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*Review*

# Single-Cell Sequencing and Spatial Transcriptomics Reveal Functional Plasticity of Lymphatic Endothelial Cells in Disease

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**Abstract:** Lymphatic endothelial cells (LECs) have long been viewed as passive conduits that merely maintain tissue fluid homeostasis. But that picture has changed dramatically. Recent advances in single-cell transcriptome sequencing (scRNA-seq) and spatial transcriptomics (ST) have revealed that LECs are highly heterogeneous and remarkably plastic in their functions. This review summarizes the new research results of LECs based on the above two technologies. LECs in normal tissues have multiple types of subpopulations. In the lymph node, there are further sub-regions, such as the subcapsular sinus ceiling/floor LECs, medullary sinus LECs, valve LECs and other special subsets. Each subpopulation performs distinct functions depending on its anatomical location, ranging from lymph drainage and barrier formation to immune regulation, etc. Under pathological conditions such as cancer, chronic inflammation, cardiovascular disease, obesity, and tissue repair, LECs undergo profound transcriptional reprogramming and phenotypic remodeling. They express immune checkpoint molecules, secrete chemokines, and build ligand-receptor interaction networks. In other words, LECs transform from passive structural pipes into active signaling integration centers and immune regulatory hubs. They participate directly in immune response modulation, metastasis promotion or suppression, tissue repair, and fibrosis. These findings have fundamentally redefined the biological role of LECs in the microenvironment. Targeting LECs heterogeneity and their bidirectional cell-cell interaction networks may offer novel stromal-targeted strategies for intervening in tumor lymphatic metastasis, reversing chronic inflammation, and treating cardiovascular diseases.

**Keywords:** Lymphatic Endothelial Cells; Single-Cell Transcriptome Sequencing; Spatial Transcriptomics; Functional Plasticity; Tumor Microenvironment; Chronic Inflammation; Cardiovascular Disease; Immune Regulation

## 1. Introduction

We used to think of lymphatic vessels as little more than passive drainage networks [1]. Although the main functions of these cells are to maintain tissue fluid balance and help transport lipids [2], scRNA-seq and ST have changed this perception entirely. These technologies have driven major breakthroughs in lymphatic vessel biology, giving us a much deeper understanding of how lymphatic vessels work and how diverse their cellular makeup really is [3]. Based on the new data, the cells forming the walls of lymphatic vessels are lymphatic endothelial cells (LECs), and these cells serve as “sensors” and “signal-integration centres” in the tissue environment. They are not only observers [1].

The key insight driving this shift in thinking is the discovery that LECs possess remarkable functional plasticity [4]. This plasticity is not just a simple physiological response. Instead, it represents active phenotypic remodeling driven by changes in transcriptional, epigenetic, and metabolic networks during tissue development, homeostatic maintenance, or pathological conditions [5]. In inflammatory or tumor microenvironments, for example, LECs do far more than provide channels for immune cells or tumor cells to migrate through [6]. They actively regulate immune signals and participate in local immune responses [4, 7]. This transition from "structural conduit" to "functional node" highlights the importance of LECs as microenvironment regulators [8]. Nevertheless, a systematic understanding of LEC functional heterogeneity and its underlying molecular mechanisms remains an open question.

In the past, studying LECs presented serious technical hurdles. Lymphatic vessels make up only a tiny fraction of tissues [9]. Bulk RNA sequencing (Bulk RNA-seq) averages signals across all cells in a sample, so dominant subpopulations drown out the unique transcriptional signatures of rare LEC subsets [10]. Conventional in vitro culture cannot replicate the unique tissue microenvironment that LECs inhabit, making it impossible to truly understand how LECs interact with surrounding stromal cells, immune cells, and the extracellular matrix [10,11].

Several critical questions remained out of reach: What is the subpopulation heterogeneity of LECs? How are different subpopulations distributed in tissues? How does the local microenvironment drive plastic transformations in LECs? And how do these subpopulations dynamically reshape their transcriptomes and intercellular communication networks under disease conditions? Traditional methods could not fully answer these questions.

scRNA-seq changes the game. It can resolve the transcriptome landscape of individual lymphatic endothelial cells, identifying rare subpopulations and their marker genes [12]. This allows researchers to clarify the heterogeneity of different LEC subpopulations and track how their transcriptomes change across different diseases or different stages of the same disease [13]. ST complements this by revealing the spatial positions of different LEC subpopulations and showing which cells LECs interact with in specific disease states—interactions that can alter LEC function [14]. Therefore, this review brings together cutting-edge findings from scRNA-seq and ST. For the first time, we systematically summarize the functional plasticity of LECs in multiple diseases including cancer, chronic inflammation, and cardiovascular disease, with the aim of pointing the way for future research.

