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HETEROGENEITY AND PLASTICITY OF LYMPHATIC ENDOTHELIAL CELLS

Sunju Lee, **Inho Choi**, and **Young-Kwon Hong**
Departments Surgery and Biochemistry and Molecular Biology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California

Abstract

Endothelial cells are found in most organs and tissues in our body. Despite their apparent morphological and functional similarities, endothelial cells exhibit remarkable heterogeneity and plasticity. In a strict sense, no two endothelial cells are identical in terms of their biological, immunological, functional, metabolic, morphological and anatomical aspects. Their heterogeneity and plasticity are now known to be dependent upon and conferred by their microenvironments, arteriovenous-lymphatic cell identity, organ-specific vascular beds, fluid-dynamics, vessel sizes, anatomical locations, their physiological and pathological states and more. While abundant evidences are documented to demonstrate endothelial heterogeneity in the blood vascular system, studies of heterogeneity and plasticity of lymphatic endothelial cells are limited because of the short history of the lymphatic research. Nonetheless, a growing body of exciting begin to discover the heterogenic nature of lymphatic endothelial cells, comparable to that of blood vascular endothelial cells. In this article, we discuss heterogeneity and plasticity of lymphatic endothelial cells.

INTRODUCTION

Our body contains two major circulatory systems, the blood vascular and the lymphatic system. While the blood vascular system has been intensively and extensively studied since its discovery, the lymphatic system has been quite neglected from more than two thousand years ago when it was first described by Hippocrates as “white blood vessels”. Unlike the blood vascular system, the lymphatic system was not as readily recognized and distinctive in terms of its functions and anatomical structures. Moreover, because disorders and diseases of the lymphatic system were not as common or life-threatening as those of the blood vascular system, scientists and clinicians have not paid enough attention to and thus forgotten this mysterious secondary circulatory system for such a long time. As a result, it was only 1627 when the lymphatic system was just re-discovered by the Italian anatomist Aselli as “milky veins” [1]. In an interesting comparison, the enormous amount of knowledge was published about the blood vascular system in the next year 1628 by William Harvey in his famous book, “*An anatomical study of the motion of the heart and blood in animals*” [2]. The lack of attention in the lymphatic system continued until the early 20 century when a landmark finding was made regarding the ontogenesis of the lymphatic system. It is only past 10 years, however, that the lymphatic research began to be actively performed with various research tools of the molecular, cellular and animal models and that the mystery of the origins and development of the lymphatic systems were slowly unraveled [3]. The past decade was truly a historic era of the lymphatic research, when many important

Corresponding Author: Young-Kwon Hong, Ph.D., Assistant Professor, Departments of Surgery and of Biochemistry & Molecular Biology, Norris Comprehensive Cancer Center, University of Southern California, 1450 Biggy St. NRT6501, M/C9601, Los Angeles, CA 90033, Phone: 323-442-7825, FAX: 323-442-7844, young.hong@usc.edu.

and exciting discoveries of the lymphatic origin, development, function, diseases and disorders were made. Here, we will describe the brief history of the lymphatic research, several key lymphatic molecules and heterogeneity and plasticity of the lymphatic endothelial cells.

The Lymphatic System

Compared to the closed circulatory loop of the blood vasculature system where the fluid (blood) leaves from and returns to the same organ (the heart), the lymphatic vasculature is a linear, blunt-ended system and initiates from a network of thin-walled capillaries that collect the tissue fluid, cells and macromolecules (collectively called lymph) from the interstitial tissue spaces. This lymph is then carried through the larger collecting lymphatics and multiple lymph nodes and finally to the thoracic duct, a largest lymphatic vessel that is connected to the vein (the vena cava), for recirculation. While blood capillaries consist of membrane-surrounded endothelial cells, which are covered by smooth-muscle like pericytes, lymphatic capillaries are composed with a single layer of overlapping endothelial cells without a continuous coverage by basement membrane and pericytes. This unique loose structure enables lymph fluids to easily drain into lymphatic vessels when fluids are accumulated in the interstitial spaces. Notably, basement membranes and pericytes are only found in the collecting or bigger lymphatic vessels. The lymphatic system also includes the secondary lymphoid organs such as the lymph nodes, tonsils, Peyer's patches, spleen and thymus. The lymphatic system plays important roles in maintenance of tissue fluid homeostasis, uptake of intestinal lipids, macromolecules and proteins, and also trafficking of immune cells to the sites of inflammation and infection. In addition to these physiological functions, lymphatic vessels are used by most tumors as conduits for their metastasis to distant sites.

