

Recent Advances: Transglutaminase in Ocular Health and Pathological Processes

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Abstract

Transglutaminase (TG) is a diverse class of crosslinking enzymes involved in the regulation of cytokine production, endocytosis, cell adhesion, migration, apoptosis and autophagy. It has been implicated in inflammatory diseases, neurodegenerative processes and cancer. The eye is a specialized organ which subserves the important function of vision and has distinctive physiological and anatomical properties that differ from other body tissues. Understanding of the roles of various TGs in the eye therefore require studies specific to cells and tissues of ocular origin. We review the advances in TG research in ocular diseases, including pterygium, glaucoma, cataract and proliferative vitreoretinopathy. TG1 is a molecule important for keratinisation in many cicatrizing diseases of the ocular surface, including keratoconjunctivitis sicca. TG2, with multiple functions, has been shown to be important in inflammation and cell adhesion in various ocular diseases. The results of TG research in each region of the eye are critically assessed and the implications of these studies in the treatment of ocular diseases are discussed. By modifying wound healing process and influencing the amount of inflammation in animal models of human ocular diseases, TG-related strategies are now a possibility for selected clinical scenarios. For example, the use of retinoic acid for severe dry eye has undergone clinical trials. However, in many other areas, more research in selection of specific targets, time of intervention, specific method of delivery of interventional molecules, and safety of therapy in humans may be necessary. One promising area in the future is the use of a TG strategy to modify conjunctival wound healing to increase success rates after glaucoma surgery.

Keywords: Ophthalmology; Ocular disease; Wound healing; Inflammation; Apoptosis; Transglutaminase

Introduction

Transglutaminase (TG) is a big class of intra- and extracellular enzymes with 9 members, all of which catalyze the formation of epsilon-(γ -glutamyl) lysine isopeptide linkages between peptide substrates, except for a catalytically inactive member Band 4.1. These enzymes are tightly regulated, and involved in processes such as inflammation, re-epithelialization, neovascularization and synthesis and stabilization of a fibrous extracellular matrix (ECM) [1]. Different types of TG are found in different cellular compartments. TG1 is located in the cytosolic and membrane compartments, whereas TG2 can additionally be found in the cell nucleus. As far as we know, TG3 and TG5 are restricted to the cytosolic compartment whereas certain forms of TG, such as TG4 and factor XIII are extracellular moieties [2].

TG activity is known to be present in the eye for many years [3,4]. Recently, there has been an increase in interest in the ocular TG field due to the surge in general scientific research driven by the involvement of TG in neuro-degenerative diseases [5], cancer [6], as well as biological phenomena such as apoptosis [7,8] and more recently, autophagy [7,9-11]. The roles of TG in neuro-degeneration [5] and cancer [6] have already been described in previous reviews. In celiac disease, TG2 participates in the pathology by the generation of auto-antibody formation or modification of epitopes to further stimulate the immune response [12]. These processes eventually lead to intestinal inflammation and destruction. These patients are predisposed to uveitis, an inflammation of the internal chambers of the eye [13].

TG2 is a ubiquitous protein in living cells and therefore expressed in all types of normal ocular tissues [2]. On the other hand, TG1 is detectable only in stratified ocular surface epithelium of non-diseased eyes such as in the cornea [4]. The development of TG inhibitors has

propelled further research in this field, and a wide variety of techniques have been employed, including peptides [14], small molecules [15], molecules from bacteria [16] and lead compounds discovered by screening [17]. (Figure 1) summarises the ocular disease states that have been linked to TG.

Many abbreviations and synonyms of various types of TG have been used in the literature. For example, TG2 will be used in place of commonly encountered abbreviations such as 'TGC' and 'tTG'.

Ocular Surface Diseases

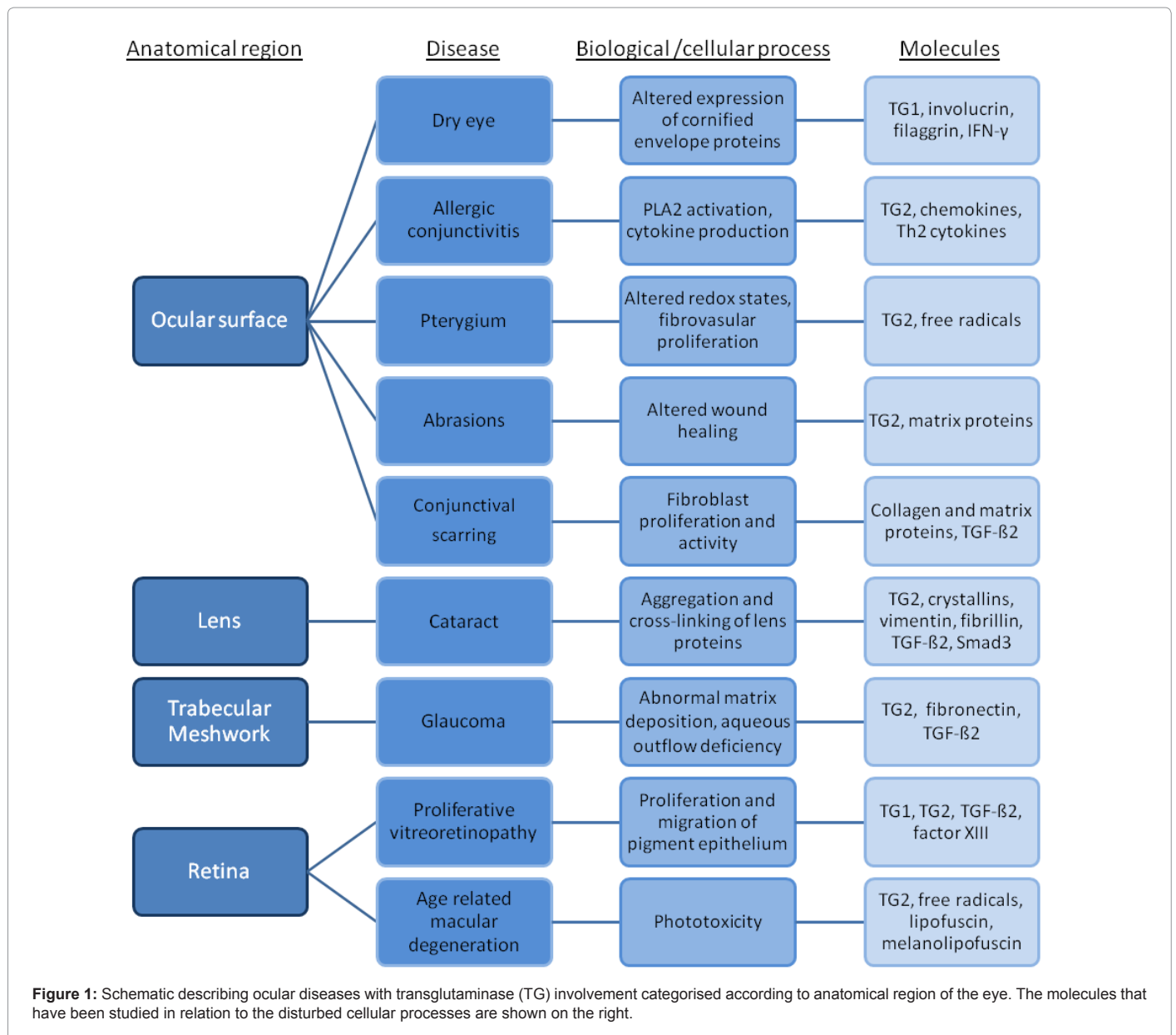
Studies that linked TG to ocular surface diseases such as pterygium [18], allergic conjunctivitis [19], dry eye [20] and cicatricial conjunctivitis [21,22] have been performed, and will be described in the following sections. In the cornea and conjunctiva, TG activity can be detected in the intercellular spaces, along the basement membranes, the cytoplasm of the epithelial cells, the superficial stromal keratocytes, as well as in the walls of the conjunctival stromal vessels [4]. Although TG2 can be found within various ocular cell types, TG1 was identified only in the suprabasal layer of the corneal epithelium.

