial spaces of tissues and organs; are transported to thicker collecting lymphatics, which are embedded with multiple lymph nodes; and are eventually returned to the blood circulation through the thoracic or lymphatic ducts that join to the subclavian veins. Microscopically, whereas blood capillaries are lined by the innermost blood vascular endothelial cells (BECs), which are covered by the basement membranes and then surrounded by smooth muscle-like pericytes, lymphatic capillaries are lined with a single layer of partly overlapping lymphatic endothelial cells (LECs) without being surrounded by the basement membrane or pericytes (Tammela and Alitalo 2010). Whereas cross sections of blood capillaries are

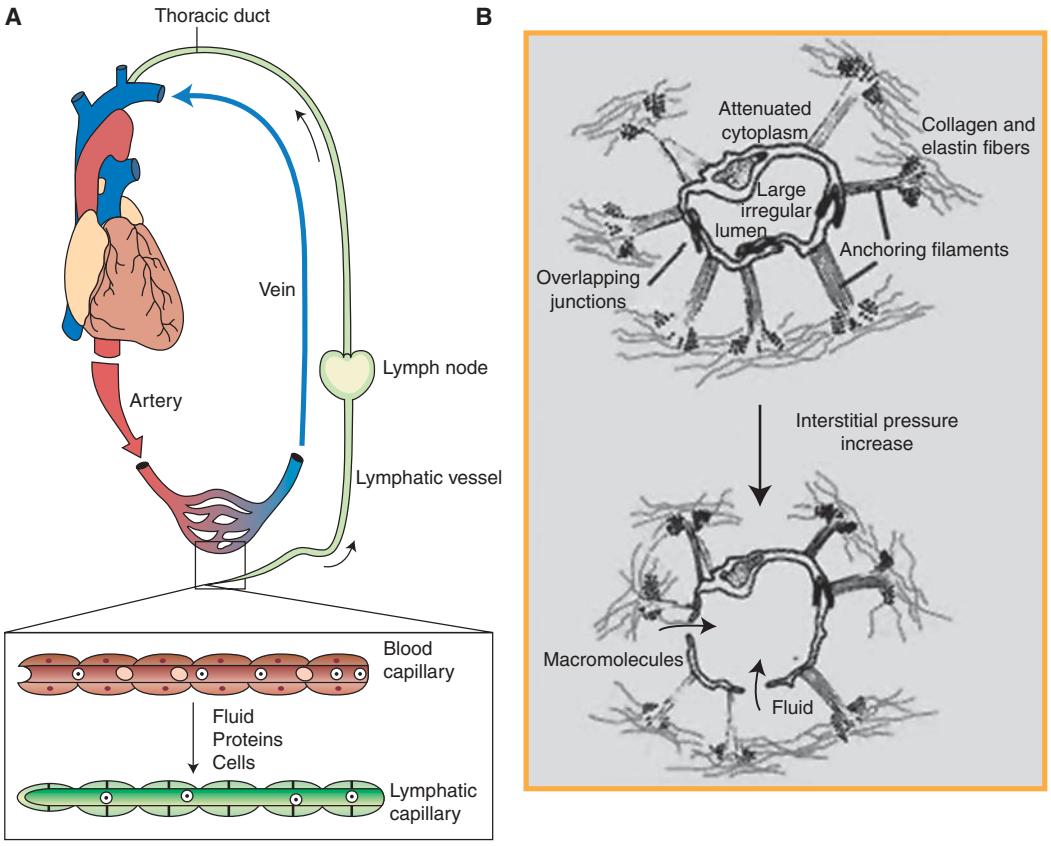

Figure 1. Macroscopic view of the blood versus lymphatic system and illustration of the structures of lymphatic capillaries. (*A*) The blood vascular system is a circular and closed system in which the fluid (blood) leaves from and returns to the same organ (heart). In comparison, the lymphatic system is a linear system in which the lymphatic capillaries at the peripheral tissues drain the fluid (lymph) containing cells, proteins, and macromolecules and transport it back to the blood vascular system through the lymphatic–blood junction at the end of the thoracic duct. (Diagram modified from Karkkainen et al. 2002.) (*B*) Microstructure of lymphatic capillaries in the skin. Lymphatic capillaries are irregular shaped and stay collapsed. When the interstitial fluid pressure increases because of fluid accumulation, the anchoring filament bundles pull lymphatic endothelial cells and open up the cell–cell junctions so that the lymph fluids can enter the lumen of lymphatic vessels for transport. (Illustration modified from Skobe and Detmar 2000.)

round and homogeneously shaped owing to hemodynamic pressure, lymphatic capillaries are irregularly shaped and usually stay collapsed (Alitalo 2002; Alitalo and Carmeliet 2002; Karkkainen et al. 2002). Lymphatic vessels can be found in all of the vascularized organs and tissues except retina, bone, and brain. (See Table 1 for the summarized comparison between BECs and LECs.)

Importantly, capillary LECs are attached by filament bundles and then directly anchored to the extracellular matrices (Leak and Burke 1966, 1968). When interstitial pressure increases, the anchoring filaments are operated to pull the cells and open up the overlapping junctions (or flaps), which allows the lymph fluids to drain into lymphatic capillaries for recirculation (Fig. 1B) (Leak and Burke 1966, 1968). Therefore, these overlapping junctions of capillary LECs mechanically function as primary valves that unidirectionally control lymph fluid drainage and are laced with discontinuous, specialized button-like intercellular adhesion points with proteins found in tight and adherens junctions (Baluk et al. 2007). In comparison, LECs of the collecting lymphatic vessels, opted for fluid transport rather than drainage, are now seamlessly aligned with each other by more tight, zipper-like junctions and ensheathed with the basement membranes

Table 1. Comparison of blood vascular endothelial cells (BECs) and lymphatic endothelial cells (LECs)

Feature	Blood vessels/BEC	Lymphatics/LEC
Constituents	Blood, blood cells	Lymph (interstitial fluid rich in protein, fat, and lipids, extravasated immune cells, and large extracellular molecules)
Gross structure	Closed, circular	Open, linear
Start/end	Heart/heart	Tissue/lymph-vein connection of the thoracic duct
Hierarchical division	Arteries, arterioles, capillaries, venules, veins	Capillaries, precollectors, collecting vessels, thoracic duct, lymph nodes
Vessel wall	Adherens and tight junctions, continuous basement membrane, pericytes, or vascular smooth muscle cells	Overlapping LECs, no tight junctions, anchoring filaments, discontinuous basement membrane, few pericytes (collecting lymphatic vessels have both continuous membranes and mural cells)
Development	Vasculogenesis and angiogenesis	Lymphangiogenesis (budding from cardinal vein)
Origin	Mesoderm, endothelial stem/precursor cells from bone marrow for adults	Mesoderm (vein) during development, lymphatic progenitor cells from bone marrow for adults
Examples of cell type–specific markers	CD34, CD105/endoglin	Prox1, LYVE-1, VEGFR-3, and podoplanin
Absence	Cartilage, cornea	Cartilage, brain, bone, spinal cord, and the retina
Functions	Hemostasis, inflammation, leukocyte trafficking, barrier function, delivery for oxygen, nutrients, and tissue wastes	Tissue fluid homeostasis, absorption of large molecules and lipids in the digestive systems, trafficking of lymphocytes and antigen-presenting cells to regional lymph nodes, transport of degraded extracellular molecules, cell debris, and lymph fluid
Heterogeneity	Well-established phenotypic heterogeneity	Comparable LEC heterogeneity was reported. LEC fate is highly plastic in response to genetic and environmental stimuli

