The Hepatic Lymphatic Vascular System: Structure, Function, Markers, and Lymphangiogenesis

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SUMMARY

Research on the lymphatic vascular system has advanced rapidly during the last decade, and lymphatic dysfunction is now implicated in the pathogenesis of multiple diseases. This review provides an overview of the lymphatic vascular system in the liver.

The lymphatic vascular system has been minimally explored in the liver despite its essential functions including maintenance of tissue fluid homeostasis. The discovery of specific markers for lymphatic endothelial cells has advanced the study of lymphatics by methods including imaging, cell isolation, and transgenic animal models and has resulted in rapid progress in lymphatic vascular research during the last decade. These studies have yielded concrete evidence that lymphatic vessel dysfunction plays an important role in the pathogenesis of many diseases. This article reviews the current knowledge of the structure, function, and markers of the hepatic lymphatic vascular system as well as factors associated with hepatic lymphangiogenesis and compares liver lymphatics with those in other tissues. (Cell Mol Gastroenterol Hepatol 2016;2:733–749; http://dx.doi.org/10.1016/j.jcmgh.2016.09.002)

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The lymphatic and blood vascular systems together constitute the circulatory system, and both have essential physiological activities. The lymphatic vascular system maintains tissue fluid homeostasis by collecting excess tissue fluid and returning it to the venous circulation. It also plays an essential role in the absorption and transport of dietary fat. Furthermore, lymphatics serve as the main conduits of antigens and antigen-presenting cells from the periphery to lymph nodes and are thus crucial for immune surveillance and acquired immunity.1–4

Lymphatic vascular research was impeded by a lack of knowledge about the markers and signaling pathways specific to the lymphatic vasculature. From 1995 to 1997, however, it was shown that vascular endothelial growth factor receptor (VEGFR)-3 is expressed in the lymphatic endothelium and that its ligand vascular endothelial growth factor (VEGF)-C promotes lymphangiogenesis.5,6 This finding identifying signaling pathways specific to the lymphatic vasculature and subsequent discoveries of other specific markers for lymphatic endothelial cells (LyECs), such as lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1),7 prospero homeobox protein 1 (Prox1),8 and podoplanin,9 significantly advanced lymphatic vascular research. As a consequence, it is now recognized that lymphatic vessel dysfunction plays an important role in the pathogenesis of various diseases.

However, in the liver, the lymphatic vascular system has been little explored. This review will provide an overview of the structure, function, and markers of the lymphatic vascular system as well as factors associated with lymphangiogenesis in the liver, highlighting both new findings and areas needing further study.

Structure of the Hepatic Lymphatic Vascular System

This section will address the structure of the lymphatic vascular system in general, followed by structural features specific to the liver. A detailed description of the anatomic structure of the lymphatic and hepatic lymphatic vascular systems is available in other review articles.3,10–12

Anatomy of the Lymphatic Vascular System

Lymphatic capillaries. Lymphatic fluid originates from plasma components leaked from blood capillaries into the interstitium and then enters lymphatic capillaries, which are blind-ended, thin-walled vessels consisting of a single layer of LyECs. Lymphatic capillaries are not covered by pericytes or smooth muscle cells and lack basement membranes.13,14 They are highly permeable, with discontinuous “button-like” junctions through which interstitial fluid, macromolecules, and immune cells can be transported.15 LyECs have anchoring filaments that are mainly composed of emilin-1 and fibrillin and bind LyECs to the surrounding extracellular matrix.14,16,17 These filaments keep lymphatic vessel...

Abbreviations used in this paper: CCl4, carbon tetrachloride; EHE, epithelioid hemangioendothelioma; HA, hyaluronan; HBx Ag, hepatitis B x antigen; HCC, hepatocellular carcinoma; IFN, interferon; IL, interleukin; LSEC, liver sinusoidal endothelial cell; LyEC, lymphatic endothelial cell; LYVE-1, lymphatic vessel endothelial hyaluronan receptor 1; mTOR, mammalian target of rapamycin; NO, nitric oxide; Prox1, prospero homeobox protein 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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lumens open, facilitating fluid intake in conditions of tissue swelling.

**Collecting vessels.** Lymphatic capillaries coalesce into collecting vessels, which are covered with smooth muscle cells and have basement membranes. Collecting vessels lack the discontinuous junctions typical of lymphatic capillaries and are thus much less permeable. Collecting vessels can be divided into smaller functional units called lymphangions that have unidirectional bicuspid valves at each end. The phasic contraction of smooth muscle cells covering lymphangions enables collecting vessels to act as pumps to drive lymphatic flow. Stimulation of smooth muscle cells causes depolarization of cell membrane and opens Ca\(^{2+}\)-influx and smooth muscle cell contraction. Smooth muscle cells also have stretch-activated Ca\(^{2+}\) channels that facilitate phasic contraction.

On the other hand, LyECs produce the vasodilator nitric oxide (NO) in response to shear stress from fluid flow, counteracting Ca\(^{2+}\)-dependent contraction. Spatiotemporal alterations of Ca\(^{2+}\) and NO levels are thereby believed to modulate the phasic contraction of lymphangions.

**Lymph nodes and lymph trunks.** Collecting vessels connect to 1 or more lymph nodes. Antigen-presenting cells including dendritic cells and macrophages in lymphatic fluid interact with lymphocytes in lymph nodes, facilitating adaptive immune responses. After reaching primary lymph nodes, lymphatic fluid flows to secondary central lymph nodes, tertiary central lymph nodes, and finally lymph trunks. Lymphatic fluid from the left side of the body, abdomen, and lower limb ultimately drains into the thoracic duct, the largest lymphatic vessel, which is connected to the left subclavian vein (Figure 1), whereas lymphatic fluid from other

![Figure 1](image_url). Schematic diagram of macro-anatomy of hepatic lymphatic vascular system. (1) Lymphatic capillaries in the portal tract coalesce into collecting vessels, which drain to lymph nodes at the hepatic hilum and the lesser omentum. Efferent lymphatic vessels (LV) from these lymph nodes connect to celiac lymph nodes, which drain to the cisterna chyli, the enlarged origin of the thoracic duct. Lymphatic fluid through the thoracic duct drains to the left subclavian vein and returns to the systemic blood circulation. (2) Lymphatic vessels along the central vein (CV) converge into large lymphatic vessels along the hepatic vein (HV), which then traverse along the inferior vena cava (IVC) through the diaphragm toward mediastinal lymph nodes. (3) Lymphatic fluid running underneath the capsule of the convex surface of the liver (3i) drains to mediastinal lymph nodes through the coronary ligament, whereas that of the concave surface (3ii) drains to lymph nodes of the hepatic hilum and regional lymph nodes. BD, bile duct; HA, hepatic artery; LN, lymph node; PV, portal vein.
parts of the body drains into the right lymph trunk, which is connected to the right subclavian vein. Lymphatic fluid that enters the subclavian veins returns to the systemic blood circulation.

Anatomy of the Hepatic Lymphatic Vascular System

A schematic diagram of the hepatic lymphatic system is shown in Figures 1 and 2. Unlike other tissues, the liver has sinusoids instead of capillaries. Sinusoids, similar to lymphatic capillaries, are distinct from blood capillaries in that they consist of 1 layer of liver sinusoidal endothelial cells (LSECs) and lack basement membranes. Hepatic lymphatic fluid is thought to originate from plasma components filtered through the fenestrae of LSECs into the space of Disse, the interstitial space between LSECs and hepatocytes. Fluid in the space of Disse primarily flows through the space of Mall, a space between the stroma of the portal tract and the outermost hepatocytes, into the interstitium of the portal tract and then into lymphatic capillaries. Some portion of the fluid in the space of Disse flows into the interstitium around the central vein, which is located in the center of the liver acinus and connected to the hepatic vein, or underneath the hepatic capsule (Figure 2).

Lymphatic capillaries in the portal tract coalesce into collecting vessels and drain to lymph nodes at the hepatic hilum, whereas lymphatic vessels along the central vein converge into 5–6 large lymphatic vessels that traverse along the inferior vena cava through the diaphragm toward posterior mediastinal lymph nodes. Lymphatic fluid running underneath the capsule of the convex surface of the liver drains to mediastinal lymph nodes through the coronary ligament, whereas that fluid running along the concave surface drains to lymph nodes in the hepatic hilum and to regional lymph nodes (Figure 1). On the basis of their locations, lymphatic vessels along the portal tract and the central vein are called the deep lymphatic system, and those along the hepatic capsule are called the superficial lymphatic system.

