Structure, Function, and Molecular Control of the Skin Lymphatic System

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The mechanisms of angiogenesis have been studied extensively over the past years. The focus, however, has been almost exclusively on blood vessels, whereas little effort has been directed toward understanding lymphangiogenesis and the role of lymphatic vessels in physiology and pathology. The lymphatic system, acting in concert with the blood vascular system, is of fundamental importance in maintaining tissue homeostasis, and disorders of the lymphatic system are common, often resulting in chronic, disabling conditions. This overview summarizes the most important aspects of the structure and function of the lymphatic system with emphasis on the skin lymphatic vasculature and the differences between blood and lymphatic vessels. Special attention has been given to the methods employed in research of the lymphatic system. Finally, we describe molecular mechanisms involved in the regulation of lymphangiogenesis. Vascular endothelial growth factor and vascular endothelial growth factor-C, expressed by distinct skin cell populations, play an important role in the molecular control of skin angiogenesis and lymphangiogenesis. ***Key words: lymphatic vessels/lymphangiogenesis/skin/VEGF-C. Journal of Investigative Dermatology Symposium Proceedings 5:14–19, 2000***

Our understanding of the biology of the lymphatic system is well illustrated by the word lymphatic itself; the derivation of the latin word *lymphaticus* signifies ''distracted and confused'' (Witte *et al*, 1997). Whereas angiogenesis involving blood vessels has been studied extensively over the past decades, the lymphatic system has received only scant attention and molecular mechanisms regulating lymphatic functions and growth have remained largely unknown. Although structurally two distinct systems, the blood and lymphatic vascular system are functionally interconnected and act together to ensure fluid and protein balance in tissues, cell nutrition, proper immunologic functioning, etc. In many ways the lymphatic system complements the blood vascular system. Thus, it is most likely that their growth and functions are regulated in concert. The regulatory mechanisms and structural elements shared by and distinct to the lymphatic *versus* blood vascular system are only beginning to be disclosed.

DISCOVERY OF THE LYMPHATIC SYSTEM

Lymphatic vessels remained unrecognized for many centuries after the first description of blood vessels. Whereas the blood vascular system and most other systems of the body have been known since ancient times, although knowledge about their organization and function was inaccurate, the lymphatic system was truly ''discovered'' (Bartels, 1909). The ancient Greeks observed structures containing colorless fluid (Hippocrates spoke of ''white blood'') but their function was not understood and the significance of the finding was not recognized. For almost 2000 years this observation fell into complete oblivion (Rusznyak *et al*, 1967). The actual discovery of lymphatic vessels dates back to the year 1622 when Gasparo Aselli, a professor of anatomy and surgery in Pavia, Italy, observed vessels in the mesentery of a well-fed dog that were filled with a white fluid (Bartels, 1909; Rusznyak *et al*, 1967; Haagensen *et al*, 1972). He instantly realized that he had made an important observation and wrote an enthusiastic description of it. Because of the milky appearance of the vessels, Aselli named them *lacteis venis* – milky veins. This first description of lymphatic vessels was published by his friends 5 years later, after his death.

Aselli mistakenly believed that the lymph flowed to the liver, where it was transformed to blood. In 1651, Jean Pecquet discovered the thoracic duct and its entry into the veins, thus becoming the first person to describe the correct route of the lymphatic fluid into the blood. While it had been assumed at the time that lymphatic vessels were the main pathway for food absorption from the intestines, Olaus Rudbeck in Uppsala revealed that the role of lymphatic vessels is not restricted to food absorption. He observed that lymphatic vessels are distributed throughout the body and recognized that these vessels form a special system, the lymphatic vascular system (Bartels, 1909; Rusznyak *et al*, 1967). Thomas Bartholin in Copenhagen, who independently made similar observations, first introduced the term *lymphaticus* in 1653 (Yoffey and Courtice, 1970). Although Rudbeck and Bartholin suggested that lymphatic vessels may be involved in the convection of fluid filtered from blood, the functions of the lymphatic system remained poorly understood until 1746 when William Hunter and his pupils at the School of Anatomy in London demonstrated that lymphatic vessels are the absorbing vessels throughout the body. Their comprehensive work

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Abbreviations: bFGF, basic fibroblast growth factor; HDMEC, human dermal microvascular endothelial cells; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; PlGF, placenta growth factor; VEGF, vascular endothelial growth factor.

laid the foundations for the modern knowledge of the anatomy, physiology and pathology of the lymphatic system.

