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Lymphangiogenesis and metastasis—A closer look at the neuropilin/semaphorin3 axis

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ABSTRACT

Metastasis is the leading cause of cancer-related deaths. Understanding how the lymphatic system responds to its environment and local stimuli may lead to therapies to combat metastasis and other lymphatic-associated diseases. This review compares lymphatic vessels and blood vessels, discusses markers of lymphatic vasculature, and elucidates some of the signaling motifs involved in lymphangiogenesis. Recent progress implicating the neuropilin and semaphorin axes in this process is discussed.

Contents

Introduction

The framework of the blood vascular system is that of a closed circulatory system that pumps blood from the heart to all organs and tissues in the body, delivering oxygen from red blood cells and nutrients from the serum. The lymphatic system is like a recycling system as it returns extra fluids that leak into the interstitial space back to the venous system. In peripheral tissues, the two systems never intermix, and homeostasis is only ensured when both systems are working properly.

Although both the blood and lymphatic systems are lined with endothelial cells (ECs), the organization and function of each are different (Kaipainen and Bielenberg, 2010). Fluid and oxygen exchange takes place at the capillary bed, not in larger vessels like arteries or veins. Mature blood capillaries are surrounded by a basement membrane and supported by pericytes—components that ensure the stability of the blood capillary. Lymphatic capillaries, on the other hand, lack a continuous basal lamina and are not associated with pericytes, as either of these components would interfere with their function (Sauter et al., 1998). In contrast, terminal lymphatic endothelial cells (LECs) are attached to the extracellular matrix via anchoring filaments (elastic fibers) (Leak and Burke, 1968). Initial lymphatic capillaries have overlapping LECs with "button-like" junctions between cells that act as a primary valve or gate regulating the uptake of fluid (Baluk et al.,

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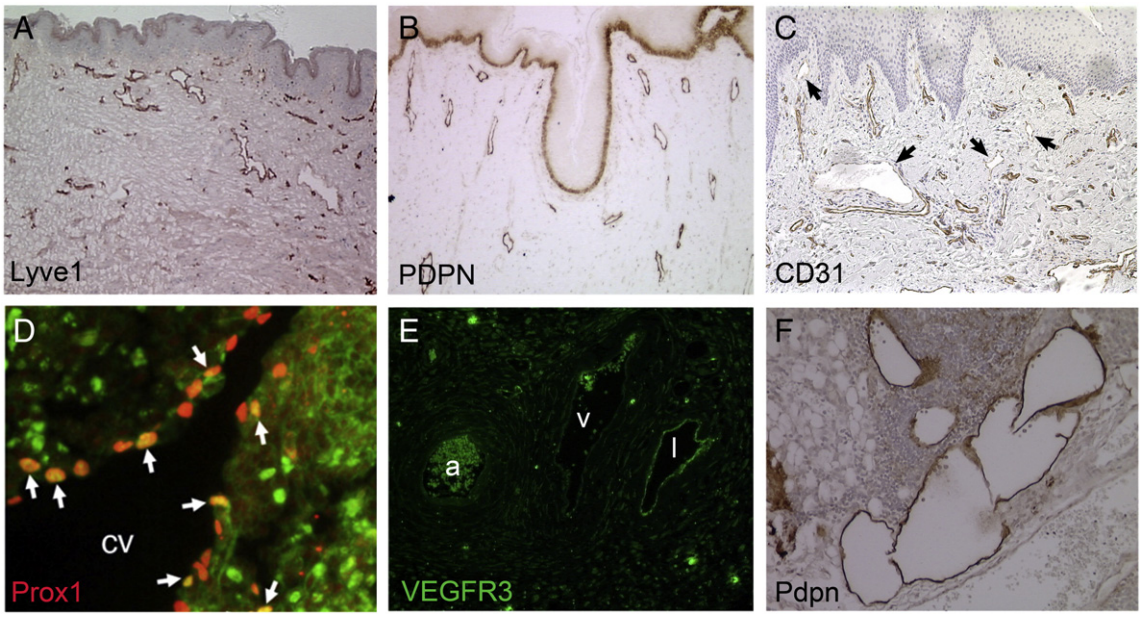

Fig. 1. Lymphatic-associated proteins. No proteins are exclusive to only LECs but several are relatively restricted in their staining profile. FFPE human skin sections were stained for LYVE1 (A), PDPN (D2-40) (B) and CD31 (C). LECs (arrows) stain weaker for CD31 than do ECs. Prox1 (red) stains the nuclei of LECs (D); CV, cardinal vein. A lymphatic vessel (l) stained for VEGFR3 (green) in a mouse skin cryosection (E); red blood cells in an artery (a) and vein (v) were auto-fluorescent. Pdpn staining outlines the LECs and the valves in a large lymphatic vessel surrounding a skin tumor in a mouse FFPE section (F). Brown color denotes positive staining in A–C and F. Sections were counterstained with hematoxylin (blue) in A, C, and F. All images in panels A–C and E–F were taken in the Bielenberg laboratory.
Panel D is taken with permission from Copyright Clearance Center from Qu et al, 2010, originally published in Development 137:1285–1295.

