VL-DUAL SHORT REVIEW

Introduction of Relationship Between the Vascular and Lymphatic Systems in Lymphedema Diagnosis and Treatment Research

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Abstract In the past, the lymphatic and vascular systems were considered to be distinct circulatory systems. Consequently, diseases related to lymphatic and vascular circulation insufficiency were considered to have entirely different pathogenesis and treatment strategies. However, recent studies have reported a close interaction between these systems, revealing that dysfunction in lymphatic circulation can affect vascular insufficiency and vice versa. This new understanding has intimated the necessity of studying body fluid circulation from an integrated perspective that encompasses both capillaries and larger vessels in the lymphatic and vascular systems. In this review, we will introduce some cases observed in our research on lymphedema, a representative lymphatic insufficiency disease, focusing on the interrelation between the lymphatic and vascular systems identified in our findings. We studied lymphatic channel sheet (LCS), which is an artificial lymphatic scaffold for lymphangiogenesis, lymphangio-dynamics, which is a method to evaluate the lymphatic function, and quantified staging using magnetic resonance (MR) lymphangiography with gadolinium contrast agents. Although these studies were initiated to diagnose and treat lymphatic circulation disorders, we could observe associations with the vascular system. Based on these cases, we aim to discuss the intricate relationship between the lymphatic and vascular systems.

Keywords lymphatic system, vascular system, lymphedema, preclinical research, lymphangiography

Introduction

The lymphatic system, a part of both the circulatory and immune systems, encompasses a large network of lymph capillaries, collecting vessels, lymph nodes, and other lymphatic organs. It serves as a home and pathway for immune cells including lymphocytes[1-3], while playing an important role in filtering body waste and external invaders as part of its vital immune functions. Furthermore, the lymphatic system plays a significant role in circulating fluidics, in conjunction with the vascular system[4-5]. The discovery of the lymphatic system has a longer history than we might imagine. The history of the lymphatic system began around the 5th century BC with the Hippocratic School's discovery of lymphatic “glands” or lymph nodes, and Herophilus of Chalcedon's identification of lymphatic vessels[6-9]. These findings marked the recognition of another circulatory organ system, responsible for transporting a

clear fluid, similar yet distinct from the vascular system. Despite the long history since those early discoveries, our knowledge of the lymphatic system is not as extensive as that of the vascular system[10]. While the vascular system generates the fluidic propulsion through the heart as its central organ, the lymphatic system moves lymphatic fluid primarily through lymphangions, which are locally independent units performing self-propelled peristalsis and it also is influenced by the surrounding environment. Typically, the lymphangion produces a positive pressure of 50-60mmHg while heart, diaphragm/breathing, and muscle contraction make an average pressure of 10mmHg[11]. Although the deeper lymphatics which have relatively large sizes and the milky 'color' of the mesenteric lymphatics could be observed easily, most of the lymphatic system has been difficult to visualize and research[12,13]. The lymphatic vessels are generally composed of nearly a single layer of cells and they carry mostly colorless and clear lymphatic fluid while vascular vessels consist of several sturdy layers and blood is distinctly red in visible light. Lymphatics exist everywhere in our bodies, yet it is almost invisible like ghosts. However, the lymphatic system has recently been found to be associated with various diseases, including lymphedema, metastatic cancer, glaucoma, Crohn's disease, myocarditis, and even neurodegenerative disorders in these days[14]. Therefore, visualizing the lymphatics and detecting lymphatic characteristic signals are becoming increasingly important[15,16]. Moreover, these efforts for observation are shifting the focus of discussion to its relationship and connections with the vascular system, another component of the circulatory system[17,18]. In this review, we will discuss the relationship between the lymphatic and vascular systems, focusing on the research on lymphatic circulation disorders that we have demonstrated or are currently undertaking. All animal experiments in this manuscript were conducted in accordance with the relevant guidelines and regulations of the Institutional Animal Care and Use Committee (IACUC) of Asan Medical Center. The IACUC abides by the Institute of Laboratory Animal Resources and Animal Research: Reporting of in vivo Experiments guidelines of the National Center for the Replacement, Refinement, and Reduction of Animals in Research.