By the end of the eighteenth century, the anatomy of the larger lymphatic truncs in the body had been correctly elucidated; however, the structure and the organization of lymphatic capillaries remained unknown. Studies of the fine structure of the lymphatic system were greatly facilitated by von Recklinghausen's discovery in 1862 that the borders of endothelial cells stained black with silver nitrate. It had been thought at the time that the lymph passed from the blood stream into the lymphatic vessels through a fine tubular system. Recklinghausen denied a direct connection between the two systems and suggested that the end of the lymphatic capillary was open and that fluid from the interstitial space passed through these open endings into the lymphatics. He very accurately described the network of lymphatic capillaries and showed that they are lined by a single layer of endothelial cells (Rusznyak *et al*, 1967).

Late in the 19th century Ludwig suggested that the lymph was a filtrate derived from blood. His concept was confirmed in 1896 by Starling, who demonstrated through a number of sophisticated experiments that the lymph is formed from the blood by filtration (Yoffey and Courtice, 1970). While Starling had realized that capillaries leak protein, it was Drinker, early in the 20th century, who developed the concept of the lymphatic system as one of absorbing vessels, the main function of which was to return to the blood stream fluid and protein molecules that escape from the blood circulation (Drinker and Yoffey, 1941).

FUNCTION AND TISSUE DISTRIBUTION OF LYMPHATIC VESSELS

The lymphatic system is of fundamental importance for the normal maintenance of fluid balance in tissues. In the course of a day, 50% of the total circulating protein escapes from the blood vessels and cannot be reabsorbed by the same. Therefore, return of the extravasated fluid and macromolecules into the blood stream is crucial for the maintenance of plasma volume and to prevent an increase in tissue pressure. Furthermore, consistent clearance of excess fluid from the interstitium assures adequate cell nutrition by keeping the distances between cells and capillaries to a minimum. The lymphatic system is also involved in the response of the organism to infection. Lymphatic vessels direct antigen presenting cells to the lymph nodes and are thus essential for the development of cellular immunity. In the skin, lymphatic vessels are an important exit path for Langerhans cells. Impairment of lymphatic functioning, e.g., inadequate transport of fluid, macromolecules, or cells from the interstitium, leads to a number of diseases that are characterized by edema, impaired immunity, and fibrosis (Ryan, 1989; Witte *et al*, 1997).

Although lymphatic vessels generally accompany blood vessels in tissues, the density of lymphatic plexus does not always parallel the abundance of blood supply. There are no lymphatic vessels in the central nervous system, and lymphatic vessels do not penetrate as far as blood vessels in several other well vascularized tissues. In lobular organs such as liver and mammary gland, lymphatic capillaries do not penetrate the lobules but are arranged around the periphery. In the spleen and the thymus, lymphatics have been observed only in the capsule and the thickest trabeculae, and in voluntary muscle they are confined to the fascial planes and do not enter the muscle bundles. Other tissues, such as the epidermis, the cornea, the crystalline lens of the eye, and cartilage are devoid of both blood and lymphatic vessels. Tissues near the surface of the body, e.g., the mucous membranes of the gastro-intestinal and respiratory tracts and the skin, are usually richly supplied with lymphatic vessels (Yoffey and Courtice, 1970).

Lymphatic vessels themselves are supplied by a rich network of nutritive blood vessels. In fact, a blood capillary network is present on the surface of all lymphatic vessels except the lymph capillaries. Abundant blood supply probably reflects the relatively high oxygen requirements of cells due to lymph vessel contractility (Evans, 1907).