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Dry eye

Much of the TG literature in ocular surface diseases refer to TG1 up-regulation in cicatrizing diseases and dry eye in humans [21-23]. Mutations in the TG1 gene results in congenital ichthyosis, which is a systemic skin condition associated with eyelid and ocular surface abnormalities [24]. The normal ocular surface epithelium is not keratinized. In advanced stages of dry eye, a common ocular surface disease, the ocular surface becomes keratinized and skin-like. This relatively thickened epithelium is detrimental to the health of the ocular surface for various reasons. Firstly, in advanced dry eye such as those related to Steven-Johnson syndrome, keratinisation is often associated with inflammation, post-inflammatory scarring and adhesion [21,22]. Secondly, the thickened epithelium is relatively opaque, affecting vision if it involves the central cornea or the visual axis [25]. Thirdly, the relative impermeability of this epithelium [26] may affect oxygen and nutrient supply from the human tears and damage cells in the deeper

layer of the epidermis or stromal cells. Lastly, the formation of the cornified cell envelope may directly result in the death of many ocular surface epithelial cells, a process which occurs naturally in the skin development [27]. In severe cicatrizing diseases, the conjunctiva can grow over and cover the cornea in a process called conjunctivalisation [22]. In one study, 4 patients in each of 3 groups with Stevens-Johnson syndrome, ocular cicatricial pemphigoid, and chemical injury were evaluated [21]. The authors found increased levels of TG1, involucrin, filaggrin, and the cytokeratin 1/cytokeratin 10 pair in the keratinized epithelium compared to un-involved age-matched subjects.

TG1, as a crosslinking protein, is able to facilitate the formation of the sodium dodecyl sulfate (SDS) insoluble cell envelope by incorporation of cornified envelope protein precursors, such as involucrin and filaggrin. In a vitamin A deficiency model of dry eye, the transcript and protein levels of TG1 were observed to rise with time and with severity of the disease [20]. In dessicating stress in mice, there

is up-regulation of cornified envelope proteins in corneal epithelium and also increased TG activity, suggesting that the two phenomena are related [28]. Even though TG1 is regarded as important in the above-mentioned ocular surface diseases, it is still unclear if it plays a role in triggering the ocular surface damage, or if it is a relatively passive player, a part of the compensatory changes that occur in these eyes. A study in rats [20] and other studies have confirmed the role of retinoic acid in the expression of TG1 [29] and TG2 [30]. Since TG1 is detrimental in keratinization, elucidation of upstream triggers such as interferon gamma (IFN- γ) [31] may be medically important. IFN- γ was found to be involved in the ocular surface keratinization of patients with Sjögren's syndrome [32], upregulating TG1 and other keratinization associated proteins in conjunctival cells/tissues from patients with Sjögren's syndrome [33] supporting the importance of the IFN- γ -TG1 pathway in the ocular surface pathology.

Allergic eye diseases

Chronic allergic conjunctivitis in humans is an immunological disorder manifested by episodic or chronic inflammation and has several clinical sub-types such as vernal, atopic or seasonal keratoconjunctivitis. The current treatment of choice is immunosuppression with corticosteroids or cyclosporine. The disadvantages of chronic treatment with corticosteroids include occurrence of glaucoma and cataracts, both of which are sight threatening complications by themselves [34]. In a guinea pig allergic conjunctivitis model, use of novel peptide inhibitors to TG2 reduced the clinical inflammation as well as eosinophilic infiltration [19]. One of the soluble peptides used in the topical treatment (KVLGDGQDP) was more effective than dexamethasone (a corticosteroid) and anti-histamine drops in preventing the eosinophilic infiltration. These findings suggest that inhibition of TG2 may be part of a novel strategy in ocular surface allergies [35]. The many signaling pathways that involve TG2 that can play a role in the regulation of inflammation are shown in Figure 2 and will be mentioned in other parts of this paper.

