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REVIEW

The Proteins of Keratoconus: a Literature Review Exploring Their Contribution to the Pathophysiology of the Disease

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ABSTRACT

Introduction: Keratoconus (KC) is a complex, genetically heterogeneous multifactorial degenerative disorder characterized by corneal ectasia and thinning. Its incidence is approximately 1/2000–1/50,000 in the general population. KC is associated with moderate to high myopia and irregular astigmatism, resulting in severe visual impairment. KC structural abnormalities primarily relate to the weakening of the corneal collagen. Their understanding is crucial and could contribute to effective management of the disease, such as with the aid of corneal cross-linking (CXL). The present article critically reviews the proteins involved in the pathophysiology of KC, with particular emphasis on the characteristics of collagen that pertain to CXL.

Methods: PubMed, MEDLINE, Google Scholar and GeneCards databases were screened for relevant articles published in English between January 2006 and June 2018. Keyword combinations of the words "keratoconus," "risk

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factor(s),” “genetics,” “genes,” “genetic association(s),” “proteins”, “collagen” and “cornea’’ were used. In total, 272 articles were retrieved, reviewed and selected, with greater weight placed on more recently published evidence. Based on the reviewed literature, an attempt was made to tabulate the up- and down-regulation of genes involved in KC and their protein products and to delineate the mechanisms involved in CXL.

Results: A total of 117 proteins and protein classes have been implicated in the pathogenesis and pathophysiology of KC. These have been tabulated in seven distinct tables according to their gene coding, their biochemistry and their metabolic control.

Conclusion: The pathogenesis and pathophysiology of KC remain enigmatic. Emerging evidence has improved our understanding of the molecular characteristics of KC and could further improve the success rate of CXL therapies.

Keywords: Collagen; Corneal biomechanical characteristics; Corneal cross-linking; CXL; Ectasia; Keratoconus proteins; Ophthalmology

INTRODUCTION

Keratoconus (KC) is a corneal dystrophy that seriously affects the quality of life of KC patients [1]. Its prevalence ranges between 1/2000 and 1/50,000 in the general population. The condition is characterized by moderate to substantial visual impairment due to the development of corneal protrusion and thinning [2–6]. In KC, visual acuity decreases due to progressive myopia, irregular astigmatism and, often, corneal apical opacification [4, 6, 7]. The key underlying factor for these pathological changes is weakening of collagen tissue in corneal stroma [6, 8]. Although KC is generally a bilateral disorder [2, 9, 10], rare unilateral cases have also been described [11].

METHODS

The present review is based on literature search that utilized the PubMed, MEDLINE, Google Scholar and GeneCards databases for articles related to KC. The keywords used were “keratoconus,” “risk factor(s),” “genetics,” “genes,” “genetic association(s),” “proteins,” “collagen” and “cornea’’ and all their relevant combinations. The search focused on articles written in English from January 2006 until June 2018. A total of 177 articles were identified and reviewed, and both their text and references were analyzed. The analysis of these references revealed an additional 95 relevant articles, which were also subsequently reviewed. The current article is based on previously conducted studies and does not contain studies on human subjects or animals performed by any of the authors; its aim is to summarize current knowledge on the proteins involved in KC. It also represents an attempt to map and categorize the proteins that are up- or down-regulated in KC according to their corneal stromal location.

RESULTS

Histopathology, Biochemistry and Biomechanics of KC Corneas

Histologically, KC is characterized by degradation of the basal membrane of the corneal epithelium, diminishing of the number and density of collagen fibrils, thinning of the corneal stroma, and keratocyte apoptosis with necrosis [2–4, 9]. Keratocyte apoptosis and necrosis involves the central anterior stroma and Bowman’s lamina and, typically, weakens the corneal tissue [12, 13]. Slit-lamp examination reveals subepithelial and anterior stromal scars [14] due to degeneration of epithelial basal cells, as well as fine folds in the posterior stroma at the corneal apex (Vogt’s striae). Endothelial damage is rarely visible. The presence of Fleisher’s ring, which is formed by the accumulation of hemosiderin around the base of the corneal cone [2, 15], is also frequent. In contrast, breaks of the Descemet membrane with subsequent leakage of aqueous humor into the corneal stroma, leading to corneal edema and decompensation (acute hydrops), are rare. Finally, evident corneal nerves together with a

decrease in sub-basal nerve density are prominent features of advanced KC [2, 15, 16].

