The corneal epithelium: clinical relevance of cytokine-mediated responses to maintenance of corneal health

O epitélio da córnea: relevância clínica das respostas mediadas por citocinas para manter a saúde da córnea

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ABSTRACT

We review the growth factor receptor-mediated cell signaling events that induce the responses required for the maintenance of corneal epithelial health. Our focus is to show how such responses contribute to sustaining corneal transparency and deturgescence, so basic to the pathogenesis of corneal diseases. Furthermore, we point out how alterations of receptor-mediated control of these responses account for losses in corneal transparency. In particular, the roles of growth factors in the mediation of normal corneal function, including epithelial cell proliferation, prevention of compromise of the barrier function of the cornea, and maintenance of normal renewal processes are discussed in relation to clinical entities involving the cornea.

Keywords: Corneal epithelium; Intercellular signaling peptides and proteins; Cytokines; Dry eye syndromes; Wound healing

INTRODUCTION

The cornea is a highly specialized transparent structure, the functions of which are crucial to normal vision. Among its important roles are refraction of impinging light onto the retina, maintenance of transparency, and provision of a barrier against environmental insults. Compromise of any of these functions results in loss of corneal epithelial health and impairment of vision. This article will discuss the role of growth factor receptor-mediated signaling in eliciting responses essential to the maintenance of corneal integrity by the corneal epithelium.

Structure of corneal epithelium

In humans, there are five layers consisting of basal cells, wing cells, and terminally differentiated suprabasal cells-lying atop Bowman's layer. The smooth optical surface of the mature cornea is necessary for appropriate refractive power, accounting for 75% of the total refractive power of the eye, and, in turn, normal vision. The tear film layer coats the cornea and is responsible for wetting and nourishing the corneal epithelium, and sustaining its smooth optical surface[1]. Contributing to tear film formation, is the corneal epithelium-mediated net NaCl trans-epithelial transport (efflux), which is osmotically coupled to fluid flow[2-4]. Under stimulated conditions, e.g., stimulation of protein kinase A (PKA), this process is responsible for up to ~25% of the total dehydrating function of both the epithelial and endothelial layers[5-6]. This efflux process contributes to the

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maintenance of deturgescence and formation of the tear film layer which hydrates, nourishes, and provides protection against invasion of pathogens into the cornea. In addition, the tear film is needed to sustain a smooth optical surface for appropriate refraction of impinging light. With its complements of lysozyme and other proteolytic enzymes, the tear film protects the corneal surface by destroying bacteria; it also contributes to supply the oxygen needed for corneal epithelial aerobic metabolism.

The ability of the corneal epithelium to remain transparent can be modified by a variety of environmental challenges, among them foreign bodies, microorganisms, and physical and chemical insults, all of which alter the cornea's barrier function[7]. In order to better understand how the corneal renewal process functions, wound healing studies have been undertaken with the purpose of identifying the cytokine-mediated mechanisms underlying this process[7-14]. These studies are also of clinical relevance to identify strategies to hasten wound healing, thus minimizing the chances of corneal infection and scarring[15-17]. We will review the cytokine-mediated cellular signaling processes that affect wound healing and indicate their therapeutic potential for accelerating this response.

Functions of the cornea

Corneal clarity is needed for appropriate refraction of impinging light. Its barrier function is essential for protecting the cornea and other intraocular tissues from damage imposed by exposure to environmental challenges. To maintain either of these functions, the corneal epithelium undergoes continuous renewal. Therefore, both (i) dynamic cytokine regulation of tight epithelial junctional resistance and (ii) epithelial cell renewal are vital to the maintenance of all corneal functions, which are, in turn, dependent on growth factor control of proliferation, differentiation, apoptosis, and epithelial fluid transport. Knowing how these functions are maintained is essential not only to improve our understanding of the molecular biology and cell physiology of the corneal epithelium, but also to identify potential therapeutic strategies geared towards maintaining and improving corneal health.

Common problems seen in the clinical setting include dry eye, Sjögren's syndrome, bacterial and fungal keratitis, herpes zoster and herpes simplex, keratoconus, Stevens-Johnson syndrome, map-dot dystrophy, lattice dystrophy, abrasion following refractive surgery, etc. There have been advances made in alleviating some of the symptomatologies associated with these diseases[18-25]. Through studies on how cytokines induce wound healing, potential drug targets have been identified to hasten this response. However, they are chronic conditions that cause recurrent tissue breakdown and loss of normal vision. Currently, symptomatic relief can only be provided in a clinical setting through the use of cytokine-based therapy.

