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Conjunctival goblet cells: Ocular surface functions, disorders that affect them, and the potential for their regeneration

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Abstract

Conjunctival goblet cells (CGCs) are specialized cells that produce and secrete soluble mucins to the tear film that bathes the ocular surface. CGC numbers and functions are affected in various ocular surface diseases including dry eye disease with diverse etiologies. In this review we will (*i*) summarize the important functions of CGCs in ocular surface health, (*ii*) describe the ocular surface diseases that affect CGC numbers and function, (*iii*) provide an update on recent research outcomes that elucidate CGC differentiation, gene expression and functions, and (*iv*) present evidence in support of the prediction that restoring CGC numbers and/or functions is a viable strategy for alleviating ocular surface disorders that impact the CGCs.

Keywords

Conjunctiva; Goblet cells; Ocular surface; Cornea; Tear film

1. Introduction.

The ocular surface consists of a continuous epithelial tissue with regional specializations that covers the transparent cornea, bulbar and palpebral (also called tarsal) conjunctiva, meibomian glands, lacrimal and accessory lacrimal glands. The ocular surface serves as a

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protective barrier for the rest of the eye, with the cornea providing the transparent and refractive functions that enable proper focusing of light on retinal photoreceptors. The entire anterior surface of the ocular surface is covered by the tear film that consists of components secreted from specialized regions of the ocular surface. The ocular surface functional unit is integrated by neural, endocrine, vascular, and immune systems, which work together to promote a healthy cornea, that is essential for sight (Fig. 1a) [1, 2]. Ocular surface disorders account for the bulk of the primary eye care services in the U.S.A., where dry eye alone affects about 6 million women and 3 million men with moderate to severe symptoms and an additional 20 to 30 million people with mild symptoms [3–5]. It is necessary to fill the persistent knowledge gap in our understanding of the ocular surface development, maturation and maintenance to lower the burden of ocular surface disorders on our healthcare system.

Conjunctival goblet cells (CGCs) are specialized cells that produce and secrete soluble mucins for the tear film, facilitating its lubricant and protective functions. CGC numbers and functions are affected in various ocular surface diseases including dry eye disease with diverse etiologies. In this review, we summarize our current understanding of the (*i*) structure and functions of CGCs, (*ii*) ocular surface diseases that affect GC numbers and function, and (*iii*) signaling and gene regulatory networks that regulate GC differentiation, gene expression and functions, and present evidence in support of our prediction that reviving CGC numbers and/or functions is a viable strategy for alleviating ocular surface disorders that impact the GCs.

2. Structure and function of the tear film.

The tear film that lubricates and keeps the ocular surface moist, nourishes and protects it from microbial infections is a complex multi-layered fluid. It is comprised of a superficial lipid layer derived from the meibomian glands, central aqueous layer derived mostly from the lacrimal gland (with residual amounts derived from the conjunctival epithelium) and a basal glycocalyx comprised of mucins (Fig. 1b). The tear fluid has a dynamic structure that responds to environmental conditions and is renewed upon every blink [6]. The superficial lipid layer prevents excessive evaporative loss and ensures a uniform surface that minimizes scatter and enables accurate refraction of light [7]. The aqueous component derived largely from the lacrimal glands is supplemented by CGC-derived soluble mucins [2, 8], trefoil factors [9] and defensins, and solutes, growth factors, antibodies and other proteins from the serum [10]. The basal glycocalyx comprised of the transmembrane- and secreted-mucins converts the hydrophobic lipid membranes of the ocular surface epithelial cells to a hydrophilic surface that is capable of retaining the tear film efficiently [2]. We predict that the glycocalyx to gradually transition into the aqueous layer with no distinct boundary in between. Thus, secreted mucins may also be found in the aqueous compartment in monomeric, non-gel form (Fig. 1b).

Tear fluid composition reflects the health of different components of the ocular surface from which it is derived: the meibomian and lacrimal glands, conjunctival and corneal epithelial cells, and the CGCs. Large scale proteomics studies have identified hundreds of proteins in varying amounts within the tear fluid collected from healthy or diseased ocular surface,

setting the stage for developing novel diagnostic markers and therapeutic targets for ocular surface pathologies [11–16]. Though powerful, these large-scale proteomics studies tend to miss small peptides such as SLURP1 that serves important immunomodulatory function in the ocular surface [17–22]. Additionally, tear biomarkers have been sought for systemic disorders such as Parkinson's disease [23], Alzheimer's disease [24] and multiple sclerosis [25]. Thus, the tear fluid is a useful and accessible source for evaluating ocular surface health and several systemic diseases, and their response to treatment. Among tear fluid components, CGC-secreted mucins take a prominent spot as they serve important functions and are decreased in several disorders of the ocular surface including dry eye disease [8, 26, 27].

Mucins, members of a family of high molecular weight, heavily O-glycosylated proteins derived from CGC, have long been recognized as integral components of the tear fluid that help protect the ocular surface against dryness, pathogens and trauma [1, 2, 8, 27–31]. There are two classes of mucins at the ocular surface: secreted (e.g., Muc2 and Muc5Ac) and membrane-associated (e.g., Muc1, Muc4 and Muc16). The secreted gel-forming mucins derived from the CGCs can homo-multimerize through D domains to form viscous mucin gel [1, 29, 32]. The corneal and conjunctival epithelial cell membrane-associated mucins that form the base of the glycocalyx layer of the tear film have a single membrane-spanning domain and a short cytoplasmic tail. Their extracellular domains are constitutively shed into the tear film. When present in optimal amounts, CGC-produced soluble and secreted mucins together improve tear fluid stability, ocular surface hydration and lubrication, and clearance of pathogenic bacteria as well as particulate contaminants, providing a smooth and refractive corneal surface that minimizes scatter and facilitates clear sight[29].