The stroma in KC is characterized by a decrease in the number of collagen lamellae and a reduction in the amounts of microfibrillar and fine granular material [17]. Changes are also observed in the arrangement of fibrils of the anterior stroma [15, 18], together with abnormal distribution of collagen fibers. This results in the decrease of corneal mechanical resistance [15, 18].

Overall, the thinning of the corneal stroma itself is associated with keratocyte apoptosis, alterations in the extracellular matrix (ECM), and changes in the activities of several enzymes [12, 19] that involve the activation of degrading enzymes and the promotion of cell death, both of which are mostly due to oxidative stress [20, 21]. Although the mechanisms of tissue breakdown remain obscure [21], over the last 25 years there has been strong evidence that collagens are susceptible to oxygen-free radical damage [20, 22]. In addition, collagen is the only protein susceptible to fragmentation by superoxide anions, a process during which small 4-hydroxyproline-containing-peptides are liberated [20]. In the presence of oxygen, hydroxyl radicals cleave collagen into small peptides; the cleavage is, apparently, specific to proline or 4-hydroxyproline residues [20]. In contrast, hydroxyl radicals, in the absence of oxygen, do not induce fragmentation of collagen molecules, but they trigger the polymerization of collagen through the formation of new cross-links such as dityrosine or disulfide bridges [20].

Thinning of the stroma and alterations of ECM in KC affect the biomechanical properties of the cornea. Comparisons of uniaxial tensile strength between KC and normal corneas have shown that maximum load and stress, maximum stiffness, and relative energy absorption were smaller in keratoconic corneas than in normal ones [2, 23]. Furthermore, load and stress values at corresponding strain values were smaller in keratoconic compared to normal corneas both for the initial (exponential) and for the linear parts of the curves [23]. In addition, no differences between KC and normal corneas have been detected in uronic acid or hydroxyproline concentrations [23]—an indication that the alteration of corneal biomechanical properties in KC are the result of collagen cleavage into smaller peptides [23]. This conclusion is further supported by the observation that the solubility of pepsin-treated collagen from KC corneas is greater than that from normal corneas—apparently due to the breakdown of KC corneal collagen into smaller peptides that has already occurred [23].

Collagen

The amount of collagen, which is a major corneal protein [2, 24, 25], is reduced in KC [12, 26]. The decrease in collagen types I and III is related to alterations in the ECM basement membrane [26], while the decrease in collagen type IV is related to alterations in the basement membranes, of which collagen type IV is the major structural component [24, 27, 28]. In addition to the decrease in the amount of collagen in the cornea, the shape and transparency of the cornea also change due to re-orientation of the collagen molecules and fibrils [29, 30]. The combination of changes in collagen orientation and the reduction of total collagen contribute to corneal thinning in KC [30].

Type IV collagens are major structural components of basement membranes and consist of six proteins encoded by six genes (COL4A1–COL4A6). Interestingly, these genes are organized in pairs in a head-to-head conformation so that each gene pair shares a common promoter [31]. Thus, the COL4A6 gene is organized in a head-to-head conformation with the COL4A5 (alpha 5 type IV collagen) gene. Similarly, the gene pairs for COL4A1 and COL4A2 and for COL4A3 and COL4A4 are also conformed head-to-head [32]. This means that deletions in one member of the pair of genes extend into the other; it also means that alternative splicing results in multiple transcript variants encoding different isoforms [24, 33].

Collagen type IV α1/α2 chains have been reported in KC patients [34], while COL4A1 has been shown to be down-regulated in KC corneas [35]. The chromosomal loci of COL4A1 and COL4A2 are close to 13q32 [32], with the

COL4A1 gene consisting of 52 exons and COL4A2 gene of 48 exons [28]. Mutations in COL4A3 and COL4A4 may be related to decreases in collagens I and III which contribute the increase of the KC risk [28]. It is noteworthy that there are several reports that COL4A3 has been found to be associated with KC in at least two European populations [28, 36]. Furthermore, genomic loci that differ between ethnic groups and are associated with variation in central corneal thinning [37, 38] are found in the genes that encode COL1A1, COL1A2, COL8A2 and COL5A1 [37, 38].