Markers of Lymphatic Vessels

Lymphatic vessel markers generally refer to those specific to LyECs. The markers LYVE-1, podoplanin, Prox1, and VEGFR-3 are most commonly used for microscopic imaging of lymphatic vessels. Identification of more specific markers for the liver is needed because the most common LyEC markers, LYVE-1 and Prox1, are also expressed in LSECs and hepatocytes, respectively. Table 1 summarizes LyEC markers histologically examined in the liver.

**Figure 2.** Schematic diagram of the micro-anatomy of the hepatic lymphatic vascular system. Blood flow (red arrows) from the portal vein (PV) and hepatic artery (HA) enters the liver. Plasma components are filtered through LSECs into the space of Disse, the interstitial space between LSECs and hepatocytes, and are regarded as the source of lymphatic fluid. Lymphatic fluid in the space of Disse mostly flows through the space of Mall, the space between the stroma of the portal tract and the outermost hepatocytes, into the interstitium of the portal tract and then into lymphatic capillaries (1). Some portion of the lymphatic fluid in the space of Disse flows into the interstitium around the central vein (2) or underneath the hepatic capsule (3).
| Marker | Liver | Other organs/cells | Hepatic expression in pathologic conditions | Reference |
|--------|-------|--------------------|---------------------------------------------|-----------|
| LYVE-1 | Sinusoidal endothelial cells | A portion of macrophages, pulmonary capillaries, epididymal adipose tissue, mesentery, eye (cornea, sclera, choroid, iris, and retina), wounded skin, and malignant tumors (melanoma and insulinoma) | In chronic hepatitis and liver cirrhosis in humans, LYVE-1(+) lymphatic vessels increase, but LYVE-1(+) sinusoidal endothelial cells decrease. | 38, 40-43, 105, 159–164 |
| Prox1  | Hepatocytes | Adrenal medulla, megakaryocytes, and platelets | Intrahepatic CCC, ductular cells in cirrhotic livers, and HCC in humans. | 8, 52, 58, 59 |
| Podoplanin | Cholangiocytes | Inflammatory macrophages, mesothelial cells, cardiomyocytes, FRCs, follicular dendritic cells, TH17 cells, and osteoblasts | Podoplanin(+) lymphatic vessels increase in decompensated cirrhosis in humans. Podoplanin(+) FRCs increase in livers of primary biliary cirrhosis patients. EHE and angiomyolipoma in humans. | 32, 72, 75–79, 165, 166 |
| VEGFR-3 | Cholangiocytes | A portion of macrophages, proliferating blood vessels, and fenestrated capillaries in endocrine glands, choroid plexus, kidney, and small intestine | HBx Ag–positive HCC and hepatic progenitor cells in primary biliary cirrhosis in humans. | 80, 83–85, 102, 167, 168 |
| CCL21  | Sinusoidal endothelial cells | A portion of dendritic cells, HEVs of lymph nodes and Peyer’s patches, T-cell areas of spleen, lymph nodes, and Peyer’s patches | Lymphoid tissue in primary biliary cirrhosis and primary sclerosing cholangitis in humans. | 169–171 |
| MMR1   | Sinusoidal endothelial cells and Kupffer cells | A portion of macrophages, sinusoidal endothelial cells in bone marrow and spleen, perivascular microglia, and glomerular mesangial cells | Unknown | 172–175 |
| Desmoplakin | Basolateral plasma membrane of hepatocytes and cholangiocytes | Esophagus, intestine, colon, salivary gland, mammary gland, sweat gland, thymus, and endocervix | Entire plasma membrane of HCC cells | 174, 176–179 |
| Integrin α9 | Hepatocytes | Airway epithelial cells, keratinocytes, muscle cells (smooth/skeletal/cardiac), neutrophils, osteoclasts, and oocytes | Unknown | 174, 180 |

CCC, cholangiocellular carcinoma; CCL21, C-C motif chemokine ligand 21; FRC, fibroblastic reticular cell; HEV, high endothelial venules; MMR, macrophage mannose receptor 1.
Its structural features suggest that LYVE-1 may be involved in the transport of HA across the entire luminal and abluminal surfaces of LyECs, even on the fine filopodia of growing vessels during lymphangiogenesis.

No definite alterations in lymphatic vessel structure and function were reported for LYVE-1<sup>−/−</sup> mice. However, diphtheria toxin-induced LYVE-1 depletion in mice caused acute loss of lymphatic lacteals in intestinal villi and lymphatic vessels in systemic lymph nodes. These changes resulted in the structural distortion of blood capillaries and villi, leading to death due to sepsis within 60 hours after LYVE-1 depletion. These observations indicate that LYVE-1 plays an important role in the maintenance of the lymphatic vascular system, especially lacteals in intestinal villi and lymph nodes. Compensatory mechanisms in the setting of congenital loss of LYVE-1 may explain the relatively mild phenotype of these mice.

In the liver, LYVE-1 is expressed not only in LyECs but also in LSECs, as shown in mice and humans. However, LYVE-1 positivity in LSECs was reported to diminish in inflamed human livers such as those of chronic hepatitis and cirrhosis. Expression levels of LYVE-1 in human hepatic tumours, especially LSECs, as shown in mice and humans. Prox1 is expressed in the nucleus in contrast to other lymphatic markers that are expressed in the cytoplasm or on the plasma membrane.

Prox1 is essential for budding of lymphatic endothelial sacs; Prox1<sup>−/−</sup> mice lack a lymphatic vascular system and die at approximately E14.5. Prox1 heterozygote mice die a few days after their birth and demonstrate dysfunction of lymphatic vessels with chylous ascites. Several lines of Prox1 promoter-directed reporter mice have recently been established as research tools (GFP, mOrange, and Prox1 promoter-directed reporter mice). These mice present with leaky lymphatic vessels and transient inflammatory cells, in the lung, spleen, and lymph nodes. Its expression is regulated by Prox1. Podoplanin is also a ligand of C-type lectin receptor CLEC-2, which is highly expressed in platelets and immune cells and promotes platelet aggregation and activation.

Podoplanin<sup>−/−</sup> mice die at birth as a result of respiratory failure. These mice have congenital lymphedema caused by lymphatic vessel defects, although blood vessel formation is normal. Podoplanin heterozygote mice are healthy and fertile, with a partial incomplete lymphatic vessel network. Keratinocyte-specific podoplanin-deficient mice and a tamoxifen-inducible podoplanin depletion mouse model have recently been developed.

Histologic analysis of normal mouse livers showed expression of podoplanin in cholangiocytes in addition to LyECs. In humans, podoplanin-positive lymphatic vessels were increased in the livers of patients with compensated cirrhosis, and podoplanin-positive fibroblastic reticular cells were increased in livers of patients with primary biliary cirrhosis. Podoplanin has proven to be a useful histologic marker for diagnosing patients who have vascular tumors with lymphatic differentiation, such as epithelioid hemangioendotheliomas (EHEs) and angiomylipomas.

**Vascular endothelial growth factor receptor.** VEGFR-3 is a membrane-anchored tyrosine kinase and the receptor for VEGF-C and VEGF-D. It plays a crucial role in lymphangiogenesis. In early embryogenesis before LyEC differentiation, VEGFR-3 is expressed in most endothelial cells, but in the later stages of development, its expression becomes mostly restricted to the lymphatic endothelium.

VEGFR-3<sup>−/−</sup> mice have lymphatic vessel defects and die at approximately E10.5, whereas VEGFR-3 heterozygous mice present with leaky lymphatic vessels and transient chylous ascites. A mouse line (Vegfr3<sup>F<sub>GFP</sub>Luc</sup>) in which a dual reporter for fluorescence and luminescence is expressed under VEGF-3-promoter was established recently, enabling luminescence imaging of tumor-induced lymphangiogenesis.

VEGFR-3 is expressed by cholangiocytes in normal rat livers and is increased in cholestatic rat livers after bile duct ligation. Hepatic progenitor cells were also found to express VEGFR-3 in patients with primary biliary cirrhosis. Hepatitis B x antigen (HBx Ag), one of the antigens of hepatitis B virus, promotes hepatocarcinogenesis by upregulating expression of genes associated with proliferation of hepatocytes; upregulation of VEGFR-3 expression was observed in HBx Ag–positive human HCC, and the prognosis of patients with VEGFR-3–positive HCC was worse than for those with VEGFR-3–negative HCC.

**Lymphangiogenesis**

This section addresses the mechanism of lymphangiogenesis in the postnatal stage and factors that affect lymphangiogenesis, including inflammatory cells, in the lymphatic system in general and then summarizes the
implications of lymphangiogenesis in the pathophysiology of liver diseases.

