Fibrosis in the Anterior Segments of the Eye

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Abstract: The anterior segment of the eye ball, i.e., cornea and conjunctiva, serves as the barrier to the external stimuli. Cornea is transparent and is a “window” of the light sense, while conjunctiva covers the sclera, the main part of the eyeball. Fibrosis/scarring in cornea potentially impairs vision by the reduction of its transparency and the alteration of the regular curvature. Fibrotic reaction in conjunctiva is also of clinical importance because inflammatory fibrosis in this tissue affects the physiology of the cornea and also a problem of post-eye surgery. In this review, we discuss the topic that is quite critical in vision. Although, various growth factors have been considered to be involved in, focus was put on the roles of transforming growth factor (TGF\textbeta).

Keywords: Fibrosis, Cornea, Conjunctiva, TGF\textbeta.

FIBROTIC DISORDERS/SCAR OF ANTERIOR OCULAR SEGMENTS

The fibrosis in the eye is similar to that of fibrotic disorders in other organs of the human body. The response of the anterior ocular segment i.e., cornea and conjunctiva, to traumas resembles the response of non-central nerve system tissues, such as skin than that of traumatized posterior ocular segment, e.g., retina. In the same way as the healing of injured skin, numerous cellular events take place sequentially resulting from injuries. An early event is inflammation characterized by the infiltration of inflammatory cells by production of cytokines and growth factors from the injured tissues and surrounding ocular surface tissues as well. Ocular surface stromal cells are normally quiescent and become activated at wound site in response to various cytokines produced by inflammatory cells, and subsequently enter cell cycle, migration and transformation to myofibroblasts that remodel injured tissues, e.g., degradation and synthesis of extracellular matrix components. However, tissue remodeling by myofibroblasts seldom repairs damaged extracellular matrix for the restoration of tissue functions, due to excess accumulation of ECM consisting of an interlocking mesh of fibrous proteins, such as collagen and proteoglycans containing glycosaminoglycans (GAGs), a characteristic of granulation tissues accompanied by the presence of inflammatory cells, neovascularization, and altered vascular permeability. Remodeling of the ECM of the granulation tissues occurs and may ultimately lead to some sort of resolution of the damaged tissue and restore certain tissue functions in some vertebrates, but it hardly takes place in human. Myofibroblasts are derived from mesenchymal cells, i.e., subconjunctival fibroblasts or keratocytes (corneal fibroblasts) at the site of injured tissue. At fibrosis/scar lesion, persistent inflammation and myofibroblasts, must be subdued for the restoration of normal tissue functions [1, 2] (Fig. 1).

FIBROSIS/SCARRING AND TREATMENT IN THE CONJUNCTIVA

Fibrosis/scar in the conjunctiva causes several undesirable outcomes of wound healing following ocular surgeries e.g., filtering surgery for treating glaucoma. For example, the loss of flexibility of conjunctiva due to fibrosis makes it difficult to completely cover the surface of surgical wound site, and makes the tissue vulnerable to microbial infection and impairs wound healing. These phenomena lead to poor surgery prognosis especially in glaucoma surgery. Glaucoma surgery via surgical fistula in the sclera is a drainage surgery, which filtrates abundant fluid from intraocular aqueous humor to underneath the conjunctiva. (Fig. 2). If the surgery is successful, a bleb can be seen in conjunctiva. To achieve favorable prognosis of glaucoma surgery, there should be enough flexible space under conjunctiva in which aqueous humor can be pooled, if the fistula persists. Therefore, fibrosis/scar in conjunctiva, which often disrupted the aqueous humor drain to the bleb, is an undesirable complication following filtering surgery.

Currently, adjunctive application of mitomycin C after filtering surgery is performed to attenuate postoperative subconjunctival fibroblasts proliferation for suppressing excessive bleb scarring. However, excess inhibition of cell proliferation and cell death leads to the formation of acellular and avascular blebs, which are susceptible to leakage and microbial infection [3, 4].