There are seven polymorphisms (M1327 V, V1516 V and F1644F in COL4A4, and P482S, P141L, D326Y and G895G in COL4A3) that are associated with KC under recessive, dominant or additive models [28, 39]. Finally, the expression of collagen types XII, XIII, XVIII and XV is altered in KC, although no relationships have been identified between mutations in these genes and their expression [40, 41]. It should be noted that collagen types XIII, XV, and XVIII are mainly expressed in basal corneal cells, and that these types are involved in the adhesion between corneal epithelial cells as well as between corneal epithelial cells and the basement membrane [35, 41].

Thus, changes in collagen have a vital role in the progression of KC. Ensuring collagen stability is the main target in corneal cross-linking (CXL), a relatively new, minimally invasive outpatient procedure used for the management of KC. Before the advent of CXL, there was no treatment to modify the underlying pathophysiology and arrest corneal ectasia. The introduction of this method to ophthalmology goes back to 1997 [42]. Subjective and objective results following this method seem to be promising. CXL is a photo-induced reaction, used to increase the rigidity of the corneal collagen and its resistance to ectasia, ensuring the mechanical and biochemical stability of the stromal tissue. Ultraviolet light A at 370 μm excites riboflavin (vitamin B_2) which functions as a photosynthesizer. Free radicals and oxidizing substance cause the formation of new covalent bonds between collagen fibrils within the cornea which stabilize stroma [43].

Other Proteins Involved in KC

There are more than 1500 different peptides and proteins in the human tears, both of intra- and extracellular origin. Their anatomic location, the genes that code for them and their potential implication in KC are summarized in Tables 1, 2 and 3. They include cytokines and small molecules and are followed by lipids and metabolites [44, 45]. Among them, one can distinguish lactoferrin, secretory immunoglobulin A, tear lipocalin, lysozyme, lipophilin, proline-rich proteins and serum albumin, all of which help maintain the health of the ocular surface [44–46]. Since abnormal levels of enzymes and inflammatory molecules are found in KC patients [44, 47, 48], the deregulation of proteins has been linked to the thinning of the cornea [14, 49]. Changes in the structural integrity of the cornea including changes in the collagen content (e.g., the reduction in the number of collagen lamellae) [2, 9], and alterations in enzymes have been reported in KC patients [14, 49]. The biochemical abnormalities observed in the KC cornea (epithelium and stroma) include the increase in the level of degradative enzymes and the decrease of the protease inhibitors [50].

Recent clinical studies imply the existence of an inflammatory component in the pathogenesis of KC [47, 51]. This means that tissue degradation in KC involves the expression of pro-inflammatory cytokines, inflammatory mediators, cell adhesion molecules and matrix metalloproteinases (MMPs) [47, 51, 52]. Inflammation in KC is characterized by the presence of many inflammatory cells and markers such as IL-1, IL-6, MMP-9, TGF-β, and TNF-a [47, 51–54]. Moreover, inflammatory factors like IL-4, IL-5, IL-6, IL-8, TNF-α, TNF-β, MMP-1, MMP-3, MMP-7, MMP-9, MMP-13 and cathepsins are increased in the tears of KC patients [47, 51, 52], a finding that suggests the deregulation of the underlying molecular pathways [55]. Table 4 summarizes the proteins that have been identified as definitive or potential biomarkers in KC together with the genes that are known to code for them.

Table 1 Proteins (and their genes) of the corneal epithelium and their regulation in keratoconus

Up-regulated		Down-regulated	
Protein	**Gene [reference]**	**Protein**	**Gene [reference]**
S100 calcium-binding protein A4 (S100A4)	S100A4 [44]	Calpain small subunit 1 (CAPNS1)	CAPNS1 [14, 44]
Cytokeratin	KRT [44]	FTH 1 (ferritin heavy chain protein 1)	FTH1 [14, 44]
Gelsolin	GSN [44]	Annexin A2	ANXA2 [14, 44]
Alpha enolase (ENO1)	ENO1 [44]	Heat shock protein beta 1 (HSPB1)	HSPB1 [14, 44]
Keratin-5 (KRT5)	KRT5 [14, 44]		
Annexin A8	ANXA8 [14, 44]		
L-lactate dehydrogenase (LDH)	LDH [14, 44]		
Serum albumin	ALB [14, 44]		
SFRP1 (secreted frizzled-related protein 1)	SFRP1 [44]		