The strategy of using small peptide inhibitors of TG2 has been inspired by the natural anti-inflammatory system offered by pro-elafin. Pro-elafin is an extracellular matrix molecule and a cornified envelope protein that exists in 2 forms, the free 6 kDa form and the covalently linked 9.9 kDa molecule incorporated into the cell envelopes [36]. The molecule contains two distinct functional domains (amino acids 23-60 and 61-117) and subserves the anti-inflammatory function in two ways.

First, on commencement of inflammation, proteases secreted by leukocytes cleave pro-elafin to release the two domains. The N-terminal cementin domain (amino acids 23-60) serves as a substrate and anchoring site for TG2 [36] and hence less TG2 is available for crosslinking and activation of phospholipase (PL) A2, a pathway that will otherwise increase inflammation. Second, the proteinase inhibitor domain (amino acids 61-117) serves to inhibit pro-inflammatory proteins elastase and proteinase-3 [37]. In experiments, the synthetic short peptides GQDP and PVKG, without the entire cementin domain, were sufficient to inhibit cornified envelope formation [38], therefore the authors proposed these as an anti-inflammatory strategy.

Ocular surface stress, and pterygium

Ocular surface cells respond to ultraviolet (UV) B-induced stress by dysregulation of cornified envelope proteins [8]. Inhibition of TG activity suppressed the ultraviolet UVB-induced up or down regulation of different cornified envelope proteins in cell cultures, suggesting that TG mediated the UVB-induced changes of envelope proteins [8].

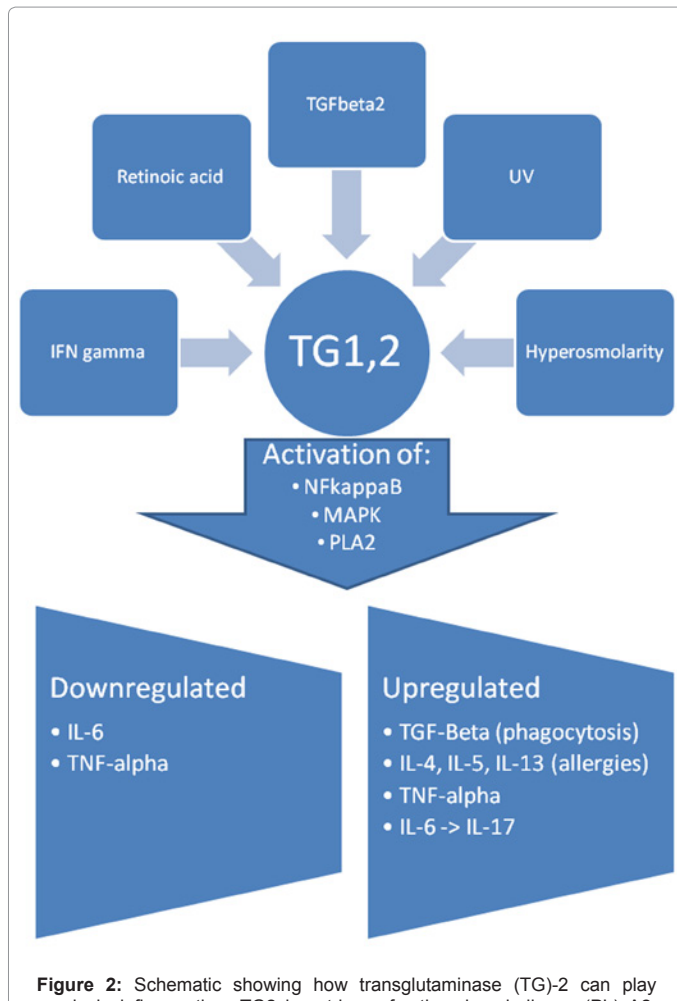
However, this action may not be relevant to clinical scenarios as the study is in-vitro, and further in-vivo studies of the UVB effect [39] is required for validation. In addition, in primary human limbal epithelial cells, hyperosmolar stimulation increased TG transamidase activity [40]. This is particularly important because hyperosmolarity may be the final common pathway in human dry eye.

Pterygium, an ocular surface disease, is exemplified by a wedge-shaped fibroblastic proliferation from the conjunctiva onto the cornea. This is a disease with unknown etiology found with increased prevalence in geographical areas with high intensities of solar ultraviolet radiation [41]. We found that aberrant DNA methylation in the TG2 promoter was associated with excised pterygium tissue, compared to uninvolved conjunctiva tissue from the same patients [42]. A significant increase in the methylation of the promoter CpG islands is consistent with a reduced transcript level through repression of gene transcription. We performed a gene expression study using the Affymetrix U133A GeneChip comparing pterygium and un-involved conjunctiva tissue (<http://www.ncbi.nlm.nih.gov/geoprofiles>, GDS1758, GSE2513 and GPL96) [43], and found that the TG2 transcript level was significantly down-regulated in pterygium compared to un-involved conjunctival tissue. In Kim et al's study [18], 21 pterygia, 2 pseudopterygia and 5 normal conjunctiva have been evaluated and the authors found TG2 staining to be strongly present in 4/23 pterygia or pseudopterygia, but negative or weak staining in normal conjunctiva. TG2 was prominent where the pterygia fibrous tissue invaded the Bowman's layer of the cornea. However, the study did not report the results for pterygium and pseudopterygium separately, and also did not examine the transcript levels, therefore precluding comparisons with the other studies. These three studies [18,42,43] are inconclusive to prove that targeting TG2 is effective to retard progression of pterygium.

Apoptosis in the ocular surface is not only present in an experimental model of dry eye [44], but contributes significantly to the disease process by reducing tear secreting components such as goblet cells and accessory lacrimal glands [45]. Previously, our research in a UVB-stimulated model has shown that TG2 contributes to the apoptosis of human corneal epithelial cells [8]. We also showed that hyperosmolarity can induce cornification in human corneal epithelial cells associated with an increase in TG activity [46]. Crosslinking of cornified envelope proteins by TG results in entrapment of goblet cells in a murine dry eye model [47]. Furthermore, we found that reducing TG2 can partially protect against the hyperosmolarity-induced mitochondrial cell death in corneal epithelial cells [48].