The Origin and Development of the Lymphatic System

In 1902, a theory on the embryonic origin of lymphatic vessels was first proposed [4, 5]. Based on ink-injection experiments into pig embryos, Florence Sabin hypothesized that initial lymph vessels are derived from embryonic veins and that these primitive lymphatics then spread throughout the entire embryo body to form lymphatic networks [4, 5]. Compared to this "centrifugal" theory by Sabin, a contrasting "centripetal" hypothesis was put forth by Huntington and McClure and proposed that lymphatics arise independently in the mesenchyme and become connected to the venous network only later during development [6]. Debates between these two competing theories continued for the next one hundred years until the dawn of this century when the animal study of the homeodomain transcription factor Prox1 demonstrated the venous origin of lymphatics during embryonic development [7, 8]. During early embryogenesis (e.g., mouse E9.5), developing venous endothelial cells display phenotypes of both blood vascular endothelial cells (BECs) and lymphatic endothelial cells (LECs); while they serve as lining cells of blood vessels, they also express important lymphatic markers such as LYVE-1 and VEGFR-3 [9, 10]. However, a subset of venous endothelial cells begins to express Prox1 upon stimulation by an inductive signal and, subsequently, buds off and migrates out to form rudimentary lymphatic vessels, called jugular lymph sacs (JLS). The Prox1-positive migrating cells undergo lymphatic differentiation by upregulating LEC-specific genes and down-regulating BEC-specific genes. In comparison, Prox1-negative cells remaining in the vein gradually lose the expression of the lymphatic markers VEGFR-3 and LYVE-1 and adopt BEC phenotypes. Importantly, Prox1-deficient mice fail to form any lymphatic system [7, 8]. Therefore, Prox1 functions as the master regulator for lymphatic system development by reprogramming endothelial cell fate from BECs to LECs. Moreover, a recent endothelial lineage tracing experiments has further demonstrated that all LECs are derived from the embryonic vein and corroborated the venous origin of mammalian lymphatic vasculature [11], supporting

Sabin's original centrifugal theory. Interestingly however, the contrasting centripetal theory, at least in part, seems to still stand for the development of the avian lymphatic system based on the chick-quail cross-species transplantation experiments [12-14]. Thus, it seems that the avian lymphatics are of dual origin: while the JLS LECs are originated from the veins, the dermal LECs may be derived from local lymphangioblasts.