THE SKIN LYMPHATIC SYSTEM

The cutaneous blood microvasculature is organized into upper and lower horizontal plexuses with the dermal capillary loops arising from the upper plexus (Braverman, 1989). Likewise, the lymphatic vessels of human skin form two plexuses (**Fig 1**) (Kampmeier, 1928; Zhdanov, 1952; Ryan, 1978). The superficial plexus extends into the dermal papillae and is found near the subpapillary arterial network. It consists of thin vessels without valves (Sabin, 1904). Whereas the bulk of the blood microcirculation resides immediately below the epidermis, lymphatic vessels are situated somewhat more distant. From the superficial plexus, branches drain vertically into a series of larger lymphatic vessels in the lower dermis and the superficial zone of the subcutaneous tissue (Forbes, 1937). The deep lymphatic plexus is situated below the second arterial network. Similar to the collecting venules of the lower dermis, the deep lymphatic vessels contain numerous valves. Whereas blood vessels are found at the junction of the fat layer and the dermis and within the fat lobules, lymphatic vessels are not contained within the subcutaneous adipose tissue (Zhdanov, 1952; Braverman and Keh-Yen, 1981). Although blood and lymphatic capillaries may lie immediately adjacent to each other they never anastomose (Clark and Clark, 1937).

The described cutaneous lymph vessel configuration is similar throughout the body; however, certain areas such as the fingers, the palm of the hand, the sole of the foot, and the scrotum appear to have a more abundant lymphatic network. The structure of the cutaneous lymphatics is dependent on the structure of the skin at the particular site and can thus vary in different areas. Generally, lymphatic vessels have a regular, uniform shape in the areas where the skin is firm and thick, whereas the shapes are more variable in regions where the skin is thin and loose.

Blood and lymphatic vessels are similar in that they form a system of tubes with a continuous endothelial lining; however, reflecting differences in their function, the structure of lymphatic capillaries is different from that of blood capillaries in several important aspects (**Fig 2**) (Casley-Smith and Florey, 1961; Leak, 1970; Daroczy, 1988). (1) The lymphatics generally possess a wider and more irregular lumen than blood capillaries. In the papillary dermis, the outside diameters of blood vessels are generally in the 17–22 μm range (Braverman, 1989), whereas lymphatic vessels can reach up to 60 μm in diameter. (2) Lymphatic capillaries are characterized by an endothelium with extremely attenuated cytoplasm, except in the

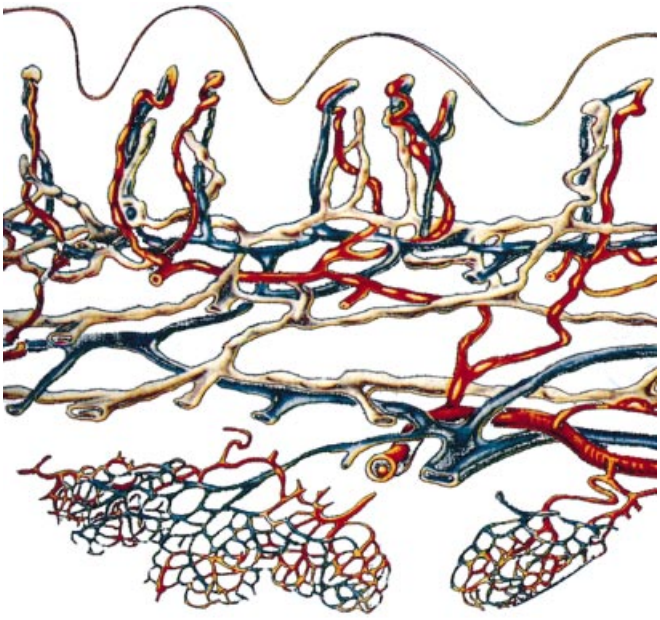

Figure 1. Relation between the lymphatic and blood-capillary network of the skin. Lymphatic vessels (white); arterial (red) and venous (blue) parts of the blood-capillary network (modified after Zhdanov, 1952).