Previous studies have found that UVB radiation reduced epithelial barrier function [49-51]. In human cornea, the exclusion of water from the corneal stroma by the tight junctions of the corneal epithelial cells plays an important role in the maintenance of tissue transparency. Therefore, ocular surface diseases can affect vision not only via disturbances of tear film regularity, but also by directly inducing corneal stromal edema, swelling and opacity. Despite these interesting findings, future studies are required to show that the *in-vivo* corneal epithelial barrier function is dependent on TG.

Corneal epithelial wounds typically heal within 2 days from an injury. In rat cornea [52], TG2 was strongly up-regulated in the early wound healing process, and this is evident in actively migrating corneal epithelial cells and stromal fibroblasts. The kinetics and distribution of TG2 was similar to that in dermal wound healing [53,54]. Early in the skin wound healing process, the TG2 elevation was noted in migrating keratinocytes and infiltrating macrophages, and later, limited to the dermoepidermal junction. Other studies have evaluated the effect



of TG in the *in-vivo* wound healing of skin, such as those after UVB radiation [55,56], but these studies may or may not be relevant to the cornea.

In summary, even though the detailed roles of TG1 and TG2 in the pathogenesis of ocular surface diseases are unclear, the above discussion (sections 2.1 to 2.3) suggests that it may be beneficial to inhibit TG function. Further studies are required to determine whether chronic treatment with TG inhibitors can be detrimental to the ocular surface. For example, inhibitors may paradoxically increase inflammation by reducing the phagocytosis of antigenic dead cells and debris [57], reducing the apoptosis of immune cells [58], or increasing production of inflammatory cytokines by macrophages [59]. Wound healing is a dynamic process with different temporal phases. By modifying the activity of extracellular matrix proteases, wound healing in the damaged ocular surface may proceed in a favorable or unfavorable manner, depending on the timing and duration of intervention.

Glaucoma

Glaucoma is a sight threatening condition in human characterized by a progressive optic neuropathy related to an inappropriate intraocular pressure. In many instances, the intraocular pressure is raised due to a decrease in outflow of the aqueous humor at the anterior chamber angle. The filtration system at the angle consists of a system of trabecular meshwork surrounding a vascular structure, the Schlemm's canal. It is believed that 'resistance' to aqueous outflow in glaucoma can be caused by abnormalities in connective tissues in the trabecular meshwork or around Schlemm's canal [60].

TG2 adversely remodels conjunctival tissue and increases scarring after wounding, and therefore predisposes to failure of glaucoma filtration surgery [61,62]. Although the study uses only cultured cells, it has been suggested that anti-sense TG2 oligonucleotides may be used to treat chronic glaucoma in human [63]. A study of primary human trabecular meshwork cells from glaucoma patients revealed an accumulation of extracellular matrix fibronectin, and up-regulation of TG2 protein and its crosslinking enzyme activity compared to control [64]. The fibronectin accumulation leads to abnormal aqueous outflow in glaucoma patients therefore elevation of intraocular pressure [64].

Other studies explored the upstream pathways of TG2 in trabecular meshwork. A recent example would be a study of endogenous transforming growth factor (TGF) β2 in cultured trabecular meshwork cells from glaucoma patients. The TGFβ2 pathway in ocular surface disease may be mediated by connective tissue growth factor (CTGF) or the lesser known non-CTGF mechanisms. The study found colocalisation of TG2 with TGFβ2 in the trabecular meshwork, and addition of exogenous TGFβ2 leads to an increase in TG2 protein and enzyme activity. This mechanism has been shown to be independent of the (CTGF) protein [65]. In contrast, the TGFβ1 pathway in conjunctival scarring mediated by conjunctival fibroblasts has been shown to be CTGF dependent [66].

In patients with pseudoexfoliation syndrome, a cause of secondary open angle glaucoma, one of the transcripts found to be consistently up-regulated was TG2 [67]. The authors concluded that abnormally regulated genes in this condition were those associated with extracellular matrix regulation or regulation of cellular stress [67]. The authors propose that TG2 may regulate a wider repertoire of gene expression and biological functions related to the matrix in pseudoexfoliation as well as the primary open angle type of glaucoma. Other genes are also up-regulated in pseudoexfoliation syndrome, these include the latent transforming growth factor binding proteins (LTBP-1 and -2) and tissue inhibitor of matrix metalloproteinase-2 [67].

The influence of TG2 in glaucoma may not be limited to the anterior segment. The remodeling of the extracellular matrix may also occur around the astrocytes in the optic nerve head, and therefore increases the susceptibility of the ganglion cell axons to pressure-induced damage [68].

Glaucoma surgery such as trabeculectomy involves the formation of a fistula from the anterior chamber of the eye to the subconjunctival space, where aqueous efflux and absorption to the vasculature can be maintained at a suitable level to control intraocular pressure. The most common reason for failure of glaucoma surgery is excessive scarring in the episcleral and subconjunctival or Tenon space where the aqueous exits the eye, in a morphological structure called the trabeculectomy bleb. Failure of filtration surgery is characterized by post-inflammatory sub-conjunctival adhesion surrounding the bleb, forming a localized Tenon cyst [69]. TG2 adversely remodels conjunctival tissue and

increase scarring after wounding, and therefore predispose to failure of filtration surgery for glaucoma [70]. Expression of TG2 and epsilon-(γ -glutamyl)-lysine, the reaction product of TG2, were present in all failed trabeculectomy blebs and immunolocalisation of TG2 was strongest at the rim of the Tenon cyst [70].

TGF- β 2 is a growth factor known to be elevated in primary open angle glaucoma, the most common type of glaucoma encountered clinically [60]. An *in-vitro* study [70] that used cultured human Tenon fibroblasts stimulated with TGF- β 2 showed an increased TG2 level and activity. The TG activity was measured by incorporation of biotinylated cadaverine into fibronectin. Cadaverine, a laboratory reagent to assess TG activity, is a substrate of TG mediated crosslinking. The authors [70] also observed colocalisation of TG2 and fibronectin, strongly suggesting the modulation of this extracellular protein. It was concluded that intervention in this pathway may be a novel method for preventing scarring in post trabeculectomy eyes.