3. Conjunctival biology and the role of GCs in ocular surface health.

The conjunctiva is a non-keratinizing stratified epithelium with interspersed GCs that covers the inner eyelids and the outer eyeball extending to the limbus that borders the cornea (Fig. 2). Unlike the 5-8 cell-layered corneal epithelium built on a clear basement membrane, the conjunctival epithelium is 2-3 cell-layered and lacks a well-organized basement layer. CGC distribution is species-specific. Mouse GCs occur in clusters enriched in the conjunctival fornix [33], while they are clustered in the nasal region of the human conjunctiva, with few individual cells in the bulbar[28], and the lid wiper region [34]. Human CGCs secrete MUC5AC, while mouse CGCs secrete both Muc5ac and Muc5b [1, 2]. In some animals including mice and rats, CGCs span the length of the 2-3 cell layered stratified epithelium, that allows them to easily convey information between the external environment and the stroma (Fig. 2). In humans though, CGCs are mostly restricted to the apical half of the conjunctiva. The GC nucleus is pushed towards the basal side, with the mucin granules filling much of the superficial side. On the stromal side, CGCs interact with antigen presenting cells (APCs), suggesting that GC can deliver antigens to APCs [35]. Tight junctions between GCs and the neighboring epithelial cells involve pore-forming claudins-2 and -10 that regulate paracellular transport in "leaky" epithelia [36, 37]. Though P2Y(2) agonists stimulate both conjunctival stratified squamous cells and CGCs, sympathetic nerves are known to stimulate stratified squamous cells, with their effect on CGCs unknown [1]. In contrast, parasympathetic nerves stimulate secretion from GCs, without influencing neighboring stratified squamous cells (Fig. 2). Though growth factors, acetylcholine, histamine and prostaglandins stimulate

GC-derived mucin secretion [38–42], their effects on conjunctival stratified squamous epithelial cells are not well studied. Thus, the presence of different cell types with highly specialized functions within the conjunctiva presents both valuable opportunities and difficult challenges to those interested in elucidating ocular surface biology.

Definitive identity and location of CGC precursors remains to be established. Pioneering studies from a couple of groups revealed that (*i*) the conjunctival cell lineage is distinct from that of the corneal epithelium [43, 44], (*ii*) CGCs are slow-cycling cells with proliferative capabilities [43, 45], (*iii*) CGCs and keratinocytes share common bipotent progenitors in the forniceal region [46], and (*iv*) a stem cell population is widely distributed but enriched in the medial canthal and inferior forniceal region in humans [47]. The suggestion that the corneal and conjunctival epithelia are equipotent with similar oligopotent stem cells in the corneal, limbal and conjunctival epithelia [48] is debated as inconsistent with the known properties of the ocular surface epithelial cells [49]. Compound niches with variable numbers of proliferating cells, slow-cycling corneal progenitor cells, and post-mitotic GCs were found to migrate from the limbal area into the cornea with limbal stem cell deficiency, facilitating formation of more GCs [50]. Collectively, these studies highlight the need for further identification of bona fide markers of conjunctival stem cells, and the developmental cues that guide them towards a GC fate.

4. Ocular surface functions of GCs.

Historically, ocular surface functions of GC were linked to the properties of mucins that they secrete: lubrication, surface wetting, and preventing microbial infection by maintaining mucosal barrier integrity (Fig. 2) [8, 51, 52]. Such a connection was supported by the reduction of CGCs observed in severe cicatrizing ocular surface diseases that often ended in corneal keratinization and opacity. Lack of overt ocular surface phenotype and the absence of excessive microbial infection in *Spdef−/−, Muc5ac−/−* or *Muc5b−/−* conjunctiva suggested that these GC functions are dispensable for normal ocular surface health in the absence of challenges [53–57]. This notion however was dispelled by the gene expression studies that argue in favor of a protective function for CGCs [53]. The expression of proinflammatory *Il1-α, Il-1β, Tnf-α*, and epithelial cell keratinization-associated *Sprr2h* and *Tgm1* which are usually up-regulated in dry eye disease, was also up-regulated in *Spdef*-null conjunctiva that lacked GCs [53]. Taken together, these results suggest a protective role for CGCs in the ocular surface.

CGCs represent a major cellular component of the innate immune system, producing and secreting gel-forming soluble mucins, trefoil factors, defensins and other anti-microbial agents to the tear film to provide a barrier that prevents encroachment by the external microbiota and limits exposure to commensals, which in turn averts chronic inflammatory responses [1, 2, 9, 58]. Though airway GCs are known to secrete cytokines and chemokines, induce Th2 responses and promote tissue restoration [59], it was not clear if the CGCs serve a similar function in the ocular surface. However, the absence of CGCs or decreased mucin secretion results in inflammatory responses, consistent with a role for GCs in maintaining immune quiescence and tolerance [60, 61]. A recent study identified CGCs as a cellular source of active TGF-β2 in ocular mucosa, implicating these cells in ocular surface

immunomodulation [35]. Moreover, CGCs facilitate tolerogenic host immune responses towards commensal microflora and clearance of pathogens by promoting adaptive immune responses against them. Collectively, these reports suggest a role for CGCs in ocular surface immune homeostasis (Fig. 2).

Additional putative functions of GCs include communicating with and responding to the connected nerves and antigen presenting cells on the matrix side, and facilitating conjunctiva-associated lymphoid tissue (CALT) functions via leaky tight junctions with neighboring keratocytes (Fig. 2) [31]. In the small intestine and the colon, GC-associated antigen passages (GAPs) have been found to facilitate luminal antigen presentation to underlying dendritic cells, aiding in development of immune tolerance [62, 63] (Fig. 2). The presence of CALT-well-organized lymphoid tissue comprised of intraepithelial lymphocytes, subepithelial lymphoid follicles, lymphatics and blood vessels-suggested the presence of similar GAPs within the conjunctiva [58, 64]. Consistent with this prediction, GAPs were recently found on the mouse conjunctiva, providing evidence that CGCs also contribute to ocular surface immune tolerance by modulating antigen distribution and antigen specific immune response [65, 66].

