Ann Vasc Dis Vol.5, No.2; 2012; pp 145–150

doi: 10.3400/avd.ra.11.00099

Review Article

Recent Developments in Morphology of Lymphatic Vessels and Lymph Nodes

Osamu Ohtani, MD, PhD, and Yuko Ohtani, MD, PhD

This paper reviews the morphology of lymphatics and lymphangiogenesis in vivo, microenvironments that promote lymphangiogenesis, and the structure and function of lymph nodes. Lymphatic capillaries consist of a single layer of lymphatic endothelial cells (LECs) and have valves, while collecting lymphatics are endowed with smooth muscle cells (SMCs) and valves besides a single layer of LECs. In the embryonic rat diaphragm, LECs first migrate presumably according to interstitial fluid flow and later join to form lymphatic vessels. SMCs of the collecting lymphatics are apparently differentiated from mesenchymal cells. LECs cultured on Cell Culture Inserts under a low oxygen condition proliferate very well and form a lymphatic network. LECs cultured on a collagen fiber network with a natural three-dimensional (3D) architecture under low oxygen rapidly form a 3D lymphatic network. The lymph node initiates an immune response as a critical crossroads for the encounter between antigen-presenting cells, antigens from lymph, and lymphocytes recruited into nodes from the blood. The node consists of spaces lined with LECs and parenchyma. High endothelial venules in the node strongly express Aquaporin-1, suggesting their involvement in the net absorption of water from lymph coming through afferent lymphatics. SMCs in node capsules seem to be involved in squeezing out lymphocytes and lymph. (*English Translation of J Jpn Col Angiol 2008; 48: 107-112.)

Keywords: lymphatic vessel, lymphangiogenesis, lymph node, metastasis, lymphedema

Introduction

The lymphatic vessels maintain the homeostasis of tissue fluid, function as an immunological surveillance mechanism in the living body, and play an important role in ingesting fat and the fat-soluble vitamins A, D, E, and K. The lymphatic vessels were first identified by an Italian anatomist, Gasparo Aselli, as "milky veins" in the canine mesentery in 1627. Since then, various techniques have been developed to investigate the lymphatic vessels to reveal their distribution and structure.[1] However, it was often difficult to identify lymphatic vessels in a tissue section because no specific marker was available. At the end of the 20th century, lymphatic vessel-specific markers, such as Prox-1,[2] podoplanin,[3] LYVE-1,[4] VEGFR-3,[5] CCL21,[6] and desmoplakin,[7] were discovered. These markers have accelerated investigations on lymphatic vessels, and should advance the diagnoses and treatments of various lymphatic disorders. The lymph node is an important intersection of antigen-presenting cells, antigens carried by the lymphatic vessels, and lymphocytes supplied by the blood. The lymph node consists of a lumen surrounded by lymphatic endothelial cells and parenchyma, featuring a reticular structure filled with lymphocytes, etc. The water of lymph from the afferent lymphatic vessels is absorbed into the lymph node to

Department of Anatomy, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Toyama, Japan

Received: December 13, 2011; Accepted: March 5, 2012
Corresponding author: Osamu Ohtani, MD, PhD. Department of Anatomy, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630, Sugitani, Toyama, Toyama 930-0152, Japan
Tel: +81-076-434-7205, Fax: +81-076-434-5010
E-mail: osmotani@med.u-toyama.ac.jp
*This article is English Translation of J Jpn Col Angiol 2008; 48: 107-112.

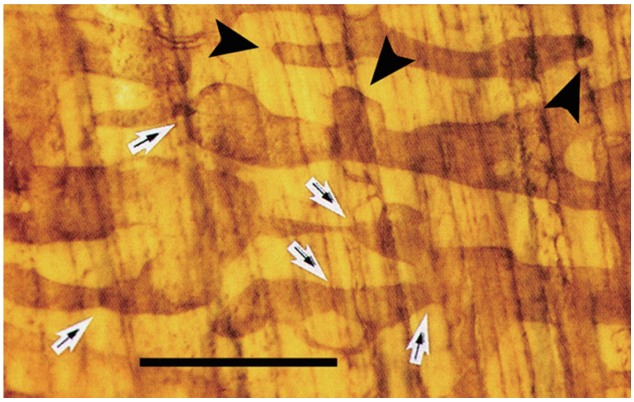

Fig. 1 Lymphatic capillaries in the rat diaphragmatic pleura. There are many blind ends (arrowheads) and valves (arrows). Enzyme histochemistry for 5′-nucleotidase. Scale bar =200m (From Ohtani et al., 1993[18])