Lens and crystallin research

The most common type of human cataract is idiopathic and age-related, characterized by opacity and altered refractive properties of the crystalline lens. This is the most common surgically correctable cause of visual morbidity in the world, and cataract surgery is the most common ophthalmic surgical procedure performed in many countries. Depending on the morphology of the cataract, it can be divided into clinical subtypes such as nuclear, cortical, posterior sub-capsular and polar cataracts [71]. The presence of TG in the mammalian crystalline lens was first published in 1981 [72], but the relevance of TG in the pathology of cataract formation has been debated over a few decades. In the section below we will describe the possible ways that TG may affect cataract formation.

The crystallins, major structural and stress related proteins found in the human crystalline lens are also found elsewhere in the body, as described in a recent review [73]. These proteins contribute to 90% of water soluble proteins in the lens, and are believed to maintain lens transparency. The importance of crystallins in cataracts is supported by the finding that mutations in these molecules are associated with inherited cataracts. Short range molecular interactions between crystallin subunits are pivotal for lens transparency. These interactions are likely to be affected by inappropriate covalent cross-linkages between proteins, or the excessive aggregation of these proteins. Crystallins have been the focus of intensive research in the characterization and search for potential amine donors that can be used by TG [74-77]. *In-vitro* studies showed that crystallins are substrates for TG, with γ -crystallin as the most effective amine acceptor [78]. In addition to transamidation, TG2 has also been reported to catalyze deamidation of glutamines in lens crystallins [79].

The chemistry of lens proteins is difficult to investigate. In cataractous lens, the amount of water soluble proteins decreases, whilst the insoluble proteins increase. Techniques such as western blots require the solubilisation of proteins and their detection by antibodies. As a result, changes in soluble protein levels detected by these blots may or may not reflect the global protein changes in cataract formation. This type of analysis showed that high molecular weight β -crystallin proteins (about 43 kDa) are increased, and 29-kDa and 31-kDa β -crystallins and 21-kDa γ -crystallin were decreased in cataractous lens [80]. Despite the limitations, these findings suggest that the crystallin composition of cataractous lens differs from those of the normal lens.

It is known that cortical cataract formation is related to TG [14].

Anterior polar cataracts were also found to have increased TG2 expression [81], although a genechip experiment did not reveal it as one of the differentially regulated genes in age-related cataract compared to clear crystalline lens [82]. The cell type-specific activation of TG by oxidative stress [83] caused by UV radiation is consistent with what is known about epidemiologic association of UV with cataracts [84]. In the laboratory, oxidative stress can be induced by hydrogen peroxide. Exposure of various lens proteins, including β L-crystallins, to oxidative stress induced by hydrogen peroxide increased their susceptibility to TG transamidation [85]. Inhibition of crystallin crosslinking can be explored in treatment strategies. For example, it has been recently shown that exogenous addition of spermidine to rabbit eye lens causes an increase in mono (γ -glutamyl) spermidine, thereby inhibiting crosslinking of lens crystallins and preserving lens transparency [86].

Apart from crystallins, other substrates of TG may also be crosslinked to induce cataract formation. A study has investigated vimentin, a cytoskeletal protein as a substrate for TG [87]. The crosslinking of the vimentin by TG is calcium dependent. Addition of calcium alone to lens homogenates can produce a variety of high molecular weight vimentin species. These reactions can be inhibited by the TG inhibitor dansyl cadaverine. Furthermore, the endogenous cytosolic TG-catalyzed crosslinking reactions can be reproduced and studied *in-vitro*, using purified bovine lens vimentin or recombinant human vimentin preparations. Using such methods, glutamine acceptor sites and lysine electron donor sites on vimentin have been discovered [87].

The crosslinking of microfibril-associated glycoprotein by TG has previously been studied, [88-90] but not in the context of diseases. In pseudoexfoliation syndrome and Marfan syndrome, zonular defects occur in the eye [67]. This may result in the dehiscences of suspensory ligaments or frank dislocation of the crystalline lens (ectopia lentis). In Marfan syndrome, the mutations in fibrillin may have disrupted or affected the crosslinking sites of TG [4,91,92], suggesting that the normal mechanical strength of the zonules is dependent on fibrillin cross-linkages. It is currently uncertain whether sub-clinical cases of zonular weakness in pseudoexfoliation syndrome can be detected prior to cataract surgery using a method of screening for fibrillin mutations.

The Smad protein is a family of proteins involved in the translocation of signals from cell surface TGF β receptors to cell nuclei. In a recent study involving TG2 silenced mice, oxidative stress was shown to trigger TGF β expression, resulting in nuclear translocation of the Smad3 molecule, which then activated TG2. Consequently, TG2 catalyzed aggregation of lens proteins, leading to cataract formation [93].

In summary, a variety of experimental approaches have suggested that TG function may be responsible for at least part of the cataractogenesis process. However, cataract formation is a very gradual process in human, and it is likely that multiple factors accumulating over time contribute to this disease rather than a single molecule having a major contribution. Nevertheless, a TG targeted anti-cataract molecule is currently the focus of research. For some time now, the 2-[(2-oxopropyl)thio]-imidazolium derivatives have been used as TG inhibitors against the crosslinking of lens proteins [94], however, such research has been successful only in whole lens and lens proteins *in-vitro* and not in the treatment of cataracts in animals or humans.

Retinal disease and research

Various types of TG predispose the posterior segment of the

eye to scarring and degenerative conditions discussed below. TG2 is the focus of research in disease processes in photoreceptors, such as autophagy, and advances in posterior segment surgery have exploited TG crosslinking function.