**Factors Associated With Lymphangiogenesis**

In the postnatal stage, lymphatic vessels are mostly quiescent, and lymphangiogenesis generally occurs in pathologic conditions such as tissue repair, inflammation, and tumor-related conditions. Many cytokines and growth factors have been reported to promote lymphangiogenesis or inhibit lymphangiogenesis, as summarized in Table 2. The extent and duration of lymphangiogenesis are determined by the balance between pro- and anti-lymphangiogenic factors.

**Intracellular Signaling Pathways in Lymphangiogenesis**

Signaling pathways in lymphangiogenesis have largely been determined in studies of developmental lymphangiogenesis. Signaling via VEGF-C/D and VEGFR-3 is the most well-known pathway for lymphangiogenesis (Figure 3). VEGF-C or VEGF-D binding to VEGFR-3 results in autophosphorylation of multiple C-terminal tyrosine residues in VEGFR-3, which transduces signals through the Ras/Raf/MEK/ERK pathway. Signal transduction also occurs through the PI3K/Akt pathway, which causes phosphorylation of Akt, thereby activating mammalian target of rapamycin (mTOR) and Rac1. Activation of these signaling pathways facilitates LyEC proliferation and migration, ie, lymphangiogenesis. Chronic inflammation and malignant tumors in the liver induce several pro-lymphangiogenic growth factors including VEGF-C/D. However, a direct link between these increased pro-lymphangiogenic growth factors and lymphangiogenesis in these pathologic conditions remains to be demonstrated (Figure 3). Excellent review articles are available detailing signaling pathways in lymphangiogenesis.

**Role of Immune Cells**

Adaptive immune responses are initiated by the migration of immune cells to inflamed sites where they phagocytose pathogens and transmigrate through lymphatic vessels to lymph nodes to present antigens to T cells. However, immune cells not only migrate through lymphatic vessels but also interact with lymphatic vessels and promote lymphangiogenesis. An increase in lymphatic vessels helps infiltrating immune cells exit inflamed sites via lymphatic vessels and accelerates resolution of inflammation.

**Macrophages.** Among the various immune cells, macrophages interact most with lymphatic vessels. LyECs secrete chemotactic factors, such as C10, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1, to attract macrophages. Macrophages in turn secrete lymphangiogenic cytokines such as VEGF-C, VEGF-D, and VEGF-A, which promote tumor-associated lymphangiogenesis and inflammation-induced lymphangiogenesis, as shown in the cornea, skin, and tail. Macrophages were recently suggested to have the ability to transdifferentiate to LyECs. However, this is controversial and requires further investigation.

**Dendritic cells.** Upregulation of inflammatory cytokines such as tumor necrosis factor-α and interleukin (IL) 1β in inflamed tissues promotes expression of chemokines (eg, CCL21/CCL19 and CXCL12) and their receptors (eg, CCR7 and CXCR-4) in LyECs and dendritic cells, which enhances transmigration of dendritic cells through LyECs. Inflammatory cytokines also increase expression of adhesion molecules such as intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin in LyECs and promote dendritic cell transmigration to lymphatic vessels. Dendritic cells have also been reported to secrete VEGF-C and promote lymphangiogenesis.

**T cells.** In a mouse model of tail lymphedema, nude mice exhibited less edema than wild-type mice, concomitant with decreased lymphangiogenic cytokines and increased anti-lymphangiogenic cytokines. The balance between these cytokines was modulated by T-cell-mediated inflammation. T cells negatively regulated lymph node lymphangiogenesis by secreting interferon (IFN)-γ in mice.

**B cells.** B cells promote lymphangiogenesis in inflamed lymph nodes by secreting a robust amount of VEGF-A in mice given keyhole-limpet hemocyanin emulsion in complete Freund’s adjuvant (an experimental model of inflamed lymph nodes). Interestingly, VEGF-C was not detected in this study. Another study that used transgenic mice overexpressing VEGF-A specifically in B cells showed increased lymphangiogenesis as well as angiogenesis.

**Neutrophils.** Neutrophils are reported to contribute to lymphangiogenesis by modulating the bioavailability and bioactivity of VEGF-A and by secreting VEGF-D. The bioavailability of VEGF-A is increased by the secretion of matrix metalloproteinases 9 and heparanase. Depletion of neutrophils in mice resulted in skin inflammation in response to immunization or contact hypersensitization, and lymphangiogenesis was decreased in these mice with increased local inflammation, suggesting that neutrophils play a role in lymphangiogenesis and that lymphangiogenesis is helpful for reducing inflammation.

**Lymphangiogenesis in the Liver**

Because 25%–50% of lymph passing through the thoracic duct originates in the liver, the liver can be considered the most important organ for lymphatic fluid production. However, the lymphatic vascular system in the liver has been minimally explored. A small number of studies have reported on hepatic lymphangiogenesis in pathologic conditions such as chronic hepatitis, liver fibrosis/cirrhosis, portal hypertension, malignant tumors, and post-transplantation. This section summarizes these studies.

**Chronic hepatitis, liver fibrosis, and cirrhosis.** Resistance to sinusoidal blood flow increases in cirrhotic livers because of architectural deformations including around the portal and central veins. Consequently, sinusoidal hydrostatic pressure is elevated, and plasma components filtrated through sinusoids (which form
| Experimental model | Remarks | Reference |
|--------------------|---------|-----------|
| **Lymphangiogenic factors** | | |
| VEGF-A | Mouse corneal lymphangiogenesis VEGF-A recruits macrophages, which promote lymphangiogenesis by secreting VEGF-C/VEGF-D. | 103 |
| | Mouse subcutaneous immunization model VEGF-A expression is upregulated concomitantly with lymphangiogenesis in LNs of immunized mice. | 117 |
| | Oxazolone sensitized delayed-type hypersensitivity in mouse ear Systemic blockade of VEGF-A attenuates lymphangiogenesis in draining LNs. | 181 |
| | HSV-1 infection of cornea HSV-1 causes lymphangiogenesis by promoting infected cells to secrete VEGF-A. | 182 |
| VEGF-C, VEGF-D | VEGF-C transgenic mouse VEGF-C promotes LyEC proliferation and LV enlargement in the skin. | 6 |
| | Isolated LyEC VEGF-C stimulates survival, growth, and migration of LyEC. | 91 |
| | FGF-2-induced corneal lymphangiogenesis VEGFR-3 blockade cancels lymphangiogenesis. | 183 |
| | Chronic airway inflammation VEGFR-3 blockade cancels lymphangiogenesis. | 184 |
| | LPS-induced peritonitis VEGF-C and VEGF-D promote lymphangiogenesis in diaphragm. | 185 |
| Ang 2 | Mouse corneal lymphangiogenesis Ang 2 is upregulated in inflamed cornea, and Ang2 blockade inhibits inflammatory lymphangiogenesis. | 186 |
| | Mouse corneal lymphangiogenesis Ang 2 is expressed in lymphatic vessels and macrophages in inflamed cornea. Inflammatory lymphangiogenesis of cornea is suppressed in Ang2 knockout mice. Ang2 blockade inhibits LyEC proliferation and capillary tube formation. | 187 |
| HGF | Canine primary LyEC, rat tail lymphedema HGF promotes proliferation and migration of LyEC. Weekly HGF gene transfer improves lymphedema in vivo. | 188 |
| LT | CCL21 transgenic mouse, RAG knockout mouse defective in T and B cell LT overexpression by CCL21 transgene promotes lymphangiogenesis in thyroid. T-cell depletion cancels this phenomenon. | 189 |
| | LTα knockout mouse, LTα transgenic mouse LTα gene deletion decreases LV. Ectopic LTα expression causes lymphangiogenesis in tertiary lymphoid organs. | 190 |
| IL1β | Mouse corneal lymphangiogenesis IL1β promotes lymphangiogenesis by upregulating expression of VEGF-A, VEGF-C, and VEGF-D. | 191 |
| IL7 | Breast cancer cell lines, subcutaneous injection of Matrigel and/or IL7 and/or breast cancer cell lines IL7 promotes VEGF-D expression of cell lines in vitro and promotes lymphangiogenesis in vivo. | 192 |
| | HECV cell line (originated from human umbilical cord), subcutaneous injection of Matrigel and/or IL7 and/or HECV cell IL7 promotes expression of Prox1, LYVE-1, and podoplanin and proliferation, migration, and tubular formation of LyEC via upregulation of VEGF-D. | 193 |
| IL8 | Human primary LyEC, IL8 transgenic mouse and Prox1-GFP mouse IL8 promotes proliferation, migration, and tube formation of LyEC. IL8 overexpression promotes lymphangiogenesis in vivo. | 194 |
lymphatic fluid) increase. In cirrhotic patients, lymphatic fluid produced in the liver increases up to 30-fold, and liver surface lymphatic vessels dilate, as shown by peritoneoscopic observation.