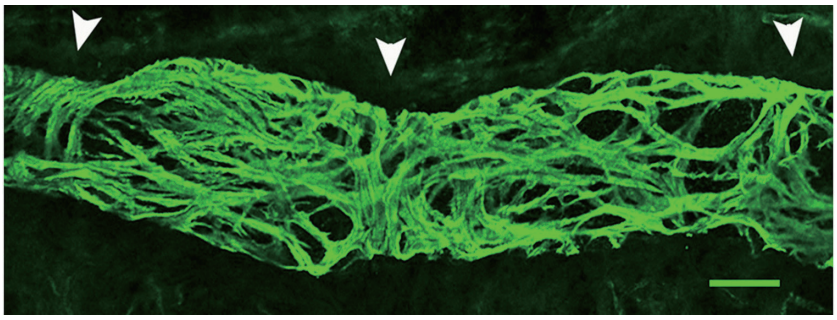

Fig. 2 Smooth muscle cells around the collecting lymphatic vessel in the rat diaphragm. They tend to run circumferentially around the valves (arrows) and obliquely or helically between valves. Scale bar = 50m (From Ohtani et al., 2001[19])

concentrate proteins. Lymph node metastasis of a tumor is the main negative prognostic factor. However, lymphadenectomy for cancer treatment causes lymphedema. We herein describe the morphology, structure, and functions of lymphatic vessels and lymphangiogenesis.

Morphology of Lymphatic Vessels

There are two kinds of lymphatic vessel: lymphatic vessels of origin, or lymphatic capillaries and lymphatic aggregates. The lymphatic capillary consists of a layer of lymphatic endothelial cells (LECs). Most lymphatic capillaries have valves at intervals of hundreds of microns to several millimeters to allow one-directional lymphatic flow (**Fig. 1**). Unlike blood vessels, the lymphatic capillary features a poorly-developed basal membrane and no pericyte. The lymphatic vessels are bound to the surrounding extracellular matrix through an anchoring filament[8] containing fibrillin.[9] Doubly or triply overlapping adhesive regions and gating non-adhesive micro valves exist between LECs. A pressure rise in the interstitial fluid increases the tension of the anchoring filaments attached to the LECs, thereby dilating the lymphatic capillary. Then, the non-adhesive regions between LECs open to allow liquid, macromolecules, and cells to enter the lymphatic vessels.

The lymphatic aggregates have valves and smooth muscles. Smooth muscles contract to cause lymphatic transport. Smooth muscles tend to run circularly around the valves and obliquely between the valves (**Fig. 2**). The smooth muscles in the lymphatic aggregates develop depending on the regions and species.

The human thoracic duct has three layers of well-developed smooth muscles. Smooth muscles run longitudinally in the inner layer and circularly in the middle layer. The smooth muscle bundles run obliquely or spirally in the

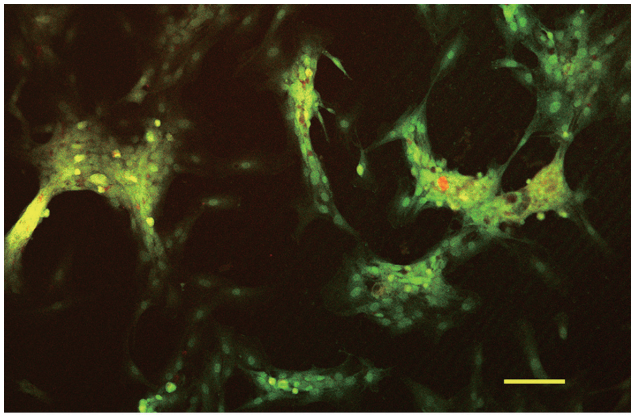

Fig. 3 LECs expressing LYVE-1 from the thoracic duct of the green rat (transgenic SD rats containing fluorescent genes; Amersham, Tokyo) cultured on an insert under a low oxygen condition proliferate rapidly and form a lymphatic-like network. Scale bar = 100m