Proliferative vitreoretinopathy

Proliferative vitreoretinopathy (PVR) is a complication of long standing retinal detachment, as well as a cause of failure in many posterior segment surgeries. This condition is essentially represented by scar formation within the posterior segment, with clinical features ranging from retraction of the vitreous to catastrophic retinal contraction, detachment and folding. Retinal pigment epithelial (RPE) cells may proliferate in the sub-retinal space beneath the neural retina, as well as migrate through retinal breaks to and along the epi-retinal surface, and then along vitreous fibrocellular membranes. It is believed that RPE transformation occurs in PVR, and native RPE cells may adopt fibroblastic characteristics or even myofibroblastic characteristics [95].

Crosslinking activity and adhesion-mediated functions of TG may affect PVR, as shown in the following studies. Various molecular interactions (Figure 3) can be interrupted for treatment of PVR.

a. Role of Transglutaminase-1 in proliferative vitreoretinopathy:

TG1 is instrumental in the scarring in the posterior segment of the eye in PVR [95], in an analogous fashion to its role in skin and conjunctival scarring on the surface of the eye [21]. The immunohistochemical localisation and colocalisation of fibronectin with TG1 were observed in both PVR [95] and the ocular surface [21]. The expression of TG1 in RPE cells, as detected by Northern and Western blots, was increased by TGF- β 2 stimulation [95].

b. Role of Transglutaminase-2 in proliferative vitreoretinopathy:

In another study, it has been found that TG2 protein and TG activity were detectable in all PVR membranes [96]. Detection of TG2 was most marked at the border of the membranes. The enzyme was colocalized with connective tissue substrates epsilon-(γ -glutamyl)-lysine and fibronectin. There were no differences between the characteristics of the TG2 expression in epi- and subretinal membranes.

Cultured RPE cells were found to express a basal level of TG2 mRNA, which increased with the stimulation by TGF- β 2 treatment. Since TGF- β 2 has been found in the vitreous of patients with PVR, this may have significance in the disease mechanism. Crosslinking activity was also increased in concert with the increase in TG2 transcript [96]. These findings suggest that TG2 play a role in the altered phenotype of RPE cells in PVR.

TG2 interaction with different cell surface and matrix entities can mediate distinct adhesion mechanisms (Figure 3). TG2 may influence the adhesion of matrix and cell components, encouraging the adherence of PVR membranes to each other and to the neural-retina. However, the migration of RPE cells may also be highly dependent on TG [97]. After stimulation of RPE cells by TGF- β 2, cell surface TG2 increases, and this facilitates the attachment of cells to fibronectin and collagen type I.

The adhesion functions of TG2 in PVR may occur through different interactions with fibronectin (Figure 3), a typical matrix component. Fibronectin is composed of two important fragments of differing lengths which have different functional interactions [98]. Anti-TG2 antibodies blocked the cell surface TG2 and inhibited the adhesion, migration and spreading of RPE on full length fibronectin or the 45 kDa

gelatin binding fragment, but not the 110 kDa cell binding fragment of fibronectin [97]. In contrast, in a non-ocular context, the 110 kDa cell binding fragment of fibronectin has been shown to interact with cell surface integrins (namely the α 1 β 5 integrins) via TG2 [99] (Figure 3).

c. Role of factor XIII in proliferative vitreoretinopathy: Co-localisation of blood coagulation factor XIII, a plasma TG and two of its substrates thrombospondin and fibronectin were observed in PVR [100]. Factor XIII is the crosslinking enzyme that stabilizes blood clot in the final stages of the blood coagulation cascade. This suggests that extracellular TG, in addition to its physiological role in blood coagulation, may also play a role in diseases such as PVR.

In an in vitro model of Matrigel tube formation, factor XIII also has a pro-angiogenic effect [101]. The proliferation and migration of vascular endothelial cells were responsive to factor XIII in a dose dependent fashion, without any alteration of levels of vascular endothelial growth factor (VEGF), a relatively more established pro-angiogenic mediator.

This angiogenic effect of factor XIII is dependent on TG function, as the effect was lost when the crosslinking activity of factor XIII was inactivated. The proliferation of blood vessels may provide further influx of factor XIII to PVR membranes, providing more opportunity for interaction of this TG with connective tissue substrates. Since factor XIII is a calcium dependent TG, it is not clear at present why the relatively high levels of calcium in the blood do not constantly activate its transamidase activity.

Age related macular degeneration and diabetic retinopathy

Age-related macular degeneration is a common, multi-factorial retinal disease known to be a leading cause of blindness in the world. An exudative form of this condition is characterized by choroidal neovascular membranes, which can be treated by photodynamic therapy

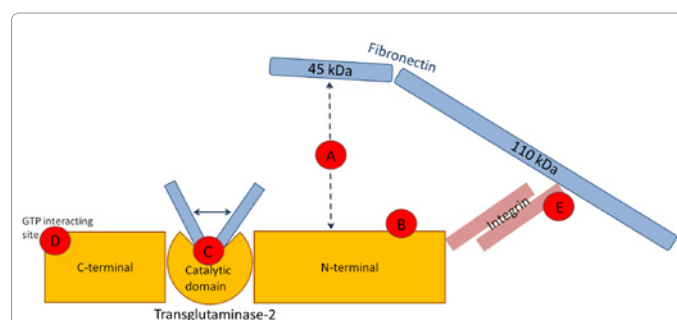


Figure 3: This is a schematic of the transglutaminase (TG)-2 matrix interactions relevant in retinal pigment epithelial cell adhesion. First, TG2 can interact with fibronectin independently of integrin (A). Second, inhibition of TG2-fibronectin interaction involves the N terminal sandwich region of TG2 (B) rather than the transamidase (C) and GTP/GDP related activities (D) subserved by other parts of TG2. However crosslinking activity of matrix molecules by TG2 (C) is relevant in scar formation and diseases like PVR. Crosslinking activity of TG2, via the recruitment of elastase inhibitors such as elafins and cystatins, can stabilize the extracellular matrix. Lastly, TG2 can interact with fibronectin domain through integrin (E) but also independently of crosslinking and G protein function. This association occurs at focal adhesion complexes to mediate integrin signaling, again independently of its transamidase. In order to inhibit proliferative vitreoretinopathy using a TG2 based intervention, it may be necessary to target these different interfaces or interactions.

