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Review Article

Corneal neovascularization and contemporary antiangiogenic therapeutics

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Abstract

Corneal neovascularization (NV), the excessive ingrowth of blood vessels from conjunctiva into the cornea, is a common sequela of disease insult that can lead to visual impairment. Clinically, topical steroid, argon laser photocoagulation, and subconjunctival injection of bevacizumab have been used to treat corneal NV. Sometimes, the therapies are ineffective, especially when the vessels are large. Large vessels are difficult to occlude and easily recanalized. Scientists and physicians are now dedicated to overcoming this problem. In this article, we briefly introduce the pathogenesis of corneal NV, and then highlight the existing animal models used in corneal NV research—the alkali-induced model and the suture-induced model. Most of all, we review the potential therapeutic targets (i.e., vascular endothelial growth factor and platelet-derived growth factor) and their corresponding inhibitors, as well as the immunosuppressants that have been discovered in recent years by corneal NV studies.

Keywords: angiogenesis; antiangiogenesis; corneal neovascularization; immunosuppressant; vascular endothelial growth factor

1. Introduction

The cornea is the outermost part of the eye, constituting the most important refractive structure of the visual system. Avascularity and transparency of the cornea are vital to normal vision. From the outermost to the innermost layer, the cornea can be divided into five parts: the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium. The epithelium is composed of five to seven layers of cells that are continuous with the conjunctival epithelium. The epithelium is responsible for maintaining the smoothness and integrity of the anterior surface of the cornea. The stromal part is the thickest part of the cornea. Its transparency depends on strictly arranged collagen fibers and endothelial pumping function. Endothelial cells are responsible for water and solute transport between the corneal stroma and the aqueous humor, but are unable to regenerate in humans.

In most tissues, blood and lymphatic vessels are needed to supply oxygen and nutrients, drain extracellular fluid, and

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protect them against pathogens. However, the cornea is a unique tissue without vascularization. The glucose diffusing from the aqueous humor and oxygen diffusing through the tear film meet the metabolite demand of the cornea, so it can remain avascular in a healthy eye. In some diseased conditions, pathologic corneal hemangiogenesis or lymphangiogenesis occurs, which decreases corneal transparency. Situations leading to corneal angiogenesis are mostly associated with hypoxia, infection, inflammation, and poor limbal barrier function.[1,2] Accordingly, patients with transplanted cornea, infectious keratitis, ocular chemical or traumatic injury, autoimmune diseases, and chronic contact lens wear are at risk of developing corneal neovascularization (NV). We summarize the etiologies of corneal NV in Table 1.[1–3]

Angiogenesis, the formation of new vessels from preexisting vascular structures, is different from physiological vasculogenesis. Vasculogenesis means the formation of new blood vessels from bone marrow-derived angioblasts, which mainly occurs during embryogenesis.[4] Angiogenesis results from an imbalance between angiogenic and antiangiogenic factors.[5] These factors promote the migration and proliferation of vascular endothelial cells, finally forming a capillary tube as a part of the wound-healing process.[6,7]

Corneal NV has three clinical patterns. The first one, vascular pannus, results from ocular surface disease. The second is stromal NV, which results from stromal keratitis or alkaline injury. The third is deep NV overlying Descemet's membrane, which can be observed in herpes or interstitial keratitis.[1,2,5,8,9] Mixed patterns are often seen clinically. Researchers have been working on different strategies for the anti-(lymph) angiogenic therapies of different patterns.

2. Animal models of corneal NV

In order to survey the pathogenesis of corneal hemangiogenesis, lymphangiogenesis, and tissue responses to therapies, scientists have developed some platforms to simulate these conditions. Many different methods have been used to induce corneal NV in animals. Among these, alkali burn and suture placement are the two animal models that are widely accepted. In the alkali-induced model, corneal NV can be induced by a paper disc soaked with 1N NaOH placed on the animal ocular surface for 10 seconds. The paper disc is later removed, and the ocular surface is washed with normal saline. In the suture-induced model, corneal NV can be triggered by directly suturing two stitches with 10-0 nylon onto the temporal cornea. In both models, corneal NV will appear and progressively extend its occupying area within 2 weeks. Giacomini and colleagues[10] compared these two models in C57BL/6 mice and FVB mice. They found that hemangiogenesis was similar between these two models in C57BL/6 mice, and in the sutured models between C57BL/6 and FVB. Corneal lymphangiogenesis was more pronounced in the sutured group than in the alkali burn group of C57BL/6 mice. More prominent lymphatic vessels were noted in the FVB strain, compared with both models in C57BL/6 mice. Thus, the suture model may be more appropriate for the survey of corneal lymphangiogenesis. Jia and coworkers[11] compared the genome-wide gene expression between suture- and alkali-induced murine models by microarray assay. The results pointed out that the overlapping upregulated genes were associated with chemotaxis and immune response, whereas downregulated genes were associated with oxidation reduction and programmed cell death. In both models, vascular endothelial growth factor (VEGF) was upregulated, while pigment epithelium-derived factor (PEDF) remained stable.

Another frequently used method for the *in vivo* survey of corneal NV is corneal micropocket assay, which needs to create two adjacent micropockets on the animal cornea. VEGF or basic fibroblast growth factor (bFGF) is implanted in one micropocket, while the target antiangiogenic agent is implanted in the other. The degree of corneal NV can be measured using a slit-lamp stereomicroscope.

Table 1
Causes of corneal neovascularization.

Categories	Cause
Hypoxia	Contact lens wearing
Infectious keratitis	Viral
	Fungal
	Bacterial
	Parasitic
Inflammatory disorder	Mucous membrane pemphigoid
	Stevens–Johnson syndrome
	Atopic conjunctivitis
	Rosacea
	Lyell's syndrome
	Corneal graft rejection
Loss of limbal barrier function	Limbal stem cell deficiency
	Chemical burn, thermal burn, or other injury
Other disorders	Ocular surface neoplasia (papilloma, and conjunctival or corneal intraepithelial neoplasia)
	Pterygium

3. Potential targets of therapy

There are many potential targets for antiangiogenic therapies. With new techniques to enhance or suppress these targets, we can reduce corneal NV and maintain corneal transparency. We depicted several important targets and summarized their related therapies in Table 2.[19–33,36,38,42–46,48,49,54–57,60–62,66,67,71,73,74,77,78,80–87]

3.1. Vascular endothelial growth factor

VEGF is the most important target for antiangiogenic therapies. The so-called VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor.[12] VEGF-A can induce hemangiogenesis through VEGF receptor (VEGFR)-2, whereas VEGF-C and VEGF-D can stimulate lymphangiogenesis through VEGFR-2 and VEGFR-3, respectively.[13–16] Macrophages in corneal stroma can also produce VEGF-A, VEGF-C, and VEGF-D after injury or inflammation.[17] Therapies that block VEGF may reduce corneal hemangiogenesis and lymphangiogenesis through regulating the signaling pathway of receptor tyrosine kinases,