Tight junctions (zonulae occludens), which are specializations of the epithelium, provide a semipermeable barrier against high molecular weight pathogens. Tight junctions permit net transport of solutes out of the cornea, since their relatively high resistance impedes back diffusion of higher molecular weight solutes from the tears into the stroma.

Renewal of the corneal epithelium

Proliferation, differentiation, apoptosis are under the control of cytokine receptor-mediated events. After compromise of the corneal surface, it is of therapeutic importance and clinical relevance to expedite corneal epithelial renewal in order to avoid loss of transparency and vision. Expeditious cell renewal is required to maintain stable and continuous contact of the corneal outer layer with the tear film layer, as continuous contact assures a smooth refractive surface. Renewal is also required to sustain tight junctional barrier integrity, necessary for preventing infiltration by pathogenic agents.

Numerous cytokines activate cognate receptors and cell signaling pathways linked to the effectors that underlie each of these renewal responses (i.e., proliferation, differentiation, migration, and apoptosis). The various cell signaling mechanisms interact with one another through crosstalk to modulate the time dependence and signaling strength induced by receptor activation[26-28]. In order for corneal epithelial renewal to sustain tissue function, cytokine-mediated events have to be coordinated with one another. For example, basal cell proliferation must be exquisitely regulated in order to prevent tissue hyperplasia. It is now evident that cytokine-induced signaling acts in a "push-pull" manner so that a mitogenic signal is offset by an opposing "turn-off" cue, which, instead, induces migration. Such feedback between the signaling pathways linked to receptor activation is possible because of crosstalk between parallel signaling pathways. Therefore, through studies targeted at understanding how crosstalk signaling is mediated, it will be possible to identify strategies for optimizing wound healing responses to corneal epithelial injury.

Proliferation

Growth factor-mediated epithelial cell proliferation occurs in the basal cell layer, which is attached to the underlying basement membrane via hemi-desmosomes. Basal cells are the source of all of the more superficial corneal cell layers. Following injury (surgery or environmental insult), expression of epidermal growth factor (EGF), a mitogen, is up-regulated, promoting increased cell migration and mitosis, and, thus, wound repair[29]. Clinical application of topical EGF in eye drops can hasten healing of corneal epithelial erosions resulting from suppression of EGF receptor activation[30]. Certain hydrogels used in the treatment of dry eye have been demonstrated to stimulate EGF receptors and promote wound healing through stimulation of mitogenesis[31].

Experimental models of wound healing are of great value in delineating the molecular mechanisms responsible for healing following injury. EGF induces increases in proliferation and migration through activation of parallel signaling

pathways that interact with one another through crosstalk. The basis for this realization stems from earlier studies of alkali-induced corneal wound healing in rabbits. In that situation, human EGF was shown to hasten healing of the induced corneal wounds within a specific concentration range, whereas it had an opposite effect at higher concentrations[32]. Such complexity suggests that EGF stimulation of its cognate receptor has diverse effects on the signaling pathways that control the various responses associated with wound healing, i.e., reepithelialization. One such EGF-elicited response is stimulation of corneal epithelial cell migration, which has been demonstrated at low cell densities in vitro[32]. In vivo studies indicate that topical application of EGF at certain concentrations (10-20 ng/ml) improves the rate of corneal wound healing through mitogenesis. Since EGF is present in tears, there are autocrine- and paracrine-mediated controls by this growth factor, which contributes significantly to the maintenance of normal corneal health[33-35].

Differentiation

Differentiation of basal cells into squamous cells occurs following their upwards displacement from the basal layer into the outer layers, where they are attached to one another via desmosomes. Molecular mechanisms that could potentially interfere with normal differentiation include loss of specific cytokines, e.g., transforming growth factor (TGF)-β, which underlies losses in normal vision resulting from corneal haze. This occurs through TGF-β stimulation of stromal keratocyte trans-differentiation into myofibroblasts, a phenotypic change that induces visual distortion as result of increases in light scattering. Furthermore, the ensuing stromal scarring may distort normal vision. Consequently, research efforts are directed towards inhibiting the effects of TGF-β on stromal keratocytes. One approach is to reduce TGF-β gene expression whereas another is to inhibit its activation of cell signaling events leading to scarification[36-38].