Blue bars: extracellular matrix molecules, including fibronectin. Pink: integrin molecules. Yellow: TG2 protein domains, note that C terminal is simplified as in reality it consists of 2 domains.

or anti-angiogenic therapy [102]. A recent study shows that a mutation in factor XIII (G185T) is associated with worsening of the response of choroidal neovascularisation to photodynamic therapy [103]. Age-related macular degeneration may be precipitated or exacerbated by the cumulative free radical damage from light toxicity. Ultraviolet radiation may initiate free radical chain reactions in photoreceptor outer segments, and modify the normal cellular molecules such that they can no longer be recognized by lysosomal enzymes. This results in the accumulation of lipofuscin and melanolipofuscin in the photoreceptors and the phagocytosing RPE cells, followed by cell dysfunction or even rupture [102]. Autophagy, a process which is regulated by TG2 [9,10], also mediates the accumulation of lipofuscin in RPE.

Apoptosis in the retina occurs during normal development, where critical synaptic connections are established to enable normal visual function [104]. In another context, it can be triggered by light in the presence of specific mutations in photoreceptor proteins. During photic injury, the crosslinking of proteins by intracellular TG is crucial for the induction of apoptotic damage in photoreceptors [105]. Normal rat retina has constitutive level of TG2 expression in the photoreceptor outer segments. With increasing dose of light exposure, the measured TG activity in photoreceptors was increased, with a concomitant increase in nuclear TG2 [105]. This situation seems to be reminiscent of the effect of UVB in cultured corneal epithelial cells [8]. TG2 may therefore be a sensor of stressful environment, not only on the ocular surface, but also in the posterior segment. In rats intrinsically susceptible to retinal degeneration, TG2 activity increases with increased age (60 days), whereas in normal Lewis rats, continuous exposure to light over 2 days can have the same effect on TG2 activity level. Rat photoreceptor damage was observed in both instances, and this was likely due to elevated TG2 activity. These processes may mimic the sequence of events in human age related macular degeneration [105]. With regard to the role of TG in apoptosis in the retina, many questions remain. It is unclear whether TG2 is an effector or trigger of apoptosis. This is not merely an academic question. Transfection of neuroblastoma cells with plasmids expressing full length TG2 invokes apoptosis and reduced cellular proliferation [106]. This would be clearly undesirable in human eyes where a critical number of functional retinal elements is necessary to sustain vision. On the other hand, if TG2 is only an effector in apoptosis, it can serve to cross-link critical molecules in the dying cells in apoptotic bodies and limit the leakage of antigens, hence preventing post-inflammatory damage. Such considerations should be resolved before employing clinical strategies to enhance or inhibit TG function in the retina. Nevertheless, the above findings point to the possible relevance of TG2 in the mechanism of age related macular degeneration.

Endocrine diseases such as diabetes mellitus have various ocular manifestations [107]. These include complications such as diabetic retinopathy, which can be sight threatening. A study has found that in TG2 deleted mice, administration of glucose intraperitoneally resulted in glucose intolerance [108]. TG2 in cell membranes play a role in secretion of insulin stimulated by glucose. Interestingly, TG2 silenced mice demonstrated a tendency for hypoglycemia after administration of exogenous insulin. This is because TG2 silencing may result in phosphorylation of receptor substrate (IRS)-2, leading to enhanced insulin sensitivity. A missense mutation (N333S) in the TG2 gene active site has been found in Italians with maturity onset diabetes of the young [108]. Factor XIII and fibronectin have been found in pre-retinal membranes in diabetic retinopathy, suggesting a role for TG, at least in the advance stages of this disease [100].

Research in retinal photoreceptor and autophagy

For many years, it has been known that disc shedding and autophagy are normal physiological processes in the photoreceptors [109,110]. Recently, TG2 has been implicated in the inhibition of autophagy in the context of cancer cells [9,10]. It is currently not known if TG2 also affects autophagy in photoreceptors. Phototransduction in photoreceptors is an energy dependent process [111]. In view of the rapid depletion of ATP levels in cardiac tissue in a TG2 dependent way [112], it is theoretically possible that phototransduction may be affected by TG2 function as well.

TG is an invaluable tool in molecular research of the photoreceptor pigment. It can be used to insert a fluorescein probe into rhodopsin in photoreceptors [113]. Certain specific residues of rhodopsin in rod photoreceptors can be chemically crosslinked by erythrocyte TG [114]. Monodansyl cadaverine, a TG inhibitor, specifically labels the autophagic vacuoles and not other endosomes [11]. As a result, TG researchers have a valuable tool to investigate autophagy in living cells of the retina and other parts of the eye.

Advances in retinal surgery

TG2 may have various applications in retinal surgery. In retinal detachment surgery, one of the important aims is to establish adhesion of the neural retina to the underlying RPE [115]. Traditionally vitrectomy, scleral buckling, drainage of subretinal fluid, cryotherapy and laser photocoagulation or a combination of these procedures are used, with or without the aid of further tamponade from intraocular space-filling agents. At least in theory, one of the ways that relatively rapid adherence can be achieved is by means of a tissue adhesive. Most tissue adhesives however, require a dry surface to function optimally. A TG2 based adhesive has been proposed to aid reattachment of the retina as a form of adjunctive therapy in retinal detachment surgery [46]. This adhesive used a microbial TG that is calcium independent, in place of factor XIIIa, which is calcium dependent. Histological studies show no evidence of cellular toxicity two weeks after injection of the adhesive into the vitreous cavity of rat eyes. In addition, this adhesive also binds to bovine retinal tissue even under wet conditions.