and pericytes/smooth muscle cells that propel drained lymph fluids back to recirculation (Baluk et al. 2007). Like the veins, collecting lymphatic vessels and ducts are equipped with bileaflet secondary valves to prevent retrograde flow of the lymph, and optimal lymph flow is effectively controlled by multiple factors including lymphatic muscle contractions (Dougherty et al. 2008; Muthuchamy and Zawieja 2008).

In addition to the tissue fluid homeostasis, the lymphatic system serves as a conduit for trafficking of lymphocytes and antigen-presenting cells to regional lymph nodes, where the immune system encounters pathogens, microbes, and other immune elicitors. Lymph-node lymphatic vessels, which uptake various antigens from peripheral tissues, are positively regulated by chemokines/cytokines secreted by B cells, macrophages, and dendritic cells during inflammation (Jeon et al. 2008; Kataru et al. 2009; Kim et al. 2009). Toll-like receptor (TLR)-4 is highly expressed in LECs and contributes to lipopolysaccharide (LPS)-induced lymphangiogenesis by chemotactic recruitment of macrophages (Kang et al. 2009). Notably, a recent study showed that lymph nodes in athymic nude mice have excessive lymphatic vessels and that interferon-γ released by T lymphocytes negatively regulates lymph-node lymphatic vessel formation by suppressing key lymphatic molecules (Kataru et al. 2011). Importantly, lymphatic vessels can perform functions beyond passive conduits for immune response. Inflamed LECs can directly modulate inflammatory response by suppressing dendritic cell maturation and function through an Mac-1/ICAM-1–dependent mechanism (Podgrabinska et al. 2009). Inhibition of lymphatic-specific vascular endothelial growth factor receptor (VEGFR)-3 increased inflammatory edema formation and inflammatory cell accumulation along with inhibition of lymphangiogenesis in the inflammatory skin (Huggenberger et al. 2010) and arthritis (Guo et al. 2009), and, conversely, activation of lymphangiogenesis by genetic delivery of the lymphangiogenic factor VEGF-C suppressed chronic skin inflammation, suggesting that induction of functional lymphangiogenesis could be a novel strategy to treat chronic inflammatory disorders (Huggenberger et al. 2010, 2011). Moreover, LECs modulate inflammatory response by secreting CCL21, a chemokine for recruitment of the CCR7-positive dendritic cells to lymphatic vessels (Saeki et al. 1999; Randolph 2001; Wiley et al. 2001; Vigl et al. 2011). These studies indicate that lymphatic vessels are not mere passive conduits for immune cells but actively participate in modulating the immune responses. Interestingly, while lymphatic vessels transport lymph fluids, both interstitial pressure and fluid flow can also activate LECs, thus increasing fluid/solute permeability and uptake (Swartz et al. 2008; Miteva et al. 2010), and regulate lymphatic morphogenesis in vitro and in vivo (Boardman and Swartz 2003; Goldman et al. 2007; Helm et al. 2007; Lund and Swartz 2010).

Moreover, lymphatic vessels such as lacteals in the intestines absorb and transport large molecules, fats, and lipids in the digestive system mainly in the form of lipoprotein such as chylomicrons—large lipoprotein particles that are created by the enterocytes of the intestine and consist of triglycerides, phospholipids, cholesterol, and proteins. Notably, lymph fluid and chylomicrons can stimulate adipocyte differentiation (Nougues et al. 1993; Rosen 2002). This finding is consistent with the fact that chronic lymphedema is often associated with tissue fibrosis and accumulation of fat and adipose tissues (Rockson 2000, 2009). A mouse genetic study showed that when lymphatic vessel integrity is compromised, the lymph fluids rich in fat and lipids leaked out from mispatterned or ruptured lymphatic vessels and activated fat accumulation, leading to adult-onset obesity (Harvey et al. 2005).

Unfortunately, many malignant tumors take advantages of the lymphatic system for their dissemination. A large number of in vitro animal and human studies have shown a causal relationship between lymphatic vessel density and tumor metastasis (Skobe et al. 2001; Stacker et al. 2001; Das and Skobe 2008), and more scientific and clinical attention is needed to prevent and intervene tumor metastasis through lymphatic vessels.

DEVELOPMENT OF THE LYMPHATIC SYSTEM

Phylogenetic Standpoints

From the phylogenetic standpoint, the lymphatic system is believed to have first appeared in vertebrates (Rusznyak et al. 1967). Zebrafish has served as a great experimental tool for vascular research and was thought to lack lymphatic circulation for a long time. However, a primitive lymphatic system with evolutionarily conserved structural and cellular features was recently discovered in zebrafish (Yaniv et al. 2006) and has been enormously valuable in various genetic studies. Amphibians, reptiles, and flightless birds have also developed a lymphatic system with a specialized lymph heart that drives lymph drainage and transport. The lymphatic system further evolved in flying birds and mammals to lose the lymph heart and instead acquire lymph nodes for the immune functions.

Historical Perspectives

Hippocrates (460–377 B.C.) first described the lymphatic vessel as "white blood" and coined the term "chyle" (from the Greek *chylos*, meaning juice) (Grotte 1979; Chikly 1997). Chyle is a milky tissue fluid consisting of emulsified fats and free fatty acids, collectively called lymph, which is formed in the digestive system and taken up by the specialized lymph vessels known as lacteals. Subsequently, the lymphatic system was further illustrated by Greek physicians Herophilus (335–280 B.C.) and Erasistratus (304–250 B.C.), two founding fathers of human anatomy who are believed to have performed—for the first time—systematic dissections of human cadavers (Lord 1968; Leeds 1977; Chikly 1997). After the Hippocrates era, the lymphatic system was largely forgotten until 1627, when an Italian anatomist, Gaspare Aselli, rediscovered the lymphatic system (mesentery lymphatic vessels) as the "*venae albae et lacteae* (milky veins)" from a well-fed dog, while studying the diaphragm (Asellius 1627). He postulated that foods were digested and fragmented into numerous droplets and then transported via the "chyliferous" vessels (Lord 1968; Leeds 1977; Chikly 1997). Following this discovery of the mesentery lymphatics, additional anatomical structures of the lymphatic system such as the collecting lymphatic vessels and thoracic ducts were identified and characterized.