## 2. scRNA-seq Reveals Normal Lymphatic Endothelial Cell Heterogeneity

LECs are the main cellular component of lymphatic vessel walls [15], and their anatomical structure and function vary considerably across different levels of the lymphatic system [16]. Primary lymphatic vessels, also known as lymphatic capillaries, are blind-ended channels located in tissue spaces [2]. They collect interstitial fluid and direct it into collecting lymphatic vessels [17]. The endothelial cells of primary lymphatic vessels are flat or cuboidal in shape and arranged loosely [18]. Unlike vascular endothelial cells, primary lymphatic endothelial cells connect to each other through "button-like" junctions with spacing of approximately 3 μm [16,19]. These button-like junctions form valve-like openings that serve as channels for interstitial fluid and immune cells to enter lymphatic vessels. A thin, discontinuous basement membrane surrounds these structures [20,21].

Collecting lymphatic vessel endothelial cells differ from their primary counterparts. They are arranged more tightly and adhere more strongly to each other through zipper-like junctions [22]. This structure prevents leakage of lymph fluid during transport. Collecting lymphatic vessels also contain multiple valves that prevent backflow [23]. Both button-like and zipper-like junctions share similar constituent proteins [18]. During embryonic development, the button junctions of primary lymphatic vessels are converted from zipper junctions. Under disease conditions or specific experimental manipulations (genetic or pharmacological), button-like junctions can transform into zipper-like junctions [22].

With the advent of scRNA-seq technology, researchers have captured expression profiles from thousands of individual LECs and constructed high-resolution molecular maps of human lymphatic vessels. These maps reveal substantial heterogeneity in both anatomical structure and functional specialization [13,24] (**Figure 1a**). Lymph nodes serve as central hubs for immune responses, and the heterogeneity of LECs within them is particularly complex [25].

Takeda et al. performed scRNA-seq on four axillary lymph nodes and two head-and-neck lymph nodes to construct a human lymph node atlas [26]. They identified six LEC subpopulations within lymph nodes (**Table 1**): subcapsular sinus ceiling lymphatic endothelial cells (cLECs, LEC I), subcapsular sinus floor lymphatic endothelial cells

(fLECs, LEC II), medullary capsule-lining lymphatic endothelial cells (cLEC1, LEC III), Capillary lymphatic endothelial cells (LEC IV), valve lymphatic endothelial cells (valve LECs, LEC V), and Medullary sinus (MS) and cortical sinus (CS) lymphatic endothelial cells (MS/CS LECs, LEC VI) [26].

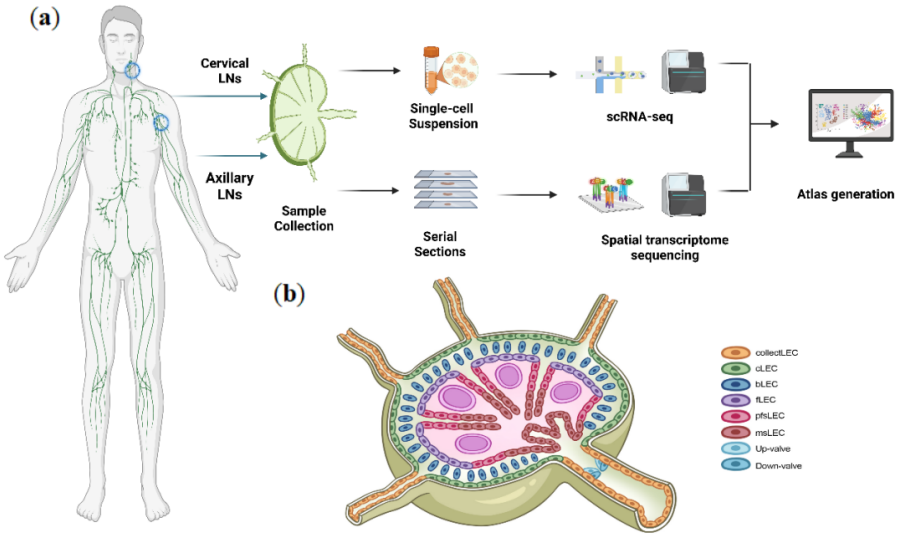


**Figure 1.** Single-cell and spatial transcriptomics reveal heterogeneity of lymphatic endothelial cells within lymph nodes. (**a**) Advances in scRNA-seq and ST have revolutionized our understanding of the heterogeneity within LECs. By performing scRNA-seq and ST profiling of lymph nodes from specific anatomical sites, such as the cervical and axillary regions, researchers have successfully mapped a high-resolution atlas of the lymphatic endothelium. (**b**) Integrating the single-cell landscapes described by Abe et al. and Takeda et al., human lymph node LECs can be broadly categorized into eight major subtypes.