MOLECULAR PLAYERS IN LYMPHATIC DEVELOPMENT

Prox1

The master control gene for lymphatic development Prox1 was originally isolated by its strong amino acid sequence homology to the *Drosophila* protein Prospero [15-17], which plays an essential role in development of the nervous system [18]. During mouse embryogenesis, Prox1 is expressed in the developing CNS, the eye lens and retina, the pancreas, the liver, the skeletal muscles and heart, in addition to the lymphatic vessels [15]. Human and mouse Prox1 genes encode a protein of 737 amino acids with an estimated molecular weight of 84-kDa and contain the homeodomain and the Prospero domain at the C-terminus [17]. Sequence comparison of Prospero and Prox1 revealed that their C-terminal 240 amino acids show a strong similarity [19]. This region of Prospero alone was shown to be sufficient for activating transcription of a reporter gene by binding to specific DNA sequences [20, 21]. Similarly, Prox1 directly binds to specific DNA sequences in the promoter of several lymphatic-associated genes including FGF receptor (FGFR)-3 and activates their LEC-specific expression [22]. Prox1 is sufficient to induce lymphatic reprogramming of post-developmental BECs; when ectopically expressed in BECs isolated from human foreskin, Prox1 can override BEC-phenotype by repressing BEC-specific markers and adopt lymphatic phenotypes by upregulating LEC-specific genes [23, 24]. Together, Prox1 acts as the key regulator for a differentiation program specifying LEC-fate during and post development. Prox1 has been found to be as a binding partner and co-regulator of four orphan nuclear receptors; liver receptor homologue (Lrh)-1/NR5A2, hepatocyte nuclear factor (HNF)-4α/NR2A1 and ff1b, a zebrafish homolog of mammalian steroidogenic factor (SF)-1/NR5A1 and COUP-TFII/NR2F2 [25-30]. Recently, it was shown that the transcription factor Sox18 (SRY (sex determining region Y) box 18) can directly activate Prox1 transcription, that overexpression of Sox18 in BECs upregulates Prox1 and other LEC-markers and that Sox18-null embryos showed a complete blockade of LEC-differentiation from the cardinal vein [31, 32], suggesting a critical role of Sox18 in developmental lymphangiogenesis.

VEGF-C/VEGF-D/VEGFR-3

Vascular endothelial growth factor (VEGF)-C was the first molecule to be identified as a LEC-specific growth factor [33]. VEGF-C binds to the receptor VEGFR-3 [34, 35], which is selectively expressed on the surface of LECs [36]. VEGF-C plays an essential role during embryonic lymphatic development by regulating the sprouting of the initial lymphatic vessels from the cardinal vein [37]. The second molecule identified as a LEC-specific growth factor is VEGF-D, which shares about 61% amino acid homology with VEGF-C and is also capable of binding to VEGFR-3 for its function [38]. Similar to VEGF-C, overexpression of VEGF-D in transgenic mouse skin also induces lymphangiogenesis [39]. Surprisingly however, VEGF-D deficient mice did not exhibit any lymphatic phenotypes, suggesting either an insignificant role of VEGF-D in normal lymphatic development or compensation by VEGF-C for the lack of VEGF-D [40]. VEGFR-3, a receptor for both VEGF-C and VEGF-D, is a member of the fms-like receptor tyrosine kinase family and was identified as the first lymphatic-specific gene [36]. Activation of VEGFR-3 enhances proliferation, migration and survival of LECs [41] and is sufficient to induce lymphangiogenesis *in vivo* [39]. VEGFR-3 knockout mice display embryonic lethality at

embryonic day 9.5 due to abnormal vasculatures with tissue fluid accumulation and cardiovascular failure [42]. Moreover, functional blocking of VEGFR-3 by skin-specific expression of a soluble form of VEGFR-3 in transgenic mice caused lymphatic vessel regression and lymphedema without affecting the blood vasculature [43]. VEGFR-3 activation results in receptor dimerization and induction of various signaling pathways, including activation of the p42/p44 MAP kinase signaling cascade and induction of Akt phosphorylation [41].

VEGFR-2

VEGF receptor (VEGFR)-2 is a receptor for VEGF-A, VEGF-C and VEGF-D. VEGFR-2 is expressed in BECs and LECs *in vitro* and *in situ* [9, 44, 45]. Although the role of VEGFR-2 signaling in angiogenesis has been well studied, its contribution to lymphangiogenesis is currently a matter of controversy. Importantly, VEGF-A can promote proliferation, migration and tube formation of LECs *in vitro* [9, 44] and a local injection of VEGF-A-adenovirus into mouse ears induces pronounced *in vivo* lymphangiogenesis [46]. Moreover, transgenic mice overexpressing murine VEGF-A_{164} in the skin showed promoted lymphangiogenesis and angiogenesis during tissue repair and skin inflammation [9, 47]. Consistently, inhibition of VEGFR-2 function using a neutralizing antibody suppresses both angiogenesis and lymphangiogenesis in healing wounds, indicating the contribution of VEGFR-2 in lymphangiogenesis *in vivo* [46].