5. Gene expression during CGC differentiation.

Although CGCs have long been recognized as a major source of the soluble mucins such as Muc2 and Muc5ac in the tear, molecular mechanisms that regulate CGC differentiation and function remain poorly studied. A unified picture emerging from the studies involving differentiation of CGCs, as well as gut and lung GC establishes the involvement of Notch and Wnt pathways (Fig. 3). Conditional inhibition of canonical Notch signaling by over-expression of dominant negative mastermind-like 1 (Maml1) affected CGC differentiation, induced epithelial hyperplasia and aberrant desquamation [67]. Ablation of Notch-regulated zinc finger transcription factors *Klf4* or *Klf5* resulted in loss of GCs in the conjunctiva [68–73] and the gut [74, 75]. Additional defects were noted in the *Klf4*- and *Klf5*-deficient ocular surface, suggesting that the influence of KLF4 and KLF5 is not limited to GCs, and that they regulate the whole ocular surface epithelial differentiation [68, 70, 71, 76–81].

We catalogued the changes in gene expression during GC development by comparing the conjunctival forniceal transcriptome at post-natal day- 9 (PN9), PN14 and PN20, when CGCs are absent, developing and present, respectively [82]. Prominent among the transcription factors whose expression is significantly affected in the mouse conjunctiva during early postnatal development are the members of the forkhead box (Fox), SRY-related HMG box (Sox), Ets and Krüppel-like family members (Table 1). We also compared gene expression patterns in corresponding regions of the *Klf4*-deficient conjunctiva, to identify the Klf4-target genes that play essential roles in CGC differentiation and function [82] (Fig. 3). Concurrent with GC differentiation, pathways related to glycoprotein biosynthesis, mesenchymal-epithelial transition, mucosal immunity, endocytic and neural regulation were increased [82].

Sterile alpha motif-pointed domain ETS factor (SPDEF), a Wnt responsive transcription factor[54], is a key regulator of GC differentiation [53, 83]. A subtractive microarray analysis

comparing wild type and *Spdef−/−* conjunctival epithelial gene expression identified Wnt pathway genes Frzb, Dixdc1, Wnt5b and Wnt11 as downregulated in the *Spdef−/−* conjunctiva lacking GCs [53]. Consistently, GC numbers in *Frzb−/−* mice are significantly reduced [53]. Transient transfection studies established that KLF4 and KLF5 trans-activated the Spdef promoter activity, placing them upstream in the heirarchical network of transcription factors regulating GC differentiation (Fig. 3). Conditional deletion of TGF-β in Krt14-positive conjunctival cells upregulated *Spdef* expression and induced epithelial and GC hyperplasia suggesting that TGF-β suppresses *Spdef* promoter activity [83]. Consistently, Smad3 was demonstrated to bind *Spdef* promotor and prevent *Spdef* transcription [83] (Fig. 3). The forkhead box protein A3 (Foxa3) also influences GC differentiation. Spdef and Foxa3 reciprocally regulate each other In the airway epithelia [56,57], and the conjunctiva [83]. Foxa3 induces GC metaplasia in airway epithelia [84], and is downregulated in *Spdef* null lung [56] and conjunctiva [53] suggesting that Foxa3 collaborates with Spdef in regulation of GC differentiation (Fig. 3). While these studies have begun to identify GC-enriched transcription factors with a role in their differentiation, additional studies involving trans-differentiation approach are essential to define the minimal set of transcription factors required to establish the CGC lineage, as in other systems [85–93].

6. Effect of ocular surface disorders on GCs.

Inflammatory diseases of the ocular surface such as keratoconjunctivitis sicca (KCS; dry eye), blepharitis, Stevens Johnson Syndrome (SJS), Sjogren's Syndrome, Ocular Cicatricial Pemphigoid (OCP), and Graft Versus Host Disease (GVHD) result in decreased GC numbers with concurrent decrease in Muc5ac and other GC products in the tear film (Fig. 4) [26, 32, 94]. In general, the number of GCs is negatively correlated with the extent of inflammation in these diseases. Though mouse models of dry eye have been useful for studying the influence of inflammation on GC numbers [95], underlying molecular mechanisms that drive the reduction in GC numbers in these inflammatory diseases remain elusive. In a rabbit model of dry eye induced by topical administration of commonly used preservative benzalkonium chloride (BAC) for 5 weeks, decreased GC density and other KCS symptoms persisted for 2 weeks after BAC removal [96]. Inflammatory cytokines such as TNF-α, IFN-γ mediate the decrease in GC numbers and their secretory function in Sjögren's syndrome-associated ocular surface inflammation [38]. Elevated expression of TNF-α and IFN-γ in the conjunctiva of *Tsp1* deficient mouse model of Sjögren's syndrome affects GC functions [38, 4]. These reports demonstrate that CGC survival and function is negatively influenced by the ocular surface inflammatory disorders with diverse etiologies (Fig. 4).