Ascites formation in association with cirrhosis is one of the most recognized clinical manifestations of lymphatic vascular disorders. How ascites is formed still remains to be elucidated. Although several theories have been put forward, the most accepted currently is “the peripheral arterial vasodilation theory”, also known as “the forward theory”. According to this theory, splanchnic arterial vasodilation caused by portal hypertension results in underfilling of the splanchnic arterial circulation (hypovolemia). In moderate stages, the hypovolemia is compensated for by renal retention of sodium and water. However, in severe portal hypertension with splanchnic arterial vasodilation, sodium and water retention is persistent and leads to leakage of fluid into the peritoneal cavity. When its amount exceeds the absorption capacity of lymphatic vessels, ascites results.

Table 2. Continued

| Experimental model | Remarks | Reference |
|--------------------|---------|-----------|
| IL17 Cornea micro pocket assay, autoimmune ocular disease mouse | IL17 promotes proliferation of LyEC via upregulation of VEGF-D. Blockade of IL17 decreases corneal lymphangiogenesis. | 195 |
| IL20 Human telomerase-transfected dermal LyEC | IL20 promotes proliferation, migration, and tubular formation of LyEC via PI3K and mTOR pathways. | 196 |

Anti-lymphangiogenic factors

TGF-β

Human dermal lymphatic microvascular endothelial cells | TGF-β inhibits LyEC proliferation, cord formation, migration, expression of lymphatic markers (LYVE-1, Prox1), and lymphangiogenesis by VEGF-A/C via TGF-β type I receptor. | 197 |

Mouse tail skin excision and lymphatic vessel ligation | TGF-β1 inhibition promotes lymphatic vessel regeneration. TGF-β1 inhibits LyEC proliferation and fibrosis. | 198 |

Biopsy specimens from limbs of secondary lymphedema patients and mouse tail skin excision | TGF-β1 positive cells increase 3-fold in human lymphedema specimens. TGF-β1 inhibition decreases fibrosis, increases lymphangiogenesis and lymphatic function. | 199 |

BMP2 Zebrafish BMP2 transgenic model | BMP2 inhibits LyEC differentiation from cardinal veins via inhibition of Prox1 expression. | 200 |

IFN-α, IFN-γ LyEC isolated from pig thoracic duct | IFN-α or IFN-γ decreases LyEC proliferation and migration. Treatment with both IFN-α and IFN-γ promotes LyEC apoptosis. | 201 |

Cervical LNs of T-cell-deprived mouse | T cells inhibit lymphangiogenesis in LNs by secreting IFN-γ. | 116 |

IL4, IL13 Mouse LyEC isolated from LNs, human dermal LyEC, mouse asthma model | IL4 and IL13 inhibit expression of Prox1 and LYVE-1 and tube formation of LyEC. Blockade of IL4 and/or IL13 increases the density and function of lung LVs in asthma model. | 202 |

IL27 Human dermal lymphatic microvascular endothelial cells | IL27 inhibits LyEC proliferation and migration via STAT1/CXCL10, CXCL-11 axis. | 203 |

Activin A Subcutaneous injection of melanoma cell line to mouse | Activin A reduces lymphangiogenesis in melanoma model and inhibits sprouting of LyEC via phosphorylation of SMAD2. | 204 |

FGF-2, fibroblast growth factors-2; HGF, hepatocyte growth factor; HSV-1, herpes simplex virus 1; LN, lymph node; LPS, lipopolysaccharide; LT, lymphotoxin; LV, lymphatic vessel; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; STAT, signal transducer and activator of transcription; TGF, tumor growth factor.
On a related note, impaired lymphatic drainage in the splanchnic and peripheral regions was reported in cirrhotic rats with ascites. This was accompanied by increased activity of endothelial NO synthase and production of NO by LyECs in these regions. In addition, smooth muscle cell coverage of lymphatic vessels in these regions was significantly decreased. Treatment of these cirrhotic rats with an NO synthase inhibitor improved lymphatic drainage, decreased ascites volume, and increased smooth muscle cell coverage. This study thus demonstrates a role for NO in the impairment of lymphatic vessels in splanchnic and peripheral regions and in the development of ascites. It is not known whether lymphatic vessels in human cirrhotic livers show similar pathologic features.

The occurrence of hepatic lymphangiogenesis was reported for the first time in liver fibrosis and cirrhosis by Vollmar et al. in 1997. They found lymphatic vessels to be increased and enlarged in rat liver cirrhosis induced by carbon tetrachloride (CCL4). These observations were confirmed the following year in patients with chronic viral hepatitis/cirrhosis.

Microarray analysis demonstrated a 4-fold increase in VEGF-D expression in endothelial cells from CCL4-induced cirrhotic rat livers as compared with control rat livers. Because VEGF-D is a well-known lymphangiogenic factor that binds to VEGFR-3, which is also highly expressed in the LyECs of these cirrhotic rats, increased VEGF-D could be associated with the lymphangiogenesis observed in liver cirrhosis (Figure 3).

Lymphangiogenesis also occurs in idiopathic portal hypertension in human patients. It was presumed that increased lymph production that was due to increased
Portal pressure caused lymphangiogenesis. In 2 rat models of portal hypertension (portacaval shunt and portal vein ligation), upregulation of Vegfr-3 expression was observed, leading us to speculate the occurrence of lymphangiogenesis. However, the significance and mechanism of hepatic lymphangiogenesis, including in chronic hepatitis and liver fibrosis and cirrhosis, remain unknown.

**Malignant tumors.** Lymphatic vessels play a pivotal role in the pathogenesis of malignant tumors by serving as a pathway through which tumor cells metastasize. The incidence of lymph node metastasis differs among tumors. For example, it is 5.1% in HCC and 45.1% in intrahepatic cholangiocarcinoma. The prognosis of tumor-bearing patients with lymph node metastasis is worse than in cases without such metastasis. Many malignant tumors secrete lymphangiogenic factors such as VEGF-C and VEGF-D and promote lymphangiogenesis in their adjacent tissues, which helps tumor cells to metastasize to lymph nodes, and many studies have demonstrated that tumor-associated macrophages play a vital role in lymphangiogenesis in malignant tumors by secreting VEGF-C and VEGF-D. In intrahepatic cholangiocarcinoma, the lymphatic vessel density of surgically resected tumors was positively correlated with the incidence of lymphatic metastasis. In HCC, VEGF-C expression was positively correlated with the size of tumors and the number of extrahepatic metastases and was negatively correlated with disease-free survival time. Thus, blockade of VEGF-C may be a potential therapeutic strategy against malignant tumors. A VEGF-C neutralizing antibody (VGX-100) is now the subject of a Phase I clinical trial for adult patients with advanced or metastatic solid tumors (NCT01514123).

**Post-transplant lymphangiogenesis.** In solid organ transplants, lymphatic vessel connections between the graft and the recipient are interrupted. Because lymphatic vessels are essential for adaptive immunity, the association between lymphangiogenesis and graft rejection has received considerable attention. Post-transplant lymphangiogenesis in grafts was associated with acute cellular graft rejection in transplants of various organs (kidney, heart, and lung) in humans. However, the pathologic role of post-transplant lymphangiogenesis in graft rejection remains unclear. Post-transplant lymphangiogenesis could be detrimental if newly formed lymphatic vessels promote antigen presentation in draining lymph nodes and provoke alloimmune responses that result in graft rejection. On the other hand, these newly formed lymphatic vessels could be beneficial if they efficiently clear immune cells. In a rat model of liver transplantation, post-transplant lymphangiogenesis in grafts was associated with long-term survival of recipients for more than 90 days. In addition, rats that had failed grafting by 11 days with acute cellular rejection and antibody-mediated rejection showed disappearance of lymphatic vessels from severely rejected areas, suggesting that lymphatic vessels have an important role in mitigation of inflammation at least in the early stage of transplantation. Further investigations to determine the mechanism and the time course of clearance of infiltrating immune cells by lymphatic vessels, especially in the early post-transplant period, may help increase transplant success.