Lymphangiogenesis and angiogenesis in lymphatic channel sheet (LCS)

Prevention of lymphedema is as important as treatment. Considering that approximately 21.4% of patients develop the condition of breast cancer-related lymphedema following breast cancer surgery including lymph node dissection[19], a technique to reconnect broken lymphatic vessels during the surgery can be a novel treatment strategy. There is a surgical prevention technique known as LYMPHA (Lymphatic Microsurgical Preventive Healing Approach). However, it is a highly specialized microsurgical method that is difficult to apply universally and there is a lack of research on its effectiveness[20,21]. Artificial lymphatic structures for maintaining lymph flow and inducing lymphangiogenesis could be a relatively accessible alternative approach, and the Lymphatic channel sheet (LCS) is one of them. The LCS is an artificial micro-fluidic structure designed to maintain lymphatic flow and induce lymphangiogenesis, particularly in the context of breast cancer-related lymphedema[22]. The LCS was made from polydimethylsiloxane (PDMS) and features a two-dimensional multi-channel design to increase the possibility of lymphangiogenesis and facilitate easy fixation to tissues. The LCS was designed to be applied by cutting it to the size of a lymph node at the site where the lymph node has been excised and then fixing it between the severed lymphatic vessels on both sides. We demonstrated that the LCS has been shown to successfully transport lymphatic fluid in place of removed lymph nodes, preventing swelling and abnormal lymphatic drainage in the upper limb of small animal models using near-infrared fluorescence indocyanine green lymphangiography (NIRF-ICG L). Additionally, we demonstrated that the lymphatic flow passed through the interior of the channel and reached the proximal axillary lymph nodes based on NIRF-ICG L imaging and direct observations during surgical procedures.

Next, we needed to prove whether this flow was due to newly formed lymphatic vessels within the channels or just a fluid flow into the channels. Therefore, the implanted LCS was extracted after 8 weeks post-transplantation, and the tissue inside channels was examined histologically. The number of newly formed lymphatic vessels at the tens of microns diameter were identified inside the channels (Fig 1a.)[22]. The lymphatic vessels connected on either side of the removed lymph nodes were approximately 200 microns in diameter as collecting lymphatic vessels but they were connected by numerous small-sized lymphatic vessels generated inside the LCS after lymphangiogenesis. It is known from previous research that the direction of lymphangiogenesis is regulated by Piezo1 mechanotransduction signaling, following the direction of lymphatic fluid flow. Therefore, the LCS positioned between severed collecting lymphatic vessels became a pathway for the continuous lymphatic flow, leading to lymphangiogenesis along the direction of the channels.

Interestingly, angiogenesis around the newly formed lymphatic vessels also occurred in the same direction as the lymphatics. As can be seen in Figure 1b[22], numerous blood vessels of similar size to the lymphatic vessels, marked by the endothelial cell marker CD31, were near lymphatics. Moreover, these newly formed blood vessels shared the same directionality as the newly formed lymphatics. While

our study did not reveal the mechanism by which lymphatic and blood vessels share directionality during regeneration, we were able to observe that lymphangiogenesis and angiogenesis are more closely related and share directional neogenesis more than previously known.

Venous enhancement during magnetic resonance lymphangiography (MRL) in lymphedema animal model

When we injected gadolinium-based contrast agents (GBCA) into the interstitial space of the paw for MR lymphangiography in the animal experiment, we could observe the visualization of not only lymphatic vessels but also the blood vessels. Similarly, this challenging issue lies with MR lymphangiography using GBCA, where vascular images need to be separated and removed for accurate lymphatic circulation diagnosis in clinical settings[23,24]. Particularly, there is a high likelihood of interpretative challenges in cases of superficial venous enhancement because it is difficult to distinguish between lymphatic vessels and veins[25].