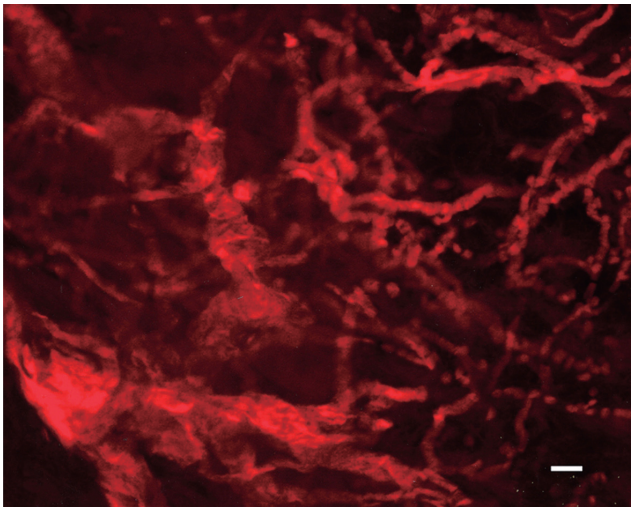

Fig. 4 LECs from the rat thoracic duct cultured on a collagen fiber sheet from the human thoracic duct rapidly form a three-dimensional lymphatic network. Scale bar = 25m

outer layer.[10] The middle layer is the thickest, and the inner and outer layers are relatively thinner. These layers cause peristaltic movement to allow lymphatic transport against gravity.

Lymphangiogenesis

In 1902, Sabin (Florence) proposed that primitive lymph sacs are formed through sprouting from the veins in an early developmental stage, from which the endothelium sprouts into the surrounding tissues and organs to form peripheral lymphatic vessels.[11] Oliver (2004) employed genetically-engineered mice to demonstrate Sabin's hypothesis that mammalian lymphatic vessels develop from embryonic veins.[12]

VEGF-C promotes the proliferation and migration of cultured LECs through VEGFR-3.[13] Furthermore, VEGF-A, VEGF-C, and VEGF-D promote lymphangiogenesis through VEGF receptor or neuropilin 2. The molecular regulatory mechanisms of lymphangiogenesis have been rapidly elucidated. For details, see a review.[14]

Environmental factors that promote LEC proliferation and lymphangiogenesis remain unclear. LECs collected from the rat thoracic duct more rapidly reached confluency when cultured under hypoxic conditions than when cultured under a 5% CO^2 atmosphere. LECs cultured on an insert under hypoxic conditions proliferate in layers to form lymphatic vessels (**Fig. 3**). Cells collected from the human thoracic duct and diaphragm were dissolved. Only the collagen fiber network was removed, with its natural three-dimensional structure maintained.[15,16] LECs cultured on the network under hypoxic conditions rapidly formed lymphatic vessels in three dimensions (**Fig. 4**). These findings suggest that a hypoxic environment and three-dimensional scaffold are critical for lymphangiogenesis.[17]

Only a few studies have been conducted on the in vivo development of lymphatic vessels. We have investigated lymphatic vessel development in the rat diaphragm.[18–20] Lymphatic vessels can be clearly recognized in the thoracic region of the diaphragm from embryonic day 16. After a while, they also appear in the peritoneal region. Lymphatic vessels develop mainly through sprouting. However, we previously suggested that LECs discretely migrated to form a line in regions where lymphatic vessels may be formed and the LECs aggregated to form lymphatic vessels (**Fig. 5**).[17] Alternatively, the LECs discretely migrate in the direction of interstitial fluid flow and subsequently aggregate to form lymphatic vessels.[21]

Little attention has been paid to the development of smooth muscles in lymphatic aggregates. Our study on the rat diaphragm[19] revealed many spindle-shaped cells expressing α-smooth muscle actin (α-SMA) until two weeks after birth. Subsequently, α-SMA-positive cells wrapped with elongated extensions were observed around the lymphatic vessels. After a while, typical smooth muscles circularly or spirally bound to the lymphatic vessels were observed. These findings suggest that the smooth muscles of lymphatic vessels differentiate from mesenchymal cells.[19]