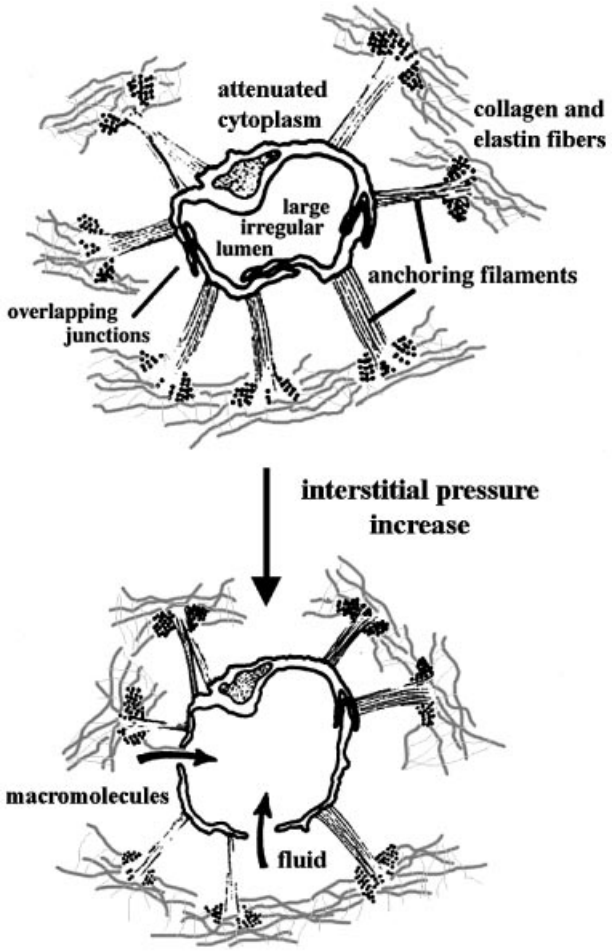

Figure 2. Structural features of a dermal lymphatic capillary. Lymphatic endothelial cells are firmly attached to the collagen and elastin fibers in tissues by anchoring filaments. An increase of the interstitial pressure caused by an increased fluid volume in tissue stretches the fibers and expands the lymphatic lumen. Furthermore, overlapping intercellular junctions facilitate opening of the intercellular channels, allowing easy passage of fluid and macromolecules into the vessel (modified after Leak, 1970).

perinuclear region. Whereas postcapillary venules in the horizontal dermal plexus that are comparable in size with the lymphatic vessels of the area possess a vessel wall thickness of 3.5–5 μm, the vessel walls of lymphatic capillaries measure 50–100 nm, except in the nuclear region where the vessel wall thickness can reach up to 6 μm. (3) Lymphatic endothelial cells contain numerous fine cytoplasmic filaments whose longitudinal orientation implicates their contractile function. (4) In contrast to blood vessels, lymphatic capillaries have either no or only a poorly developed basal lamina and (5) they are not encircled by pericytes. (6) Tight junctions and adherens junctions, which are major types of intercellular junctions in blood vessels, are not as frequently seen in lymphatics. Nevertheless, they are implicated in maintaining firm cell–cell adhesion along the lymphatic vessel. In lymphatic vessels these adhesive molecules represent focal points of adhesion instead of connecting adjacent endothelial cells over entire cell boundaries as in blood vessels. The fact that skin blood and lymphatic capillaries do not anastomose suggests the possible existence of lymphatic endothelium specific cell adhesion molecules. (7) One of the most striking characteristics of lymphatic capillaries is that they come into an intimate association with the adjacent interstitial areas. The end of the lymphatic capillary is open for free passage of fluid and particles into the vessel, and lymphatic endothelial cells are closely connected to the surrounding tissue by fine strands of reticular fibers and collagen (Pullinger and Florey, 1935). These anchoring filaments are attached to the abluminal surface of the cells and extend deeply into the connective tissue, thereby firmly attaching lymphatic endothelium to interstitial collagen fibers and to the network of elastic fibers (**Fig 2**). Elastic fibers represent a low resistance path for the transinterstitial transport of fluid and are thus regarded as prelymphatic pathways (Ryan, 1989). In the skin, they are ideally arranged for directing fluid into the lymphatics; in the upper dermis fibers are oriented perpendicularly whereas they are oriented horizontally in the lower dermis.

Lymphatic capillaries respond to increased demands for fluid transport by widening their lumina. An increase in the interstitial fluid volume stretches the connective tissue fibers and pulls the lymphatic vessels open, despite an increase in the interstitial pressure. Greater demand for fluid uptake is also accommodated by opening of intercellular junctions. (8) Overlapping intercellular junctions formed by extensive superimposing of adjacent endothelial cells are a property unique to lymphatic vessels. By being loosely apposed to each other over long distances, lymphatic endothelial cells cast intercellular clefts. When interstitial fluid pressure rises and exceeds the pressure within the lymphatic vessel, anchoring filaments open the intercellular channels by pulling adjacent endothelial cells apart, thus permitting easy passage of fluids and particles into the vessel.