In contrast, exposure to allergic conditions stimulates CGC survival, proliferation, and secretory function, suggesting that GC numbers and functions are tightly regulated in response to external cues. Allergic conjunctivitis and inverted mucoepidermoid papilloma result in increased GC numbers and hypersecretion of mucins to the tear film. Histamines, leukotrienes, prostaglandins and other allergic mediators stimulate mucin secretion [31]. Histamine receptors transactivate EGFR, which leads to phosphorylation of AKT and increased intracellular Ca2+ and stimulate mucin secretion (Fig. 4). Chronic injury or allergy causes lung GC hyperplasia and mucus hypersecretion, mediated by interleukins

IL-4, IL-13, and EGF signaling [56]. In the airway epithelium, GC metaplasia that accompanies recurrent viral infections depends on IL-13-mediated elevated activity of the transcription factor Foxa3 [84]. The natural killer T cell-derived IL-13 stimulates GC numbers via upregulation of transcription factor SPDEF, that induces GC differentiation [57, 97]. Collectively, these reports suggest that CGC numbers and functions are fine-tuned in response to the specific nature of the external stimuli (Fig. 4).

The reports summarized above demonstrate that GC numbers and functions are altered in response to (*i*) ocular surface genetic disorders, (*ii*) dysregulated immune responses as in GVHD, (*iii*) external stimuli in the form of environmental allergens and (*iv*) chronic injury due to dry eye or other cicatrizing disorders. In view of the importance of GC functions in ocular surface health, it is predictable that a better understanding of the molecular mechanisms that regulate GC differentiation and functions, and how they are altered in diverse disease conditions, will facilitate development of novel approaches for therapeutic modulation of GC numbers and functions. Below, we discuss some of the current efforts in this direction.

7. Tissue- and cell-based approaches for restoration of CGC numbers and functions.

As described above, the conjunctiva becomes damaged in a variety of ocular surface disorders. In these situations, although a healthy conjunctival autograft is ideal for regeneration of conjunctival epithelium, it is not always feasible prompting consideration of other mucosal epithelia as alternatives. As oral mucosa does not contain GCs it does not supplement the tear film. Nasal mucosal grafts contain GCs and may be useful in extreme dry eye situations [98]. Thin and translucent amniotic membrane possesses anti-inflammatory and antiangiogenic properties, and is widely used in ocular surface reconstruction as it supports conjunctival re-epithelialization when conjunctival stem cells remain in the recipient and repopulate during the respite [99].

Conjunctival cells have been cultivated ex vivo on scaffolds paving the way for obtaining conjunctival grafts for transplants in patients with conjunctival diseases [100–104]. Co-culturing subconjunctival fibroblasts with conjunctival epithelial cells supported the progenitor cells, providing a useful approach to expand conjunctival progenitor cells for potential clinical use [105]. In cases where limbal stem cell deficiency also leads to significant CGC deficiency, a combined conjunctival limbal autograft and living-related conjunctival limbal allograft was found to maximize the amount of healthy limbal stem cells while also minimizing the antigenic burden [106]. In a mouse model of inflammation-mediated dry eye, periorbital administration of mesenchymal stem cells decreased the ocular surface expression of inflammatory cytokines and infiltration of CD4(+) T cells while increasing tear production and the number of GCs [107]. However, progenitor cell-derived epidermal sheets cultured on amniotic membranes failed to regenerate GCs when used for ocular surface reconstruction in monkeys, highlighting the need to further refine these cell and tissue-based approaches for CGC regeneration [108].

8. Small molecule therapeutics for improving CGC functions.

The pathologies that relate to loss of GCs and their mucin production can be ameliorated by enhancing the secretion from the fewer remaining GCs, and/or sustaining GC survival or stimulating their genesis.

8.1. Targeting Resolvins.

Resolvins are metabolic byproducts of omega-3 fatty acids that belong to a class of polyunsaturated fatty acid (PUFA) metabolites termed specialized pro-resolving mediators (SPMs). Resolvins are grouped into sub-classes based on the straight chain PUFA from which they are formed. Resolvins actively terminate inflammation that is a part of the etiology of dry eye disease among other means by regulating GC mucin secretion, and therefore are attractive as a novel treatment option for sustaining ocular surface homeostasis in dry eye disease [109]. Resolvin-D1 preserves the mucous layer and maintains homeostasis by stimulating GC secretion via phospholipase-A2, -C, -D, ERK1/2 and Ca2+/CamK [110]. Resolvin-D2 on the other hand elevates cAMP to increase intracellular [Ca2+] and stimulate GC secretion [111]. Chronic inflammatory diseases such as allergic conjunctivitis cause GC hyperplasia and increased mucin secretion. Resolvins-D1 and -E1 controlled inflammatory leukotriene-stimulated mucin secretion by preventing the increase in intracellular [Ca2+] and activation of ERK1/2 [112]. These results demonstrate that resolvins hold the promise to be effective modulators of CGC function and deserve additional attention.

8.2. Targeting Notch signaling.

The means to increasing GC numbers could focus on directing resident stem cells toward GC differentiation; this could occur by directing the molecular signals of cell fate. Notch signaling plays a major role in decisions leading to the formation of either secretory or absorptive cells in the intestinal epithelium [113]. Blocking the Notch cascade, which is dependent on cleavage of the intracellular domain to transit to the nucleus, with a γ -secretase inhibitor resulted in conversion of proliferative crypt cells into post-mitotic GCs in the small intestine [114, 115]. Intraperitoneal injection of Dibenzazepine, a γ-secretase inhibitor resulted in GC hyperplasia in the gut[114]. GCs fail to develop upon conditional ablation of *Klf4*, a Notch-regulated transcription factor in the intestine [75] and conjunctiva [70]. In the *Klf4*-conditional null cornea, Notch1 expression is upregulated roughly 2-fold [69]. Conversely, inhibition of Notch signaling increases Klf4 expression and GC differentiation, and reduces proliferation and tumor formation suggesting reciprocal regulation between Notch signaling and KLF4 [116]. Consistently, cell cycle arrest and accumulation of GCs in the gut upon γ–secretase inhibitor treatment was mediated by upregulation of KLF4, a negative regulator of cell cycle [115]. Based on these studies, it is predicted that suppressing the Notch pathway in the conjunctival epithelial cells will promote the formation of GCs, providing a new avenue for alleviating the discomfort of ocular surface disorders associated with GC loss. Cautionary to this approach, the finding that tissue-specific inducible and irreversible inactivation of Notch-1 in adult corneal epithelium resulted in hyperkeratosis and hyperplasia, leading to the formation of a hyperproliferative, vascularized and opaque plaque also calls for an abundance of caution in such attempts [117]. Thus, this avenue of increasing GC numbers via directed differentiation will require further study, determining

whether Notch inhibition limited in extent or duration can avoid these confounding side effects.