It is well known that a wound healing reaction is orchestrated by a variety of signals derived from endogenous soluble factors. For example, transforming growth factor (TGF) b mediates scarring in the conjunctiva. Members of TGF\textbeta super family consist of several functional regulatory peptides that modulate many cellular functions, i.e., proliferation and differentiation. TGF\textbeta plays a key role in wound-
healing and scar tissue formation by promoting transformation of mesenchymal cells (fibroblasts) to myofibroblasts, which often synthesize excess ECM components, such as collagens and proteoglycans in healing ocular tissues. However, administration of neutralizing antibody against TGFβ2 failed to yield favorable outcomes in suppressing fibrosis/scar after filtering surgery [5]. Aqueous humor contains abundant TGFβ2, whereas TGFβ1 and β2 are produced by cells residing in the filtering bleb tissue [6]. Thus, it may be necessary to target all TGFβ family members rather than each individual TGFβ isoform for achieving desirable therapeutic outcomes. bb. For example, it was shown by blocking TGFβ receptor with siRNA effectively suppressed the fibrogenic/inflammatory reaction in vitro and in vivo [7]. However, this strategy may potentially impair the healing of conjunctival epithelium because TGFβ/p38 mitogen-activated kinase (MAPK) signaling is essential for epithelium migration during wound healing. It is known that each TGFβ isoform propagates its signal through the activation of TGFβ receptors serine/threonine kinases at the cell surface, and signal transduction networks involving MAPK/Erk, p38MAPK, C-Jun-N-terminal kinase (JNK), and Smads (Fig. 3). Therefore, blocking TGFβ stimuli at receptor level may potentially compromise epithelial healing on the ocular surface. Alternatively, it might be better to block only the fibrosis specific signals such as TGFβ/Smad pathways within mesenchymal cells and preserve the TGFβ signaling required for the maintenance of epithelium homeostasis.

We focused on Smad pathway because of its close correlation with fibrosis induced by TGFβs. Regardless of the ligand isoform, blocking Smad2/3 signal will suppress TGFβ/Smad signaling pathways, allowing us to bypass the tissue-specific distribution of each TGFβ isoform in situ. Smad7 is one of the most important key players that suppress the TGFβ signaling mediated by Smads. There has been development of promising strategies to induce Smad7 expression by small molecule agents, e.g., interleukin-7 and interferon-gamma and gene therapy to preventing conjunctival fibrosis/scarring [8-11]. Indeed, Smad7 over-expression by adenoviral vector inhibits TGFβ1 induced up-regulation of fibrogenic and inflammatory cytokines in cultured human subconjunctival fibroblasts [11]. Moreover, Smad7 gene transfer attenuates the fibrogenic reaction in a healing, incision-injured, mouse conjunctiva, suggesting that this strategy

![Fig. (1). Myofibroblast plays an important role in the process of tissue fibrosis. Myofibroblast is derived from either fibroblasts cells (mesenchymal cells) or fibrocytes (bone-marrow derived cell), and exerts a central role in cell repopulation, inflammation and extracellular matrix reconstruction in the process of fibrosis.](image)

![Fig. (2). Glaucoma surgery (trabeculectomy) is a drainage surgery, which filtrates abundant aqueous humor from intraocular to under the conjunctiva, via surgical fistula in the sclera. Post glaucoma surgery, aqueous humor which includes TGFβ2, fibroblasts and macrophages which localize at wound site play an important role in wound healing. *Iris is usually excised (iridectomy) to avoid the impaction to fistula.](image)
might have a therapeutic potential in the prevention of excess scarring in the filtering bleb post-trabeculectomy [11].

Connective tissue growth factor (CTGF), another fibrogenic cytokine, is upregulated by TGFβ and mediates TGFβ action. CTGF protein is required for the development of persistent fibrosis induced by TGFβ [12]. This cytokine, therefore, could also be a target to prevent scarring in the filtering bleb. CTGF is induced by TGFβ signaling (but not via Smad signal in general), which stimulates fibrogenic reaction in tissue, thus blocking CTGF may effectively prevent the scar tissue formation [13, 14]. Moreover, this strategy of blocking CTGF has been applied to treat conjunctival scarring disorders, e.g., vernal conjunctivitis or Stevens-Johnson syndrome [15].