Optic Nerve

In the optic nerve, TG2 may induce the differentiation of neuronal stem cells and precursors to neuronal elements. After severing the optic nerve in rats, central nervous system putative 'stem cells' such as oligodendrocytes might be able to differentiate into neuronal precursors with rudimentary visual function. This was evidenced by some electroretinographic recovery following injection of TG2 [116]. After transection of the optic nerve in goldfish, there was notable up-regulation of a neural TG during the axonal elongation stage (10 to 30 days) of nerve regeneration [117,118]. The increase in TG was only found in the ganglion cells and not in other layers of the retina. The authors successfully cloned and produced a recombinant TG from the goldfish retina. In explant cultures that are exposed to this recombinant TG, enhancement of neurite growth was observed. This effect was specific to TG addition, because short interfering RNA against this TG was able to suppress the neurite outgrowth. The authors concluded that the novel TG is a key regulator of central nervous system regeneration [117,118].

Orbit and Eyelids

Detection of TG protein in soft tissue tumors such as necrobiotic xanthogranulomas [119] has been reported, but it remains unclear if

TG is responsible for the formation or progression of lesions. Such lesions in the ocular adnexa are not only prone to recurrence, but are also associated with systemic malignancies such as multiple myelomas.

Lamellar ichthyosis is a rare, autosomal recessive, genetically heterogeneous skin disease caused by mutations in the TG1 gene [120]. Eye abnormalities in lamellar ichthyosis include bilateral ectropion of the lower eyelids, chronic blepharitis, and nuclear cataract. The skin of such affected individuals has greatly reduced TG activity. In lamellar ichthyosis, molecules apart from the cornified envelope proteins may be incorporated into the pericellular cornified envelope by TG1. A protease inhibitor such as the plasminogen activator inhibitor-2 has also been incorporated [121]. Other targets for crosslinking by TG include the C1 inhibitor [122], the protease inhibitors cystatin [123] and human procarboxypeptidase U [124]. Taken together, these studies suggest that the protease pathway in the extracellular matrix and the formation of fibrin in vasculature can be modulated by TG. For TG to exert its effect in the extracellular matrix, there should be a secretory pathway that transfers the protein from the cell interior outwards. Currently, however, this pathway is not fully understood [125].

Transglutaminase as a Target for Drugs

Understanding of the signaling pathways that involve TG in ocular tissues (Figure 2) is a crucial step to target TG in eye diseases. The development of new and better inhibitors to TG is an active area of research. Older inhibitors include cell permeable dansylcadaverine [126] and 3-halo-4,5-dihydroisoxazoles [127]. Newer inhibitors include LDN-27219 [17], which was discovered by screening 100 000 compounds. Another inhibitor, cystamine [15], was able to reduce TG2 levels in NZB/W F₁ mice with lupus-like autoimmune disease. The effect of TG2 inhibition by cystamine in such mice includes the decrease of MMP9, TIMP1 and 2, TNF- α and β , and more importantly, anti-cardiolipin autoantibodies. This suggests that anti-TG strategy has great potential in treatment of systemic lupus erythematosus, a devastating systemic inflammatory disease with ocular complications [15]. A high molecular weight inhibitor (Y-200) produced by bacteria has also been reported [16].

Since TG2 activity was increased in the aqueous humor of uveitis patients and in a rat model of ocular inflammation, it was suggested that TG2 inhibitors could be effective to treat uveitis [128]. An octapeptide with anti-TG properties was able to reduce lung inflammation in guinea pigs [129]. This may be a potential application in other mucous membrane diseases such as those in the ocular surface.

In the sclera, a previous study indicated that TG activity was mainly localized to the episcleral vessel walls [4]. Myopia is a refractive condition in humans characterized by abnormal scleral remodeling as a result of inappropriate visual stimuli [130]. TG1, 2, 3 and 5 are expressed in human scleral fibroblasts [131]. Previous research has shown that TG activity can be increased after agonistic stimulation of muscarinic receptors [132]. Anti-muscarinic receptor drugs are used in treatment of myopia in humans [133,134]. This suggests that antagonism of TG via muscarinic receptors may be a plausible method of intervention in scleral remodeling and possibly myopia retardation [131,135].

It is well known that TG1 expression in the keratinization process can be negatively regulated by the action of retinoic acid [136]. The mouse TG promoter is activated by all-trans retinoic acid and by retinoic acid receptor (RAR)-specific and retinoid X receptor (RXR)-specific retinoids [137]. Retinoic acid has been shown to oppose TG1

expression induced by interferon gamma [31]. One attractive idea in hyperkeratotic disorders is to treat with retinoic acid associated drugs [138]. Clinical trials in skin diseases such as psoriasis have used drugs that alter TG1 or TG activity in skin tissue [136,139,140]. In one randomized placebo controlled study based on the use of a drug that elevates retinoic acid, psoriasis can be significantly improved compared to placebo [141]. In a randomized controlled parallel group clinical trial, treatment with vitamin A (a retinol which can be converted to retinoic acid) and cyclosporine A 0.05% eyedrops were found to be equally efficacious in the treatment of dry eye syndrome [142].

Conclusions

Greater understanding of the biology and biochemistry of TG, especially TG2, is now possible because of advances in molecular biology, genomics and proteomics techniques. Some cellular processes affected by TG2, such as stress regulatory pathways and inflammation are now better understood (Figure 2), as TG2 mediated diverse molecular pathways, it may be anti-inflammatory or pro-inflammatory depending on the context.

Systemic inflammatory diseases may have ocular complications which can be improved by systemic treatment. Scientists are beginning to appreciate the importance of TG in the ocular surface, anterior and posterior segments of the eye. Taking advantage of the understanding of TG in critical processes such as wound healing and modulation of inflammation, translational research in ocular diseases will be greatly enhanced. A myriad of ocular diseases are bound to benefit from the recent advances, including dry eye, allergic conjunctivitis, glaucoma, cataract and retinal degeneration. Clinical trials in dermatology that interfere with TG1 function have already been attempted. Mediators of keratinisation such as retinoic acid related molecules in the ocular surface have progressed into clinical trial. There is no doubt that in the coming years, more light will be shed on the roles of these enzymes in the eye, and more treatment strategies related to these proteins will be developed in ophthalmology.

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