Table 2 Proteins (and their genes) found in both epithelium and stroma and their regulation in keratoconus

Up-regulated		Down-regulated	
Protein	**Gene [reference]**	**Protein**	**Gene [reference]**
Increased epithelial and stromal		Decreased epithelial and stromal	
Keratin type I cytoskeletal 14 (KRT14)	KRT14 [14, 44]	Transketolase	TKT [14, 44]
Keratin type I cytoskeletal 16 (KRT16)	KRT16 [14, 44]	Pyruvate kinase	PKLR [14, 44]
Tubulin beta chain (TUBB)	TUBB [14, 44]	Stratifin (14-3-3 sigma isoform)	SFN [14, 44]
Lamin-A/C (LMNA)	LMNA [14, 44]	Phosphoglycerate kinase 1 (PGK 1)	PGK1 [14, 44]
S100 calcium-binding protein A4 (S100A4)	S100A4 [14, 44]	NAD (P)H dehydrogenase (quinone) 1	NQO1 [14, 44]
Heat shock cognate 71 kDa protein (HSPA8)	HSPA8 [14, 44]		

Protein Changes in KC

Changes in proteins that might contribute to corneal thinning include the increase of degradative enzymes such as acid phosphatases, acid esterases and acid lipases [24]. In eyes with KC, the levels of enzymes such as cathepsin B and cathepsin G increase, while protease inhibitors like the a1 proteinase inhibitor (a1-PI) and the a2 macroglobulin decrease [14, 50]. Corneal thinning therefore is caused by the up-regulation of cellular proteases and the down-regulation of their inhibitors [14]. Both enzyme up-regulation and enzyme inhibitor down-regulation involve the mRNA as well as the protein levels of expression [56]. The increase of corneal proteinase activity is also involved in the pathophysiology of KC [50]. As a result, KC may

Table 3 Proteins (and their genes) exclusive of the stroma and their regulation in keratoconus

Up-regulated		Down-regulated	
Protein	**Gene [reference]**	**Protein**	**Gene [reference]**
Vimentin	VIM [14, 44]	β-actin (ACTB)	ACTB [14, 49, 71]
Keratocan (KTN)	KERA [14, 44]	TGF-beta (transforming growth factor beta) induced ig-h3 (Bigh3) protein	TGFBI [14, 44]
Decorin	DNC [14, 44]	Meprin A-5 protein, and receptor protein-tyrosine phosphatase mu (MAM domain)	14, 44
Keratin 12	KRT12 [14, 44]	Serotransferrin	TF [14, 44]
Haptoglobin precursor (HP)	HP [14, 44]	HuR (human antigen R)	ELAVL1 [14, 49, 71]
Apolipoprotein A-IV precursor (APOA4)	APOA4 [14, 44]	Isoforms 2C2A of collagen alpha-2 (VI) chain	COL6A2 [14, 44]
ALDH3A1 (aldehyde dehydrogenase 3A1)	ALDH3A1 [14, 44]		
Lipoprotein Gln	LPL [14, 44]		
Prolipoprotein	14, 44		
TIMP3	TIMP3 [81, 142]		
TIMP1	TIMP1 [14]		

develop because of defective regulation of the proteinase activity in the cornea [50]. Nitrotyrosine malonaldehyde, glutathione S transferase, and inducible nitric oxide synthase levels increase, while aldehyde dehydrogenase and superoxide dismutase levels decrease [14]; in addition, there is an accumulation of GAG (glycosaminoglycan) polyanions in keratoconic corneas [57], while analysis of the KC corneal proteome indicated a decrease in decorin, keratocan, lumican, and biglycan [58]. Keratan sulfate (KS) antigenicity appears to decrease in the central, thinned region of the keratoconic cornea [59], while the KS organization in corneas with advanced KC is markedly different from that in healthy corneas [60]. Interestingly, there are molecular alterations in proteoglycans in some connective tissue disorders that are associated with KC [61, 62].