At the beginning of the 20th century, researchers proposed two competing theories on the histogenetic origin of the lymphatic system. One hypothesis argued for the blood vascular origin of lymphatics, in which the lymphatic system is derived from the blood vascular system during early development (the "centrifugal model"). In a sharp contrast, the other theory, more widely accepted then, claimed that LECs are independently differentiated from mesenchymal cell–derived lymphangioblasts and that the primitive lymphatic plexuses are formed by these lymphatic stem cells first and then gain connections to the embryonic vein only later (the "centripetal model"). In 1902, the American anatomist and medical researcher Florence Rena Sabin, based on experiments on ink injection into the veins of pig embryos, demonstrated that the lymphatic system is derived from the early embryonic vein (Sabin 1902, 1904). Although the Sabin experiments largely resolved the scholastic debate, the presence of lymphatic progenitor cells (lymphangioblasts) and their critical roles have been further validated in embryonic development of the nonmammalian lymphatic system (Wilting et al. 2001, 2003, 2006) and during postdevelopmental lymphangiogenesis in mammals including rodents and human (Kerjaschki et al. 2006; Lee et al. 2010a).

Initial Steps for Lymphatic Specification and Differentiation

Although an in-depth comparative study on blood versus lymphatic vascular systems was first initiated about half a century ago (Leak and Burke 1966), the dearth of lymphatic-specific molecular and cellular markers significantly hampered efforts. However, a series of landmark discoveries made in the late 1990s has opened a new door to lymphatic research. One of the most groundbreaking findings was the identification of the LEC-specific vascular endothelial growth factor receptor (VEGFR)-3

(Kaipainen et al. 1995). VEGFR-3 is a member of the VEGF receptor family and structurally related to VEGFR-1 and VEGFR-2. VEGFR-3 expression is detectable in a majority of vascular endothelial cells during early development but restricted to lymphatic plexuses at later stages of development and postdevelopment (Kaipainen et al. 1995). Thus, VEGFR-3 is the first lymphatic-specific marker and has been extensively used in the field of lymphatic research. However, genetic deletion of VEGFR-3 resulted in defective blood vessel development with abnormally organized large vessels and fluid accumulation in the pericardial cavity at mouse embryonic day 9.5 (E9.5), when the lymphatic development is about to begin (Fig. 2). Thus, the role of VEGFR-3 specifically in early lymphatic development has not been readily studied (Dumont et al. 1998). Instead, knockout studies of VEGF-C, a ligand for VEGFR-3, have provided a wealth of important information for the initial steps in early lymphatic development (Karkkainen et al. 2004). In VEGF-C-deficient mice, endothelial cells are still committed to the lymphatic lineage but unable to form rudimentary lymphatic vessels. This mutant phenotype was rescued by VEGF-C and VEGF-D, another VEGFR-3 ligand, but not by VEGF-A, showing VEGFR-3 specificity. The knockout embryos lack a functional lymphatic system and die prenatally from fluid accumulation in tissues. Notably, VEGF-C heterozygote mice developed cutaneous lymphatic hypoplasia and lymphedema, indicating the essential roles of VEGF-C in normal lymphatic development (Karkkainen et al. 2004).

The controversy regarding the origin of the lymphatic system continued for a hundred years until 1998, when the first mutant mouse with failed lymphatic development supported Sabin's hypothesis of the blood vascular origin of the lymphatic system (Wigle and Oliver 1999; Wigle et al. 2002). Wigle et al. discovered that deletion of a homeodomain transcription factor, Prox1, resulted in arrest of lymphatic endothelial differentiation at an early stage and that Prox1 knockout mice fail to develop a lymphatic system. Moreover, Prox1 is expressed in a subset of endothelial cells in the cardinal vein at mouse E9.5, and the Prox1-positive endothelial cells bud off and migrate out to form the rudimentary lymphatic vessels, known as jugular lymph sacs (Fig. 2). Importantly, the lymphatically differentiating, budding endothelial cells gradually up-regulate LEC-signature genes and progressively down-regulate BEC-specific genes as embryos develop (Wigle and Oliver 1999; Wigle et al. 2002). Thus,

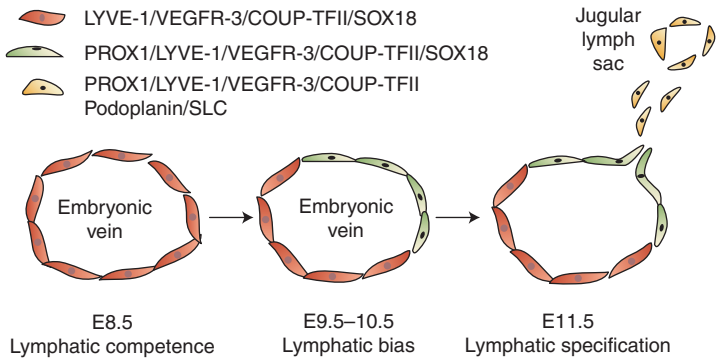

Figure 2. Current model of a stepwise embryonic development of the mammalian lymphatic system. At mouse E8.5, endothelial cells in the vein express LYVE-1, VEGFR-3, COUP-TFII, and SOX18 and are potentially capable of lymphatic differentiation ("lymphatic competence"). At E9.5–E10.5, Prox1, the master regulator for lymphatic development, is up-regulated in a subset of venous endothelial cells by an inductive signal ("lymphatic bias"). These Prox1-positive cells begin to migrate out and form the rudimentary lymphatic vessels, known as jugular lymph sacs, at E11.5, expressing additional lymphatic molecules such as podoplanin and SLC ("lymphatic specification"). (Diagram modified from Oliver and Detmar 2002.)

Prox1 expression is restricted in LECs, serving as an LEC signature. Furthermore, Prox1 was shown to induce lymphatic reprogramming postdevelopmentally when overexpressed in BECs and to be required continuously to maintain LEC phenotypes (Wigle and Oliver 1999; Hong et al. 2002; Petrova et al. 2002; Wigle et al. 2002; Johnson et al. 2008), gaining the honorable name: "the master control gene for lymphatic development."

Following the identification of VEGFR-3 and Prox1 as the LEC-signature markers, two additional LEC-specific molecules, lymphatic vessel endothelial hyaluronan receptor (LYVE)-1 and podoplanin, were simultaneously reported in 1999. LYVE-1 was originally isolated in an effort to identify a receptor for hyaluronan (HA), an extracellular matrix glycosaminoglycan that is an abundant component of skin and mesenchymal tissues and facilitates cell migration during wound healing, inflammation, and embryonic morphogenesis (Banerji et al. 1999). It turned out that LYVE-1 is selectively expressed in LECs and is a homolog of CD44, another HA receptor mainly found in blood vessels (Jackson 2009). Like VEGFR-3, LYVE-1 expression is detected in embryonic veins at an early stage of development and is later restricted in LECs (Oliver 2004). LYVE-1 expression in LECs becomes diminished as the capillary lymphatic plexus matures to collecting lymphatic vessels (Petrova et al. 2004; Makinen et al. 2005). Interestingly, both LYVE-1 single and LYVE-1/CD44 double knockout mice still develop a normal lymphatic system without any significant functional defects in tissue fluid homeostasis (Gale et al. 2007; Luong et al. 2009). Although LYVE-1 is used as an important LEC marker, it is also expressed in activated tissue macrophages and sinusoidal endothelial cells of the liver and spleen (Jackson 2003, 2004).