**Table 1.** Molecular features of different human lymph node endothelial cell.

| Abe et al. [13] Classification (8 Subsets) | Takeda et al. [26] Classification (6 Subsets) | Human Markers | Anatomical Location and Function |
|---|---|---|---|
| cLECs | LEC I (cLECs) | ACKR4, EDN1, FABP4 | Location: Ceiling of the subcapsular sinus (SCS), directly adjacent to the LN capsule [23,27].<br>Function: Establishes chemokine gradients, serves as a physical barrier, and scavenges lipoproteins [28–30]. |
| bLECs | Likely included in original LEC I | PAI1 (SERPINE1), ANKRD1 | Location: Covers collagen trabeculae traversing the sinus; physically connects the outermost SCS ceiling to the cortex-adjacent floor [13].<br>Function: Provides structural support and tension, aids in drainage, and participates in tumor microenvironment remodeling [13]. |
| fLECs | LEC II (fLECs) | CXCL1, CXCL2, CXCL3, CXCL5, VCAM1 | Location: Floor of the subcapsular sinus [12].<br>Function: Facilitates immune cell drainage, antigen transport, and sorting; maintains the macrophage barrier and contributes to immunomodulation and tolerance [12,31,32]. |
| pfsLECs | LEC VI (MS/CS LECs) | MARCO, LYVE1, FABP5 | Location: Cortical region, specifically surrounding B cell follicles [13].<br>Function: Acts as a rapid exit route for lymphocytes; involved in antigen clearance/presentation and regulation of B cell responses [33]. |
| collectLECs | LEC III (cLEC1) | MFAP4, NTS, FOXC2, GJA1/GJA4 | Location: Walls of collecting lymphatic vessels external to or connecting to the LN [27,34].<br>Function: Responsible for active lymph transport, preventing backflow, and maintaining tight junction barriers [22,35]. |
| msLECs | LEC IV (capillary LECs) | PTX3, CCL21, PDPN, LYVE1, STAB2, ITGA2B | Location: Sinuses of the deep medulla and paracortical regions [36].<br>Function: Facilitates lymphocyte egress, lymphangiogenesis, and innate immune regulation; supports plasma cell survival [24,30,37]. |
| Up-valve LECS | LEC V | GJA4, CLDN11, GATA2 | Location: Within collecting vessels, upstream of valve leaflet attachment points [38].<br>Function: Senses and responds to mechanical signals; guides valve morphogenesis, maintains barrier integrity, and regulates lymphatic pumping [39–41]. |

**Table 1.** *Cont.*

| Abe et al. [13] Classification (8 Subsets) | Takeda et al. [26] Classification (6 Subsets) | Human Markers | Anatomical Location and Function |
|---|---|---|---|
| Down-valve LECs | LEC V | ANGPT2, GJA1, SCG3 | Location: Posterior surface of valve leaflets and the endothelium immediately downstream [38].<br>Function: Adapts to disturbed flow microenvironments; involved in immune cell recruitment/regulation and sensing pressure gradients [29,42]. |

Abe et al. subsequently performed scRNA-seq on nine tumor-free human lymph nodes and identified eight subpopulations [13] (**Figure 1b**). The classification and annotation largely matched Takeda's work, but with some important additions.

Abe's team discovered a new subpopulation located in the perifollicular sinus—perifollicular sinus lymphatic endothelial cells (pfsLECs) [13]. They also identified bridge lymphatic endothelial cells (bLECs) and confirmed the presence of PAI1+ bLECs through immunofluorescence [13]. These cells anatomically connect different parts of the subcapsular sinus.

Additionally, Takeda et al. had classified LEC IV as capillary lymphatic endothelial cells [26]. However, Abe et al. used PTX3 staining to confirm that these cells actually reside in the medullary region of lymph nodes, namely as medullary sinus LECs (msLECs) [13]. Takeda et al. had identified LEC III as medullary capsule-lining lymphatic endothelial cells [26], but Abe et al. found through MFAP4 staining that these cells mainly exist in the afferent and efferent collecting lymphatic vessels connected to lymph nodes. They reclassified them as collecting vessel LECs (collectLECs) [13]. Abe et al. further divided valve lymphatic endothelial cells into Up-valve LECs and Down-valve LECs. Up-valve LECs highly express GJA4, while Down-valve LECs highly express ANGPT2 and GJA1 [13].