**FIBROSIS/SCARRING AND TREATMENT IN CORNEA**

The cornea is a vascular tissue, which provides the major refractive power for vision and must remain transparent and maintain an appropriate curvature. An organized ECM structure consisting of proper ratio of collagen types I, III, and V and proteoglycans is essential for the formation and maintenance of a transparent cornea that provides major and refractive power for vision [16]. Although, the cornea lacks vasculature, the molecular and cellular mechanisms involved in corneal wound healing are similar to those of skin, e.g., re-stratified epithelium and deposition of a collagenous matrix by underlying mesenchymal cells. Various growth factors/cytokines are involved in the pathogenesis of ocular surfaces scarring diseases, i.e., Stevens–Johnson’s syndrome, post-alkali burn. In the majority of these diseases the components of the disease process, include inflammation, fibroblast/myofibroblast transformation and excess ECM accumulation [17, 18]. Deficiency in the limbal stem cells often leads to the formation of vascularized scar tissue on the corneal surface. Transparency of the cornea is reduced by stromal fibrosis/scarring, leading to the poor vision [19, 20]. Such severe corneal fibrosis could be treated by the transplantation of an epithelial cell sheet grown on amniotic membrane [21].

TGFβ activates both macrophages and corneal fibroblasts (keratocytes) as well, initiating transformation of these cell types to myofibroblasts found in granulation tissues. TGFβ has a positive feedback loop of its expression and thus induces higher levels of TGFβ expression as well as other fibrosis-related cytokines, i.e., CTGF, by inflammatory cells. Other growth factors/cytokines are known to modulate TGFβ’s action in tissue. For example, interleukin-7 or Peroxisome proliferator activated receptors gamma are known to induce Smad7, an inhibitory Smad, which blocks the activation of Smads signaling pathways and consequently results in the suppression of ECM production induced by TGFβ1 [10, 22]. It has been reported that blocking type II TGFβ receptor suppressed scarring and neovascularization in alkali burned rat corneas [23]. On the other hand, TGFβ-
activated p38 MAPK is critical for the migratory activity of corneal epithelial cells during tissue repair [24]. Therefore, it is beneficial in treating injured corneas with the strategies that maintain p38 MAPK signaling for epithelium migration, while specifically block Smad signaling in mesenchymal cells. The latter often yielded unfavorable scarring and development of neovascularization in an alkali-burned cornea. To examine the efficacy of this treatment regimen, we have demonstrated that topical adenoviral gene transfer of Smad7 cDNA produces a strong therapeutic effect in suppressing fibrosis/scarring and neovascularization of an alkali-burned mouse cornea, which is characterized by significant decreases in the expression of profibrogenic and proinflammatory cytokines usually seen in alkali burns [25]. Smad7 not only blocked TGFβ/Smad signaling in corneal (myo) fibroblasts, but also inhibiting macrophage recruitment into the injured tissue, resulting in a reduction of macrophage-derived growth factors. We showed that over-expression of Smad7 does not inhibit phosphorylation of the p65 subunit (RelA) of NF-kB [26], an inflammatory signal transmitter, but does block its nuclear translocation. It was confirmed that inhibiting NF-kB signaling by a peptide inhibitor, SN50, also produced a therapeutic effect on alkali-burned corneas in mice. The mechanisms of action of SN50 include suppression of the inflammatory response and also acceleration of epithelial cell proliferation through cross-activation of TNFα/JNK signal pathways [27].

ECM itself also plays an important role in wound healing. Corneal ECM is abundant in collagen and proteoglycans, two small class II leucine-rich proteoglycans, lumican and keratocan, which are synthesized and deposited by stromal keratocytes. We showed that injured mouse cornea epithelium ectopically and transiently expressed lumican at early stage of wound healing [28]. Lumican null mice show a delay of corneal wound healing compare to wild type mice. Moreover, purified lumican from human amniotic membrane promotes mouse corneal epithelium wound healing in organ culture [29]. These observations suggest that lumican may modulate cell behaviors, such as cell migration and proliferation, and contribute to healing of epithelial debridement.

In conclusion, further understanding of the roles of growth factors and ECM behavior which concern with TGFβ in physiological and pathological processes of the eye is needed to develop new strategies in the treatment of ocular fibrosis.

REFERENCES