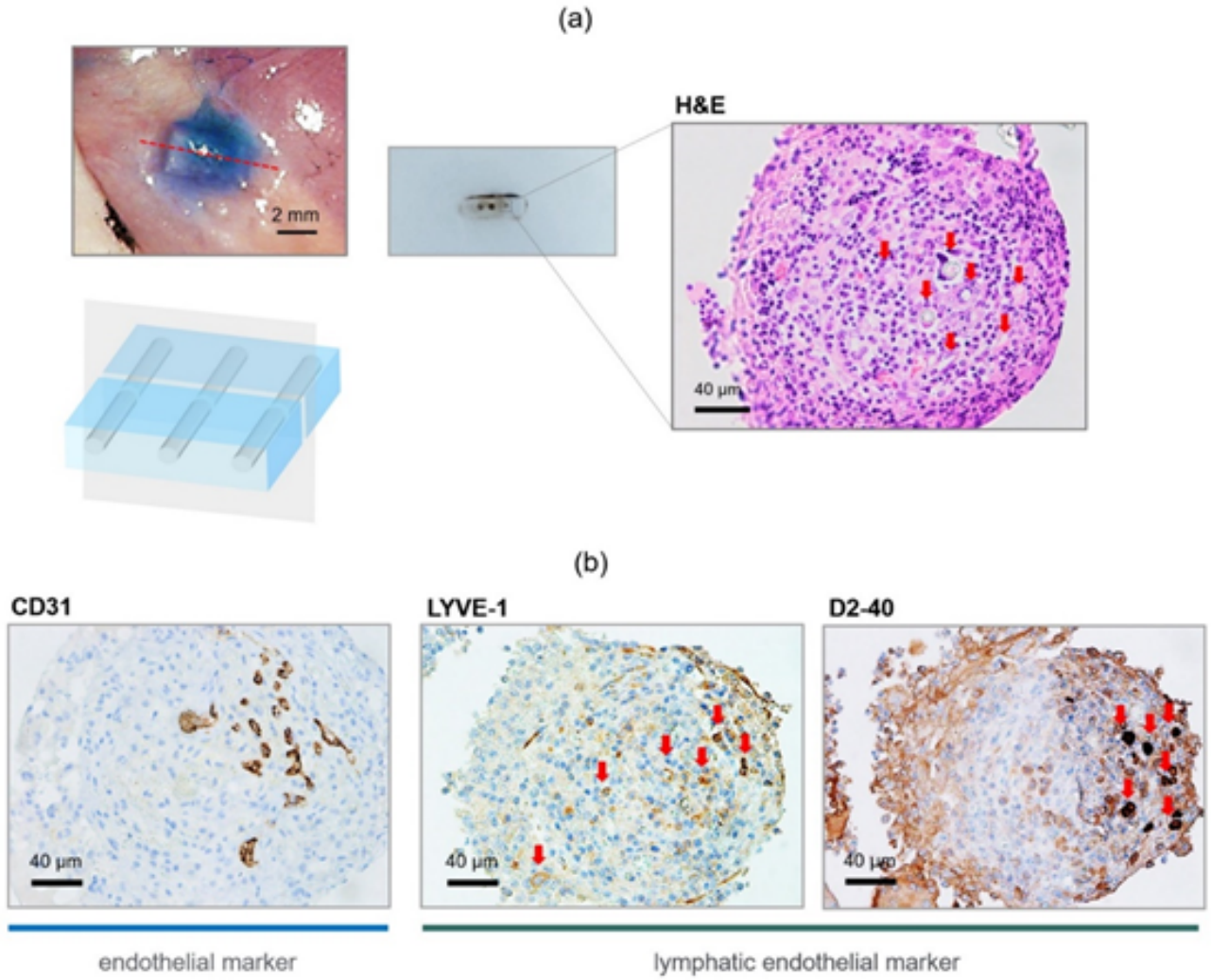

Figure 1 - After 8 weeks of implantation, the lymphatic channel sheet (LCS) underwent histological examination. (a) Sections of the LCS were taken at the midpoint, perpendicular to the channel orientation, and subjected to H&E staining to analyze the cross-sectional structure, with red arrows indicating the lumens. (b) Immunohistochemical analysis was performed to evaluate the regeneration of lymphatic and blood vessels within the channels, revealing multiple lymphatic and blood vessels with diameters ranging from 10 to 20 μm inside the LCS channels. This work is licensed under a Creative Commons Attribution 4.0 International License (Cheon H, et al., John Wiley & Sons Inc.)