8.3. Targeting CXCR3-Ligands.

Very recently it has been reported that activation of the CXCR3 chemokine receptor improves GC numbers after conjunctiva damage during trabeculectomy, when compared to diluent alone [118]. Subconjunctival injection of an activating chemokine resulted in more GCs in the rabbit eye after the surgical procedure. The mechanism for this increase is not described but may involve either direct effects on GC precursors, or indirectly by modulating the inflammatory environment. Suppression of fibrosis by these chemokines [119–121] would be consistent with allowing a regenerative healing with GC repopulation. As the cycle of injury and chronic inflammation leading to fibrosis contributes to DED, disrupting this should limit the extent or chronicity of disease. This is supported by the finding that suppression of inflammation may rescue GCs after cataract surgery [122]. Further, the CXCR3 ligand CXCL10 is produced by M1-type macrophages [123], suggesting that this may be part of the physiologic response to wounding that protects precursor or stem cells in the face of death ligands or early inflammation. Even though quite preliminary and speculative at present, this report suggests that modulation of conjunctival inflammation may provide relief from DED via rescue of GCs.

9. Conclusions.

Ocular surface mucosal epithelium serves a vital barrier function against external assaults. This protective function is aided by CGCs that produce and secrete mucins, trefoil factor, and defensins. CGC-secreted mucins play a key role in maintaining the ocular surface mucosal epithelial homeostasis. These mucins not only promote tear film integrity to maintain a moist and appropriately refractory film but also serve as anti-microbial and anti-inflammatory substances. When inflammatory ocular surface disorders affect CGC numbers and/or functions, the resulting decrease in secreted mucins leads to a dysregulated feed-forward cycle that further drives the pathology increasing the risk of vision loss.

Despite the obvious importance of CGCs for ocular surface health, several gaps remain in our understanding of CGC biology, impeding with the development of new therapeutic strategies for devastating ocular surface diseases. Molecular mechanisms that regulate the mucin production and secretion by CGCs in healthy and disease-ravaged ocular surface remain incompletely understood despite recent efforts to elucidate the network of genes involved in CGC differentiation and function. Though CGC-enriched transcription factors and their roles in GC differentiation have been identified, mucin gene regulation itself remains poorly studied. Another understudied area relates to the events leading up to decreased CGC numbers and functions in cicatrizing ocular surface diseases. Further studies elucidating the molecular mechanisms involved in inflammatory cytokine-mediated destruction, and similar studies defining mechanisms of survival or expansion of CGCs are essential to fully realize the potential of promising leads for modulating CGC numbers and functions in disease conditions.

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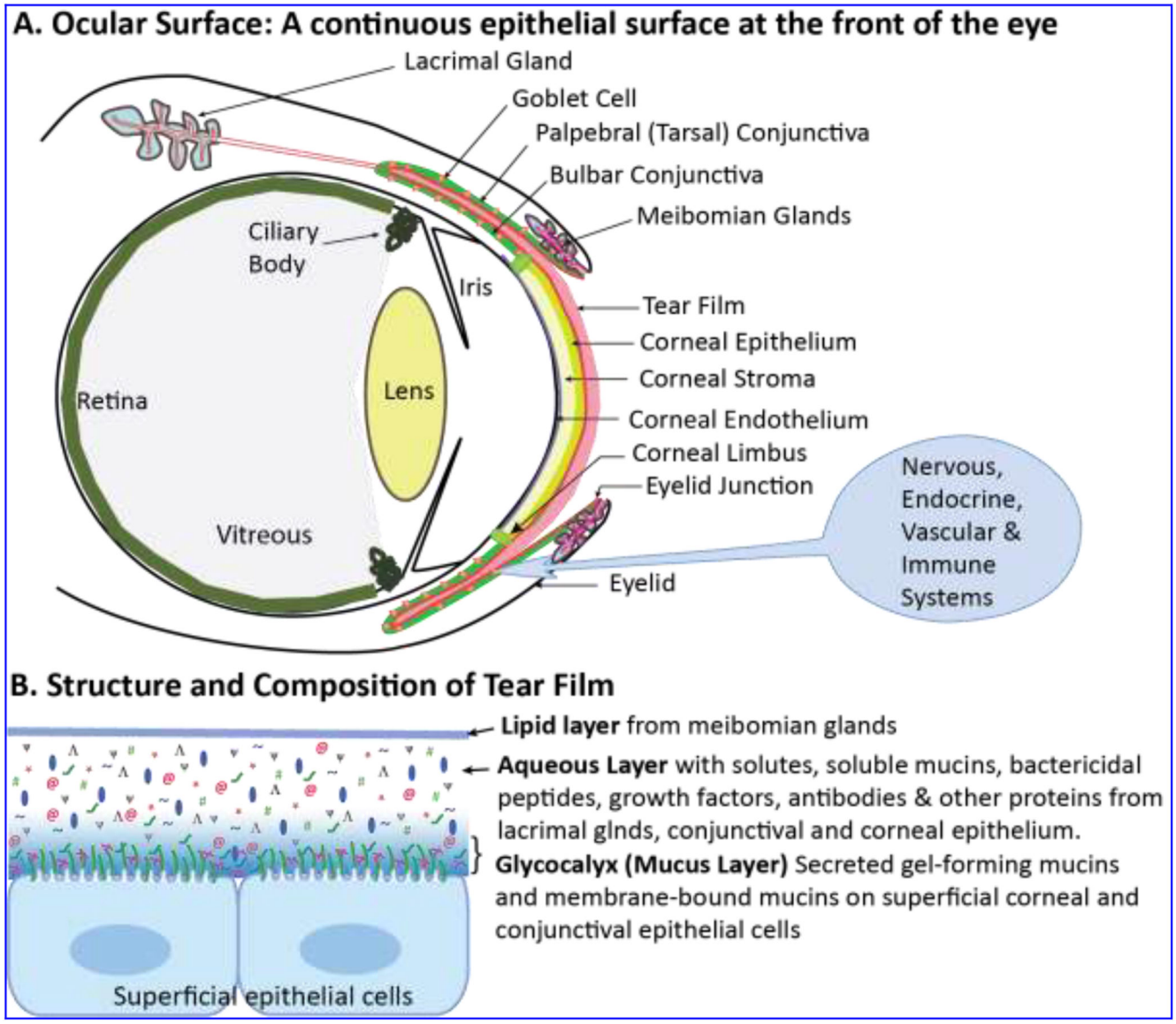

