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Major review

Understanding lymphangiogenesis in knockout models, the cornea, and ocular diseases for the development of therapeutic interventions

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ABSTRACT

A major focus of cancer research for several decades has been understand the ability of tumors to induce new blood vessel formation, a process known as angiogenesis. Unfortunately, only limited success has been achieved in the clinical application of angiogenesis inhibitors. We now know that lymphangiogenesis, the growth of lymphatic vessels, likely also plays a major role in tumor progression. Thus, therapeutic strategies targeting lymphangiogenesis or both lymphangiogenesis and angiogenesis may represent promising approaches for treating cancer and other diseases. Importantly, research progress toward understanding lymphangiogenesis is significantly behind that related to angiogenesis. A PubMed search of "angiogenesis" returns nearly 80,000 articles, whereas a search of "lymphangiogenesis" returns 2,635 articles. This stark contrast can be explained by the lack of molecular markers for identifying the invisible lymphatic vasculature that persisted until less than 2 decades ago, combined with the intensity of research interest in angiogenesis during the past half century. Still, significant strides have been made in developing strategies to modulate lymphangiogenesis, largely using ocular disease models. Here we review the current knowledge of lymphangiogenesis in the context of knockout models, ocular diseases, the biology of activators and inhibitors, and the potential for therapeutic interventions targeting this process.

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1. Introduction

The lymphatic vasculature is responsible for collecting excess fluid and macromolecules from capillary beds, returning these elements to the blood circulation, and capturing and delivering antigens to lymph nodes to induce immunologic responses.[43] Thus, lymphangiogenesis, the generation of new lymphatic vessels from preexisting

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lymphatics, serves important functions during embryonic development and wound healing, but disruption of the fine balance between prolymphangiogenic and anti-lymphangiogenic factors can cause certain pathologies. For example, excess lymphangiogenesis can result in tumor metastasis,[100,241] and deficient lymphangiogenesis can lead to lymphedema. In the eye specifically, transmission of immunogenic stimuli from a corneal graft through both lymphatic and blood vessels can lead to graft rejection, and the presence of lymphatic vessels in the host before corneal transplantation has been shown to be a key predictor of poor outcome, thus demonstrating the importance of lymphatics in transplantation.[7,9,37,49,62] Because the identification and visualization of normally invisible lymphatic vessels was difficult, the contribution of these vessels in graft rejection was largely overlooked; however, the discovery of molecular markers for lymphatic vessels has helped advance lymphangiogenesis research in the past 20 years, including the recent discovery of a classical lymphatic drainage system in the central nervous system.[168]

Normally, the cornea lacks lymphatic vessels and can tolerate foreign antigens without mounting a systemic immune response, a concept termed "immune privilege."[48,55] This makes corneal transplant acceptance possible without human leukocyte antigen matching.[247] The eye has an anterior chamber-associated immune deviation through which inflammatory and immune cells are naturally suppressed when foreign antigens are introduced into the anterior chamber, preventing a systemic immune response.[247] When a corneal graft is introduced, it forms the anterior surface of the anterior chamber, and through anterior chamber-associated immune deviation, rejection can be avoided.[247] In the presence of corneal neovascularization, however, the integrity of the anterior chamber-associated immune deviation is lost, and the risk associated with graft rejection increases significantly because immune privilege is no longer applicable.[58]

The normal adult cornea is both avascular and alymphatic and is, therefore, an ideal model for easily assessing both forms of vessel formation.[48,55,215] Lymphangiogenesis in the cornea occurs when lymphatic vessels either grow from pre-existing vessels in the limbus of the eye or form *de novo*.[53] Under hypoxic and inflammatory conditions, various members of the vascular endothelial growth factor (VEGF) family are released by inflammatory cells to stimulate both angiogenesis and lymphangiogenesis.[28,235,238] Elucidating the mechanisms by which lymphangiogenesis occurs in the cornea can lead to the development of therapeutics targeted at reducing corneal neovascularization and may be extrapolated to the prevention of tumor cell metastasis in cancer patients.[17,35,118]

In this review we first provide an overview of lymphatic markers and knockout models currently used to study lymphatic development and emphasize why the cornea serves as a great model for studying lymphangiogenesis. We also provide updated descriptions of the normal ocular surface anatomy, factors involved in regulating lymphangiogenesis, and ocular diseases associated with lymphangiogenesis. Finally, we discuss strategies for modulating lymphangiogenesis in the context of disease.

2. Corneal lymphangiogenesis and angiogenesis

The cornea is normally avascular and serves as an ideal model in which to study both angiogenesis and lymphangiogenesis.[37,43,48,53] In various ocular pathologies, however, corneal angiogenesis and lymphangiogenesis are induced, and corneal transparency is lost.[263] The presence of newly formed blood and lymphatic vessels induces leakage of proteins, lipids, and calcium within the cornea, which results in a reduction in visual acuity and increases the risk of graft rejection after corneal transplantation.[7,9,37,49,62] Before the discovery of lymphatic markers, corneal neovascularization was believed only to involve blood vessels because the lymphatic vessels are biomicroscopically undetectable[54]; however, we now know that pathologic lymphangiogenesis is usually present with angiogenesis and occurs in the setting of an inflammatory insult directly to the cornea, overriding the angiogenic and lymphangiogenic privilege of the cornea.[51] Members of the VEGF family (VEGF-A, -B, -C, and -D) are the primary mediators of both angiogenesis and lymphangiogenesis. Lymphangiogenesis is primarily mediated by VEGF-C and VEGF-D binding to VEGF receptor 3 (VEGFR-3) on lymphatic endothelial cells (LECs).[99,174] Bone marrow–derived cells such as macrophages produce both VEGF-C and VEGF-D.[48,177,230] If inflammation occurs after corneal transplantation, macrophages will enter *via* the blood vasculature in response to cytokines and other mediators of inflammation, whereas antigens are transported by antigen-presenting cells to regional lymph nodes *via* the lymphatic vasculature.[177]

3. Lymphatic markers

A major breakthrough in the study of lymphangiogenesis occurred with the introduction of lymphatic-specific markers that made lymphatic vessel visualization more accessible and facilitated significant scientific advancements. The ideal characteristic of a lymphatic-specific marker is its exclusive expression on LECs[107,151]; however, this is rarely the case because many of the lymphatic-specific markers currently used are also expressed on certain nonendothelial cells. Thus, the most important feature is that these markers are not expressed on blood vessels and can be used to distinguish lymphatic vessels from blood vessels. A summary of lymphatic-specific markers is provided in Table 1.

3.1. *LYVE-1*

Banerji and colleagues discovered lymphatic vessel endothelial hyaluronan (HA) receptor 1 (LYVE-1), a member of the Link superfamily of HA-binding proteins, by searching the expression sequence tag database for complementary DNAs homologous to CD44, the only other major member of the Link superfamily of HA receptors.[10] Although both CD44 and LYVE-1 bind to HA, CD44 is not expressed on lymphatic vessels and is instead primarily located on blood vascular endothelial cells. In contrast, LYVE-1 is highly expressed on lymphatic vessels and serves as a lymphatic-specific marker.[10] LYVE-1