This presents a significant obstacle in lymphatic research. The traditional Starling's Principle explains that the fluidic movement across the capillary walls is governed by the balance between hydrostatic and oncotic pressure gradients, and it allows to reabsorb the interstitial fluid from the arterial side, which has relatively higher pressure, to the venous circulation with lower pressure[26,27]. According to the explanation, since the movement of interstitial fluid is separated between the venous system and the lymphatic system, it explains why GBCA injected into the interstitial fluid for lymphatic imaging contaminates the venous system. However, the revised Starling's Principle renders this explanation less clear. The revised Starling's Principle suggests that interstitial fluid predominantly returns to the circulatory system through almost lymphatic circulation. The fluidic movement could not reverse into the venous system due to consistent pressure dynamics and the presence of the glycocalyx layer in the blood vessels[28]. The glycocalyx layer is a dense matrix of glycoproteins and proteoglycans coating the luminal surface of endothelium lining the capillaries. It produces an oncotic pressure gradient across the endothelial surface, and the forces controlling fluidic movement are affected by also the hydrostatic and oncotic pressures within the subglycocalyx space. Injecting a contrast agent solution can increase the pressure in its injection area, which might lead to a collapse of "consistent pressure" in the localized interstitial space, potentially explaining venous contamination. However, this explanation loses its persuasiveness in situations of lymphedema where the pressure within the lymphatic system is extremely high. Certainly, the traditional/revised Starling's principle is a theory explaining the circulation of fluids in a normal condition. However, because lymphedema represents a state of fluidic imbalance in the interstitium, it is useful for understanding our body's circulation and related diseases from a microscopic view.

In our animal model of Sprague-Daweley (SD) rats weighing 250 - 300 g, we surgically removed the popliteal and inguinal lymph nodes which are major lymphatic drainage pathways in the lymphosome of the hindlimb, and incised the skin up to the fascia along the circumference of the limb, creating a 2-mm gap of skin fixed to the muscles on both sides. Two days post-surgery, the surgical site was irradiated with 20 Gy of radiation to induce tissue fibrosis. Although the surgical wounds healed with scarring after approximately 1 week, the small epidermal lymphatics included in the skin were severed and disconnected by a massive dam of fibrotic tissue. This method of model formation causes extreme disruption of lymphatic circulation in the distal part of the limb including the paw, and induces hindlimb lymphedema[29].

In this setting, we injected one of the GBCAs, gadoterate meglumine (Dotarem®), in a volume of 0.02 ml into the interstitial space of the paw in the lymphedema animal models and acquired MRL images through a 3D heavily T2-weighted sequence. Additionally, by comparing with magnetic resonance venography (MRV) images obtained using the same method but with the agent injected into a vein, we were able to ascertain the positions of lymphatic and blood vessels. The results showed that vein enhancement could be observed alongside lymphatics imaging even in lymphedema limbs (red arrows in Figure 2). This suggests that under lymphedema conditions, the interstitial fluid was easily reabsorbed into the venous system due to the high pressure within the interstitium. It indicates a dysfunction of the glycocalyx layer in the vascular endothelium and the possibility that lymphedema potentially raises venous pressure as well by increasing fluidic flow to the veins. This is seen as a phenomenon indicating a correlation between lymphedema and venous hypertension[18,28]. While further research and a deeper understanding of the mechanism are needed, long-term lymphatic circulatory disorders could potentially induce vascular diseases considering the critical role of the glycocalyx layer in vessel-wall homeostasis[30]. The discovery of the glycocalyx layer has expanded our understanding of the microfluidic dynamics in the interstitium by highlighting the complexity of the capillary barrier and the sophisticated nature of the fluidic exchange between the bloodstream and tissues. Furthermore, it allows for the observation of the interactions between the lymphatic and vascular vessels to underscore the importance of the lymphatic system's role in health and disease.

Conclusion

In this review, we discussed the correlation between lymphatic and vascular circulation from the perspectives of disease treatment and onset based on our results of the preclinical research. Although the studies we have conducted or are conducting were not directly aimed at investigating the correlation between the lymphatic and vascular systems, we have obtained several clues about their relationship in the process of basic research for lymphedema treatment and diagnosis. While more research and evidence are needed, we hope that what we have observed may serve as a significant starting point for future studies.