Podoplanin (PDPN), a mucin-type transmembrane sialoglycoprotein, is another LEC-signature molecule that has been initially identified (Breiteneder-Geleff et al. 1999). Podoplanin is expressed in all endothelial cells of the cardinal vein at mouse E11.5 and is later, similarly to VEGFR-3 and LYVE-1, progressively down-regulated in venous endothelial cells, but is continuously expressed in LECs throughout development. Despite its predominant expression in LECs but not in BECs, podoplanin is also expressed in a broad range of cell types including lung type I alveolar cells, ciliary epithelial cells of the eye, choroid plexus cells, osteocytes, and kidney podocytes and was independently cloned and differently named as, for example, OTS8, E11 antigen, RTI40, murine gp38, canine gp40, human gp36, aggrus, and murine PA2.26 (Nose et al. 1990; Wetterwald et al. 1996; Zimmer et al. 1999; Hirakawa et al. 2003; Kato et al. 2003; Ramirez et al. 2003). Consistently, podoplanin knockout mice revealed defects in multiple organs and die perinatally from respiratory failure (Ramirez et al. 2003; Schacht et al. 2003). Notably, podoplanin-deficient mice fail to develop a functional lymphatic system with a severe lymphedema in the extremities (Schacht et al. 2003). A series of recent findings has shown that podoplanin activates a novel platelet receptor, C-type lectin-like receptor (CLEC)-2 of platelet, and has an essential role in blood–lymphatic separation during lymphatic development (see more information below) (Fu et al. 2008; Kato et al. 2008; Bertozzi et al. 2010a,b; Suzuki-Inoue et al. 2007, 2010; Uhrin et al. 2010; Suzuki-Inoue 2011). Moreover, some lymphatic vessels were found to express a high level of podoplanin ($LEC^{podo\text{-}high}$) and the others a low level of podoplanin ($LEC^{podo\text{-}low}$), and these two subpopulations differentially recruit CCR10-positive T lymphocytes during the inflammation response (Kriehuber et al. 2001; Wick et al. 2008).

Chicken ovalbumin upstream transcription factor (COUP-TF) II is an orphan nuclear receptor expressed in the embryonic veins from E8.5 (Lin et al. 2010). The critical role of COUP-TFII in vascular development was identified from a knockout mouse study (You et al. 2005). COUP-TFII deletion resulted in abnormal arterialization of the veins by misexpression of the Notch pathway genes in the venous compartment, whereas ectopic COUP-TFII overexpression in vivo induced the excessive venous endothelial phenotype at the expense of arterial endothelial cells (You et al. 2005). Importantly,

COUP-TFII has been identified as an interacting partner of Prox1 in vitro and in vivo, and the functional interaction between the two cell-fate regulators was proposed to constitute an essential part in the program specifying LEC fate (Lee et al. 2009; Yamazaki et al. 2009; Srinivasan et al. 2010). Supporting this notion, conditional ablation of COUP-TFII at an early embryonic stage blocked the LEC-fate specification of the venous endothelial cells and formation of lymphatic vessels (Lin et al. 2010). COUP-TFII deficiency at a late developmental stage not only reversed the LEC differentiation program, ending up regaining the blood endothelial cell fate, but also impaired lymphatic vessel sprouting, indicating the essential roles of COUP-TFII in lymphatic development (Lin et al. 2010). Notably, it has been shown that the VEGF coreceptors neuropilin (NRP)-1 and NRP-2 are differentially expressed in endothelial cell lineages: Whereas NRP-1 is selectively expressed in the arterial compartment, NRP-2 is predominantly in venous and lymphatic endothelial cells (Herzog et al. 2001; Neufeld et al. 2002; Yuan et al. 2002). COUP-TFII plays a key part in this endothelial cell-lineage-specific expression of NRP-1 and NRP-2 (You et al. 2005; Lee et al. 2009; Lin et al. 2010).

FOXC2, a Forkhead family transcription factor, is one of the few causative genes associated with human lymphatic disorder and malformation to date and is found to be responsible for lymphedema–distichiasis (double row of eye lashes) syndrome by multiple groups (Fang et al. 2000; Erickson 2001; Erickson et al. 2001; Finegold et al. 2001; Bahuau et al. 2002; Brice et al. 2002; Traboulsi et al. 2002; Kriederman et al. 2003; Fabretto et al. 2010). Foxc2 is expressed in both arterial and lymphatic endothelial cells and, along with Foxc1, is required for arterial specification and normal lymphatic sprouting from the vein during development (Dagenais et al. 2004; Seo et al. 2006; Kume 2009). Importantly, Foxc2 and Vegfr3 were found to cooperate in lymphatic vascular patterning during lymphatic development, and Foxc2-deficient mice showed abnormal lymphatic patterning, increased mural cell investment, absence of lymphatic valves, and lymphatic dysfunction (Petrova et al. 2004). Lymphatics in Foxc2 knockout mice up-regulated PDGF-B, abnormally recruited excessive pericytes and smooth muscle cells (SMC), and were surrounded by a thicker layer of basement-membrane protein collagen type IV (Petrova et al. 2004). Moreover, Foxc2 has been shown to have a key role in lymphatic maturation (Norrmen et al. 2009) (see below for details). Altogether, Foxc2 has important roles throughout lymphatic development.

Prox1: The Master Regulator for Lymphatic Development

Prox1 was originally isolated by its protein sequence homology to the *Drosophila* protein Prospero (Oliver et al. 1993; Tomarev et al. 1996). The *Prospero* gene was first cloned by its mutant phenotype in neuronal lineage cell-fate specification (Doe et al. 1991; Vaessin et al. 1991). When a neuroblast cell divides during development, one daughter cell remains as a stem cell, and the other daughter cell undergoes neuronal differentiation to become a neuron or glia. During this process, only one daughter cell receives Prospero protein through an asymmetrical segregation of the protein and undergoes further differentiation (Doe et al. 1991; Vaessin et al. 1991). Despite a high protein sequence similarity between Prospero and Prox1, the amino acid motif responsible for this asymmetric segregation of Prospero seems not present in Prox1 (Hong and Detmar 2003), and asymmetric segregation of Prox1 has not been reported to date. Like Prospero, Prox1 is also expressed in many other organs, such as the liver, brain, pancreas, heart, eye lens, ear sensory epithelium, taste bud, and retina (Oliver et al. 1993; Wigle et al. 1999; Wigle and Oliver 1999; Sosa-Pineda et al. 2000; Govindarajan and Overbeek 2001; Miura et al. 2003; Bermingham-McDonogh et al. 2006; Dudas et al. 2006; Edqvist et al. 2006; Laerm et al. 2007; Kirjavainen et al. 2008; Risebro et al. 2009). Interestingly, the cell-fate-specifying function of Prospero seems to have been inherited by Prox1 and appears to be the common functional theme of Prox1 in such diverse cell types. For example,