LYVE-1

Another important LEC-specific marker LYVE-1 (*Ly*mph vessel endothelial hyaluronan receptor-1) is a receptor for hyaluronic acid (HA), a large mucopolysaccharide polymer that is an abundant component of the extracellular matrix and maintains the structural integrity of connective tissues [48, 49]. Extracellular HA undergoes a constant turnover and released HA is transported through lymphatic vessels to the lymph nodes and the liver for hydrolysis. LYVE-1 was identified by its high amino acid homology to the leukocyte receptor CD44 that is predominantly expressed in BECs [50]. The lymphatic marker LYVE-1 is absent from most of BECs, but is expressed by activated tissue macrophages and sinusoidal endothelium of the liver and the spleen, where HA is absorbed and degraded [48, 49]. LYVE-1 has been also implicated in the trafficking of cells within lymphatic vessels and lymph nodes. Despite the important role of LYVE-1 as LEC-marker, LYVE-1 knockout mice appear normal without obvious lymphatic phenotypes or abnormalities [51].

Podoplanin

Podoplanin is a mucin-type transmembrane and predominantly expressed in LECs, but not BECs [24, 44, 45, 52, 53]. Podoplanin is also expressed in a broad range of other cell types including lung type I alveolar cells, choroid plexus cells, ciliary epithelial cells of the eye, osteocytes and kidney podocytes [52-57]. Because of its expression in various cell types, it has been cloned by a number of groups and independently named as OTS8, E11 antigen, RTI40, murine gp38, canine gp40, human gp36, aggrus and murine PA2.26 [52-57]. During mouse development, podoplanin is first expressed at around E9 in the central nervous system and the foregut [53, 54, 58] and at E11.5 - E12.5, its expression becomes apparent in endothelial cells in the cardinal vein including the budding Prox1-positive cells [53]. Like other LEC-markers VEGFR-3 and LYVE-1, podoplanin expression is continuously maintained in the lymphatic lineage cells, but is progressively downregulated in venous ECs [53]. Podoplanin-knockout mice die perinatally because of lung failure [53, 54]. Importantly, mutant pups display lymphedema in the limbs, impaired cutaneous lymphatic transport and abnormal lymphatic vasculature [53].

CCL21

CCL21 (CC chemokine ligand 21), also known as SLC (secondary lymphoid-tissue chemokine), exodus-2 and 6Ckine, is predominantly expressed in LECs, high endothelial venules (HEV) and the T-cell area cells in lymph nodes and Peyer's patches. CCL21 binds to the chemokine receptor 7 (CCR7) and promotes adhesion and migration of various immune cells including thymocytes, T-lymphocytes, macrophages and neutrophils via high-affinity binding to the receptor. CCL21 is the first chemokine known to mediate homing of lymphocytes and migration of antigen-stimulated dendritic cells into secondary lymphoid organs [59, 60].

COUP-TFII

Chicken ovalbumin upstream promoter transcription factors (COUP-TFs) are members of the steroid/thyroid hormone receptor superfamily. To date, COUP-TF homologues have been cloned from fly to human and their sequences are highly conserved and two COUP-TF proteins (COUP-TFI/NR2F1 and COUP-TFII/NR2F2) have been isolated and characterized in mammals. Because they share an exceptionally high homology at the amino acid level and their tissue expression patterns are distinct, they are thought to carry out redundant functions [61-63]. Deletion of COUP-TFII in mice resulted in embryonic lethality with severe defects in cardiovascular development [62]. Importantly, COUP-TFII has been shown to be expressed specifically in venous, but not arterial endothelial cells, and that endothelium-specific ablation of COUP-TFII enables the veins to acquire the arterial characteristics, including the expression of the arterial markers and generation of hematopoietic cell clusters [64]. Furthermore, ectopic expression of COUP-TFII in endothelial cells resulted in failure of arteriovenous specification by forming the fusion of veins and arteries in transgenic mouse embryos [64]. COUP-TFII counteracts Notch signaling and plays an essential role in maintaining the vein identity during development [64]. Interestingly, COUP-TFII has been recently shown to be also expressed in LECs and physically and functionally interact with Prox1 for regulation of several LEC-markers and LEC-fate specification [29, 30].