Under normal circumstances, lymphatic capillaries are generally collapsed. An elevation of interstitial pressure from values of –7 to +2 mmHg distends lymphatic vessels and increases lymph flow. When the interstitial fluid pressure is raised to more positive values, however, there is no further increase in flow. Thus, the inability of the lymphatic system to accommodate excess fluids leads to edematous conditions as the interstitial fluid pressure approaches +2 mmHg (Guyton, 1965; Taylor *et al*, 1973). The concept of anchoring filaments explains why venules are compressed in inflammatory reactions and other conditions associated with increased fluid accumulation, whereas lymphatics are greatly dilated (Pullinger and Florey, 1935). The functional state of lymphatic vessels, however, cannot be determined by their morphology, as lymphatics can be dysfunctional when overdistended, but also when they are collapsed. Extensive degradation of the extracellular matrix, e.g., by hyaluronidase, induces a collapse of lymphatic vessels and renders them nonresponsive to the changes in the interstitium. Hence, the functioning of lymphatic vessels is critically dependent on the extracellular matrix composition, geometry, and integrity.

LYMPHATIC IMAGING

One major reason for our incomplete understanding of lymphatic physiology has been the lack of convenient methods to visualize lymphatic vessels. Classical methods to visualize lymphatics are interstitial injections of an agent that readily enters the lymphatic capillaries, spreads throughout the plexus and drains into the collecting ducts and regional lymph nodes. Anatomists in the last centuries performed postmortem lymphography by using injection materials such as water, gelatine, wax, oil, ink, prussian blue, and mercury. More recently, vital dyes such as Trypan blue, Evans blue, or patent blue have been used extensively. In most of the earlier experiments, contrast medium was injected into the tissue from which it then entered the lymphatic vessels. Later, injection of a dye was performed first to delineate the peripheral lymphatic vessels that were then cannulated and infused with a contrast agent directly into the lumen (Yoffey and Courtice, 1970). Conventional lymphography is based on the use of a direct oil contrast and has been extensively used to visualize lymphatic vessels in living subjects; however, this technique has notable limitations because it is frequently accompanied by serious side-effects. More recently, significant advances in lymphatic imaging have been achieved by introduction of water-soluble contrast agents, isotopes, and fluorescent tracers. Fluorescence micrography, providing superior contrast and resolution, is particularly suitable for visualization of microlymphatics. FITC-labeled tracers that do not readily enter

blood vessels, e.g., high molecular mass dextranes, have been successfully used to visualize lymphatic capillaries in experimental models (**Fig 3**). Lymphangioscintigraphy (isotope lymphography) that also allows functional and quantitative insights into the lymph flow kinetics, currently represents the preferred method for imaging lymphatics in patients. Other promising techniques include computed tomography and magnetic resonance imaging, the latter being particularly promising for visualization of dermal lymphatics (Witte *et al*, 1993).

Research of the lymphatic system has been hampered by the lack of positive markers that reliably distinguish blood from lymphatic endothelial cells. Lymphatic vessels, in particular capillaries, have been mostly identified by certain features that they either lack or express at lower levels when compared with blood vessels (**Table I**). The most ubiquitous endothelial cell marker, CD31 (PECAM-1), is expressed in both blood and lymphatic vessels (Albelda *et al*, 1991; Erhard *et al*, 1996). Based on their poorly developed basement membrane (Leak and Burke, 1966; Leak, 1970), lymphatic vessels can be identified by double immunostainings with antibodies directed to the CD31 antigen and to components of the basement membrane such as collagen type IV or laminin (Nerlich and Schleicher, 1991). Likewise, lymphatic vessels can be identified as CD31+/PAL-E-vessels because the PAL-E antigen is a specific marker for blood vasculature (Schlingemann *et al*, 1985, 1991). Lymphatic vessels appear not to express ICAM-1, VCAM-1, and E-selectin, adhesion molecules commonly expressed in blood vascular endothelium (Erhard *et al*, 1996). Desmoplakin and plakoglobin, cell adhesion molecules contained within *complexus adhaerentes* junctions, are found in lymphatic but not in blood vessels, yet they are also expressed in desmosomes of stratified squamous epithelium (Schmelz *et al*, 1994). High activities of the enzymes 5′-nucleotidase, adenylate, and guanylate cyclase have been reported specifically in lymphatic endothelial cells (Nishida and Ohkuma, 1992, 1993; Weber *et al*, 1994). Moreover, the surface charge distribution appears to be reversed in lymphatic vessels as compared with blood vessels, with a high density of anionic sites along the luminal side (Leak, 1986).