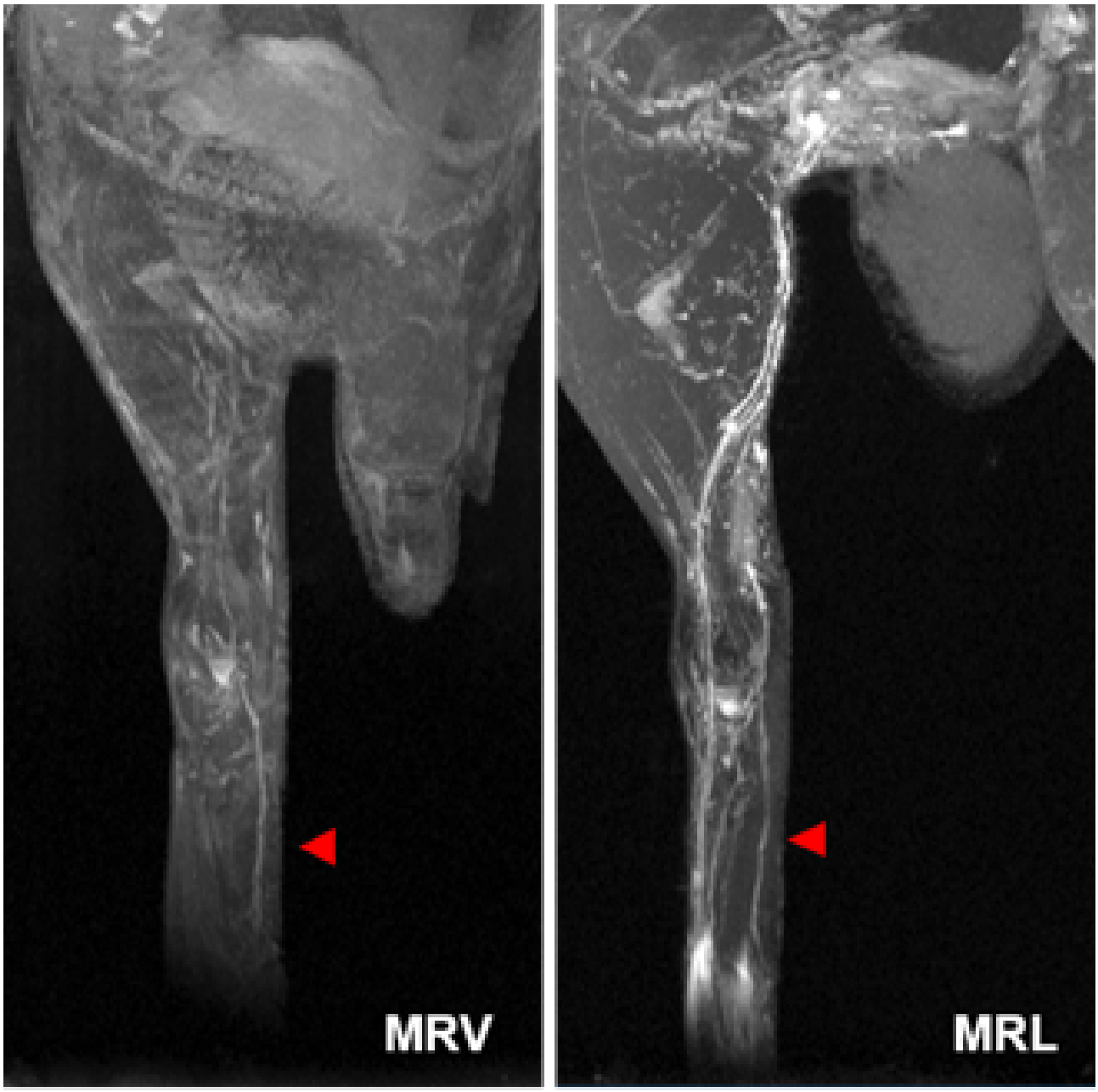

Figure 2 - The images obtained from direct injection of Dotarem® into a vein (MR venography, MRV) and from injection into the interstitial space (MR lymphangiography, MRL). The vein enhancement appeared in both sets of images (red arrows).

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