HETEROGENEITY AND PLASTICITY OF LYMPHATIC ENDOTHELIAL CELLS

Unstable Phenotypes of Endothelial Cells

It has been repeatedly documented that the endothelial phenotypes are rather unstable and thus tend to change when they are dissociated from their original tissue microenvironments and cultured *in vitro*. Researchers have witnessed that isolated and cultured endothelial cells progressively lose some or significant portion of their original phenotypes and cell identities [65, 66]. Cultured endothelial cells have been shown to lose their blood-brain barrier, fenestration, morphology and electrical properties as they go through several passages [67-69]. Moreover, murine ear vessels could express genes characteristic of heart vessels when heart tissue was transplanted to the ear [70] and when aortic endothelial cells were cultured in lung extracellular matrix [71] or on matrix derived from kidney cells [67], they displayed the lung or kidney-specific features, respectively. In fact, when high endothelial venule endothelial cells were isolated and cultured outside the lymphoid tissue microenvironment, they rapidly lost their phenotypes and undertook dedifferentiation [72]. All these studies suggest that endothelial cells may have a constant bidirectional communication with their environment to optimally maintain their phenotypes and cell identity.

This phenomenon was also observed with LECs. Defined cultures of human dermal LECs were first established only a decade ago and cultured LECs have significantly contributed to the current lymphatic research [24, 44, 45, 73]. Isolation and characterization of dermal

BECs and LECs revealed that the cultured cells were initially found to be stable and maintain their lineage-specific gene expression patterns based on genome-wide transcriptional profile studies [24, 44, 45, 73]. Cultured primary dermal LECs continue to express most of lymphatic markers such as Prox1, LYVE-1, VEGFR-3 and podoplanin, suggesting that their LEC-identity was mostly retained. Moreover, when dermal LECs were immortalized with telomerase, their LEC-specific gene expression pattern was largely maintained with an extended life span [74, 75]. Despite this stability of LEC-identity, a more detailed transcriptomal comparative study of human dermal LECs *ex vivo* and *in vitro* has demonstrated that about two thirds of genes that were shown to be differentially expressed between BECs and LECs *in vitro* (total ~950 genes) was in fact due to cell culture effects and that only one third were truly differentially expressed genes both *in vitro* and *ex vivo* [76]. Another study taking the similar approach has further demonstrated that > 50~65% of *in vivo*-expressed BEC/LEC-defining gene were silenced by *in vitro* culture condition, indicating that blood and lymphatic-specific cell differentiation programs are stringently controlled by the tissue microenvironment [77]. The study has newly identified a list of BEC- or LEC-specific genes and also found that the expression of MHC class II genes is a hallmark feature of the *in vivo* differentiation program of BECs that is strictly regulated by microenvironment and can be subject to change by *in vitro* culturing [77].

Heterogeneity in Lineage-Specific Gene Expression of Lymphatic Endothelial Cells

While extensive studies have been performed on heterogeneity of BECs, only a small number of studies have been done on tissue-specific heterogeneity of LECs. Although limited, the studies have demonstrated that LECs also exhibit astonishing heterogeneity. For example, LECs from initial/capillary (micro) versus collecting (macro) lymphatic vessels display a significant difference in gene expression patterns. Structurally, while initial or capillary lymphatic vessels are composed of a single layers of overlapping endothelial cells lacking valves and mural cell coverage, collecting lymphatics have basement membranes and mural cell (pericytes) surrounding the endothelial cells.