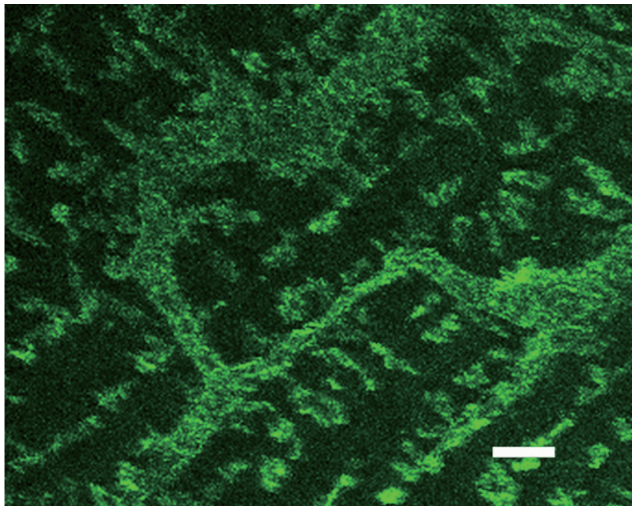

Fig. 5 Developing lymphatic vessels in the rat diaphragm. Numerous single LECs are in lines in the places where lymphatic vessels are expected to be formed. L: lymphatic vessels already formed. Scale bar = 50m

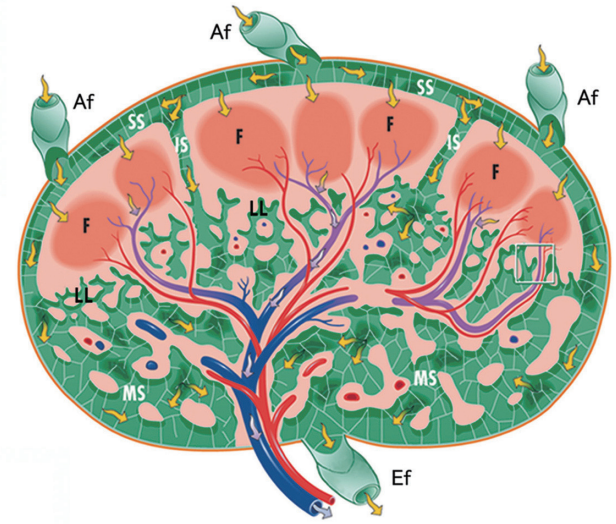

Fig. 6 Schematic diagram of the rat lymph node showing an overview of lymphatic pathways (green), an artery (red) and veins (violet: HEVs; blue: ordinary vein) of the lymph node. Arrows indicate the direction of fluid flow. Cross lines in subcapsular sinuses (SS), intermediate sinus (IS), and medullary sinuses (MS) indicate the networks of intraluminal reticular cells (i.e., lymphatic endothelial cells). F: follicle, Af: afferent lymphatic vessels, Ef: efferent lymphatic vessels, LL: lymphatic labyrinth. (From Ohtani et al., 2003[22])

Structure and Functions of Lymph Nodes

The lymph node is an important intersection of antigen-presenting cells, antigens carried by the lymphatic vessels, and lymphocytes supplied by the blood to initiate an immune response. The lymph node consists of a lumen surrounded by lymphatic endothelial cells and parenchyma, featuring a reticular structure filled with lymphocytes, etc.[22] In the parenchyma, the cortex is connected to myelin through the deep cortex. The afferent lymphatic vessels flow into the cortical lymphatic sinuses. Fluid and cells enter the cortex through the pores on the bed of the cortical lymphatic sinuses (**Fig. 6**). A lymphatic labyrinth filled with lymphocytes exists in the deep cortex. The lymphatic labyrinth is connected to the medullary sinuses. The medullary sinuses feature a large internal diameter with a well-developed beam column surrounded by LECs and many macrophages entangled. The cortical lymphatic sinuses are also connected to the medullary sinuses through the intermediary sinuses. The intermediary sinuses have the same structure as the medullary sinuses. High endothelial venules (HEVs) develop in the deep cortex. Circulating lymphocytes enter the lymph node parenchyma through the HEV wall (**Fig. 7**). On the other hand, lymphocytes from the parenchyma enter the lymphatic labyrinth in the deep cortex (**Fig. 7**). CCR7 is expressed in the lymphatic labyrinth.

Lymph from the afferent lymphatic vessels is absorbed into the lymph nodes to concentrate proteins.[23] This mechanism remains unclear. Water may be absorbed from the HEV wall into the blood vessels, because a water channel called Aquaporine-1 is strongly expressed near the surface of the endothelial cells of HEVs.[22] This suggests a functional intravenous shunt of lymphatic vessels in the lymph node.