Apoptosis

While apoptosis is rare in the corneal epithelium, it can be induced in vitro by exposure of the corneal epithelial cells to a hypertonic challenge[39]. Nevertheless, cells are lost into the tears following terminal differentiation of the uppermost superficial layers of the corneal epithelium, resulting in cells sloughing off into the tears. These lost cells are continuously replaced through basal cell layer proliferation. Appropriate synchronized release of a host of cytokines is needed to maintain deturgescence and transparency, and, hence normal vision. Clinically, situations that compromise the physical integrity of the cornea potentially lead to infection and development of opacities. One cytokine that is protective against cell loss through apoptosis is neural growth factor (NGF)[40], while pro-inflammatory cytokines, i.e., tumor necrosis factor (TNF)α, act to promote apoptosis[41]. On the other hand, in vitro TNFα supports human corneal epithelial cell survival[42].

Osmolyte transport and dry eye

Osmolyte transport studies of corneal epithelium in various species have shown that the corneal epithelium mediates net osmolyte transport from the corneal stroma outwards into the tears[43]. Coupled to net transport of various solutes is osmotically coupled fluid transport[44]. Such efflux of fluid provides a critical dehydrating function, without which the stromal ground substance would swell (due to its physiochemical properties, it has a natural tendency to imbibe fluid), resulting in loss of transparency. This entire process is modulated through hormonal and cytokine cognate receptor control. Adrenergic and serotonergic receptor activation induces increases in net sodium chloride transport towards the tears through increases in cyclic AMP and activation of PKA[45-46]. Similarly, there is also muscarinic receptor expression eliciting control of this transport process[47-48]. Indications are that muscarinic receptor control is essential to corneal health due to the fact that the cornea has higher endogenous concentrations of this neurotransmitter than any other tissue in the body. However, there is limited information about which responses are regulated by muscarinic receptors. Nevertheless, there is a report that acetylcholine stimulates wound closure in vitro[49].

There are only limited studies on the corneal epithelial layer regarding the role of cytokines in eliciting control of net ion transport. One study indicated that endothelin-1 (ET-1) inhibits net fluid transport across isolated rabbit corneal layers, despite the fact that ET-1 is a mitogen and hastens corneal wound healing in live rabbits[50-51]. However, acceleration of wound healing by ET-1 is less effective than that by EGF, suggesting that ET-1 has limited clinical potential.

Another important consequence of receptor-mediated control of net fluid transport by corneal epithelial cells is compensation for tear film anisoosmolarity. For example, tear film hypertonicity can develop from either loss of meibomian gland and/or lacrimal gland function[52]. In response to hypertonicity, it is paramount that the epithelial layer mediates a regulatory response called regulatory volume increase (RVI). RVI restores isotonic cell volume by stimulating osmolyte uptake, which is followed by an increase in fluid uptake by the cells. RVI may restore isotonic cell volume, thereby maintaining the normal close cell-to-cell attachments that are necessary for sustaining normal epithelial barrier function and optical integrity. The RVI response is dependent on the activation of anion transporter called the Na:K:2Cl cotransporter activation which is dependent on Na:K pump stimulation, which steepens the inwardly directed Na concentration gradient[53]. As a consequence of Na:K pump stimulation, there is an elevation of the intracellular K^+ concentration, since the Na:K pump has a variable Na:K stoichiometry. For Na:K pump activation to persist, there must be a parallel increase in basolateral membrane K^+ conductance. These membrane ion transport changes are under the control of PKA and PKC, activations of which, in turn, are elicited through surface membrane receptors that include adrenergic component subtypes and EGF[53-54].

As tear film hypertonicity is often associated with dry eye, it is conceivable that this condition is a consequence of dysfunctional RVI responses. Under this condition, hypertonicity-induced cell shrinkage results in loss of barrier function, which cannot be offset by the RVI response. Accordingly, penetration by environmental pathogens through leaky tight junctions into the stroma could ensue. This permeation would then result in persistent inflammation resulting from infiltration of inflammatory cells from surrounding capillaries into the cornea. Therefore, one potential target for ameliorating dry eye symptoms would be to improve RVI responses to a hyperosmotic tear film. A potential line of investigation to achieve this goal would be to use appropriate agents to activate the cell signaling pathways that mediate increases in the activity of ion transporters underlying RVI. Unfortunately, we still do not know how to rescue losses in tight junctional resistance. Knowing this could, in turn, offset losses in cell-to-cell contact caused by hypertonicity-induced cell shrinkage.