Prox1 induces cell cycle arrest in developing lens and directs differentiation of the lens fiber cells (Wigle et al. 1999). Similarly, Prox1 induces the exit of retinal progenitor cells from the cell cycle and promotes horizontal cell differentiation (Dyer et al. 2003). Prox1 overexpression resulted in enhanced neuronal differentiation, whereas knockdown of Prox1 impaired the generation of neurons (Karalay et al. 2011). Notably, Prospero has been shown to regulate cell cycle progression because loss of Prospero induced aberrant expression of cell cycle genes such as *cyclin A* (*cycA*), *cyclin E* (*cycE*), and *string* (*srg*) and resulted in increased mitotic activity (Li and Vaessin 2000). Conversely, overexpression of Prospero causes repression of multiple cell cycle genes and premature termination of cell division (Li and Vaessin 2000). Although Prox1 regulates the expression of CDKN1C/$p57^{kip2}$, a major cell cycle inhibitor, in LECs as well as other cell types (Wigle et al. 1999; Govindarajan and Overbeek 2001; Petrova et al. 2002; Pan et al. 2009), there has not been any report detailing cell cycle regulation during Prox1-induced LEC differentiation.

Recent studies have provided more information on the regulation and function of Prox1. SRY-related HMG-box 18 (Sox18), a member of the F-group of Sox transcription factors, has been identified to bind upstream of *PROX1* and up-regulate Prox1 expression (François et al. 2008). Moreover, mice carrying a natural Sox18-dominant-negative mutation fail to initiate Prox1 up-regulation in venous endothelial cells during development and to develop a normal lymphatic system (François et al. 2008). Importantly, humans with mutations in Sox18 develop a blood/lymphatic vascular disease, hypotrichosis–lymphedema–telangiectasia (HLT), which is characterized by alopecia, hemorrhage, and lymphedema (Irrthum et al. 2003). Sox7 and Sox17 were also found to regulate lymphatic development as modifiers of Sox18 (Hosking et al. 2009), and mutation in Sox17 has suggested it to be a candidate gene for human primary lymphedema (Ferrell et al. 2008).

In addition to Sox18, NF-κb (Flister et al. 2010, 2011), TGF-β (Oka et al. 2008), interleukin-3 (Groger et al. 2004), and VEGF-C (Sivakumar et al. 2008) have been shown to regulate Prox1 in endothelial cells, but their roles in lymphatic development need to be established. Moreover, regulators of Prox1 have been reported in other types of cells. In colon cancer cells (Petrova et al. 2008) and hippocampus cells (Karalay et al. 2011), Prox1 is regulated by the Wnt signal through two binding sites of β-catenin/TCF found in the Prox1 enhancer region at −49 kb and −43 kb upstream of the Prox1 start codon, which induce colon cancer progression and neuronal cell differentiation, respectively. Mash1 (Torii et al. 1999) and Foxe3 (Medina-Martinez et al. 2005) were found to regulate Prox1 expression in the nervous system and lens, respectively.

A recent study has identified that the Prox1 mRNA contains an extraordinarily long 3′ untranslated region (UTR) (Yoo et al. 2010). Whereas the human Prox1 open reading frame (ORF) is ∼2.2 kb long and encodes a 737-amino-acid-long protein (Oliver et al. 1993; Tomarev et al. 1998), the PROX1 gene is ∼50 kb long and expresses ∼8-kb-long transcripts in most organs and tissues, except retina (Tomarev et al. 1998) and testis (Steffensen et al. 2004), which express an ∼2.3-kb Prox1 mRNA. Detailed molecular analyses show that Prox1 mRNA harbors an ∼5.4-kb-long 3′ UTR and that this evolutionarily conserved region of the gene is found to be subjected to posttranscriptional regulation by HuR (Yoo et al. 2010) and microRNAs (Kazenwadel et al. 2010; Pedrioli et al. 2010), indicating that Prox1 may be regulated by multiple physiological and pathological signals and stimuli.

Separation of Blood and Lymphatic Vessels

After migration of the lymphatically committed venous endothelial cells, the next key developmental process is a coordinate separation of the rudimentary lymphatic vessels from blood circulation to avoid blood–lymphatic mixing. Interestingly, blood–lymphatic mixing phenotypes have been reported in several mutant mice lacking SYK (Cheng et al. 1995; Turner et al. 1995), SLP-76 (Abtahian et al. 2003), or

PLCγ-2 (Wang et al. 2000). These findings have provided important insights into the essential roles of the hematopoietic compartment in lymphatic development because the expression of these genes (SYK and SLP-76) is restricted in hematopoietic lineage cells. Supporting this notion, RUNX1 knockout mice that fail to undergo definitive hematopoiesis also reveal similar blood–lymphatic mixing phenotypes during early lymphatic development (Srinivasan et al. 2007). However, various genetic and cell lineage tracing experiments ruled out the possibility of direct incorporation of hematopoietic cells into growing lymphatic vessels during development (Srinivasan et al. 2007). Therefore, the question of how the hematopoiesis genes control lymphatic development, especially lymphatic separation from blood circulation, remained unanswered until two reports brought forward a critical clue that links podoplanin to platelet activation: Suzuki-Inoue and colleagues demonstrated a novel Syk-dependent mechanism of platelet activation by CLEC-2 (Suzuki-Inoue et al. 2006) and *O*-glycan-dependent physical interaction of podoplanin with CLEC-2 (Suzuki-Inoue et al. 2007). These pioneering findings were followed by a series of molecular and genetic studies confirming the role of podoplanin/CLEC-2 interaction in lymphatic development. Notably, endothelial cell *O*-glycan deficiency caused blood/lymphatic misconnection in mouse (Fu et al. 2008), and podoplanin or CLEC-2 knockout mice showed the blood–lymphatic mixing phenotype of mice lacking SYK, SLP-76, or PLCγ-2 (Bertozzi et al. 2010a,b; Suzuki-Inoue et al. 2010; Uhrin et al. 2010). Together, these studies show that the platelets mediate blood and lymphatic separation by activation of the CLEC-2 receptor following interaction with the podoplanin ligand found on the surface of LECs.