Figure 1. Tear film lubricates, nourishes and protects the ocular surface.

A. The ocular surface (red) is a continuous epithelial surface that covers the cornea, conjunctiva and the ductal surfaces of the meibomian and lacrimal glands. Despite being comprised of different cell types, the ocular surface is a single functional unit whose functions are integrated by nervous, vascular, endocrine and immune systems. **B.** Structure and composition of tear film that covers the ocular surface. The superficial lipid layer derived from the meibomian glands, central aqueous layer derived mostly from the lacrimal glands with soluble mucins from CGCs, and the basal glycocalyx (mucus layer) of secreted gel-forming mucins (red squiggly lines) and membrane-bound mucins (green squiggly lines connected to the superficial epithelial cells) are shown. We believe that the glycocalyx layer gradually transitions into the aqueous layer with no distinct boundary in between. Thus, the gel-forming mucins may also be present in lower concentrations (in non-gel form) in the aqueous layer.

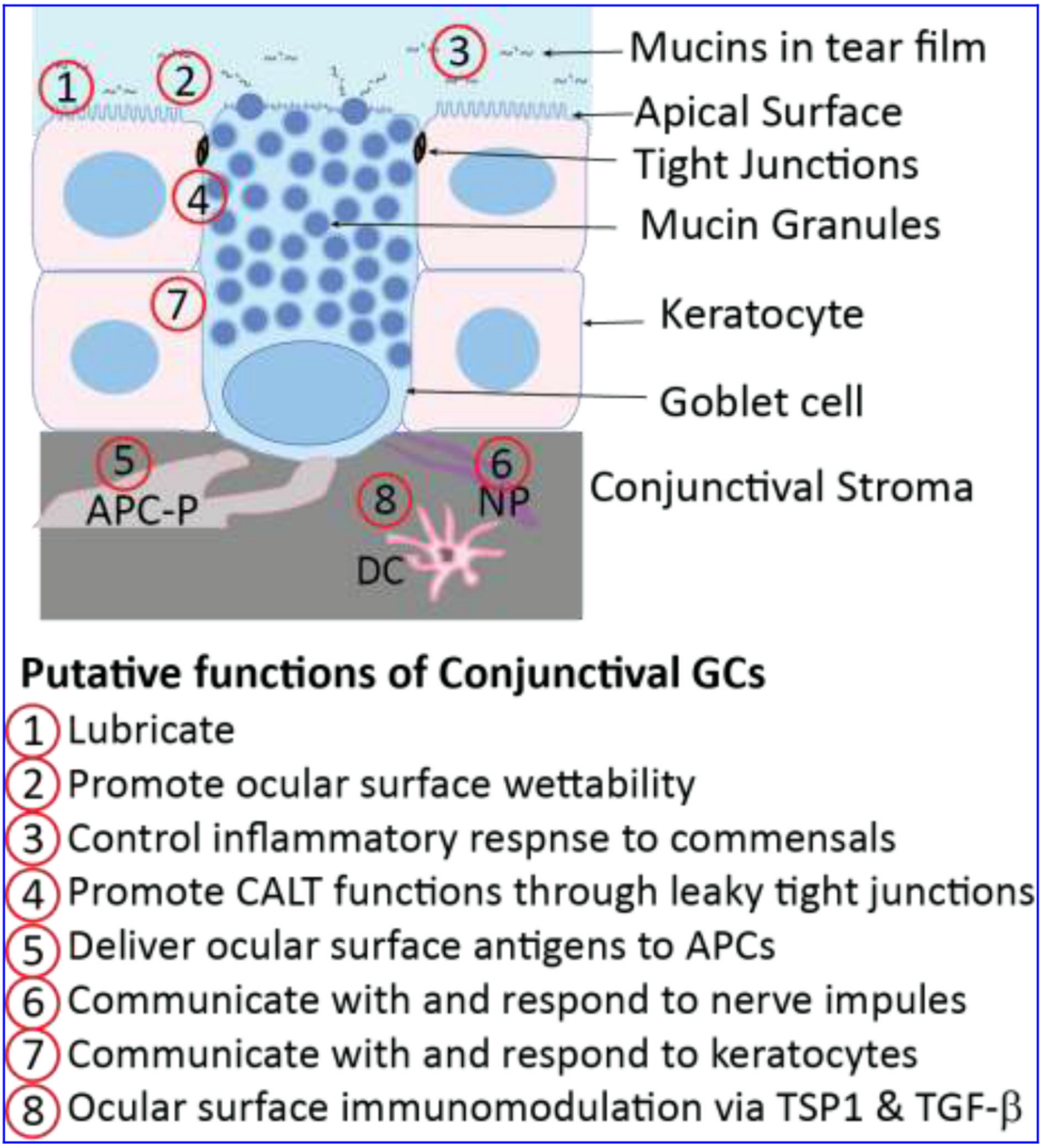

Figure 2. Structure and functions of CGCs.