LYVE-1 and podoplanin are two important lymphatic-specific markers that have been utilized from the early stage of the lymphatic research [50, 78]. However, it is worth to know that whereas capillary LECs express LYVE-1 and podoplanin, LECs of collecting lymphatic vessels express only podoplanin, not LYVE-1 [79, 80]. Similarly, while the arterial marker ephrinB2 is expressed predominantly in LECs of collecting lymphatic vessels, the venous molecule EphB4 is found to be express in both capillary and collecting lymphatic vessels [79]. Moreover, a marked heterogeneity in the immunoreactivity of NO synthase and cyclooxygenase (COX) was also reported with collecting lymphatics showing a significantly higher level [80]. Moreover, capillary LECs displayed higher proliferative activity and less oxygen sensitivity compared to LECs in collecting lymphatics [80].

In addition, a recent study reported that there are two distinct subpopulations of LECs that show differential expression of the LEC marker podoplanin and they were referred as podo-high and podo-low LECs [81]. These two LEC populations are predominantly found in capillary and pre-collector lymphatic vessels in the skin and display a different expression pattern for several pro-inflammatory factors. For example, in comparison to the high-podoplanin lymphatics, podo-low capillary lymphatics were found to be associated with a lower expression of CCL21 and a higher level expression of Duffy blood group antigen receptor (DARC) and chemokine CC motif ligand 27 (CCL27) that recruits the pathogenic $CCR10^+$ T-lymphocytes in human inflammation [81]. Thus, the podo-low capillary lymphatics were proposed to constitute a specialized segment of the initial lymphatic vasculature that plays an important role in trafficking of $CCR10^+$ T-lymphocytes during skin inflammation [81].

Notably, LYVE-1 was initially documented to be exclusively expressed in LECs, but not in BECs [48, 49]. However, it was later found that LYVE-1 is also present in normal hepatic blood sinusoidal endothelial cells in mice and humans, but is absent in the angiogenic blood vessels of liver tumors and only weakly present in regenerative hepatic nodules in cirrhosis [82]. Moreover, the expression of LYVE-1 in sinusoidal endothelium was reported to be reduced in chronically inflamed human livers [83].

Lymphatic Heterogeneity in Cell-To-Cell Junction

Lymphatic drainage of lymph fluid is mainly driven by hydrostatic and colloidal osmotic pressure gradients. Endothelial cells of capillary lymphatics have incomplete or no intercellular junctions [84, 85]. When interstitial fluid pressure accumulates, LECs are pulled by attached anchoring filaments to open up their overlapping junctions, which allows the lymph to flow along its pressure gradient into lymphatics [86]. This suggests the presence of specialized cell-cell junctions in capillary lymphatics for fluid drainage and cell trafficking. Indeed, distribution and composition of junctional proteins in capillary lymphatics are significantly different compared to those of the conventional intercellular junctions in collecting lymphatics and blood vessels [87]. Interestingly, overlapping flaps of capillary LECs lack junctions at the tip, but are anchored by discontinuous button-like junctions that are different from the conventional, continuous, zipper-like cell junctions of LECs in collecting lymphatics [87]. Notably, growing tips of lymphatic sprouts are found to have zipper-like cell junctions, not button-like junctions, indicating that button-like junctions are not immature cell junctions, but rather specialized junctions, which open and close without disrupting junctional integrity for efficient lymphatic drainage [87].