Growth factors and renewal of corneal epithelium

Corneal epithelial renewal is a very complex and continuous process. For its functional properties to be retained, diverse growth factors interact with one another to synchronize the events needed for maintenance of corneal transparency and normal refraction. Multiple growth factors are expressed, any of which may elicit a host of autocrine responses depending on concentrations. For example, in vitro EGF is an effective mitogen through its stimulation of the extracellular-regulated kinase (ERK) pathway, whereas at higher concentrations, it becomes inhibitory through a cyclic AMP-mediated negative feedback effect on its activation[55]. In other cases, TGF-β inhibits epithelial cell proliferation promoted by keratinocyte growth factor (KGF) in a dose-dependent manner[56]. Through endeavors to understand mechanisms of expression and function of growth factors in the cornea, drug targets need to be identified to accelerate wound healing and minimize the risk of scar formation during this process.

Epidermal growth factor (EGF)

EGF, with a molecular weight of ~6 kDa, is a very effective mitogen within a specific concentration range. It is present in the tears, and is produced by both corneal epithelial cells and lacrimal gland. This growth factor binds to both low affinity (K_D=1-2 nM) and high affinity (K_D=10-50 pM) EGF receptor (EGFR) sites[57]. EGF's binding to EGFR activates intrinsic tyrosine kinase receptor activity, leading to DNA synthesis and production of extracellular matrix (ECM) molecules (including fibronectin and hyaluronic acid). At low cell densities, EGF is an effective mitogen, whereas this property is suppressed by contact inhibition at higher concentrations. EGFR phosphorylation induces increases in cell proliferation and migration through concomitant ERK mitogen-activated protein kinase (MAPK), p38 MAPK, and phosphoinositol-3 kinase (PI3-K) pathway activation[55,58]. The mitogenic response is dependent on EGFR-induced increases in plasma membrane Ca^{2+} influx resulting from activation of phospholipase C-mediated increases in inositol triphosphate (IP_3) formation. This second messenger interacts with IP_3 receptors on intracellular store (ICS) membranes, an interaction that leads to Ca^{2+} release from ICS. Such depletion elicits feedback activation at the plasma membrane level[59]. In other tissues that mediate capacitative calcium entry (CCE), emptying of ICS Ca^{2+} induces clustering of a sensor protein (called STIM) on the ICS membrane. Through a protein-protein interaction with another protein (called ORAI) located in the plasma membrane, the transient receptor potential (TRP) tetrameric Ca^{2+} channel complex is activated. The Ca^{2+} channel selectively responding to ICS Ca^{2+} emptying is a member of the TRP protein family. Channel activation occurs resulting in refilling of the depleted ICS through stimulation of an ICS membrane, ATP-dependent Ca^{2+} pump[60]. These channels are referred to as store-operated Ca^{2+} channels (SOCs). One of the TRP isoforms forming such a channel in the corneal epithelial membrane is the TRPC4 isoform[61]. Therefore, propagation of the signaling that elicits a mitogenic response to EGF involves coordinated time-dependent activation of Ca^{2+} signaling subsequent to IP_3-induced ICS Ca^{2+} emptying, followed by activation of the ERK MAPK, p38 MAPK and PI3-K pathways. As a consequence of wave-like chain-linked phosphorylation, stimulation of these two parallel concatenated pathways induces stimulation of nuclear transcription factor turn-on of early response genes leading to increases in proliferation and migration. These two responses are regulated in a push-pull manner in that there is an inverse relationship between changes in the rates of proliferation and migration.

Transforming growth factor (TGF)

The TGF-β family consists of three isoforms, all of which have an approximate molecular weight of 25 kD and are secreted along with a dimeric-associated peptide-latent – activated peptide (LAP) – in an inactive complex. This binding occurs with high affinity to the ECM to create pools of the latent growth factor. Dissociation of TGF-β from the complex by extracellular- or cell membrane-bound enzymes activates this cytokine[62]. TGF-β binds to cognate TGF-β receptor glycoproteins. Several linked signaling pathways are, in turn, activated, thus mediating a diverse – often cell-specific – set of responses, including production of ECM, changes in cell shape, and regulation of cell growth and differentiation[63-64]. TGF-β has been shown to inhibit epithelial, endothelial, and leukocyte cell growth as well as to promote trans-differentiation of fibroblasts into myofibroblasts.