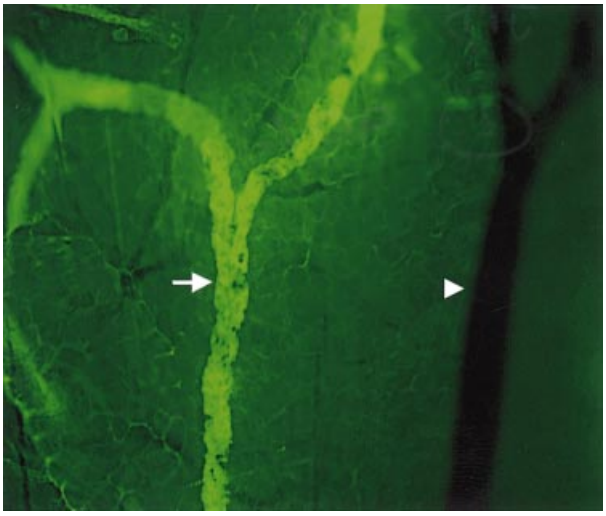

Figure 3. Visualization of skin lymphatic vessels by uptake of FITC-dextrane. High-molecular weight FITC-dextrane (MW 500 000) was injected intradermally into mouse skin (10 μL of a 2% solution in 0.9 M NaCl). Skin, turned upside down, was examined 30 min after injection by using a Leica DM IRBE microscope. Lymphatic vessels (*arrow*) have taken up FITC-dextrane from the interstitium and exhibit bright fluorescence. Blood vessels (*arrowhead*) are unable to absorb the high-molecular mass dextrane and remain unlabeled. Note that the lymphatic vessel runs in parallel with a blood vessel.

Very recently, several novel molecules have been identified that allow a more precise distinction between lymphatic and blood vascular endothelium. An antibody to the receptor for the vascular endothelial growth factor-C (VEGF-C), VEGFR-3 (flt4), has been shown to selectively label lymphatic endothelia in several normal tissues and in vascular tumors of lymphatic origin (Jussila *et al*, 1998). Likewise, podoplanin has been found to be selectively expressed in the normal lymphatic endothelium of the skin and kidney, and in vascular tumors of lymphatic origin (Breiteneder-Geleff *et al*, 1999; Weninger *et al*, 1999). Most recently, a novel hyaluronan receptor termed LYVE-1 has been shown to be restricted to lymphatic vessels in a number of normal tissues (Banerji *et al*, 1999). In human skin, strong expression of LYVE-1 is detected specifically in the CD31+/PAL-E-vessels, revealing a remarkably rich lymphatic network (**Fig 4**).

MOLECULAR REGULATION OF LYMPHANGIOGENESIS IN THE SKIN

Lymphatic capillaries possess a great capacity to regenerate (Daroczy, 1988; Witte *et al*, 1997). Growth of lymphatic vessels has been observed in various normal and pathologic processes such as wound healing, inflammation, and tumor growth. During wound healing, lymphangiogenesis is as prominent as angiogenesis of blood vessels. Generally, regeneration of lymphatics succeeds that of blood vessels, sometimes with a considerable delay. Studies using the rabbit ear chamber technique showed that blood vessels invaded the wound area in an average time of 7 days, whereas the ingrowth of lymphatic vessels was only observed after 19 days. The cause of this disparity in the growth rate is unclear (Yoffey and Courtice, 1970; Witte *et al*, 1997). The fact that the regeneration of lymphatic and blood vessels is spatially and temporally coordinated implies the existence of mutual regulatory mechanisms; however, the delayed outgrowth of lymphatics suggests different responsiveness to the stimuli involved.