Organ-specific heterogeneity of LECs

A previous large scale microarray study has shown that BECs are highly heterogeneous depending on anatomical locations and organs [88]. Similarly, recent studies show that LECs also display a significant organ-specific heterogeneity: LECs isolated from lymph node, spleen, thymus, palatine tonsil and iliac lymphatic vessels display differential expression patterns of LEC and vascular markers [89, 90]. Moreover, a knockout mouse of the PIK3R1 gene, a member of the phosphoinositide 3-kinase (PI3K) family, shows interesting organ-specific lymphatic malformation, arrested lymphatic sprouting and maturation defects without a major impact on blood vessels [91]. Notably, while lymphatic sprouting toward the diaphragm was arrested, lymphatics invaded the gut, where remodeling and valve formation were impaired. Therefore, the PIK3R1 gene products exert their functions in distinct steps of embryonic lymphangiogenesis in different organ microenvironments without affecting normal angiogenesis [91]. In addition, a recent genome-wide comparative study of intestinal LECs versus dermal LECs of human revealed that although the two cell populations display similar gene expression profiles, > 200 genes were found to be differentially expressed [92]. Among them, the LAR protein-tyrosine phosphatase-interacting protein liprin β1 was found to be expressed in lacteals, skin and mesenteric collecting lymphatics and their valves, but not in the skin lymphatic capillaries. Ablation of liprin β1 disrupted the normal formation of the dorsal and ventral caudal lymph vessels and significantly compromised the integrity of lymphatic vasculature [92].

Plasticity and Heterogeneity of Lymphatic Endothelial Cell Fate

Studies of the arteriovenous endothelial fates provide clear examples of the plasticity of endothelial cell fates. Notch signal genes has been reported to be predominantly expressed in the arterial compartment and direct the arterial endothelial cell phenotypes [93, 94]. When ectopically expressed in the venous compartment, Notch can induce arterialization of the venous compartment by upregulating the arterial endothelial cell markers. Conversely, when Notch signal is inhibited, the arterial compartment loses the arterial cells fate and

upregulates the venous endothelial cell markers [93, 94]. Similarly, when the venous endothelial cell-specific nuclear receptor COUP-TFII is genetically abolished, the venous compartment exhibits the arterial-specific features such as upregulation of the arterial markers and functional generation of hematopoietic cell clusters [64]. This kind of endothelial cell fate plasticity was also discovered in the lymphatic compartment. Studies of genome-wide transcriptional profiling of human dermal BECs versus LECs revealed that > ~95% of genes are comparatively expressed in two subtypes of endothelial cells [24, 44, 73]. As discussed above, Prox1 directs lymphatic differentiation of endothelial cells: When ectopically overexpressed in BECs, Prox1 induces lymphatic reprogramming of BECs by upregulating LEC-specific genes and downregulating BEC-specific genes [23, 24, 44]. Conversely, inhibition of Prox1 in either embryonic or post-developmental LECs results in loss of lymphatic phenotypes both *in vivo* and *in vitro* [29, 95]. Therefore, LEC-identity appears to be highly plastic and reversible and Prox1 is required to maintain LEC-identity [29, 95].

Moreover, while Notch is selectively expressed in the arteries and COUP-TFII in the veins, the lymphatics express all three cell fate regulators [7, 8, 29, 96]. This finding puts forward a new concept that all three endothelial cell fates may co-reside in LECs and a subtle alteration can result in a significant change in LEC-fate. In fact, we elucidated a molecular basis to verify this concept by establishing a cross-control mechanism among these regulators (Kang et al in preparation). We found that activated Notch or Notch ligands downregulates Prox1 and COUP-TFII through Hey1 and Hey2 in LECs and that ectopic expression of Notch suppress the lymphatic phenotypes and induces the arterial cell fate (Kang et al in preparation). On the contrary, Prox1 and COUP-TFII attenuate VEGF signaling, which is known to induce Notch, by repressing VEGFR-2 and neuropilin-1. We also found that previously reported podoplanin-based LEC-heterogeneity is strongly associated with differential expression of Notch1 in human cutaneous lymphatics. Together, the three key endothelial fate regulators seem to be under an exquisite feedback regulatory network in LECs and their regulatory “equilibrium” may play an important role in the arteriovenous-lymphatic cell fate specification and LEC-plasticity.