## 3. Tumor Microenvironment Drives LEC Remodeling

LECs in the tumor microenvironment actively reshape local immune and metastatic niches [7]. scRNA-seq and ST technologies provide evidence at both single-cell and spatial resolution for dissecting LEC subpopulation heterogeneity, spatial niches, and intercellular interactions in tumors [43]. Below we discuss how LECs undergo functional remodeling across different cancer types.

### 3.1. Breast Cancer

Compared with normal human lymph nodes, scRNA-seq analysis of metastatic lymph nodes (MLNs) from human breast cancer revealed a new and significantly enriched LEC subpopulation marked by CD200+HEY1+ [44]. At the same time, fLECs and msLECs were markedly reduced [44]. Monocle trajectory analysis suggested that both CD200+HEY1+ LECs and fLECs originate from CCL2+ cLECs, but the tumor metastatic environment preferentially drives differentiation toward CD200+HEY1+ LECs rather than fLECs [44]. This indicates that tumor metastasis can push LECs toward an immunosuppressive phenotype.

ST further confirmed that CD200+HEY1+ LECs show a capillary-like distribution and wrap around tumor cells, while immunoregulatory subpopulations such as TNFRSF9+PD-L1+ fLECs are spatially depleted [44]. These observations suggest that tumors actively remodel the normal spatial architecture of LECs. CD200 is a known immune checkpoint molecule [45]. In vitro co-culture experiments confirmed that CD200 expressed by LECs significantly suppresses T cell activation. This subpopulation may induce local immune tolerance in metastatic lymph nodes, helping cancer cells evade immune attack [46]. These cells also highly express MGP, and follow-up experiments showed that high MGP expression in LECs promotes adhesion between LECs and cancer cells [44]. Thus, CD200+HEY1+ LECs may directly promote the retention and migration of cancer cells by the MGP molecule.

### 3.2. Melanoma

Changes in the composition of tumour-draining lymph node LECs have also been found in both human and mouse melanoma [32,47]. In human metastatic lymph nodes, the Notch ligand DLL4 on the surface of LECs can activate the Notch3 receptor of melanoma cells and thus induce WNT5B expression [47]. WNT5B then acts in a paracrine manner on LECs to promote their sprouting and reduce the barrier function. Together, these findings

constitute a feedback loop for Notch/DLL4/WNT5B signalling [47].

Transcriptomic remodelling of the fLEC subpopulation has also been found in mouse melanoma-draining lymph nodes [32]. Several genes were significantly overexpressed, such as Pdpn, CD200, BST2, Tenascin [32]. Notably, in Pdpn conditional knockout mice for LECs, the number of macrophages in the medullary sinus of lymph nodes was also reduced. Therefore, fLECs probably regulate macrophage adhesion and positioning by Pdpn expression and suppressing T cell immunity [32]. Additionally, CD200 is known as an immune-inhibitory molecule [48], and LECs have been reported to suppress T cell responses via PD-L1 in lymph nodes [49]. Together, these findings suggest that fLECs shape the local immune microenvironment in tumor-draining lymph nodes by modulating both macrophage localization and immune checkpoint pathways.

### 3.3. Head and Neck Squamous Cell Carcinoma

Some studies have identified ligand-receptor interactions between SPP1-positive tumour-associated macrophages (TAMs) and LECs via the SPP1-ITG alpha9 beta1 and FN1-ITG alpha2 beta1 signaling axes [50,51]. Multiplex immunohistochemistry (mIHC) further showed that in metastatic lymph nodes, SPP1+ TAMs preferentially located within 20 μm of LECs, and their density decreased with an increasing distance from LECs [50]. Analysis of the TCGA cohort also shows that a high score for the SPP1+TAM gene signature is associated with a poor prognosis and an increased risk of lymph node metastasis [50,52]. Together, the above studies show that LEC-TAM interaction creates a spatial niche that helps promote the spread of lymph node metastasis in head and neck squamous cell carcinoma.

### 3.4. Bladder Cancer

scRNA-seq analysis of bladder cancer identified ESM1 as specifically enriched in LECs [53,54]. High ESM1 expression correlates significantly and negatively with tumor microenvironment immune scores and stromal scores [53]. Mechanistic studies confirmed that ESM1 activates the SPP1 signaling pathway to suppress T cell receptor signaling and Fc gamma R-mediated immune pathways [54]. This reduces infiltration of CD4+ T cells, CD8+ T cells, macrophages, and dendritic cells, thereby mediating immune evasion in bladder cancer [53,55].