Table I. Expression of vascular markers in blood and lymphatic vessels

	Microvasculature		References
	Lymphatic	Blood	
CD31 (PECAM-1)	+	+	Albelda *et al*, 1991; Erhard *et al*, 1996
PAL-E	–	+	Schlingemann *et al*, 1985; Erhard *et al*, 1996
basal lamina	+/–	+	Leak, 1970; Nerlich and Schleicher, 1991
desmoplakin	+	–	Schmelz *et al*, 1994
5′-nucleotidase	+	–	Weber *et al*, 1994
adenylate/guanylate cyclase	+	–	Nishida and Ohkuma, 1992; Nishida and Ohkuma, 1993
ICAM-1	–	+	Erhard *et al*, 1996
VCAM	–	+	Erhard *et al*, 1996
E-selectin	–	+	Erhard *et al*, 1996
VEGFR-3 (flt-4)	+	–	Jussila *et al*, 1998
podoplanin	+	–	Breiteneder-Geleff *et al*, 1999; Weninger *et al*, 1999
LYVE-1	+	–	Banerji *et al*, 1999

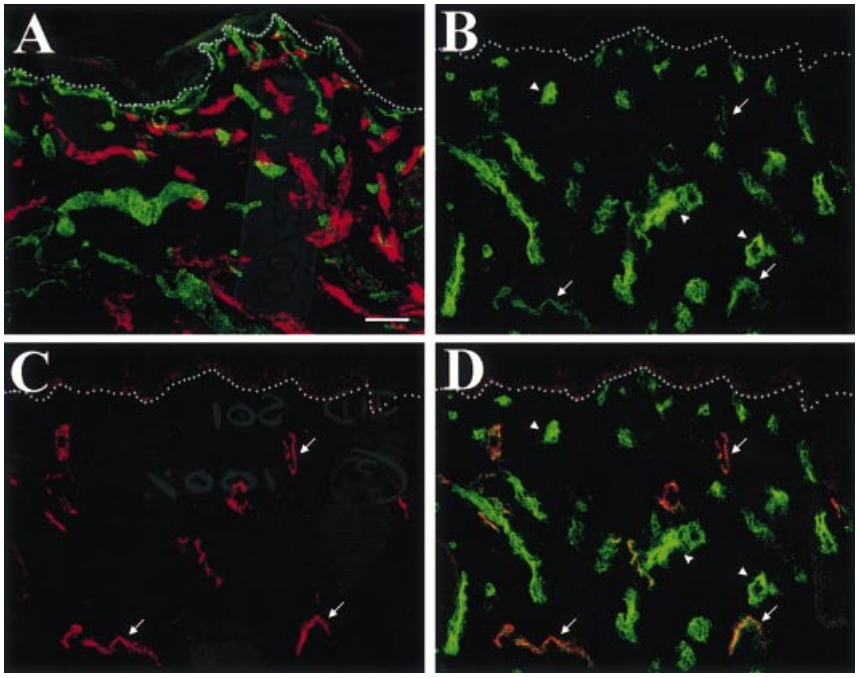

Figure 4. Expression of the endothelial cell markers LYVE-1, PAL-E, and CD31 in the cutaneous vasculature. (*A*) Double immunofluorescent staining of LYVE-1 receptor (red) and PAL-E antigen (green) in 50 μm thick section of human foreskin. The stainings are mutually exclusive, indicating high specificity of the LYVE-1 receptor for lymphatics and of PAL-E for blood vessels. Note the high lymphatic vessel density. (*B–D*) Double immunofluorescent staining of 6 μm thick skin sections with antibodies to (*B*) CD31 and (*C*) LYVE-1; (*D*) overlapping image of (*B*) and (*C*). CD31 is clearly expressed in both lymphatic (*arrows*) and blood (*arrowheads*) vessels. Note that all lymphatic vessels express CD31. Dots indicate dermal–epidermal junction. *Scale bar*: 20 μm.