Tumor-Associated Heterogeneity of Lymphatic Endothelial Cells

In addition to the physiological condition, endothelial cells exhibit a marked heterogeneity under the pathological condition. VEGFR-3 is one of the first lymphatic markers determined to be expressed in LECs, but not BECs and considered to be a major regulator of lymphangiogenesis in normal tissues [33]. However, abnormal VEGFR-3 expression in BECs has been documented in various malignant tumors and granulation tissues [97-100]. Accordingly, VEGFR-3 expression in BECs was proposed to be a new microvascular progression marker that mediates lymphangiogenic factor-induced neovascularization [101]. Conversely, genome wide transcriptome studies revealed that tumor-associated LECs differentially express ~800 genes compared to LECs of normal, inflammatory cytokine, or mitogen-activated LECs [102, 103]. Most notably, tumor-associated LECs upregulate functionally significant molecules such as the tight junction regulatory protein endothelial-specific adhesion molecule (ESAM), TGF-β coreceptor Endoglin (CD105), leptin receptor and CD200. In particular, although exclusively expressed in BECs in normal tissue, ESAM was found to be upregulated in tumor lymphatics and associated with nodal metastasis [102]. In addition, another BEC-specific marker CD34 was found to be expressed by tumor-associated LECs in human [104]. Notably, CD34 was reported to be expressed by LYVE-1+/podoplanin+/Prox1+ tumor-associated LECs in colon, breast, lung, and skin tumors and > 80% of detectable intratumoral lymphatics showed complete co-localization of CD34 with LEC markers [104].

Lymphatic Reprogramming of Vascular Endothelial Cells by Kaposi Sarcoma Herpes Virus

One of the best studied example of pathological heterogeneity and plasticity of endothelial cells is Kaposi's sarcoma (KS). KS is the most common neoplasm among HIV-positive individuals and the proliferating KS tumor cells are known to be originated from endothelial cells [105]. Since its first description by Moritz Kaposi in 1872 [106], KS has not received much attention until AIDS became endemic in 1980s. Human herpes virus (HHV)-8 or Kaposi's sarcoma associated herpes virus (KSHV) was identified and characterized to be the causing agent for KS in 1994 [107]. Since then, intensive and extensive studies of the pathogenesis of KS and KSHV have been performed. Although the proliferating host cells of KSHV were initially reported to be endothelial cells about forty years ago [108], the precise histogenetic origin has been a matter of controversy for many years mainly due to the mixed gene expression profile of KS tumor cells. KS tumor cells were originally found to exhibit various BEC-specific gene expression pattern and functional phenotypes [105]. As new lymphatic research tools such as novel lymphatic markers and defined LEC cultures are available from the late 1990s, lymphatic characteristics of KS tumor cells became evident and apparent. However, the mystery of the histogenetic origin of KS was largely unraveled when KS's dual phenotypes of BECs and LECs was attributed to lymphatic reprogramming of BECs by KSHV [109-111]. It was found that when KSHV virus infects BECs, it activates the expression of Prox1, the master control gene of lymphatic differentiation, and subsequently induces lymphatic reprogramming of BECs [109-111]. In fact, this oncogenic virus re-activates the otherwise silenced embryonic endothelial differentiation program in adult cells. It is not yet understood why and how KSHV induces the Prox1-mediated lymphatic reprogramming and elucidation of the molecular mechanism underlying the KSHV-mediated endothelial cell fate reprogramming will surely advance our understanding of endothelial cell plasticity and heterogeneity in health and disease.

Closing Remarks

Heterogeneity and plasticity are two astonishing features of endothelial cells. While endothelial heterogeneity has been defined by pathophysiological observations, endothelial plasticity has been established by molecular, cellular and genetic studies. Importantly, these two characteristics are inseparable and heterogeneity and plasticity may be the two sides of a coin. Endothelial heterogeneity can not be comprehended without a good understanding of their plasticity because plasticity is the main mechanism that enables heterogeneity. Although accumulated knowledge on the blood vascular system has significantly helped the lymphatic research to blossom in the past decade, much more studies are definitely needed to characterize both common and distinct features between two vascular systems. It will be exciting to see more investigations to be performed to define the molecular and cellular mechanism underlying the heterogeneity and plasticity of endothelial cells in the lymphatic system.

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