### 3.5. Kaposi Sarcoma

Two scRNA-seq and ST studies of Kaposi sarcoma (KS) found that the proportion of LECs in KS lesions can increase dramatically from 0.7% in normal skin to 33.3% in nodular lesions [56]. Moreover, 77.7% of Kaposi's Sarcoma-associated Herpesvirus (KSHV) positive cells are LECs, and CD34+ precursor-like LECs were identified as the main target cells of this virus [56].

KSHV+ LECs no longer maintain a pure lymphatic endothelial phenotype. Instead, they simultaneously express vascular endothelial markers, fibroblast markers, and macrophage markers, demonstrating significant transcriptional reprogramming and cell identity remodeling [56]. ST showed that STC1 and VEGFR3 are significantly upregulated in KSHV-infected LECs, and KSHV gene expression shows strong positive correlation with LEC markers [57]. In vitro, knockdown of VEGFR3 in primary LECs reduces STC1 expression [57]. Spatially, STC1-high regions show negative correlation with macrophage markers, suggesting that KSHV may suppress macrophage chemotaxis through the VEGFR3/STC1 pathway, thereby mediating immune evasion [57].

In summary, LECs in the tumor microenvironment are far more than passive lymphatic drainage conduits. They are remodeled into highly heterogeneous, spatially polarized "multifunctional stromal hubs" with active immune regulatory capabilities [32]. Cross-cancer studies show that LECs undergo profound transcriptional reprogramming and phenotypic remodeling under tumor assault [58].

On one hand, by upregulating immune checkpoint or immunosuppressive molecules such as CD200, PD-L1, ESM1, and STC1, LECs directly suppress T cell activation and weaken macrophage chemotaxis, constructing a local immune tolerance barrier [45,53]. On the other hand, through ligand-receptor axes such as CCL21-CCR7, Notch/DLL4/WNT5B, and SPP1-integrin, LECs actively guide tumor cell chemotactic migration, adhesion, and retention, while compromising endothelial barriers, thereby systematically promoting lymphatic metastasis [47,53,59].

Furthermore, scRNA-seq and ST have confirmed that this functional remodeling does not occur uniformly. It displays significant subpopulation specificity and spatial niche dependence. From the CD200+HEY1+ capillary-like LECs wrapping cancer cells in breast cancer, to the SPP1+ TAM microenvironment forming gradient distributions around LYVE1+ LECs in head and neck squamous cell carcinoma, to the precursor-like LECs acquiring mixed vascu-

lar endothelial, fibroblast, and macrophage phenotypes driven by KSHV in KS—all these examples suggest that tumors "educate" LEC subpopulations to spatially reconstruct the immune-metastasis interaction interface [44,50,57]. Therefore, targeting LEC heterogeneity and their bidirectional cell-cell interaction networks may overcome the limitations of current therapies that target only tumor cells or immune cells, offering novel stromal-targeted strategies for intervening in tumor lymphatic metastasis and reversing immunosuppressive microenvironments.

## 4. Chronic Inflammation Drives LEC Remodeling

Similar to the tumor microenvironment, chronic inflammatory microenvironments can also drive LEC transcriptional reprogramming and phenotypic remodeling [30,31,60]. LECs transform from passive conduit structures into active "multifunctional hubs" that participate in immune regulation, inflammation amplification, and tissue remodeling [61–63].

### 4.1. Inflammatory Lymph Nodes

In a mouse imiquimod (IMQ)-induced skin inflammation model, researchers performed scRNA-seq on LECs from inflammatory lymph nodes and identified three subpopulations: floor-lining LECs (fLECs), ceiling LECs (cLECs), and medullary sinus LECs (medullary LECs) [64]. Among them, fLECs had the most significant changes in transcriptomes in inflammatory lymph nodes, with 161 differentially expressed genes. CD200 and Anxa2 were significantly elevated in fLECs and thus likely involved in the suppression of immune responses [64].

By ligand-receptor interaction prediction, scholars have also identified a large number of cell-to-cell communication networks among LECs and different immune cells [24,30]. Notably, this study shared approximately 50% of differentially expressed genes with the oxazolone inflammation model, suggesting that different inflammatory stimuli can induce partially conserved transcriptional responses in LECs [64]. These findings demonstrate that in mouse chronic inflammatory lymph nodes, LECs can actively shape the local immune microenvironment by upregulating immunoregulatory molecules.