Plasticity of Lymphatic Endothelial Cell Fate—Lymphatic Equilibrium

Heterogeneity and plasticity are the two remarkable features of endothelial cells (Lee et al. 2010b). Various genetic and molecular studies using gain-of-function (GOF) or loss-of-function (LOF) mutations have shown that endothelial cells maintain astonishing flexibilities in electing their arteriovenous–lymphatic cell fates. For example, ectopic Notch expression in the venous compartment induces the arterial phenotypes at the expense of the veins, and, conversely, inhibition of Notch brings abnormal venous phenotypes onto the arterial vessels (Lawson et al. 2001, 2002; Lin et al. 2007; Benedito and Adams 2009). Similarly, when the venous endothelial cell–specific nuclear receptor COUP-TFII is genetically deleted, the venous compartment displays the arterial-specific characteristics such as up-regulation of the arterial markers and functional generation of hematopoietic cell clusters (You et al. 2005). This kind of endothelial cell fate plasticity can be also discovered in the lymphatic compartment. Studies of the genome-wide transcriptional profiles of human dermal BECs versus LECs revealed that more than ~95% of genes are comparatively expressed in two subtypes of endothelial cells (Petrova et al. 2002; Podgrabinska et al. 2002; Hirakawa et al. 2003). However, when overexpressed in BECs, Prox1 induces lymphatic reprogramming of the cells by up-regulating LEC-specific genes and simultaneously down-regulating BEC-specific genes (Hong et al. 2002; Petrova et al. 2002; Hirakawa et al. 2003). Conversely, Prox1 inhibition in embryonic or postdevelopmental LECs results in loss of lymphatic cell fate in vivo and in vitro (Johnson et al. 2008; Lee et al. 2009). Therefore, LEC identity appears to be highly plastic and reversible, and Prox1 is required to maintain LEC identity (Johnson et al. 2008; Lee et al. 2009).

In fact, it has been proposed that the three endothelial cell fate regulators—namely, Notch (arterial) (Shawber et al. 2007), COUP-TFII (venous) (You et al. 2005), and Prox1 (lymphatic) (Wigle and Oliver 1999)—are all expressed in LECs and cross-regulate one another (Lee et al. 2009; Kang et al. 2010). Notch, which is selectively expressed in arterial endothelial cells and acts as a downstream effector of VEGF-induced arterialization signal (Lawson et al. 2001, 2002; Weinstein and

Lawson 2002; Lanner et al. 2007; Siekmann and Lawson 2007), represses the expression of COUP-TFII, Prox1, and podoplanin through Hey1 (Kang et al. 2010). COUP-TFII, a nuclear receptor that is selectively expressed in the venous compartment, has been shown to interact physically and functionally with Prox1 in LECs to direct a developmental program that specifies LEC fate (Lee et al. 2009; Yamazaki et al. 2009; Kang et al. 2010; Srinivasan et al. 2010). Interestingly, Prox1 and COUP-TFII concertedly suppress VEGF signaling by down-regulating the expression of the major VEGF receptors VEGFR-2 and NRP-1 (Kang et al. 2010). Consistent with these in vitro findings, podoplanin and Notch1 are found to be conversely regulated in lymphatic vessels in vivo: LECs with low Notch1 expression tend to express more podoplanin protein, and vice versa. Interestingly, however, knockdown of Notch or its ligand Dll4 in zebrafish embryos impairs lymphangiogenesis, in particular, the initial budding of lymphatically differentiating venous endothelial cells, and lymphatic vessel navigation (Geudens et al. 2010), suggesting that despite its repressive role of lymphatic phenotypes, Notch signal is still required for the optimal lymphatic development. Together, these studies proposed a new concept that the expression of the three cell fate controllers is regulated by an exquisite feedback mechanism working in LECs and that LEC fate may be viewed as a phenotypic consequence of Prox1-directed "lymphatic equilibrium" among the cell fate regulators.

Postnatal lymphatic plasticity also needs to be addressed. LEC-fate plasticity is most often associated with various pathological conditions (Lee et al. 2010b). For example, the expression of VEGFR-3 is detectable in BECs before lymphatic sprouting, but after then is restricted to LECs, thus serving as an important LEC-signature gene in normal tissues (Jeltsch et al. 1997). However, abnormal expression of VEGFR-3 in BECs has been reported in various malignant tumors and granulation tissues (Partanen et al. 1999; Valtola et al. 1999; Witmer et al. 2001; Nakamura et al. 2003). Thus, VEGFR-3 expression in BECs was postulated to be a new microvascular progression marker that mediates lymphangiogenic factor–induced neovascularization (Clarijs et al. 2002). In addition, tumor-associated LECs were found to express several hundreds of genes differentially compared to normal, inflammatory cytokines or mitogen-activated LECs (Clasper et al. 2008; Royston and Jackson 2009). For example, tumor-associated LECs were found to up-regulate functionally significant molecules including the tight junction endothelial-specific adhesion molecule (ESAM), endoglin (CD105), leptin receptor, and CD200. Although exclusively expressed in BECs in normal tissue, ESAM was up-regulated in tumor lymphatics and linked with nodal metastasis (Clasper et al. 2008). Moreover, another BEC-signature molecule, CD34, was reported to be expressed by tumor-associated LECs in colon, breast, lung, and skin tumors, and a majority of intratumoral lymphatics revealed a complete colocalization of CD34 with various LEC markers such as LYVE-1, podoplanin, and Prox1 (Fiedler et al. 2006).

Lymphatic Vessel Maturation and Postdevelopmental Remodeling

In addition, Foxc2 has recently been shown to control formation and maturation of lymphatic collecting vessels through cooperation with NFATc1 (Norrmen et al. 2009). As lymphatic vessels mature during development, the lymphatic capillary plexus undergoes substantial morphological remodeling and acquires distinct characteristics of the collecting lymphatic vessels such as even diameter, mural cell recruitment, intraluminal valves, and little branching (Norrmen et al. 2009). Moreover, maturing lymphatic vessels were found to down-regulate some of the lymphatic capillary markers: For example, the high expression of Foxc2 and Prox1 can be continuously detected in LECs of bileaflet luminal valves but is somewhat reduced in collecting lymphatic vessel endothelial cells (Norrmen et al. 2009).

In addition to its role in the arteriovenous–lymphatic cell fate specification, the Notch signal pathway is also known to regulate vascular

morphogenesis by controlling angiogenic sprouting (Noguera-Troise et al. 2006; Ridgway et al. 2006; Hellstrom et al. 2007; Hofmann and Iruela-Arispe 2007; Siekmann and Lawson 2007). The main role of Notch in endothelial cells appears to be to regulate blood vessel quiescence and laterally inhibit new sprouting partly by down-regulating VEGFR-2 during angiogenesis, placing Notch signal as a negative regulator of VEGF-stimulated angiogenesis (Phng and Gerhardt 2009). This function of Notch in angiogenesis has been recently extended to the lymphatic compartment. Notch negatively regulates postdevelopmental lymphangiogenesis: Notch inhibition synergizes VEGF-induced lymphatic sprouting in vitro and in vivo, and forced expression of its ligand Dll4 in LECs promoted adoption of the tip cell position, suggesting that the Notch signal pathway negatively regulates lymphatic sprouting and induces stalk cell specification (Niessen et al. 2011; Zheng et al. 2011). Importantly, Notch pathway components are found more abundantly expressed in LECs over BECs, and this higher baseline activity of Notch was hypothesized to lead to a lower sensitivity of LECs than BECs to VEGF-activated vascular sprouting (Zheng et al. 2011).