**Conclusions and Perspective**

The lymphatic vascular system has been poorly studied in the liver. To drive research in this area, it is essential to identify better markers for LyECs that do not overlap with markers for LSECs, hepatocytes, and other liver cells. The development of experimental models for studying the lymphatic vascular system in postnatal livers will be important in examining its role and molecular mechanisms in physiological and pathophysiological conditions. Although this field is wide open, it may be helpful to identify specific questions particularly in need of study.

First, the mechanism of hepatic lymphangiogenesis is largely unknown. The VEGF-C/VEGFR-3 axis is considered the most potent signaling pathway that regulates lymphangiogenesis in other organs. However, cellular sources of VEGF-C and VEGFR-3 have not been fully identified in the liver. Furthermore, as shown in Table 2, many other molecules are reported to regulate lymphangiogenesis. These molecules are mostly observed in the liver in physiological and pathophysiological conditions. It would be worth characterizing these molecules in relation to hepatic lymphangiogenesis.

Second, although the relationship between the lymphatic vascular system and metastasis is well-known and the growth of lymphatic capillaries in liver tumors has been observed, the role of lymphatic capillary growth in the development and progression of liver tumors is largely unknown. As for angiogenesis, it would be interesting to investigate lymphangiogenesis in liver cancer.

Third, inflammation is closely related to the development of many liver diseases, and infiltrating immune cells are drained to lymphatic vessels. Thus, it would be interesting to examine lymphangiogenesis in relation to inflammation in the liver. It is also unknown how immune cells recognize lymphatic vessels at the time of migration. Elucidation of these mechanisms may help in the development of anti-inflammatory strategies that facilitate immune cell clearance.

Fourth, although LyECs are derived from cardinal veins and LSECs are derived from the septum transversum, LyECs and LSECs have many similarities. Both LyECs and LSECs express LYVE-1, a type II transmembrane protein that supports leukocyte adhesion, and reelin, a glycoprotein that is associated with embryonic development, are also expressed in both LyECs and LSECs. Furthermore, under normal conditions, neither LyECs nor LSECs are associated with basement membranes. Examining the similarities and differences between these 2 types of endothelial cells could help to understand endothelial cell–related liver function.

In summary, the lymphatic vascular system in the liver is a large open area for investigation. More research will significantly advance our understanding of liver physiology and pathophysiology and in turn contribute to the...
development of new therapeutic strategies for many liver diseases.

References

1. Chung C, Iwakiri Y. The lymphatic vascular system in liver diseases: its role in ascites formation. Clin Mol Hepatol 2013;19:99–104.
2. Tammela T, Alitalo K. Lymphangiogenesis: molecular mechanisms and future promise. Cell 2010;140:460–476.
3. Schulte-Merker S, Sabine A, Petrova TV. Lymphatic vascular morphogenesis in development, physiology, and disease. J Cell Biol 2011;193:607–618.
4. Koltowska K, Betterman KL, Harvey NL, et al. Getting out and about: the emergence and morphogenesis of the vertebrate lymphatic vasculature. Development 2013;140:1857–1870.
5. Kaipainen A, Korhonen J, Mustonen T, et al. Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. Proc Natl Acad Sci U S A 1995;92:3566–3570.
6. Jeltsch M, Kaipainen A, Joukov V, et al. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science (New York, NY) 1997;276:1423–1425.
7. Banerji S, Ni J, Wang SX, et al. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J Cell Biol 1999;144:789–801.
8. Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. Cell 1999;98:769–778.
9. Breiteneder-Geleff S, Soleiman A, Kowalski H, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am J Pathol 1999;154:385–394.
10. Trutmann M, Sasse D. The lymphatics of the liver. Anat Embryol (Berl) 1994;190:201–209.
11. Ohnishi O, Ohtani Y. Lymph circulation in the liver. Anat Rec 2008;291:643–652.
12. Pupulin LF, Vilgrain V, Ronot M, et al. Hepatic lymphatics: anatomy and related diseases. Abdom Imaging 2015;40:1997–2011.
13. Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. Nature 2005;438:946–953.
14. Maby-El Hajjami H, Petrova TV. Developmental and pathological lymphangiogenesis: from models to human disease. Histochem Cell Biol 2008;130:1063–1078.
15. Baluk P, Fuxe J, Hashizume H, et al. Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med 2007;204:2349–2362.
16. Danussi C, Spessotto P, Petrucco A, et al. Emilin1 deficiency causes structural and functional defects of lymphatic vasculature. Mol Cell Biol 2008;28:4026–4039.
17. Solito R, Alessandrini C, Fruschelli M, et al. An immunological correlation between the anchoring filaments of initial lymph vessels and the neighboring elastic fibers: a unified morphofunctional concept. Lymphology 1997;30:194–202.
18. Breslin JW. Mechanical forces and lymphatic transport. Microvasc Res 2014;96:46–64.
19. Shirasawa Y, Benoit JN. Stretch-induced calcium sensitization of rat lymphatic smooth muscle. Am J Physiol Heart Circ Physiol 2003;285:H2573–H2577.
20. Davis MJ, Scallan JP, Wolpers JH, et al. Intrinsic increase in lymphangion muscle contractility in response to elevated afterload. Am J Physiol Heart Circ Physiol 2012;303:H795–H808.
21. Bohlen HG, Gasheva OY, Zawieja DC. Nitric oxide formation by lymphatic bulb and valves is a major regulatory component of lymphatic pumping. Am J Physiol Heart Circ Physiol 2011;301:H1897–H1906.
22. Shyy JY, Chien S. Role of integrins in endothelial mechanosensing of shear stress. Circ Res 2002;91:769–775.
23. Kunert C, Baish JW, Liao S, et al. Mechanobiological oscillators control lymph flow. Proc Natl Acad Sci U S A 2015;112:10938–10943.
24. Forster R, Braun A, Worbs T. Lymph node homing of T cells and dendritic cells via afferent lymphatics. Trends Immunol 2012;33:271–280.
25. Jeltsch M, Tammela T, Alitalo K, et al. Genesis and pathogenesis of lymphatic vessels. Cell Tissue Res 2003;314:69–84.
26. Wake K, Sato T. “The sinusoid” in the liver: lessons learned from the original definition by Charles Sedgwick Minot (1900). Anat Rec (Hoboken) 2015;298:2071–2080.
27. Mall FP. A study of the structural unit of the liver. American Journal of Anatomy 1906;5:227–308.
28. Munoz SJ, Fenkel JM, Kiley K. The liver in circulatory failure. In: Schiff ER, Maddrey WC, Sorrell MF, eds. Schiff’s diseases of the liver. Hoboken: Wiley-Blackwell, 2011;924–933.
29. Ross MH. Histology: a text and atlas. 3rd ed. Philadelphia: Lippincott Williams and Wilkins, 1995.
30. Prevo R, Banerji S, Ferguson DJ, et al. Mouse LYVE-1 is an endocytic receptor for hyaluronan in lymphatic endothelium. J Biol Chem 2001;276:19420–19430.
31. Jackson DG. Biology of the lymphatic marker LYVE-1 and applications in research into lymphatic trafficking and lymphangiogenesis. APMS 2004;112:526–538.
32. Schacht V, Ramirez MI, Hong Y-K, et al. T1α/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. EMBO J 2003;22:3546–3556.
33. Wigle JT, Harvey N, Detmar M, et al. An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype. EMBO J 2002;21:1505–1513.
34. Duncan MK, Cui W, Oh D-J, et al. Prox1 is differentially localized during lens development. Mech Dev 2002;112:195–198.
35. Wilting J, Papoutsii M, Christ B, et al. The transcription factor Prox1 is a marker for lymphatic endothelial cells in normal and diseased human tissues. FASEB J 2002;16:1271–1273.
36. Baluk P, McDonald DM. Markers for microscopic imaging of lymphangiogenesis and angiogenesis. Ann N Y Acad Sci 2008;1131:1–12.

37. Neame PJ, Barry FP. The link proteins. Expert Rev 1993;49:393–402.

38. Gale NW, Prevo R, Espinosa J, et al. Normal lymphatic development and function in mice deficient for the lymphatic hyaluronan receptor LYVE-1. Mol Cell Biol 2007;27:595–604.

39. Jang JY, Koh YJ, Lee SH, et al. Conditional ablation of LYVE-1+ cells unveils defensive roles of lymphatic vessels in intestine and lymph nodes. Blood 2013;122:2151–2161.

40. Mouta Carreira C, Nasser SM, di Tomaso E, et al. LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. Cancer Res 2001;61:8079–8084.

41. Lalor PF, Lai WK, Curbishley SM, et al. Human hepatic sinusoidal endothelial cells can be distinguished by expression of phenotypic markers related to their specialised functions in vivo. World J Gastroenterol 2006;12:5429–5439.