2007; Yao et al., 2012). When interstitial pressure increases, the anchoring filaments pull on the LECs to cause a small gap between cells. Oncotic and hydrostatic pressures drive fluid entry. Immune cells and tumor cells can also enter lymphatic capillaries with fluid uptake.

The lymphatic capillaries then drain the fluid through larger channels called collecting ducts. Collecting lymphatics are lined with LECs containing "zipper-like" adherens junctions that stain positively for VE cadherin (Baluk et al., 2007). Collecting vessels are surrounded by a basement membrane and smooth muscle cells and do not have anchoring filaments or openings. An important feature of collecting lymphatics is the presence of valves that prevent fluid backflow (Fig. 1F). These ducts are arranged into units called lymphangions—defined as the distance between two consecutive valves. Interestingly, the valve region of the lymphangion lacks smooth muscle coverage, presumably to ensure proper function of the valve. Ducts channel the fluid into the lymph node (LN) through afferent vessels into the sinus region. Immune and tumor cell entry into the LN may be an active and rate-limiting step depending on the cell type (Von Andrian and Mempel, 2003; Das et al., 2013). Fluid exits the LN through a single efferent vessel. Lymph may be filtered through several LNs before returning to the venous system through large lymphatic ducts which empty into subclavian veins. The thoracic duct, also called the left lymphatic duct, is the largest lymphatic vessel and drains into the left subclavian vein, while the right lymphatic duct drains into the right subclavian vein.

The structure and morphology of the lymphatic system are crucial to its function. Lymphatics regulate interstitial fluid volumes to prevent edema, absorb dietary fats in the intestines, transport antigens to the LN, and facilitate immune cell traffic to and from LNs. However, the structure of the lymphatic vasculature, especially that of lymphatic capillaries, makes them susceptible to invasion by migrating cancer cells and thus key players in the process of metastasis.

Lymphatic vessels

Lymphatic vessel detection

In the last two decades, the understanding of the biology of the lymphatic system has advanced considerably due to the identification of relatively specific protein markers for lymphatic endothelial cells. The first marker thought to be restricted to lymphatic vessels was vascular endothelial growth factor receptor three (VEGFR3) (Kaipainen et al., 1995). Today, many lymphatic markers are used for detection including lymphatic vessel endothelial hyaluronan receptor-1 (LYVE1), podoplanin (PDPN), prospero homeobox protein 1 (prox1), CD31, and neuropilin 2 (NRP2) (see staining in Figs. 1, 3). Antibodies to these proteins have enabled the detection of lymphatic vessels in tissues using immunohistostaining techniques. It is now appreciated that none of these markers is unique to lymphatic vessels, and their presence on other cell types can confound lymphatic detection.

LYVE1 is a cell surface receptor for hyaluronan found on LECs (Banerji et al., 1999). Macrophages also express LYVE1. PDPN is a mucin-type transmembrane glycoprotein with extensive O-glycosylation expressed by LECs (Breiteneder-Geleff et al., 1999). Podocytes and some epithelial cells, especially those in the epidermis and mammary fat pad, also express PDPN. Platelet endothelial cell adhesion molecule-1 (PECAM1), also called CD31, is a pan-endothelial cell stain. CD31 stains blood ECs and, to a lesser extent, LECs and macrophages. NRP2 is not exclusive to LECs and is expressed by ECs, neurons, melanocytes and visceral smooth muscle (Bielenberg and Klagsbrun, 2007; Bielenberg et al., 2012). Prox1 is a transcription factor found in many cell types. Prox1 is a master regulator of many lymphatic vessel-associated genes (Wigle and Oliver, 1999). Nuclear staining for prox1 is useful for distinguishing lymphatic vessels from blood vessels, which lack prox1 (Fig. 1D).

Another method to detect lymphatic vessels and to measure their functionality is lymphangiography. This technique involves injecting a dye (either colored or fluorescent) or a radioactive tracer into the tissue