LYMPHEDEMA AND LYMPHATIC DISEASES

"Lymphedema" refers to chronic tissue swelling in the face, arms, legs, or abdominal walls caused by accumulation of interstitial fluids mainly due to lymphatic dysfunction caused by lymphatic dysplasia, malformation, misconnection, and obstruction, as well as absence of functional lymphatic valves. Lymphedema is classified as primary (genetic) or secondary (acquired) lymphedema (Browse and Stewart 1985; Fonkalsrud 1994; Mortimer 1998; Rockson 1998; Child et al. 1999).

Primary Lymphedema

Primary lymphedema arises from genetic defects that interfere with normal lymphatic development and, despite its highly variable clinical manifestation, can be traditionally divided into three groups depending on the age of onset: congenital lymphedema (at birth), lymphedema praecox (early onset), and lymphedema tarda (late onset) (Allen 1934).

Congenital lymphedema includes all forms of lymphedema that are clinically evident at birth and accounts for 10%–25% of all primary lymphedema. Congenital lymphedema manifests more often with females than males, in lower than upper extremities, and in single than both legs. A subgroup of congenital lymphedema, namely, Milroy disease (Milroy 1892, 1928), has been initially linked to the VEGFR-3 locus on distal chromosome 5q, and later various mutations in the VEGFR-3 gene have been identified in patients with this disorder (Evans et al. 1999; Irrthum et al. 2000; Connell et al. 2009). Mice with VEGFR-3 mutations were shown to develop primary lymphedema, confirming the underlying etiology of the disease (Karkkainen et al. 2000).

"Lymphedema praecox," often called Meige's disease, collectively refers to all lymphedema with onset at ages 1 through 35, most often at puberty, and thus includes the majority (60%–80%) of primary lymphedema. Notably, females are affected four times more often than males. Generally, Meige's disease patients display dysplastic, smaller, and fewer lymphatic vessels compared to healthy individuals. Because the classification for lymphedema was established based on the onset age of the diseases, not on their genetics, pathophysiology, or etiology, the terms "lymphedema praecox" or "Meige's disease" have been confusing and impeding to better understand, diagnose, and treat the diseases. In fact, hereditary lymphedema praecox is frequently associated with several other anomalies including distichiasis, extradural cysts, vertebral anomalies, cerebrovascular malformation, yellow nails, and sensorineural hearing loss (Wheeler et al. 1981).

Lymphedema–distichiasis syndrome, a subset of lymphedema praecox, is a rare autosomal dominant disease characterized by swollen limbs and double rows of eyelashes (Robinow et al. 1970; Hoover and Kelley 1971; Jester 1977) and has been genetically linked to the FOXC2 gene, which encodes a member of the

Forkhead/Winged-Helix family of transcription factors that are involved in diverse developmental pathways (Fang et al. 2000; Bell et al. 2001; Finegold et al. 2001). Consistently, Foxc2-deficient mice display lymphatic dysfunction, including abnormal mural cell coverage on lymphatic capillaries, defective valves of the collecting lymphatic vessels, and irregular lymphatic patterning (Petrova et al. 2004). In addition, hypotrichosis–lymphedema–telangiectasia syndrome, another subset of lymphedema praecox, has been described to be associated with hypotrichosis and telangiectasia (Irrthum et al. 2003; Hosking et al. 2009). Mutations of the SRY-related transcription factor SOX18, which acts upstream of PROX1, were found to be responsible for both recessive and dominant forms of this disease. Recently, two more genes (GJC2 and CCBE1) were identified to be causally associated with lymphedema. The GJC2 gene encodes connexin 47, an intercellular gap junction protein, and mutations in the protein were postulated to cause impaired gap junction activities and result in defective lymphatic flow (Ferrell et al. 2010). CCBE1 has been shown to play a role in lymphatic sprouting during zebrafish development, and mutations in CCBE1 were found in patients with Hennekam lymphangiectasia–lymphedema syndrome (Alders et al. 2009; Hogan et al. 2009).

The third type of primary lymphedema is called lymphedema tarda, which manifests the lymphedema symptom only at late stages of life, usually after age 35, and constitutes ~10% of all primary lymphedema (Ohara and Taneichi 1973; Segal and Turner 1976; Vieras and Boyd 1976; Majeski 1986; Burgos and Luginbuhl 2009). This form of lymphedema displays a tortuous, hyperplastic pattern of lymphatic vessels characterized by an increase in diameter and number. Notably, the patients with this form of lymphedema often lack functional lymph valves.

Despite its historical significance and convenient organization, this traditional onset-age-dependent classification bears significant limitations and drawbacks because it does not convey the precise information of the disease and thus can be confusing and even hampering to basic research and clinical practice for lymphedema patients, especially in the era of molecular medicine. Luckily, several attempts have been made to establish new classifications and categorizations of primary lymphedema based on clinical phenotypes, familiar history, onset age, associated abnormalities, local and systemic involvement, and underlying genetics (Szuba and Rockson 1998; Miller et al. 1999; Board and Harlow 2002; Northup et al. 2003; Honnor 2008; Connell et al. 2010).

Secondary Lymphedema

Secondary or acquired lymphedema is caused by functionally compromised lymphatics owing to infection, surgery, radiation, or compression. In the advanced countries, the majority of secondary lymphedema is observed among cancer patients who undergo various radiation therapies following lymphadenectomy. Lymphadenectomy is a surgical lymph node dissection and a common procedure for assessing the stages of tumors. Although it is an essential practice, it inevitably destroys and obstructs lymphatic flows and thus renders patients at a high increased risk of lymphedema. Studies report that 25%–56% of breast cancer patients develop mild-to-severe lymphedema after cancer treatment (Pezner et al. 1986; Kiel and Rademacker 1996; Hinrichs et al. 2004; Ozaslan and Kuru 2004). Despite recent adoption of the sentinel lymph node biopsy technique, which removes only tumor-draining lymph nodes and thus significantly reduces the incidence of surgical lymphedema, cancer therapy–associated secondary lymphedema remains the most common complication for cancer survivors.

Worldwide, the most common cause of secondary lymphedema is filariasis, direct infection of lymphatic vessels by mosquito-borne parasitic nematodes like *Wuchereria bancrofti* (Shenoy 2006; Hoerauf 2008; Bockarie et al. 2009; Pfarr et al. 2009). More than 100 million people in tropical Africa and Asia are currently affected by lymphedema filariasis, associated with infection-induced inflammatory responses that cause lymphatic hyperplasia and obstructions, followed by tissue fibrosis,

hypercellularity, fat accumulation, and secondary infection. Lymphedema can also be caused by vein stripping, vascular surgery, lipectomy, and burns. Lymphedema presents severe social, economic, and psychological burdens to patients and their families, and, unfortunately, no cures are currently available for this disfiguring disease.