Lymph node metastasis of a tumor is the main aggravating factor of patient prognosis. Recently, some kinds of human tumor were found to produce VEGF-A, -C, and -D. Such tumors facilitate lymphatic vessel proliferation and promote sentinel lymph node metastasis.[24] VEGF-C expression is correlated with Cox-2 expression in human stomach and colorectal cancers.[25,26] Reportedly, a Cox-2 inhibitor suppresses lymphangiogenesis when administered to an experimental tumor model. Thus, lymphangiogenesis inhibition in a tumor has been investigated as a cancer therapy strategy.

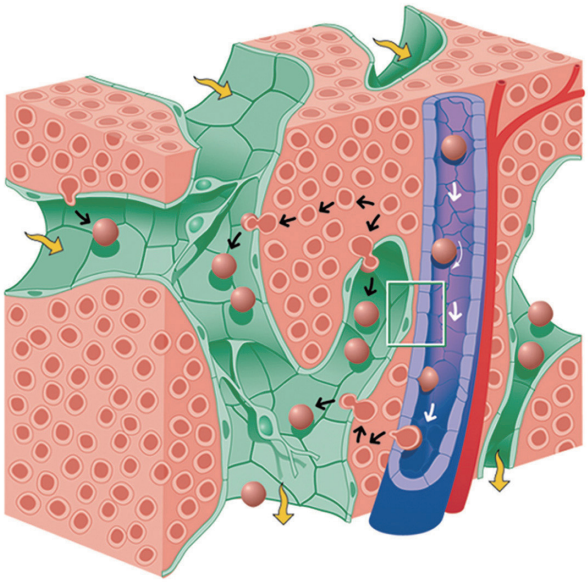

Fig. 7 Schematic of a closer view of the boxed area in **Fig. 6**. Thicker white arrows indicate a possible time sequence for the uppermost lymphocyte flowing in a HEV, which subsequently rolls on (thinner white arrow), then adheres to the luminal surface, and finally penetrates through its endothelium to enter the lymph node parenchyma. Black arrows indicate that lymphocytes in the lymph node parenchyma move towards the lymphatic labyrinths, and penetrate through their endothelium to enter the labyrinths. Yellow arrows indicate the lymph flow direction. (From Ohtani et al., 2003[22])

The mechanism of tumor cell metastasis to the lymph node remains unclear. Lewis lung carcinoma (LLC) cells, transplanted in the mouse lung, metastasize to the mediastinal lymph node.[27] GFP-expressing LLC cells were investigated as a model to demonstrate that LLC cells reached and scattered in the cortical lymphatic sinus and proliferated to form a tumor nodule. LLC cells scatter around the nodule and exist independently or form a small cell population. LLC cells that passed through the medullary sinus metastasize to other lymph nodes. Preventing tumor cells from passing through the medullary sinus may also provide an effective cancer therapy strategy.

Well-developed smooth muscles exist in the capsule of the lymph node. Smooth muscles in the capsule of the lymph node are better developed in the axillary or inguinal lymph nodes than in the cervical lymph nodes. Lymphocytes and lymph are squeezed out by the capsular smooth muscles into the efferent vessels. The lymph nodes absorb about half of the water in the lymph into the blood. Lymphadenectomy for cancer treatment often causes lymphedema. Lymphadenectomy abrogates the above two functions of the lymph nodes to cause lymph retention and lymphedema. Lymphatic-venous anastomosis, as well as conservative therapies, such as lymph drainage and donning of an elastic stocking, is effective in treating lymphedema. Autologous lymph node transplantation should be therapeutically effective.

Acknowledgment

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Basic Research B−Grant No. 16390047).