TGF-β induces proliferation and migration of corneal stromal fibroblasts, and induces alteration of ECM synthesis, the latter of which modulates the response to the growth factor after injury. In vitro, this cytokine induces rises in stromal heparin and dermatan sulfates, although keratin sulfate levels fall[65-66]. Addition of TGF-β enhances mitogenic responses by keratocytes to EGF via induction of up-regulation of EGFR[67].

On the other hand, TGF-β weakly inhibits EGF-induced increases in epithelial cell proliferation and migration, whereas such inhibition is much greater following exposure to keratinocyte and hepatocyte (HGF) growth factors[56,63].

A gene product of TGF-β expressed in the cornea is TGF-β1 also known as BIGH3. TGF-β1 and three corneal dystrophies (lattice corneal dystrophy [LCD] type I, granular dystrophy [GCD] type I and Avellino corneal dystrophy have been mapped to chromosome 5. Hence TGF-β1 is a strong candidate as the disease gene for those inherited corneal disorders that were mapped to the same locus. All of these inherited corneal disorders as well as Reis-Bucklers corneal dystrophy are caused by different mutations in TGFβ1. There are about 33 different mutations in TGF-β1. About half of them are associated with mutations at two distinct sites on the TGF-β1 gene. The realization of the genetic origin of these dystrophies has prompted studies to determine tissue specific factors that induce specific mutations in the TGF-β1 gene at specific sites in the cornea. In addition, the clinician can more readily diagnose specific dystrophies based on the results of molecular genetic analysis[68].

Keratinocyte growth factor (KGF)

KGF is a member of the fibroblast growth factor (FGF) family and is a single polypeptide of ~28 kDa. It is found in corneal epithelial cells and shares the same signaling pathway that is linked to EGF receptor stimulation[69]. KGF binds to heparin and is stored in the ECM until its release for use by other cells. KGF receptors exist only on corneal epithelial cells, but the KGF transcript is found only in stromal cells, indicating that KGF regulates corneal epithelial cells in a paracrine manner[29,56,70]. KGF selectively increases proliferation by corneal epithelial cells without having any effect on migration or differentiation. Constitutive expression of KGF by keratocytes in the unwounded cornea is important for maintenance of normal corneal epithelial integrity[29].

Hepatocyte growth factor (HGF)

HGF, a 90 kDa glycoprotein bound to the extracellular matrix (ECM), interacts in corneal epithelial cells with c-Met, a tyrosine kinase (Trk) A proto-oncogene HGF receptor (HGFR). HGFR activation induces proliferation, motility, and a transition to fibroblast-like phenotype[71-73].

HGF is produced mainly by stromal fibroblasts and affects corneal epithelial cells in a paracrine manner. HGF is present in tears (at a concentration of 200 pg/ml) and in the lacrimal gland. It, therefore, contributes to the maintenance of corneal epithelial health[73-74].

Platelet-derived growth factor (PDGF)

PDGF, a cysteine knot-containing dimer of 35 kDA, is composed of an A and B chain[75]. PDGF exists as isomers PDGF-AA, PDGF-AB, and PDGF-BB, and binding to its receptor induces mitogenic responses[56]. The PDGF-BB isoform is produced exclusively by the corneal epithelium and is bound at high levels in the basement membrane[76]. Both PDGF-AA and PDGF-BB cause chemotaxis in the presence of fibronectin.

Fibroblast growth factor (FGF)

The FGF family comprises 20 different members of ~18 kDa molecular weight. FGFs regulate proliferation, differentiation, migration, and ECM deposition as well as angiogenesis through various signaling pathways[77-78]. By binding to protective glycoproteins, FGFs are not degraded[79]. They attach to high-affinity receptor tyrosine kinases. Basic FGF (bFGF) is a mitogen expressed in corneal epithelial cells[80].