### 4.2. HBV-Related Acute-on-Chronic Liver Failure (ACLF)

Using scRNA-seq, researchers identified an LEC subpopulation (LEC2) characterized by apoptosis and dysfunction in the livers of ACLF patients [65]. This subpopulation is virtually absent in healthy controls and in patients with cirrhosis alone [65]. LEC2 highly expresses apoptosis-related genes, while expression of the lymphatic vessel core transcription factor PROX1 decreases [65]. Ligand-receptor analysis showed that infiltrating monocytes/macrophages can act on LEC2 through the SPP1-ITGB1 pathway [65].

Follow-up immunohistochemistry confirmed that intrahepatic lymphatic vessel density is significantly reduced in ACLF patients compared with cirrhosis patients [62,65]. Macrophages massively accumulate in areas with reduced lymphatic vessels in ACLF livers, consistent with the single-cell data [66]. This inflammation-macrophage-lymphatic vessel damage positive feedback loop suggests that protecting intrahepatic lymphatic vessels and promoting their repair may serve as a complementary therapeutic approach to reduce mortality in ACLF.

### 4.3. Neuroinflammation

In a mouse experimental autoimmune encephalomyelitis (EAE) model, cribriform plate lymphatic endothelial cells (cpLECs) also undergo functional remodeling [67]. Clear transcriptomic differences exist between healthy and EAE-derived cpLECs [68]. These cells can be divided into three subpopulations: quiescent healthy cells (cluster 1), proliferating transitional cells (cluster 3), and inflammation-activated cells (cluster 2) [67].

Pseudotime trajectory analysis showed that cpLECs undergo a sequential transition from quiescence to proliferation to inflammatory activation during disease progression [67]. They upregulate chemokines, IFN-gamma response genes, antigen presentation-related genes, and the immunoregulatory molecule PD-L1 in sequence [67]. Spatial validation further confirmed that in the neuroinflammatory microenvironment, cpLECs not only present autoantigens to activate T cells through MHC II, but also regulate T cell fate through IFN-gamma-dependent PD-L1 upregulation [69]. Together these mechanisms form a local regulatory system that balances immune activation and tolerance [67].

In the cribriform plate region, loss of E-Cadherin creates gaps in the arachnoid structure, allowing cpLECs to

directly contact cerebrospinal fluid without crossing an intact barrier [67,70]. Further studies showed that cpLECs can drain cerebrospinal fluid through AQP-1 water channels on their surface [67,71]. In summary, cpLECs acquire triple functions during neuroinflammation: lymphatic drainage, antigen presentation, and PD-L1-dependent immune regulation [67]. The cribriform plate serves as a unique neuroimmune interface that elevates the meningeal lymphatic system from passive drainage to active immune regulation, providing new therapeutic targets for multiple sclerosis and related diseases [72].

### 4.4. Chronic Obstructive Pulmonary Disease (COPD)

A study combining human COPD patients and a cigarette smoke-exposed COPD mouse model showed that pulmonary LECs play dual pro-inflammatory and pro-thrombotic roles in COPD [63]. In vitro experiments showed that cigarette smoke extract (CSE) reduces fibrinolytic activity in LECs while increasing expression of plasminogen activator inhibitor-1 (PAI-1) [63,73]. Thrombin stimulation induces LECs to upregulate adhesion molecules such as VCAM-1 and ICAM-1 while reducing expression of tissue factor pathway inhibitor (TFPI) [63,74]. RNA-seq analysis showed significant upregulation of pro-inflammatory pathways in LECs in both acute and chronic cigarette smoke exposure models [73].

In human COPD patients, LECs similarly show reduced TFPI and elevated VCAM-1 phenotypes [63]. These findings indicate that in the chronic inflammatory environment of COPD, pulmonary LECs acquire a dual pro-inflammatory and pro-thrombotic phenotype [75]. LECs may participate in COPD pathogenesis by promoting vascular inflammation and thrombosis [75].

This shift from passive to active, from structural support to functional participation, not only reshapes our understanding of lymphatic vessel biology but also opens new dimensions for clinical intervention. Protecting and repairing damaged LECs, targeting their immunoregulatory phenotypes, and blocking positive feedback loops of inflammation-lymphatic vessel damage may become important strategies for breaking through treatment bottlenecks in chronic inflammatory diseases.

## 5. Functional Plasticity of LECs in Cardiovascular Diseases

### 5.1. Myocardial Infarction

The combined application of scRNA-seq and ST has provided dual support at single-cell precision and spatial localization dimensions for understanding functional remodeling of cardiac LECs after acute myocardial infarction [76,77]. Studies show that LECs rapidly exit their quiescent state after acute myocardial infarction and activate lymphangiogenesis through the VEGFC/VEGFR3 signaling axis [77,78]. scRNA-seq data further reveal that repair-phase LECs display significant functional heterogeneity [76,77]. One subpopulation specifically acquires a TGF-beta1+/IL-10+ anti-inflammatory phenotype and actively promotes M2 macrophage polarization through paracrine signals [77]. This accelerates interstitial edema resolution and drainage of damage-associated molecular patterns, thereby limiting ventricular fibrosis expansion [76,77].