Lymphedema Therapy—Beyond Physical Compression

To date, several factors have been reported to stimulate lymphangiogenesis of both cultured LECs and animals, including VEGF-A, VEGF-C, VEGF-D, FGF-2, PDGF, IGF-1, IGF-2, Angiopoietin-1, and HGF (Adams and Alitalo 2007; Karpanen and Alitalo 2008; Tammela and Alitalo 2010; Lahteenvuo et al. 2011; Norrmen et al. 2011), and the list is growing rapidly. Among them, VEGF-C has been best characterized and is considered to be the most promising therapeutic agent to treat human lymphedema (Norrmen et al. 2011). Administration of a VEGF-C-expressing adenovirus enhanced generation of functional lymphatic vessels in *Chy* mutant mice (Karkkainen et al. 2001), and adenoviral delivery of VEGF-C in normal skin or at the edge of epigastric skin flaps in mice strongly induced lymphangiogenesis (Enholm et al. 2001; Saaristo et al. 2004). In addition, recombinant VEGF-C was shown to be sufficient to activate in vivo lymphangiogenesis and reversed the symptoms of lymphedema in rabbit ears (Szuba et al. 2002); VEGF-C gene therapy stimulated postnatal lymphangiogenesis and thus ameliorated secondary lymphedema in animal models (Yoon et al. 2003). More recently, using a newly established mouse model in which the axillary lymph nodes were removed to better recapitulate human breast cancer lymphadenectomy, Tammela et al. (2007) have shown that collecting lymphatic vessels could be efficiently regenerated and connected to transplanted lymph node in the lymphedema area. Adenoviral delivery of VEGF-C or VEGF-D into lymph-node-excised mice resulted in robust proliferation of lymphatic capillaries, followed by remodeling, differentiation, and maturation to establish functional collecting lymphatic vessels that are equipped with uniform endothelial cell–cell junctions and intraluminal valves, as well as mural cell investment (Tammela et al. 2007). Moreover, this experimental paradigm has been further extended to a non-rodent model: Lahteenvuo et al. (2011) evaluated the therapeutic effect of autologous lymph node transfer combined with adenoviral expression of VEGF-C or VEGF-D in a newly established porcine model of lymphedema in the inguinal area. Consistent with the data from the rodent model, both growth factors stimulated robust lymphangiogenesis in the defect area, and postoperative lymphatic drainage was dramatically improved, with the structure of the transferred lymph nodes best preserved in the VEGF-C-treated pigs. Taken together, VEGF-C provides the most promising therapeutic efficacy against lymphedema to date, and further development of VEGF-C as a therapeutic drug is eagerly awaited by human lymphedema patients.

Lymphangioleiomyomatosis (LAM)

Lymphangioleiomyomatosis (LAM) is a rare destructive lung disease that is etiologically associated with excessive proliferation of LECs (lymphangio) and smooth muscle cells (leiomyoma) throughout the lungs including bronchioles, alveolar septa, perivascular spaces, and parenchyma (Hohman et al. 2008; Darling et al. 2010; Kristof 2010; Kwiatkowski 2010; Seyama et al. 2010). The abnormal proliferation of these two types of cells (LECs and SMCs) in LAM results in both obstruction of airways and tissue fluid drainage, causing pulmonary cyst formation, pneumothorax, and chylous pleural effusion with respiratory failure, needing lung transplantation. LAM occurs in about one-third of child-bearing-age women with tuberous sclerosis complex (TSC), which is caused by various mutations in either TSC1 or TSC2 genes. Importantly, LAM patients express a high level of the potent lymphangiogenic factor VEGF-D in their blood serums, which may partly explain the excessive LEC proliferation in their lungs. Because mutations in TSC1/2

lead to abnormal activation of the downstream effector mTOR, rapamycin, a chemical inhibitor for mTOR, was found to be beneficial to some LAM patients (Darling et al. 2010; Kristof 2010).

Lymphatic Reprogramming of Vascular Endothelial Cells by Kaposi's Sarcoma Herpes Virus (KSHV)

Kaposi's sarcoma (KS) is an endothelial cell tumor and the most common neoplasm among HIV-positive individuals (Aguilar and Hong 2009). Although it was first described by Moritz Kaposi in 1872 (Kaposi 1872), KS did not receive much medical and scientific attention until the 1980s, when acquired immune deficiency syndrome (AIDS) became endemic. In 1994, Kaposi's sarcoma associated herpes virus (KSHV) was identified to be the causing agent for KS (Chang et al. 1994), and since then, studies of the pathogenesis of KS and KSHV have been extensively performed. Despite original studies that pointed to endothelial cells as the origin of KS tumor cells 40 years ago (Dayan and Lewis 1967), the histogenetic origin of KS tumor cells has been elusive to define for many years because of the mixed gene expression profile of KS tumor cells: KS tumor cells were thought to express BEC-specific genes, but as new lymphatic research tools became available from the late 1990s, lymphatic characteristics of KS tumor cells became evident (Aguilar and Hong 2009). Later, it was found that KS's dual phenotypes of BECs and LECs were attributed to lymphatic reprogramming of BECs by KSHV (Carroll et al. 2004; Hong et al. 2004; Wang et al. 2004): When KSHV infects BECs, it activates the expression of Prox1, the master control gene of lymphatic differentiation, and subsequently induces lymphatic reprogramming of BECs (Carroll et al. 2004; Hong et al. 2004; Wang et al. 2004), suggesting that this oncogenic virus reactivates the otherwise silenced embryonic endothelial differentiation program in adult cells. It is not yet understood why KSHV induces the Prox1-mediated lymphatic reprogramming of vascular endothelial cells, and studies of its molecular mechanism will surely advance our understanding of endothelial cell plasticity and heterogeneity in health and disease.

CONCLUDING REMARKS

Since its original description by Hippocrates, the lymphatic system has been neglected by both scientific and medical communities because of its vagueness in structure and function. Even after its rediscovery 400 years ago, the lymphatic system was considered a secondary vascular system that supports the blood vascular system. However, a series of landmark discoveries in lymphatic research has significantly advanced our understanding of not only the organogenesis, function, and anatomic structure of the system, but also the cellular and molecular biology of LECs. In particular, substantial attention has been given to the elucidation of the molecular control of physiological and pathological lymphangiogenesis, reevaluating its essential roles in human health and well-being. This paradigm shift simultaneously forced us to take a brand-new look at the lymphatic system as the *other*, not the secondary, vascular system. Considering the vital functions that the lymphatic system engages in and how little knowledge we have regarding the system, lymphatic research is truly a gold mine that invites ambitious young scientists and clinicians.

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