42. Arimoto J, Ikura Y, Suekane T, et al. Expression of LYVE-1 in sinusoidal endothelium is reduced in chronically inflamed human livers. J Gastroenterol 2010;45:317–325.

43. Nonaka H, Tanaka M, Suzuki K, et al. Development of murine hepatic sinusoidal endothelial cells characterized by the expression of hyaluronan receptors. Dev Dyn 2007;236:2258–2267.

44. Kitagawa K, Nakajima G, Kuramochi H, et al. Lymphatic vessel endothelial hyaluronan receptor-1 is a novel prognostic indicator for human hepatocellular carcinoma. Mol Clin Oncol 2013;1:1039–1048.

45. Hong YK, Harvey N, Noh YH, et al. Prox1 is a master control gene in the program specifying lymphatic endothelial cell fate. Dev Dyn 2002;225:351–357.

46. Ordonez NG. Immunohistochemical endothelial markers: a review. Adv Anat Pathol 2012;19:281–289.

47. Wigle JT, Chowdhry K, Gruss P, et al. Prox1 function is crucial for mouse lens-fibre elongation. Nat Genet 1999;21:318–322.

48. Dyer MA, Livesey FJ, Cepko CL, et al. Prox1 function controls progenitor cell proliferation and horizontal cell genesis in the mammalian retina. Nat Genet 2003;34:53–58.

49. Rissebo CA, Searles RG, Melville AA, et al. Prox1 maintains muscle structure and growth in the developing heart. Development 2009;136:495–505.

50. Lavado A, Oliver G. Prox1 expression patterns in the developing and adult murine brain. Dev Dyn 2007;236:518–524.

51. Burke Z, Oliver G. Prox1 is an early specific marker for the developing liver and pancreas in the mammalian foregut endoderm. Mech Dev 2002;118:147–155.

52. Dudas J, Mansuroglu T, Moriconi F, et al. Altered regulation of Prox1-gene-expression in liver tumors. BMC Cancer 2008;8:1–15.

53. Shimoda M, Takahashi M, Yoshimoto T, et al. A homeobox protein, prox1, is involved in the differentiation, proliferation, and prognosis in hepatocellular carcinoma. Clin Cancer Res 2006;12:6005–6011.

54. Song KH, Li T, Chiang JY. A Prospero-related homeodomain protein is a novel co-regulator of hepatocyte nuclear factor 4alpha that regulates the cholesterol 7alpha-hydroxylase gene. J Biol Chem 2006;281:10081–10088.

55. Charest-Marcotte A, Dufour CR, Wilson BJ, et al. The homeobox protein Prox1 is a negative regulator of ERR (alpha)/PGC-1(alpha) bioenergetic functions. Genes Dev 2010;24:537–542.

56. Dufour CR, Levasseur MP, Pham NH, et al. Genomic convergence among ERRalpha, PROX1, and BMAL1 in the control of metabolic clock outputs. PLoS Genet 2011;7:e1002143.

57. Qin J, Gao DM, Jiang QF, et al. Prospero-related homeobox (Prox1) is a corepressor of human liver receptor homolog-1 and suppresses the transcription of the cholesterol 7alpha-hydroxylase gene. Mol Endocrinol 2004;18:2424–2439.

58. Takeda Y, Jetten AM. Prospero-related homeobox 1 (Prox1) functions as a novel modulator of retinoic acid-related orphan receptors alpha- and gamma-mediated transactivation. Nucleic Acids Res 2013;41:6992–7008.

59. Martin-Villar E, Scholl FG, Gamallo C, et al. Characterization of human PA2.26 antigen (T1alpha-2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. Int J Cancer 2005;113:899–910.

60. Mahtab EA, Wijffels MC, Van Den Akker NM, et al. Cardiac malformations and myocardial abnormalities in podoplanin knockout mouse embryos: correlation with abnormal epicardial development. Dev Dyn 2008;237:847–857.
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68. Mahtab EA, Vicente-Steijn R, Hahurij ND, et al. Podoplanin deficient mice show a RhoA-related hypoplasia of the sinus venosus myocardium including the sinoatrial node. Dev Dyn 2009;238:183–193.

69. Douglas YL, Mahtab EA, Jongbloed MR, et al. Pulmonary vein, dorsal atrial wall and atrial septum abnormalities in podoplanin knockout mice with disturbed posterior heart field contribution. Pediatr Res 2009;65:27–32.

70. Ramirez MI, Millien G, Hinds A, et al. T1alpha, a lung type I cell differentiation gene, is required for normal lung lumen formation and alveolus formation at birth. Dev Biol 2003;256:61–72.

71. Bekiaris V, Withers D, Glanville SH, et al. Role of CD30 in podoplanin expression during the mesenchymal transition in liver injury. Proc Natl Acad Sci U S A 2012;109:6223.

72. Astarita JL, Acton SE, Turley SJ. Podoplanin: emerging functions in development, the immune system, and cancer. Front Immunol 2012;3:283.

73. Baars S, Bauer C, Szabowski S, et al. Epithelial deletion of podoplanin is dispensable for re-epithelialization of skin wounds. Exp Dermatol 2015;24:785–787.

74. Herzog BH, Fu J, Wilson SJ, et al. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. Nature 2013;502:105–109.

75. Li Y, Wang J, Asahina K. Mesothelial cells give rise to hepatic stellate cells and myofibroblasts via mesothelial-mesenchymal transition in liver injury. Proc Natl Acad Sci U S A 2013;110:2324–2329.

76. Yokomori H, Oda M, Kaneko F, et al. Lymphatic marker podoplanin/D2-40 in human advanced cirrhotic liver: re-evaluations of microlymphatic abnormalities. BMC Gastroenterol 2010;10:131.

77. Link A, Hardie DL, Favre S, et al. Association of T-zone reticular networks and conduits with ectopic lymphoid tissues in mice and humans. Am J Pathol 2011;178:1662–1675.

78. Fuji T, Zen Y, Sato Y, et al. Podoplanin is a useful diagnostic marker for epithelioid hemangioendothelioma of the liver. Mod Pathol 2008;21:125–130.

79. Xian ZH, Cong WM, Lu XY, et al. Angiogenesis and lymphangiogenesis in sporadic hepatic angiomyolipoma. Pathol Res Pract 2011;207:403–409.

80. Dumont DJ, Jussila L, Taipale J, et al. Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. Science 1998;282:946–949.

81. Karkkainen MJ, Haiko P, Sainio K, et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol 2004;5:74–80.

82. Martinez-Corral I, Olmeda D, Dieguez-Hurtado R, et al. In vivo imaging of lymphatic vessels in development, wound healing, inflammation, and tumor metastasis. Proc Natl Acad Sci U S A 2012;109:6223–6228.

83. Gaudio E, Barbaro B, Alvaro D, et al. Vascular endothelial growth factor stimulates rat cholangiocyte proliferation via an autocrine mechanism. Gastroenterology 2006;130:1270–1282.

84. Franchitto A, Onori P, Renzi A, et al. Expression of vascular endothelial growth factors and their receptors by hepatic progenitor cells in human liver diseases. Hepatobiliary Surg Nutr 2013;2:68–77.

85. Lian Z, Liu J, Wu M, et al. Hepatitis B x antigen up-regulates vascular endothelial growth factor receptor 3 in hepatocarcinogenesis. Hepatology 2007;45:1390–1399.

86. Paupert J, Sounni NE, Noel A. Lymphangiogenesis in post-natal tissue remodeling: lymphatic endothelial cell connection with its environment. Mol Aspects Med 2011;32:146–158.

87. Kelley PM, Steele MM, Tempero RM. Regressed lymphatic vessels develop during corneal repair. Lab Invest 2011;91:1643–1651.

88. Zampell JC, Avraham T, Yoder N, et al. Lymphatic function is regulated by a coordinated expression of lymphangiogenic and anti-lymphangiogenic cytokines. Am J Physiol Cell Physiol 2012;302:C392–C404.

89. Dixelius J, Makinen T, Wirzenius M, et al. Ligand-induced vascular endothelial growth factor receptor-3 (VEGFR-3) heterodimerization with VEGFR-2 in primary lymphatic endothelial cells regulates tyrosine phosphorylation sites. J Biol Chem 2003;278:40973–40979.

90. Ichise T, Yoshida N, Ichise H. H-, N- and Kras cooperatively regulate lymphatic vessel growth by modulating VEGFR3 expression in lymphatic endothelial cells in mice. Development 2010;137:1003–1013.