ST further indicates that early after myocardial infarction, LEC proliferative activity is significantly enhanced [76,78]. Newly formed lymphatic vessels create density gradients between the infarct core and border zone [76]. The distribution of these regions is closely associated with areas of reduced inflammation and new-forming blood vessels [78]; thus, it has been shown that the lymphatic system is also active in the immune microenvironment and tissue-repair process, rather than just serving as a conduit for returning fluid [77,78].

Single-cell atlas analysis has also resolved interaction networks between LECs and stromal and immune cells. VCAM1+ cardiac fibroblasts drive border zone lymphatic network reconstruction through paracrine VEGFC, while LYVE-1+ resident macrophages support LEC sprouting through IL-1 beta and other factors [77]. However, this remodeling is highly heterogeneous [76]. Some POSTN+podoplanin+ LEC subpopulations can promote fibrosis through TGF-beta signaling, while CD36+ LEC subpopulations dominate lymphangiogenesis through VEGFR3 phosphorylation [77]. This suggests that the functional balance between repair and fibrosis in LECs depends on the local microenvironment [77]. In summary, single-cell and spatial omics-driven research has not only redefined the immune regulation and tissue repair functions of LECs after myocardial infarction, but also provides a theoretical basis for developing precise lymphatic-targeted intervention strategies targeting specific LEC functional states [77].

### 5.2. Hypertension

In single-cell atlases of the aorta and mesenteric arteries from spontaneously hypertensive rats (SHR), researchers identified an endothelial cell subpopulation (EC sub-cluster 4) expressing classical lymphatic markers Lyve1 and Pdpn [79]. The subgroup has enriched pathways for lymphatic vessel development and shows that local LECs are present in the arterial wall [79]. Sox18 is a master transcription factor for lymphatic vessel development; therefore, it has been significantly upregulated in the hypertensive subpopulation, and thus may be directly activated to drive remodeling of the arterial wall by blood pressure [79].

LEC function is also altered in the kidney and gonads of a salt-sensitive hypertension (SSHTN) model [80]. LEC number and transcriptional diversity are both higher in hypertensive samples [81]. A total of 597 differentially expressed genes were found to be significantly enriched in inflammation-related pathways [81]. Prox1, Pdpn, Vegfr3 and Ccl21 (lymphatic vessel-specific chemokine) are all expressed at higher levels in hypertensive LECs; therefore, a more favourable microenvironment for immune cell infiltration has been formed [80].

Further studies have indicated that after reducing blood pressure with the antihypertensive drug hydralazine, the density of renal lymphatic vessels in male SSHTN mice decreased compared to the untreated group, but it was still significantly higher than that in normal controls [80]. Lymphatic vessel density in the testes did not fall after lowering blood pressure; rather, the expression of Vegf-c increased [80]. Therefore, hypertension and high-salt load may affect organs differently in their changes to LECs, and some such effects may be independent of blood pressure [80].

At the level of cell-cell interaction networks, LECs in SHR arteries interact with multiple ligands and receptors on smooth muscle cells, mesenchymal stem cells and immune cells [79]. For example, in the presence of hypertension, ICAM-1 expressed by LECs shows an enhanced binding affinity for integrins and IL-2 receptors on dendritic cells, T cells, and other immune cells [79]. Pulmonary LECs in a pulmonary arterial hypertension (PAH) mouse model are also activated at the level of antigen processing and presentation, and it has been proposed that LECs may actively participate in inflammation in chronic vascular diseases [82].

### 5.3. Atherosclerosis (*AS*)

scRNA-seq analysis of atherosclerosis shows that LECs are bimodal in response: an initial expansion of proliferation is followed by a substantial decrease in later stages due to the exhaustion of proliferative factors [83]. The two types of functional sub-populations for LECs are the immune-chemotactic LEC-1 and the lipid-processing LEC-2. Both have different transcription profiles [83,84].