GCs are present in clusters or interspersed among stratified keratocytes and form leaky tight junctions with the neighboring cells. In the mouse conjunctiva, GCs unlike keratocytes, extend from the surface to the underlying extracellular matrix, where they interact with antigen presenting cell processes. Human GCs also are elongated and span the apical half of the conjunctiva. These cells are packed with multiple mucin granules which push the nucleus towards the base. Putative functions of CGCs are listed below, with the sites of these functions indicated in the schematic. GC-derived TGF-β2 drives dendritic cells toward an

immature tolerogenic state. APC-P, Antigen presenting cell processes; NP, nerve processes; CALT, conjunctiva-associated lymphoid tissue; DC, dendritic cell; TSP1, thrombospondin-1; TGF-β, transforming growth factor-β.

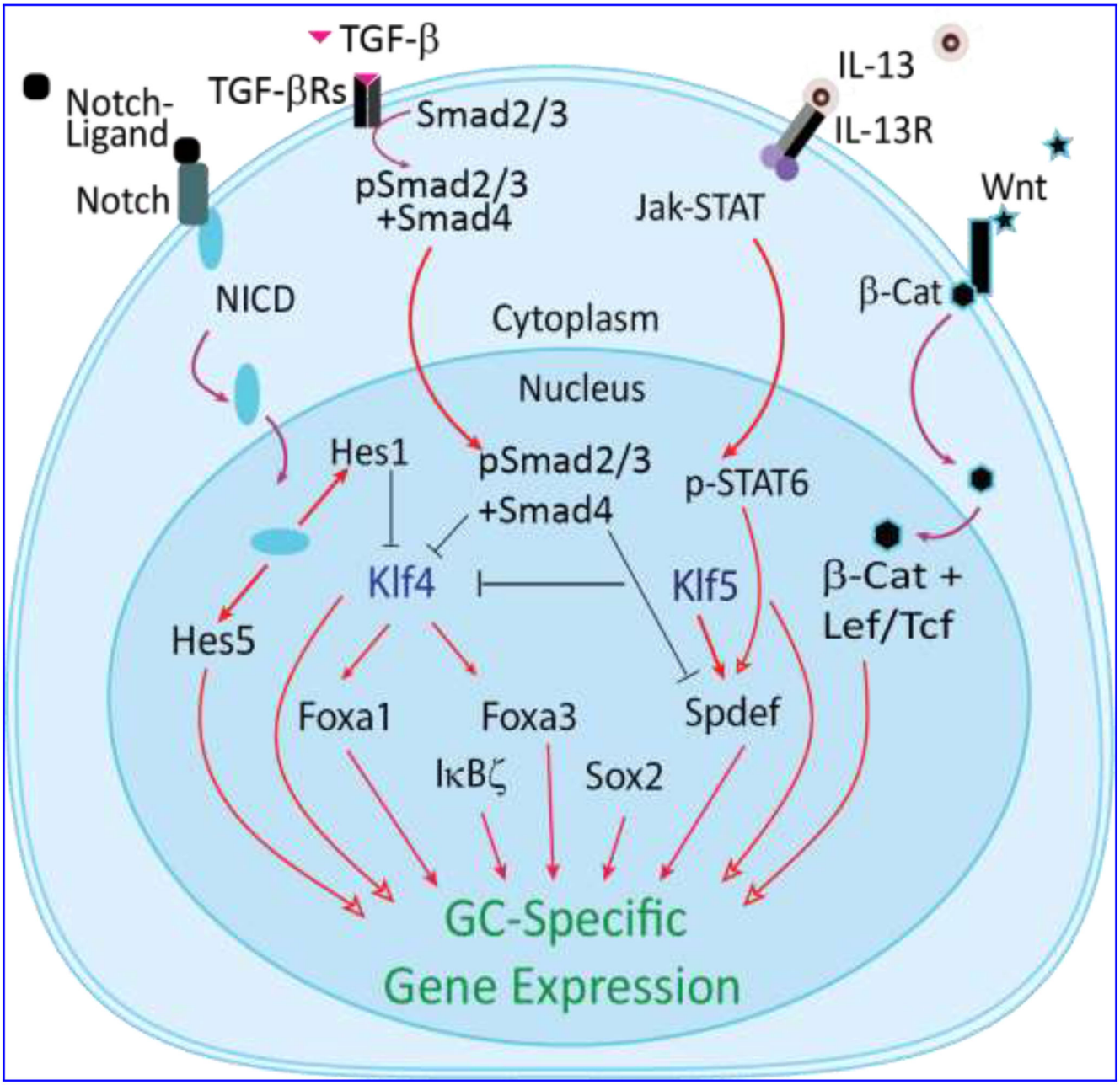

Figure 3. Gene regulatory network in GC differentiation.

Schematic shows key signal transduction pathways and genes known to influence CGC differentiation. The influence of IL-13, Notch, and Wnt signaling pathways on Spdef functions are inferred from studies on the lung and intestine GC fate determination. In pro-inflammatory conditions, TGF-β signaling suppresses Klf4 and Spdef affecting GC functions. NICD, Notch intracellular domain; Jak, Janus kinase; STAT, signal transducer and activator of transcription; pSTAT6, Phospho-STAT6; SPDEF, SAM-pointed domain ETS factor; IL-13, Interleukin-13; IL-13R, IL-13 receptor; β-Cat, β-catenin; KLF4, Krüppel-like factor-4; Lef/Tcf, Lymphoid enhancer factor/T-cell factor.