A large number of molecules have been identified that are implicated in regulating blood vascular angiogenesis, whereas molecules regulating growth and function of lymphatic vessels have remained largely unrevealed. Recently, novel members of the VEGF family of angiogenic growth factors have been identified that are distinguished by their capacity to stimulate lymphangiogenesis (Achen *et al*, 1998; Joukov *et al*, 1996; Lee *et al*, 1996). Similar to the major angiogenic factor VEGF, VEGF-C and VEGF-D activate the VEGFR-2 (KDR) receptor; however, they do not bind to the VEGFR-1 (flt-1). VEGF-C and VEGF-D also activate the receptor tyrosine kinase VEGFR-3, which has been regarded as a specific marker of lymphatic endothelium (Joukov *et al*, 1996; Lee *et al*, 1996; Achen *et al*, 1998). Indeed, overexpression of VEGF-C in the skin of transgenic mice resulted in hyperplasia of the lymphatic network without obvious effects on blood vessels (Jeltsch *et al*, 1997).

In vitro analysis of VEGF-C mRNA expression in different cells derived from human skin reveals abundant expression of VEGF-C in dermal fibroblasts and low basal expression levels in keratinocytes, melanocytes, and microvascular endothelial cells (**Fig 5**). In human keratinocytes, expression of VEGF-C is strongly upregulated by TGF-α (**Fig 5**), whereas KGF, HGF, TGF-β1, and IGF-1, potent inducers of VEGF expression, had no effect. TGF-α also potently increased VEGF-C mRNA levels in immortalized HaCaT keratinocytes and in A431 epidermoid carcinoma cells. These results reveal a distinct regulation of VEGF and VEGF-C gene expression in keratinocytes. Recently, we observed significant amounts of VEGF-C mRNA and protein in blood vascular endothelium adjacent to Kaposi's sarcomas *in vivo* and have verified this finding using human dermal microvascular endothelial cells (HDMEC) *in vitro* (Skobe *et al*, 1999). Moreover, we found that VEGF dose-dependently stimulated VEGF-C expression in HDMEC, whereas no effects were observed upon stimulation with other growth factors tested, including PDGFs, bFGF, PlGF, and TNFα. Upregulation of the lymphangiogenic factor VEGF-C by the major angiogenic factor VEGF reveals a new molecular mechanism that may regulate blood and lymphatic vessel growth in concert.

In fibroblasts, VEGF-C is expressed at higher steady-state levels than VEGF and is induced by serum and by the inflammatory cytokines IL-1α, IL-1β, and TNFα. Hypoxia and oncogenes, however, important inducers of VEGF expression, had no effect on VEGF-C expression (Enholm *et al*, 1997; Ristimaki *et al*, 1998). Because dermal fibroblasts are susceptible to alterations of conditions in the interstitium, it is conceivable that they rapidly respond to the mechanical stress caused by changes of interstitial pressure to modulate the function of lymphatic vessels. Thus, fibroblast–lymphatic endothelial cell interactions may be of particular importance in regulating lymphatic function.

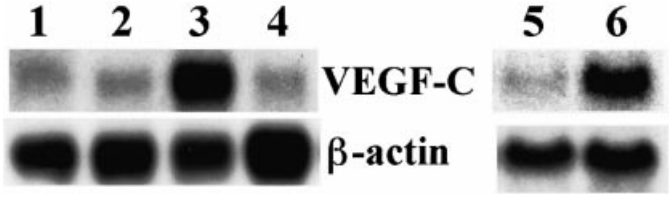

Figure 5. Northern blot analysis of VEGF-C mRNA (2.4 kb) expression in cultured cells derived from human skin. (1) Melanocytes; (2) keratinocytes; (3) fibroblasts; (4) microvascular endothelial cells; (5) untreated; and (6) TGFα treated human keratinocytes (100 ng/ml, 6 h). TGFα strongly upregulates expression of VEGF-C in keratinocytes. Hybridization with a β-actin probe served as a loading control.

In conclusion, these results indicate a distinct regulation of VEGF and VEGF-C expression in skin-derived cells. VEGF and VEGF-C appear to act cooperatively to regulate vascular permeability, lymphatic drainage, blood vascular angiogenesis, and lymphangiogenesis.

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