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Tumor lymphangiogenesis and new drug development☆

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ABSTRACT

Traditionally, tumor-associated lymphatic vessels have been regarded as passive by-standers, serving simply as a drainage system for interstitial fluid generated within the tumor. However, with growing evidence that tumors actively induce lymphangiogenesis, and that the number of lymphatic vessels closely correlates with metastasis and clinical outcome in various types of cancer, this picture has changed dramatically in recent years. Tumor-associated lymphatic vessels have now emerged as a valid therapeutic target to control metastatic disease, and the first specific anti-lymphangiogenic drugs have recently entered clinical testing. Furthermore, we are just beginning to understand the whole functional spectrum of tumor-associated lymphatic vessels, which not only concerns transport of fluid and metastatic cells, but also includes the regulation of cancer stemness and specific inhibition of immune responses, opening new venues for therapeutic applications. Therefore, we predict that specific targeting of lymphatic vessels and their function will become an important tool for future cancer treatment.

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Abbreviations: APC, Antigen presenting cells; CSC, Cancer stem cell; DFS, Disease-free survival; DSS, Disease-specific survival; ILV/PLV, Intratumoral/peritumoral lymphatic vessel; LEC, Lymphatic endothelial cell; LN, Lymph node; LVD, Lymphatic vessel density; NIR, Near-infrared; NSAID, Non-steroidal anti-inflammatory drug; OS, Overall survival; PFS, Progression-free survival; PTA, Peripheral tissue antigen; RTKI, Receptor tyrosine kinase inhibitor; SLN, Sentinel lymph node.

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1. Introduction

1.1. A brief overview of the lymphatic system

The lymphatic system is one of the two vascular systems present in the human body.

In contrast to the blood vascular system, it is blind ended and unidirectional, and lacks a central pump. Its principal functions under physiologic conditions are the drainage of interstitial fluid, the absorption of dietary fats in the gastrointestinal tract, and the transport of immune cells and antigens from peripheral to lymphoid tissues (reviewed in [1]). In order to fulfill these functions, lymphatic capillaries form an extensive network in most peripheral organs, which is particularly dense at potential entry sites for pathogens, such as the skin and the mucosae. Lymphatic capillaries merge to form larger pre-collecting and collecting vessels, which ultimately converge in the thoracic duct which is connected to the blood circulation via the jugular vein. The microanatomy of the lymphatic capillaries facilitates the entry of interstitial fluid.

Capillary lymphatic endothelial cells (LECs) form specialized, flap-like junctions, so called "primary valves", which also allow entry of immune cells such as dendritic cells, while preventing leakage of fluid back into the tissue. Capillaries have a discontinuous basement membrane and no pericyte coverage, making them even more permissive for fluid and cell entry. Anchoring filaments, connecting the capillary LECs with the surrounding extracellular matrix, prevent the collapse of capillaries, even when the external pressure is high. Pre-collecting and collecting lymphatic vessels on the other hand are generally resistant to fluid transport through the vessel wall. Collector LECs form tight, "zipper-like" junctions, are surrounded by a complete basement membrane, and are further supported by perivascular cells (reviewed in [2]). Once inside the lymphatic network, the fluid (now called lymph) is actively transported from the periphery back to the blood circulation. This is achieved by external pressure on the vessels, e.g. due to body movement and skeletal muscle activity, as well as an intrinsic pumping activity in collecting vessels, which is mediated by perivascular smooth muscle cells. Valves in collecting vessels prevent the backflow of lymph, and a specialized valve at the lympho-venous interface between the thoracic duct and the jugular vein impedes retrograde entry of venous blood into the lymphatic system.

On its way down the lymphatic tree, the lymph also passes several lymph nodes, the principal sites for encounters between antigen, antigen-presenting cells (APCs), and cells of the adaptive immune system. Antigen derived from the periphery is transported with the lymph either as free, soluble molecules, or may be taken up and transported by dendritic cells. Within the lymph nodes, APCs present processed antigen to the T- and B-cells, leading to specific immune responses or peripheral tolerance, depending on the context. Therefore, a functional lymphatic system is crucial for the initiation and regulation of adaptive immune responses.

1.2. Tumor lymphangiogenesis

Lymphangiogenesis is defined as the formation of new lymphatic vessels from pre-existing ones, and is considered the predominant mechanism of postnatal lymphatic vessel growth. In addition, lymphatic vessel remodeling can involve enlargement of preexisting lymphatic vessels. In general, lymphangiogenesis is needed for the development of the lymphatic system during embryogenesis, but does not occur in a healthy, adult organism, with a few exceptions, e.g. in the ovaries and the mammary tissue during the female reproductive cycle. However, lymphangiogenesis as well as enlargement of pre-existing lymphatic vessels are induced in various pathological conditions, most prominently during acute and chronic inflammatory conditions, wound healing, but also in various types of human cancers and experimental tumor models (reviewed in [3,4]). This is due to activation of lymphangiogenic pathways such as the VEGF-C/VEGFR-3 pathway, as outlined below (Section 3). In tumors, lymphangiogenesis may occur both within the primary tumor mass and/or in the tumor periphery, leading to formation of intratumoral (ILVs) and peritumoral lymphatic vessels (PLVs), respectively. ILVs are often small in caliber and appear collapsed in histological tissue sections, which may be explained by the high interstitial pressure within the tumor mass and/or the loss of the normal tissue architecture, impeding the function of the anchoring filaments. Consequently, ILVs have been hypothesized to be functionally compromised [5–8]. On the other hand, PLVs often appear grossly dilated, tortuous in shape, and filled with cells, and have thus been considered as the major route for fluid and cell drainage from the primary tumor (reviewed in [4]) (Fig. 1). Of note, tumor lymphangiogenesis is very heterogeneous, with some tumor types showing a very low degree or even absence of lymphatic vessel growth [9,10]. Despite this, the tumor mass may still acquire ILVs and/or PLVs by "trapping" them from the tissue into which the tumor is expanding.

1.3. Lymphangiogenesis in tumor draining lymph nodes

Lymph drained from the primary tumor is transported by the lymphatic system through one or several lymph nodes on its way back to the blood circulation, the first of which is referred to as the "sentinel lymph node" (SLN). During tumor growth, the SLN frequently expands in size and cellularity, concomitant with a dramatic lymphatic expansion within the node. This may be due to metastatic tumor cells entering the node and expressing lymphangiogenic factors. However, experimental work by our lab has shown that SLN lymphangiogenesis occurs even before the arrival of metastatic cells, and is most likely stimulated by factors drained from the primary tumor [11–13]. By this mechanism, tumors have been suggested to prepare a "pre-metastatic niche" in the SLN to facilitate later dissemination to the node. Another potential source of lymphangiogenic factors in the SLN are immune cells, such as B-cells or macrophages, which have been found to regulate LN lymphangiogenesis in inflammatory conditions [14,15]. Inflammatory cytokines drained from the primary tumor are likely to induce expression of lymphangiogenic factors in these cells, and also to induce additional recruitment of immune cells from the blood circulation.

2. Role of tumor-associated lymphatic vessels in disease progression

2.1. Lymphatic metastasis

Whereas lymphatic expansion plays an important, protective role in acute and chronic inflammatory conditions [16,17], the role of lymphatic vessels during tumor growth and progression is rather opposite. Although tumor-associated lymphatic vessels may aid in relieving interstitial fluid pressure, tumors have adapted to exploit the lymphatic system for their own benefit, including cancer cell dissemination. Metastasis to tumor draining lymph nodes is common in melanoma and in many types of epithelial cancers (carcinomas), including breast cancer and colorectal cancer, and generally correlates with distant metastasis,