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Endometrial Lymphangiogensis

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Abstract

This article briefly summarises some of the more important recent advances in understanding of lymphangiogenesis, and then reviews current knowledge of the lymphatics and lymphangiogenesis in the endometrium. The recent identification of vascular endothelial growth factor-C (VEGF-C) and VEGF-D, as well as specific lymphatic endothelial cell (LEC) markers such as vascular endothelial growth factor receptor-3 (VEGF-R3), lymphatic endothelial hyaluronan receptor-1 (LYVE-1), podoplanin, and prospero-related homeobox-1 (PROX1), has provided the tools to characterize and investigate lymphatic development and function in a wide range of tissues. There are conflicting reports on the distribution of endometrial lymphatics, with some studies reporting lymphatics in the functional zone of human endometrium, others only in the endometrial basalis, and some reporting none at all. Using immunohistochemical methods we have shown that lymphatic vessels of the functionalis were small and sparsely distributed whereas the basalis lymphatics are larger, more frequent and often closely associated with spiral arterioles. Based on comparisons of serial sections, the majority of lymphatic vessels are positive for CD31 but not FVIII or CD34. By comparing CD31 with D2–40 (labels lymphatic endothelial cells) vessel immunostaining, it was estimated that 13% of the vessel profiles in the functionalis, 43% in the basalis and 28% in the myometrium were lymphatics. The lymphangiogenic growth factor VEGF-C is immunolocalized most prominently in the glandular cells, vascular endothelium and some stromal cells in normal cycling endometrium. There is no difference in staining intensity observed between the basalis and functionalis. VEGF-D is immunolocalized throughout the endometrial and myometrial tissues, with no difference in intensity between endometrial glands and stroma or between the basalis and functionalis across the normal cycle. In conclusion, despite an apparently similar distribution of VEGF-C, VEGF-D and VEGF-R3 in endometrial functionalis and basalis, the lymphatic vascular density is 4–5 times higher in the basalis compared to the functionalis. There is also a close association between some lymphatics in the basalis and the spiral arterioles, thus identifying a potential mechanism for a vascular control feedback loop.

Keywords: Endometrium; Lymphangiogenesis; Vascular endothelial growth factor (VEGF)-C; VEGF-D; VEGF-R3

1. Introduction

The lymphatic system has always been a relatively invisible partner in microvascular research, with the vast majority of studies focusing on the blood vascular system. However, major advances in understanding of the molecular mechanisms behind lymphatic growth and development, along with the identification of a number of specific markers for lymphatic endothelial cells (LEC), has led to a recent surge of interest in the lymphatic system. This article will briefly summarise some of the more important advances in understanding of lymph vessel growth, or lymphangiogenesis, and then review current knowledge of the lymphatics and lymphangiogenesis in the endometrium.

2. Recent advances in the understanding of lymphangiogenesis

The lymphatics commence in the tissues as blind-ending capillaries which connect to and drain via collecting vessels, lymph nodes, lymphatic trunks and ultimately the thoracic

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duct into the subclavian veins. Lymphatics collect and drain protein-rich fluid that exudes from the high pressure blood vascular system, returning it to the circulation at a site where the blood pressure is close to its lowest. Loss or impairment of lymphatic function, as occurs with surgical removal of lymph nodes, can cause lymphoedema, where excessive tissue fluid accumulates and cannot drain. In addition to tissue fluid balance, the lymphatics play a central role in immune surveillance of the body. Antigen-presenting cells and lymphocytes travel through the lymphatic vessels from peripheral tissues to the lymphoid organs, where immune defence against pathogens is mounted.

The endothelium of the terminal lymphatics is discontinuous, as is the underlying basement membrane. Anchoring filaments link LEC's to the interstitial extracellular matrix (ECM) so that fluid accumulation in the tissues opens inter-LEC gaps in the terminal lymphatics and enhances the uptake of interstitial fluid. The recent identification of specific LEC markers such as vascular endothelial growth factor receptor-3 (VEGF-R3) a tyrosine kinase receptor that is activated by vascular endothelial growth factor-C (VEGF-C) and VEGF-D [1], lymphatic endothelial hyaluronan receptor-1 (LYVE-1), a transmembrane receptor that binds to glycosaminoglycan hyaluronan [2], podoplanin, a transmembrane glycoprotein that controls podocyte shape and platelet aggregation [3] and prospero-related homeobox-1 (PROX1), a transcription factor [4], has provided the tools to accurately characterise lymphatic distribution in a wide range of tissues. The functional roles of the above LEC markers, as well as a number of other molecules that influence lymphatic structure and function have recently been reviewed [5].