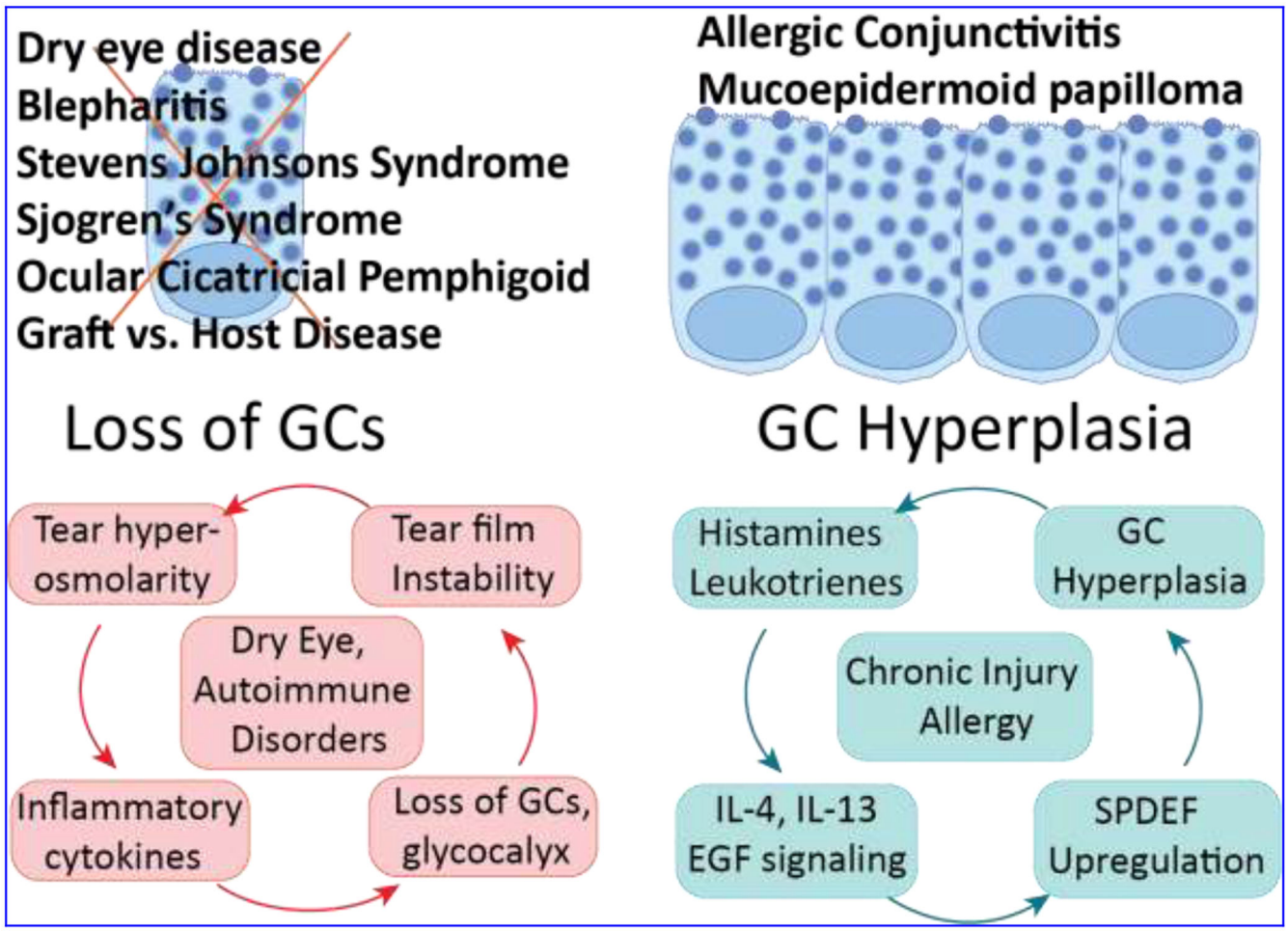

Figure 4. Effect of ocular surface disorders on GCs.

Loss of GCs is associated with dry eye disease and certain autoimmune disorders that affect the eye. In contrast, GC hyperplasia and mucin hypersecretion are reported in allergic conjunctivitis, chronic injuries and conjunctival papilloma. Cyclical nature of these events and the known pathways that influence them are indicated in the lower panel. While the numerous inputs that lead to the ongoing damage are not shown, what is central is the role of GC mucins that lubricate and protect the ocular surface. The GCs can be damaged by cytokines and toxins (such as mitomycin-C during trabeculectomies), and the tear film can be further destabilized in a dry environment. We predict that a better understanding of the molecular mechanisms that regulate normal CGC differentiation in early postnatal stages, their functions in later stages, and how they are altered in diverse disease conditions will facilitate development of novel approaches for therapeutic modulation of GC numbers and functions in the near future.

Table 1.

Transcription factors with altered expression during CGC development (From Gupta et al., 2011 [82])

Transcription factor family	Upregulated during CGC differentiation	Downregulated during CGC differentiation
Krüppel-like factors	Klf4, Klf6, Klf9	Klf13, Klf7, Klf10, Gli2
Epithelial-specific Ets (ESE)factors	Spdef, Ehf, Elf3, Elf5	Ets1
Forkhead box family proteins	Foxa3, Foxa1, Foxk1, Foxo3, Foxp1	Foxp2
High mobility group proteins	Hmgb1, Hmgb2	Hmg20b
Sox family members	Sox21	Sox4, Sox11, Sox12,
Interferon regulatory factors	Irf1	Irf3