Functionally, early LECs activate T cells via the IL-7/IL-7R axis and reduce Galectin-9-mediated immune tolerance [83,85]. The capacity of these lipids for clearance has also been reduced, and key genes such as Ldlr and Abca1 are downregulated [84]. Early proliferation of LECs is driven by the RAS pathway inside the cell, and does not require the conventional VEGF-C/D signal [83]. Trajectory analysis also found pathological transdifferentiation of fibroblasts into LECs in early atherosclerosis, but these newly formed LECs are functionally immature [83]. Advanced human coronary artery tissue has only about 0.2% LECs, and these are numerically scarce as well as functionally compromised [83]. In short, atherosclerosis modifies the immune function of lymphatic endothelial cells (LECs), inhibits lipid transport, and induces abnormal differentiation [86,87]; thus, the lymphatic system fails to reduce inflammation or remove lipids effectively [88,89]. Future therapies should target LEC functional restoration rather than simply promoting lymphangiogenesis.

## 6. Obesity Drives LEC Remodeling

LECs in adipose tissue undergo significant transcriptomic reprogramming and functional remodeling under obesity conditions [90]. At the single-cell level, LECs maintain expression of classical markers such as PROX1 and LYVE1, but their functional genes show depot-specific changes [91]. For example, LECs in mouse inguinal white adipose tissue specifically highly express neurotensin (NTS), which inhibits brown adipose thermogenesis through the NTSR2-ERK axis [91,92]. Obesity-associated weakening of sympathetic nervous regulation exacerbates this inhibitory effect [93]. In addition, VEGF-C/D-driven lymphangiogenesis can improve glucose and lipid metabolism in specific adipose tissues, but excessive activation causes systemic lymphatic vessel lesions [94]. This suggests that LEC-mediated lymphatic vessel remodeling exhibits strict dose-dependent effects [95].

Obesity can also induce LEC transformation toward pro-inflammatory and pro-fibrotic phenotypes [96]. Single-nucleus RNA sequencing shows that epididymal white adipose tissue exhibits more dramatic endothelial cell remodeling compared with subcutaneous fat [97]. LECs can acquire mesenchymal characteristics such as PDGFRA and ZEB2 through endothelial-mesenchymal transition and secrete extracellular matrix components to participate in tissue fibrosis [97].

Spatial transcriptomics and ligand-receptor analysis further confirm that spatial interaction networks between LECs and immune cells and adipocytes are reconstructed in the obese microenvironment [96]. Pro-inflammatory signals and metabolic regulatory signals interact and reinforce each other, jointly driving adipose tissue transformation from energy storage to chronic inflammatory microenvironment [96]. This demonstrates that LECs can actively sense metabolic microenvironment changes and regulate thermogenesis and immune-inflammatory responses through specific signaling networks [98]. Moreover, LEC remodeling shows some heterogeneity across different adipose tissues, providing a novel cellular biological perspective for explaining the heterogeneity of obesity-related metabolic complications, and laying an important theoretical foundation for developing precise metabolic interventions targeting LECs [99].

## 7. Conclusion

Powered by scRNA-seq and ST, LECs are undergoing a conceptual transformation from passive conduits to active participants in tissue microenvironment regulation. We now understand that LECs not only perform the structural function of tissue fluid drainage but also possess high heterogeneity and significant functional plasticity. In tumors, they reconstruct the immune-metastasis interface. In chronic inflammation, they balance immune activation and tolerance. In cardiovascular disease, they coordinate repair and fibrosis. Obese individuals sense changes in their metabolism and adjust thermogenesis and inflammation accordingly.

Therefore, based on the above results, the lymphatic system has been widely employed to study disease microenvironments, and LECs are likely to be key regulatory nodes in structural support, immune regulation, and tissue repair. In the future, addressing the heterogeneity of LECs and their bidirectional cell-cell interaction networks may be used to overcome the limitations of single-target therapies for some diseases, opening up new stromal-targeted strategies for tumor metastasis inhibition, chronic inflammation reversal, and precision treatment of metabolic diseases.

## Author Contributions

Conceptualization, Q.C., C.W. and M.L.; methodology, Q.C. and C.W.; software, S.Z.; validation, Q.C., C.W. and K.Z.; formal analysis, Q.C.; investigation, C.W.; resources, M.L.; data curation, S.Z.; writing—original draft preparation, Q.C. and C.W.; writing—review and editing, M.L. and K.Z.; visualization, K.Z.; supervision, M.L.; project administration, M.L.; funding acquisition, M.L. Q.C. and C.W. are co-first authors. All authors have read and agreed to the published version of the manuscript.

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## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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## Conflicts of Interest

The authors declare no conflict of interest.

## AI Use Statement

During the preparation of this work, the authors used Gemini and Kimi for language polishing, grammar checking, and refinement of academic expression. The authors subsequently reviewed and edited the content as necessary and take full responsibility for the final content of the published article.

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