References

1) Ohtani O, Kato S, Uchino S. Lymphatics Morphology, Function, and Development, Nishimura Shoten, Niigata, 1997. (in Japanese).
2) Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. Cell 1999; **98**: 769-78. [Medline] [CrossRef]
3) 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**: 385-94. [Medline] [CrossRef]
4) 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. [Medline] [CrossRef]
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 USA 1995; **92**: 3566-70. [Medline] [CrossRef]
6) Gunn MD, Tangemann K, Tam C, et al. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. Proc Natl Acad Sci USA 1998; **95**: 258-63. [Medline] [CrossRef]
7) Ebata N, Nodasaka Y, Sawa Y, et al. Desmoplakin as a specific marker of lymphatic vessels. Microvasc Res 2001; **61**: 40-8. [Medline] [CrossRef]
8) Leak LV, Burke JF. Fine structure of the lymphatic capillary and the adjoining connective tissue area. Am J Anat 1966; **118**: 785-809. [Medline] [CrossRef]
9) Gerli R, Solito R, Weber E, et al. Specific adhesion

molecules bind anchoring filaments and endothelial cells in human skin initial lymphatics. Lymphology 2000; **33**: 148-57. [Medline]
10) Ohtani O, Ohtani Y. Anatomy of lymphatic vessels. Jpn J Lymphol 2011; **34**: 32-5 (in Japanese).
11) Sabin FR. On the origin of the lymphatic system from the veins and the development of the lymph hearts and thoracic duct in the pig. Am J Anat 1902; **1**: 367-89. [CrossRef]
12) Oliver G. Lymphatic vasculature development. Nat Rev Immunol 2004; **4**: 35-45. [Medline] [CrossRef]
13) Mäkinen 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**: 4762-73. [Medline] [CrossRef]
14) Cueni LN, Detmar M. New insights into the molecular control of the lymphatic vascular system and its role in disease. J Invest Dermatol 2006; **126**: 2167-77. [Medline] [CrossRef]
15) Ohtani O. Three-dimensional organization of the connective tissue fibers of the human pancreas: a scanning electron microscopic study of NaOH treated-tissues. Arch Histol Jpn 1987; **50**: 557-66. [Medline] [CrossRef]
16) Ohtani O, Ushiki T, Taguchi T, et al. Collagen fibrillar networks as skeletal frameworks: a demonstration by cell-maceration/scanning electron microscope method. Arch Histol Cytol 1988; **51**: 249-61. [Medline] [CrossRef]
17) Ohtani O, Ohtani Y. Organization and developmental aspects of lymphatic vessels. Arch Histol Cytol 2008; **71**: 1-22. [Medline] [CrossRef]
18) Ohtani Y, Ohtani O, Nakatani T. Microanatomy of the rat diaphragm: a scanning electron and confocal laser scanning microscopic study. Arch Histol Cytol 1993; **56**: 317-28. [Medline] [CrossRef]
19) Ohtani Y, Ohtani O. Postnatal development of lymphatic vessels and their smooth muscle cells in the rat diaphragm: a confocal microscopic study. Arch Histol Cytol 2001; **64**: 513-22. [Medline] [CrossRef]
20) Shao XJ, Ohtani O, Saitoh M, et al. Development of diaphragmatic lymphatics: the process of their direct connection to the peritoneal cavity. Arch Histol Cytol 1998; **61**: 137-49. [Medline] [CrossRef]
21) Rutkowski JM, Boardman KC, Swartz MA. Characterization of lymphangiogenesis in a model of adult skin regeneration. Am J Physiol Heart Circ Physiol 2006; **291**: 1402-10. doi: 10.1152/ajpheart.00038.2006 [CrossRef].
22) Ohtani O, Ohtani Y, Carati CJ, et al. Fluid and cellular pathways of rat lymph nodes in relation to lymphatic labyrinths and Aquaporin-1 expression. Arch Histol Cytol 2003; **66**: 261-72. [Medline] [CrossRef]
23) Renkin EM. Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. Am J Physiol 1986; **250**: H706-10. [Medline]
24) Wissmann C, Detmar M. Pathways targeting tumor lymphangiogenesis. Clin Cancer Res 2006; **12**: 6865-8. [Medline] [CrossRef]
25) Murata H, Kawano S, Tsuji S, et al. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. Am J Gastroenterol 1999; **94**: 451-5. [Medline] [CrossRef]
26) Soumaoro LT, Uetake H, Takagi Y, et al. Coexpression of VEGF-C and Cox-2 in human colorectal cancer and its association with lymph node metastasis. Dis Colon Rectum 2006; **49**: 392-8. [Medline] [CrossRef]
27) Doki Y, Murakami K, Yamaura T, et al. Mediastinal lymph node metastasis model by orthotopic intrapulmonary implantation of Lewis lung carcinoma cells in mice. Br J Cancer 1999; **79**: 1121-6. [Medline] [CrossRef]