91. Makinen T, Veikkola T, Mustjoki S, et al. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. EMBO J 2001;20:4762–4773.

92. Vanhaesebroeck B, Stephens L, Hawkins P, PI3K signalling: the path to discovery and understanding. Nat Rev Mol Cell Biol 2012;13:195–203.

93. Zheng W, Aspelund A, Altalito K. Lymphangiogenic factors, mechanisms, and applications. J Clin Invest 2014;124:878–887.

94. Coso S, Bovay E, Petrova TV. Pressing the right buttons: inflammation resolution. Blood 2014;123:2614–2624.

95. Secker GA, Harvey NL. VEGF signalling during lymphatic vascular development: from progenitor cells to functional vessels. Dev Dyn 2015;244:323–331.

96. Kim H, Kataru RP, Koh GY. Inflammation-associated lymphangiogenesis: a double-edged sword? J Clin Invest 2014;124:936–942.

97. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol 2005;6:1191–1197.

98. Kataru RP, Jung K, Jang C, et al. Critical role of CD11b+ macrophages and VEGF in inflammatory lymphangiogenesis, antigen clearance, and inflammation resolution. Blood 2009;113:5650–5659.

99. Kataru RP, Lee YG, Koh GY. Interactions of immune cells and lymphatic vessels. Adv Anat Embryol Cell Biol 2014;214:107–118.

100. Mancardi S, Vecile E, Dusetti N, et al. Evidence of CXC, CC and C chemokine production by lymphatic endothelial cells. Immunology 2003;108:523–530.
114. Johnson LA, Clasper S, Holt AP, et al. An in

115. Gagliostro V, Seeger P, Garrafa E, et al. Pro-

116. Kataru RP, Kim H, Jang C, et al. T lymphocytes nega-

117. Angeli V, Ginhoux F, Llodra J, et al. B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. Immunity 2006; 24:203–215.

118. Shrestha B, Hashiguchi T, Ito T, et al. B cell-derived vascular endothelial growth factor A promotes lymphangiogenesis and high endothelial venule expansion in lymph nodes. J Immunol 2010;184:4819–4826.

119. Tan KW, Chong SZ, Wong FH, et al. Neutrophils contribute to inflammatory lymphangiogenesis by increasing VEGF-A bioavailability and secreting VEGF-D. Blood 2013;122:3666–3677.

120. Cain JC, Grindlay JH. Lymph from liver and thoracic duct; an experimental study. Surg Gynecol Obstet 1947; 85:558–562.

121. Nix JT, Flock EV, Bollman JL. Influence of cirrhosis on proteins of cisternal lymph. Am J Physiol 1951; 164:117–118.

122. Dumont AE, Mulolland JH. Flow rate and composition of thoracic-duct lymph in patients with cirrhosis. N Engl J Med 1960;263:471–474.

123. Dumont AE, Mulolland JH. Alterations in thoracic duct lymph flow in hepatic cirrhosis: significance in portal hypertension. Ann Surg 1962;156:668–675.

124. Witte CL, Witte MH, Dumont AE. Lymph imbalance in the genesis and perpetuation of the ascites syndrome in hepatic cirrhosis. Gastroenterology 1980; 78:1059–1068.

125. Barrowman AJ, Granger DN. Effects of experimental cirrhosis on splanchic microvascular fluid and solute exchange in the rat. Gastroenterology 1984; 87:165–172.

126. Shimada Y. Observations on hepatic superficial lymph flow. Lymphology 1979;12:11–13.

127. Atkinson M, Losowsky MS. The mechanism of ascites formation in chronic liver disease. Q J Med 1961; 30:153–166.

128. Lieberman FL, Ito S, Reynolds TB. Effective plasma volume in cirrhosis with ascites: evidence that a decreased value does not account for renal sodium retention, a spontaneous reduction in glomerular filtration rate (GFR), and a fall in GFR during drug-induced diuresis. J Clin Invest 1969;48:975–981.

129. Schrier RW, Arroyo V, Bernardi M, et al. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. Hepatology 1988;8:1151–1157.

130. De Franchis R, Salerno F. Pathogenesis of ascites and predictors of resistance to therapy. J Gastroenterol Hepatol 2002;17(Suppl 3):S242–S247.

131. Arroyo V, Colmenero J. Ascites and hepatorenal syn-

132. Gordon FD. Ascites. Clin Liver Dis 2012;16:285–299.

133. Sanyal AJ, Bosch J, Blei A, et al. Portal hypertension and its complications. Gastroenterology 2008;134: 1715–1728.

134. Ribera J, Pauta M, Melgar-Lesmes P, et al. Increased nitric oxide production in lymphatic endothelial cells causes impairment of lymphatic drainage in cirrhotic rats. Gut 2013;62:138–145.
135. Vollmar B, Wolf B, Siegmund S, et al. Lymphatic neoangiogenesis associated with immunologically active lymphocytic infiltrates in liver cirrhosis. J Hepatol 2004;41:651–657.

136. Yamauchi Y, Michitaka K, Onji M. Morphometric analysis of lymphatic and blood vessels in human chronic viral liver diseases. Am J Pathol 1998;153:1311–1317.

137. Schoppmann SF, Fenzl A, Nagy K, et al. Vascular endothelial growth factor-C in human hepatic sinusoidal structure with special reference to the Ito cells. Microsc Res Tech 1997;39:336–349.

138. Oikawa H, Masuda T, Sato S, et al. Changes in lymph vessels and portal veins in the portal tract of patients with idiopathic portal hypertension: a morphometric study. J Surg Oncol 2007;96:37–45.

139. Guerin F, Wagner M, Line A, et al. Hepatic proliferation and angiogenesis markers are increased after portal deprivation in rats: a study of molecular, histological and radiological changes. PLoS One 2015;10:e0125493.

140. Sun HC, Zhuang PY, Qin LX, et al. Incidence and prognostic value of lymph node metastasis in operable hepatocellular carcinoma. Ann N Y Acad Sci 2008;1131:235–241.

141. Das S, Skobe M. Lymphatic vessel activation in cancer. Expert Opin Investig Drugs 2016;25:1–14.

142. Guerin F, Wagner M, Line A, et al. Hepatic proliferation and angiogenesis markers are increased after portal deprivation in rats: a study of molecular, histological and radiological changes. PLoS One 2015;10:e0125493.

143. Skrobe M, Hamberg LM, Hawighorst T, et al. Concurrent induction of lymphangiogenesis, angiogenesis, and macrophage recruitment by vascular endothelial growth factor-C in melanoma. Am J Pathol 2001;159:893–903.

144. Schledzewski K, Falkowski M, Moldenhauer G, et al. Lymphatic endothelium-specific hyaluronan receptor LYVE-1 is expressed by stabilin-1-immunoreactive cells. Blood 2003;102:349–356.

145. Tampellini M, Sonetto C, Scaglioni GV. Novel antiangiogenic therapeutic strategies in colorectal cancer. Expert Opin Investig Drugs 2016;25:1–14.

146. Kerjaschki D, Regele HM, Moosberger I, et al. Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. J Am Soc Nephrol 2004;15:603–612.

147. Stuht S, Gwinner W, Franz I, et al. Lymphatic neoangiogenesis in human renal allografts: results from sequential protocol biopsies. Am J Transplant 2007;7:377–384.

148. Vass DG, Hughes J, Marson LP. Restorative and resection-associated lymphangiogenesis after renal transplantation: friend or foe? Transplantation 2009;88:1237–1239.

149. Geissler HJ, Dashkevich A, Fischer UM, et al. First year changes of myocardial lymphatic endothelial markers in heart transplant recipients. Eur J Cardiothorac Surg 2009;36:9:207–213.

150. Dashkevich A, Heilmann C, Kayser G, et al. Lymphangiogenesis after lung transplantation and relation to acute organ rejection in humans. Ann Thorac Surg 2010;90:406–411.

151. Ishii E, Shimizu A, Kuwahara N, et al. Lymphangiogenesis associated with acute cellular rejection in rat liver transplantation. Transplant Proc 2010;42:4282–4285.

152. Enzan H, Himeno H, Hiroi M, et al. Development of hepatic sinusoidal structure with special reference to the Ito cells. Microsc Res Tech 1997;39:336–349.

153. Yamamoto M, Takasaki K, Yoshihara K, et al. Lymph node metastasis in intrahepatic cholangiocarcinoma. Jpn J Clin Oncol 1999;29:147–150.

154. Enzan H, Himeno H, Hiroi M, et al. Development of hepatic sinusoidal structure with special reference to the Ito cells. Microsc Res Tech 1997;39:336–349.