Gross cystic disease fluid protein-15 (GCDFP15), a secretory glycoprotein, expressed in the proteome of human tear fluids [63], consists of 14 kDa [64] and is a potential biomarker for KC [63]. Analysis of the tear proteome shows that the key for the maintenance of a healthy corneal structure is the quality of tear fluids [65]. Three-dimensional cultures of human KC cells [66, 67] show an increase in oxidative stress levels when compared to normal human corneal fibroblast cultures [68]. Other known pathogenic factors are prolactin-induced protein (PIP) [69] which is involved in actin binding and the zinc-alpha-2-glycoprotein (AZGP1) [65]. The interplay between these two proteins (PIP-AZGP1) is apparently involved in KC pathogenesis [63].

PIP expression is regulated by transforming growth factor-β (TGF-β), which has a vital role in corneal wound healing and is implicated in the pathophysiology of KC [65]. The TGF-β signaling pathway is a complex, multibranched signal transduction cascade that may modulate ECM, making it a potential contributing factor in KC pathogenesis [21]. However, increases of TGF-β2 levels in the aqueous humor of

Table 4 Proteins that serve as biomarkers in keratoconus and their genes

Proteins	Gene [reference]
COL8A1	COL8A1 [40]
COL8A2	COL8A2 [40]
Basal corneal cells	
Collagen type XII	COL12A1 [35, 41]
Collagen type XIII	COL13A1 [35, 41]
Collagen type XVIII	COL18A1 [35, 41]
Collagen type XV	COL15A1 [35, 41]
IL1A	IL1A [119, 126, 127]
IL1B	IL1B [119, 126, 127]
SPARC (secreted protein acidic and rich in cysteine)	SPARC [81–83]
Human leukocyte antigen	81–83
TIMPs	TIMP [68, 81, 142]
SLC4A11 (sodium bicarbonate transporter-like protein 11)	SLC4A11 [91, 92]
AQP5 (water channel protein aquaporin 5)	AQP5 [93]

keratoconic patients are not confirmed by immunofluorescence studies [21].

The mRNA of human antigen R (HuR) is a very important post-transcriptional regulator of gene expression that is expressed in all proliferating cells [70]. The expression of mRNA and protein levels of β-actin and HuR are decreased on KC corneal stroma compared to normal corneas [14, 49, 71], indicating that the down-regulation of β-actin and HuR is the result of their mutual interaction [71].

KC is also associated with amyloid deposits [72, 73], and increased levels of specificity protein 1 (SP1) characterize KC corneas [74]. Species-specific tRNA processing (STP1) transcription factor is involved in tRNA maturation; STP1 transcription factor belongs to a specificity protein/Kruppel-like factor family, and it shares structural similarities and sequence homology with 25 other members of this family [75, 76]. The over-expression of SP1 is associated with neuro-degenerative diseases such as Huntington's chorea [77] and tumorigenesis [78], while the promoter activity of the human α1-PI gene in corneal cells is suppressed by the up-regulation of Sp1 [79, 80].

Other proteins such as secreted protein acidic and rich in cysteine (SPARC), human leukocyte antigen, mitochondrial complex I genes and 2q21.3 with RAB3 GTPase-activating protein subunit 1 (catalytic) are also involved in KC [81–83]. Luminac, biclycan, keratocan and decorin decrease in KC corneas [84], whereas keratocytes express heparan sulfate proteoglycans [85, 86]. In addition, bone morphogenic protein 4 and the gene and protein expression of TGF are increased in KC corneas [21, 87].

The epithelial–endothelial IL-1 system is vital, not only for the organization of the cornea but also for its ability to respond to mechanical injuries and pathogen invasions, by provoking keratocyte apoptosis [88]. The up-regulation of IL-1 in KC induces keratocyte apoptosis [89] and manifests itself as an increase in IL-1 protein levels and a simultaneous rise in the number of IL-1 binding sites [87, 90]. The IL-1 family consists of pleiotropic cytokines. The genes that encode for the IL-1 cytokines are located on chromosome 2q14 [89] and their transcription is affected by polymorphisms located in this region [89]. Finally, cytokines IL-1α and IL-1β control the immune response, the pro-inflammatory response and hematopoiesis; their effects are modulated by the IL-1 receptor antagonist [91].