Studies using transgenic mice have demonstrated that lymphangiogenesis occurs under the influence of VEGF-C [6] and VEGF-D [7] acting through VEGF-R3 [8]. VEGF-C and VEGF-D are produced as pre-pro-peptides that are proteolytically cleaved, altering their binding affinity for receptors VEGF-R2 and VEGF-R3 [9,10]. VEGF-C promotes the initial sprouting of the first PROX1-positive LECs from the jugular vein during development, and induces LEC proliferation and survival [11]. VEGF-C knockout mice fail to form primary lymph sacs, lack all lymphatic vessels, develop severe oedema and die before birth. Even the loss of one VEGF-C allele in heterozygous mice leads to lymphatic-vessel hypoplasia and lymphoedema in the skin [11]. VEGF-D also has lymphangiogenic activity but is not mandatory for lymphatic development. VEGF-C and VEGF-D signal primarily through VEGFR3, although proteolytically processed forms also interact with VEGFR2 [12,13]. These binding properties, along with the expression of VEGFR3 in blood vessel endothelial cells (BEC) helps to explain the severe vasculogenic and angiogenic defects seen during early embryogenesis in VEGF-R3 knockout mice [14,15]. Around the time of birth VEGF-R3 expression becomes increasingly confined to the lymphatic vasculature so that disruption of VEGF-C signaling selectively compromises lymphangiogenesis [16,17]. However, some inhibition of angiogenesis in tumors and wounds has also been observed, and correlates with the re-expression of VEGF-R3 in the blood vessels during these events [18]. Neuropillin-2 (NRP2), which is expressed by LEC, can interact with VEGF-R3 and bind to VEGF-C and VEGF-D, and is essential for lymphangiogenesis [19,20]. NRP2-knockout mice show reduced LEC proliferation and fail to develop small-diameter lymphatic vessels and capillaries [19]. VEGF-C and VEGF-D also act as ligands for the integrin $\alpha 9\beta 1$, which interacts either independently and/or in conjunction with VEGF-R3 to effect lymphatic endothelial cell adhesion and migration [21]. VEGF-A also stimulates lymphatic growth in experimental systems [22] but this activity might be indirect (for example, through the recruitment of inflammatory cells and increased VEGF-C expression [23]).

Mouse gene knockout and transgenic studies have helped to identify a number of other genes which play fundamental roles in the growth, maturation and function of the lymphatic system. Angiopoietin-2 (ANG2)-deficient mice have defective patterning of the lymphatic network as well as smooth muscle cell (SMC) recruitment to the collecting lymphatics. ANG1 can promote lymphangiogenesis, trigger LEC proliferation and rescue the lymphatic defects of ANG2-knockout mice [24,25]. Ephrin-B2 is involved in the angiogenic growth of both blood vessels and lymphatic vessels, as shown by LEC sprouting, lymphatic patterning and valve-formation defects in knockout mice [26]. A further feature of the ephrin-B2 mutant is the appearance of ectopic SMC coverage on cutaneous lymphatic capillaries. In these mice, pericytes and vascular SMCs fail to associate stably with blood vessels and some migrate to the lymphatics [27]. The lymphatics in platelet derived growth factor receptor-B (PDGFRB) knockout mice also acquire ectopic vascular SMC, which suggests that intact pericyte and vascular SMC chemotaxis helps to ensure that only the correct vessels acquire mural cells [27]. Similarly, FOXC2-deficient mice have dysfunctional lymphatics that express several BEC markers including PDGFB, are covered by SMC, and lack valves. Normal early lymphatic development in these mice demonstrates that FOXC2 is mainly required for the later maturation steps of lymph vessel formation [28].

3. Endometrial lymphatics

While new blood vessel growth, or angiogenesis, has been studied extensively in human endometrium during the menstrual cycle, there is only limited and very recent information on the growth of endometrial lymphatics, or lymphangiogenesis [29–31]. Initial descriptions of human uterine lymphatics are based on routine histological techniques and dye tracking. There is general agreement that the myometrium contains a network of lymphatic vessels of various sizes [32,33], however, there are conflicting reports on the distribution of endometrial lymphatics. Some studies report lymphatics in the functional zone of human endometrium [34], while others have only identified lymphatics in the endometrial basalis [33].

We have recently used the specific LEC marker podoplanin to investigate endometrial lymphatic distribution during the menstrual cycle [29]. This work used archival paraffin-embedded full thickness hysterectomy samples ($n = 23$ proliferative phase