Nerve growth factor (NGF)

Neurotrophic factors, e.g., nerve growth factor (NGF), and their receptors are present in the corneal epithelium[81] where they promote corneal epithelial proliferation and support corneal epithelial nerve function. Exogenous NGF is a protective agent that promotes epithelial healing following injury. Corneal nerves are required for corneal function, as nerve impairment leads to loss of epithelial metabolism and viability, and ulceration. Ulceration occurs in various ocular and systemic disorders, including diabetes mellitus[81-82]. NGF and its high-affinity receptor, Trk-A, have been detected in the corneal epithelium. NGF is a mitogen and stimulates differentiation in vitro, but less so than does EGF[81-84]. Furthermore, exogenous NGF has been shown to protect human corneal epithelial cells from hypertonicity-induced apoptosis in vitro[85]. During such a challenge, there is interleukin (IL)-1β up-regulation, which induces increases in NGF expression. This increased expression, in turn, induces an increase in nuclear transcription factor (NF)kB activity, and reduction in stress-activated janus nuclear kinase (JNK) phosphorylation and apoptosis. Therefore, NGF may be beneficial in reversing corneal damage resulting from the hypertonic stress encountered in some forms of dry eye diseases. NGF may also induce healing and reduce inflammation as well as neuropeptide release. Other actions include increases in TGF-β release and vascularization[81-82,86].

Other growth factors: insulin growth factor (IGF), bone morphogenetic protein (BMP), and vascular endothelial growth factor (VEGF)

Insulin growth factor (IGF) and its receptor induce cell proliferation and differentiation[80]. Bone morphogenetic proteins (BMPs), which are part of the TGF-β family and their associated receptors, stimulate stromal keratocyte chemotaxis and proliferation. Furthermore, there are constitutive levels of vascular endothelial growth factor (VEGF) in the corneal epithelium, although under normal conditions, the cornea remains avascular[80,87].

Growth factors and corneal wound healing

The up-regulation of growth factors following corneal wound healing is essential for mediation of corneal epithelial

cell proliferation, migration, differentiation, apoptosis, and intercellular communication. Increases in growth factor expression hastens restoration of corneal epithelial function and minimizes risk of tissue infection. Within minutes after injury, secretion of such cytokines as IL-1 and TNF-α by injured epithelial cells is thought to lead to stromal cell apoptosis as the first detectable response. IL-1 release also induces increases in expression of various endogenous growth factors. KGF and HGF release by keratocytes occurs for ~7 days after injury, concomitant with enhanced EGF expression[29]. PDGF release from the basement membrane promotes fibroblast chemotaxis[76]. Reflex stimulation of HGF and release of EGF from the lacrimal gland also contributes to the wound healing response[71,88]. Also occurring are increases in expression of EGFR as well as NGF and its cognate receptor. Furthermore, contributing to the wound healing process is coordinated induction of increases in growth factor release by fibroblasts[73]. Corneal epithelial cells release tissue plasminogen activator (tPA), resulting in plasmin production, and promote latent TGF-β activation, resulting in increases in fibroblast migration[89-90]. Fibronectin in the basement membrane is only detectable 8 hours following injury. Fibronectin, which is synthesized by the corneal epithelial cells and released into the tears, to promote cell adhesion and migration, and secretion of proteases, e.g., plasmin, which enables breaking and reforming of integrin-matrix attachments, thus permitting cell migration[91-92]. Topical application of KGF accelerates the epithelial wound healing process[56,88,93]. After confluence is reached, the fibronectin matrix disappears and the normal basement membrane composition of collagen and laminin returns[94]. TGF-β suppresses HGF and KGF expression, which, in turn, suppresses excess corneal epithelial proliferation[71,95].

Following injury, keratocytes initially undergo apoptosis; however, after 12-24 hours, they begin to proliferate[76]. Release of PDGF and TGF-β from the epithelium into the stroma stimulates keratocyte proliferation and migration[76,96]. There is concerted suppression by EGF, PDGF, and IGF of keratocyte apoptosis[29,97]. TGF-β release induces keratocytes to undergo trans-differentiation to myofibroblasts, a process characterized by α smooth muscle actin (SMA) expression and increased production of KGF, HGF, heparin and keratin sulfates, and collagen and matrix metalloproteinases for stromal remodeling. Once healing has been completed, myofibroblasts become quiescent by either undergoing apoptosis or reverting back to a keratocyte phenotype[98].