Sodium bicarbonate transporter-like protein 11 (SLC4A11) belongs to the SLC4 family of bicarbonate transporters [91] and may participate in KC pathogenesis, because its functional failure leads to keratocyte apoptosis [91, 92]. The SLC4A11 gene is located in the 20p13 chromosome and functions as an electrogenic, Na + coupled, borate co-transporter [91]. Also,

the water channel protein, aquaporin 5, may be a marker for KC [93].

Some HLA antigens, including HLA-A26, B40 and DR9, have been linked to early onset KC [82]. In addition, keratoconic corneas manifest a decrease of IGF-2 transcripts and over-expression of IGF-binding proteins 3 and 5 [94]. Proteins such as dual-specificity phosphatases or mitogen-activated protein kinase phosphatases (MKPs) are activated by oxidative stress in KC [95]. The same proteins regulate the immune response and dephosphorylate tyrosine and threonine residues on MKPs [96].

Proteomic analyses of KC patient epithelia using two-dimensional gel electrophoresis followed by mass spectrometry [44] identified high over-expression of S100A4, cytokeratin and gelsolin in keratoconic epithelium [44], while alpha enolase was slightly up-regulated. Other studies, using the same method, suggest that beta actin and alpha enolase are slightly expressed in corneal wing and superficial epithelial cells of KC patients [44, 49]. It is interesting that proteins like gelsolin and cytokeratins are implicated in ocular (e.g., vitreoretinopathy) and non-ocular (e.g., cystic fibrosis, cancer, steatohepatitis) disorders [44, 97].

Corneal epithelial and stromal proteins are expressed differentially in KC [14, 44], and six epithelial proteins, keratin type I cytoskeletal 14, keratin type I cytoskeletal 16, tubulin beta chain, lamin-A/C, S100-A4 and heat shock cognate 71 kDa protein, were identified using a label-free nano-ESI-LC–MS/MS method to be increased in KC. Five other proteins, transketolase, pyruvate kinase, 14-3-3 sigma isoform, phosphoglycerate kinase 1 and nicotinamide adenine dinucleotide phosphate, and dehydrogenase (quinone) 1, are decreased [14, 44].

Stromal proteins, such as vimentin, keratocan, decorin, keratin 12, haptoglobin precursor, apolipoprotein A-IV precursor, aldehyde dehydrogenase 3A1 (ALDH3A1), lipoprotein Gln and prolipoprotein, are up-regulated in KC [14, 44]. while A-5 protein, TGF-beta (transforming growth factor beta) ig-h3 (Bigh3), receptor protein-tyrosine phosphatase-μ (MAM) domain-containing protein 2, serotransferrin, meprin, and isoforms 2C2A of collagen alpha-2 (VI) chain are down-regulated [14, 44]. Of the proteins of the corneal epithelium, keratin-5, annexin A8, L-lactate dehydrogenase and serum albumin are up-regulated [14, 44], and calpain small subunit 1, Ferritin heavy chain protein 1 (FTH 1), annexin A2 and heat shock protein beta 1 are down-regulated [14, 44].

Recently, the role of β-galactoside-binding proteins galectin (Gal)-1 and Gal-3 in patients with KC and postcorneal CXL treatment was investigated in vitro [98]. These proteins are associated with various cellular responses that involve inflammation [99]. In KC, increased levels of Gal-1 and Gal-3 were detected in conjunctival epithelial cells compared to controls [98]. In addition, keratocytes of KC patients were found to release significant amounts of Gal-1 in the stroma. In vitro, CXL promoted the release of Gal-1 from keratocytes but decreased the concentration of inflammatory biomarkers, such as IL-6, IL-8, MMP-2 and MMP-9 [98]. Overall, these results suggest that CXL exerts an immunosuppressive effect on keratocytes by inhibiting the release of MMPs and cytokines and increasing the levels of anti-inflammatory Gal-1 [98].