155. Salmi M, Jalkanen S. Cell-surface enzymes in control of lymphangiogenesis and evaluation of routine lymphadenectomy. J Surg Oncol 2007;96:37–45.

156. Ikeda Y, Terashima T. Expression of reelin, the gene responsible for the reeler mutation, in embryonic development and adulthood in the mouse. Dev Dyn 1997;210:157–172.

157. Yamamoto M, Takasaki K, Yoshihara K, et al. Lymph node metastasis in intrahepatic cholangiocarcinoma. Jpn J Clin Oncol 1999;29:147–150.

158. Das S, Skobe M. Lymphatic vessel activation in cancer. Ann N Y Acad Sci 2008;1131:235–241.

159. Schledzewski K, Falkowski M, Moldenhauer G, et al. Lymphatic endothelium-specific hyaluronan receptor LYVE-1 is expressed by stabilin-1-immunoreactive cells. Blood 2003;102:349–356.

160. Thelen A, Scholz A, Weichert W, et al. Angiogenic therapeutic strategies in colorectal cancer. Expert Opin Investig Drugs 2016;25:1–14.
168. Witmer AN, van Blijswijk BC, van Noorden CJ, et al. In vivo angiogenic phenotype of endothelial cells and pericytes induced by vascular endothelial growth factor-A. J Histochem Cytochem 2004;52:39–52.

169. Gunn MD, Kyuwa S, Tam C, et al. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. J Exp Med 1999;189:451–460.

170. Heydtmann M, Hardie D, Shields PL, et al. Detailed analysis of intrahepatic CD8 T cells in the normal and hepatitis C–infected liver reveals differences in specific populations of memory cells with distinct homing phenotypes. J Immunol 2006;177:729–738.

171. Grant AJ, Goddard S, Ahmed-Choudhury J, et al. Hepatic expression of secondary lymphoid chemokine (CCL21) promotes the development of portal-associated lymphoid tissue in chronic inflammatory liver disease. Am J Pathol 2002;160:1445–1455.

172. Lee SJ, Evers S, Roeder D, et al. Mannose receptor–mediated regulation of serum glycoprotein homeostasis. Science 2002;295:1898–1901.

173. Takahashi K, Donovan MJ, Rogers RA, et al. Distribution of murine mannose receptor expression from early embryogenesis through to adulthood. Cell Tissue Res 1998;292:311–323.

174. Petrova TV, Makinen T, Makela TP, et al. Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. EMBO J 2002;21:4593–4599.

175. Linehan SA, Martinez-Pomares L, Stahl PD, et al. Mannose receptor and its putative ligands in normal murine lymphoid and nonlymphoid organs: in situ expression of mannose receptor by selected macrophages, endothelial cells, perivascular microglia, and mesangial cells, but not dendritic cells. J Exp Med 1999;189:1961–1972.

176. Gigi-Leitner O, Geiger B. Antigenic interrelationship between the 40-kilodalton cytokeratin polypeptide and desmoplakin. Cell Motil Cytoskeleton 1986;6:628–639.

177. Sawa Y, Shibata K, Braithwaite MW, et al. Expression of immunoglobulin superfamily members on the lymphatic endothelium of inflamed human small intestine. Microvasc Res 1999;57:100–106.

178. Cao Y, Chang H, Li L, et al. Alteration of adhesion molecule expression and cellular polarity in hepatocellular carcinoma. Histopathology 2007;51:528–538.

179. Ebata N, Nodasaka Y, Sawa Y, et al. Desmoplakin as a specific marker of lymphatic vessels. Microvasc Res 2001;61:40–48.

180. Hoye AM, Couchman JR, Wewer UM, et al. The newcomer in the integrin family: integrin alpha9 in biology and cancer. Adv Biol Regul 2012;52:326–339.

181. Halin C, Tobler NE, Vigi B, et al. VEGF-A produced by chronically inflamed tissue induces lymphangiogenesis in draining lymph nodes. Blood 2007;110:3158–3167.

182. Wuest TR, Carr DJ. VEGF-A expression by HSV-1–infected cells drives corneal lymphangiogenesis. J Exp Med 2010;207:101–115.

183. Kubo H, Cao R, Brakenhielm E, et al. Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2–induced lymphangiogenesis in mouse cornea. Proc Natl Acad Sci U S A 2002;99:8868–8873.

184. Baluk P, Tamnela T, Ator E, et al. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. J Clin Invest 2005;115:247–257.

185. Kim KE, Koh YJ, Jeon BH, et al. Role of CD11b+ macrophages in intraperitoneal lipopolysaccharide–induced aberrant lymphangiogenesis and lymphatic function in the diaphragm. Am J Pathol 2009;175:1733–1745.

186. Yan ZX, Jiang ZH, Liu NF. Angiopoietin-2 promotes inflammatory lymphangiogenesis and its effect can be blocked by the specific inhibitor L1–10. Am J Physiol Heart Circ Physiol 2012;302:H215–H223.

187. Yuan D, Grimaldo S, Sessa R, et al. Role of angiopoietin-2 in corneal lymphangiogenesis. Invest Ophthalmol Vis Sci 2014;55:3320–3327.

188. Saito Y, Nakagami H, Morishita R, et al. Transfection of human hepatocyte growth factor gene ameliorates secondary lymphedema via promotion of lymphangiogenesis. Circulation 2006;114:1177–1184.

189. Furtado GC, Marinovic T, Martin AP, et al. Lymphotxin beta receptor signaling is required for inflammatory lymphangiogenesis in the thyroid. Proc Natl Acad Sci U S A 2007;104:5026–5031.

190. Mounzer RH, Svendsen OS, Baluk P, et al. Lymphotixin-alpha contributes to lymphangiogenesis. Blood 2010;116:2173–2182.

191. Watari K, Nakao S, Fotovati A, et al. Role of macrophages in inflammatory lymphangiogenesis: enhanced production of vascular endothelial growth factor C and D through NF-kappaB activation. Biochem Biophys Res Commun 2008;377:826–831.

192. Al-Rawi MA, Watkins G, Mansel RE, et al. Interleukin 7 upregulates vascular endothelial growth factor D in breast cancer cells and induces lymphangiogenesis in vivo. Br J Surg 2005;92:305–310.

193. Al-Rawi MA, Watkins G, Mansel RE, et al. The effects of interleukin-7 on the lymphangiogenic properties of human endothelial cells. Int J Oncol 2005;27:721–730.

194. Choi I, Lee YS, Chung HK, et al. Interleukin-8 reduces post-surgical lymphedema formation by promoting lymphatic vessel regeneration. Angiogenesis 2013;16:29–44.

195. Chauhan SK, Jin Y, Goyal S, et al. A novel prolymphangiogenic function for Th17/IL-17. Blood 2011;118:4630–4634.

196. Hammer T, Tritsaris K, Hubschmann MV, et al. IL-20 activates human lymphatic endothelial cells causing cell signalling and tube formation. Microvasc Res 2009;78:25–32.

197. Oka M, Iwata C, Suzuki HI, et al. Inhibition of endogenous TGF-beta signaling enhances lymphangiogenesis. Blood 2008;111:4571–4579.

198. Clavin NW, Avraham T, Fernandez J, et al. TGF-beta1 is a negative regulator of lymphatic regeneration during...
wound repair. Am J Physiol Heart Circ Physiol 2008; 295:H2113–H2127.

199. Avraham T, Daluvoy S, Zampell J, et al. Blockade of transforming growth factor-beta1 accelerates lymphatic regeneration during wound repair. Am J Pathol 2010; 177:3202–3214.

200. Dunworth WP, Cardona-Costa J, Bozkulak EC, et al. Bone morphogenetic protein 2 signaling negatively modulates lymphatic development in vertebrate embryos. Circ Res 2014;114:56–66.

201. Shao X, Liu C. Influence of IFN- alpha and IFN- gamma on lymphangiogenesis. J Interferon Cytokine Res 2006; 26:568–574.

202. Shin K, Kataru RP, Park HJ, et al. TH2 cells and their cytokines regulate formation and function of lymphatic vessels. Nat Commun 2015;6:6196.

203. Nielsen SR, Hammer T, Gibson J, et al. IL-27 inhibits lymphatic endothelial cell proliferation by STAT1-regulated gene expression. Microcirculation 2013; 20:555–564.

204. Heinz M, Niederleithner HL, Puuulalaa E, et al. Activin A is anti-lymphangiogenic in a melanoma mouse model. J Invest Dermatol 2015;135:212–221.

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