Concomitantly, during the post-injury period, inflammatory cells move into the cornea from the limbal blood vessels[76]. One mediator released from the corneal epithelium that mediates this response is TGF-β, which draws fibroblasts, monocytes, and macrophages into the inflammatory focus[63,99]. Increased NGF further promotes survival of immune system cells. Inflammatory cells remove apoptotic cell debris and release PDGF, all of which help mediate the wound healing process[76,95].

Healing is a complex response that requires that the correct combination of growth factors be expressed at precisely appropriate times to synchronize this process. Following injury and wound healing, the cornea returns to a normal state through elimination of inflammatory cells and myofibroblasts in order to restore a quiescent stroma. Growth factor and cognate receptor expression along with their gene product levels return to their pre-wounding levels. Remodeling of the collagen matrix of the stroma also takes place to clear scar tissue, and the epithelium returns to a normal thickness. If, however, there are imbalances in any of the aforementioned growth factors, normal vision may not be restored. For example, prolonged expression of HGF and KGF leads to epithelial hyperplasia, which can reverse the outcome of refractive surgery[29].

Changes in cytokine levels associated with ocular diseases

In infectious and traumatic conditions, corneal edema and opacification may not resolve during the healing process. Such unfavorable outcomes may result from deposition of thickened ECM, inflammatory cell infiltration, and neovascularization[99]. TGF-β is a major player in such pathogenetic sequelae as it induces over-elaboration of ECM and inflammatory chemotaxis. Dysregulation of TGF-β expression levels is also implicated in Stevens-Johnson syndrome and ocular cicatricial pemphigoid[88]. Procedures to lower TGF-β levels are geared at diminishing edema, reducing opacification, and inhibiting inflammatory cell infiltration[100-101]. Since corneal epithelial cells produce pro-angiogenic mediators, neovascularization would also be suppressed.

Injuries resulting in loss of limbal cells can result in conjunctival cell overgrowth onto the corneal epithelial surface. This response results in angiogenesis and goblet cell coverage. Such changes are mediated by VEGF, whose receptors have been identified in normal and inflamed corneal epithelium[102]. Increases in VEGF levels may occur in diabetic corneas due to hypoxia[80]. In primary open-angle glaucoma (POAG), obstruction of aqueous humor outflow is due to excess deposition of ECM[103]. Elevated levels of total and activated TGF-β in the aqueous humor of POAG eyes versus normal eyes have been detected, along with declines in the counteracting bFGF[104]. In this situation, a rise in TGF-β levels may be a contributor to POAG since it not only inhibits proteolytic degradation, but also reduces trabecular meshwork proliferation and motility. A variety of techniques, including blocking of TGF-β receptor-linked Smad (a protein) signaling or application of inhibitors to reduce TGF-β levels, may be effective[105-106].

CONCLUSIONS

Timely growth factor expression by the ocular apparatus is essential to the maintenance of corneal health, a state that is dependent on the coordinated and synchronized production of a host of growth factors, all acting to elicit control of proliferation, differentiation, migration, and apoptosis. Subsequent to injury or infection, healing of the corneal epithelium and stro-

ma is similarly dependent on appropriate changes in levels of growth factor expression. Interactions between these myriad molecular events are highly complex and controlled at the cell signaling level through dynamic protein phosphatase modulation by extracellular receptor ligands. Dysfunctional receptor-mediated control potentially leads to various ocular disease states. Recent advances in control of growth factor release and activation indicate that certain pathological conditions may be ameliorated through promotion of wound healing. The use of gene therapy to reverse signaling events linked to inappropriate growth factor receptor activation is also a promising strategy for the clinical setting.

RESUMO

Revimos os eventos de sinalização celular mediados por receptores de fatores de crescimento, usados para manter a saúde do epitélio da córnea. O objetivo é mostrar como essas respostas contribuem para manter a transparência e a deturgescência da córnea, críticos na patogênese das doenças da córnea. Mais ainda, enfatizamos como alterações no controle mediado por receptor dessas respostas contribuem na transparência da córnea. Especificamente, o papel dos fatores de crescimento na mediação do controle funcional normal da córnea, incluindo proliferação epitelial, prevenção da quebra da função de barreira, manutenção do processo de renovação são discutidos em relação às entidades clínicas envolvidas na córnea.

Descritores: Epitélio anterior; Peptídeos e proteínas de sinalização intercelular; Citocinas; Síndromes do olho seco; Cicatrização de feridas

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