The FAS/FASLG Genes and Apoptosis

Apoptosis is considered the primary pathway of cell death in KC [13]. Diseases related to defective apoptotic mechanisms are associated with polymorphisms in the FAS [100] and FASLG [101] genes [102, 103]. The FAS-encoded protein belongs to the TNF-receptor superfamily and contains a death domain. It has a central role in the physiological regulation of programmed cell death, whereby its interaction with its ligand leads to the formation of a death-inducing signaling complex that includes the FADD (Fas-associated death domain protein) and caspases 8 and 10 and is responsible for apoptosis [100]. The FAS isoforms that lack the transmembrane domain may negatively regulate the apoptosis mediated by the full-length isoform. The FASLG gene also belongs to the TNF superfamily. It encodes a transmembrane protein which, when it binds to FAS, triggers apoptosis [101].

The FAS/FASLG system is expressed in the cornea and potentially has an important function in the physiology of the normal cornea, as well as the pathophysiology of corneal diseases [103, 104] through the modulation of keratocyte apoptosis following epithelial injury. Indeed, IL-1 stimulation induces corneal fibroblasts to produce apoptosis-associated FAS ligand. The fact that the same cells also express the FAS receptors makes them ideal candidates for inducing autocrine suicide of keratocytes in KC corneas. In addition, the development of KC is also characterized by an increase in the corneal sensitivity to apoptotic cytokines owing to abnormalities in the components of the FAS pathways [88]. This association between chronic keratocyte apoptosis and ongoing epithelial injury could be the link to previously unrecognized risk factors for KC, such as chronic eye rubbing, contact lens wear or atopic eye disease [105]. Finally, it is noteworthy that apoptosis is a common mechanism between KC and Fuchs corneal endothelial dystrophy [13, 106].

Other Genes Involved in KC Pathogenesis

KC has been associated with genes and proteins that regulate cellular and extracellular processes like wound healing, keratocyte proliferation, oxidative damage, differentiation, apoptosis and proteolysis [107, 108]. These genes are up-regulated in KC and myopic corneas [109]. In addition, KC patients present with abnormal gene expressions in at least some genes [44] identified using genome-wide scans, genome-wide association studies, studies of descent, and family-based linkage studies [110]. Additionally, more than 50 candidate genes have been excluded as contributors to KC development [111, 112]. Tables 5, 6 and 7 summarize the proteins that have been identified as targets of up- or down-regulation in KC as well as those genes that are known to code for them.

KC is characterized by genetic heterogeneity [2, 4, 8, 112]. Many distinct genetic loci have been mapped for KC, but none has been confirmed as a KC-associated genetic factor [113, 114]. Some of those variants apparently are more penetrant or associated with a more severe outcome [112, 114]. These variants have been identified in diverse populations and localities. Thus, KC chromosomal loci 16q22.3–q23.1 [115] were identified in 20 small Finnish pedigrees [115]; locus 20q12 was identified in an isolated population in Tasmania, Australia [116]; loci 5q22.23–q24.2 were identified in a large Northern Irish family [117]; loci 5q14.3–q21.1 were identified from a large four-generation white family pedigree [118]; and loci 3p14–q13 were identified in an Italian two-generation autosomal dominant pedigree [119].

Other loci associated with KC have been mapped to chromosomes 20p11.21 (KTCN1) [120], 16q22.3–q23.1 (KTCN2) [115], 3p14–q13 (KTCN3) [119], 2p24 (KTCN4) [121], 1p36.23–36.21 [122], 4q31 [116, 123], 5q14.3–q21.1 [118], 5q21.2 [124], 5q31 [123], 5q32–q33 [124], 8q13.1–q21.11 [123], 8q24 and 9q34 [123], 12p12 [123], 13q32 [125], 14p11 [116, 123], 14q11.2 [124], 14q24.3 [123], 15q2.32 [124], 15q22.23–q24, 16q22-q23, 17q24, 20q12, 21q [115, 117, 126], and 17p13 [123, 127].

Generally, there are KC-associated single-nucleotide polymorphisms (SNPs), as in the cases of COL4A3 (collagen type IV, alpha 3) at 2q36–q37, COL4A4 (collagen type IV, alpha 4) at 2q35–q37, IL-1A and IL-1B (both members of the interleukin 1 cytokine family) [28, 112, 128, 129]. It should be noted that the protein encoded by IL-1A is a pleiotropic cytokine involved in various immune responses, and is proteolytically processed and released in response to cell injury, inducing apoptosis [130]. The protein encoded by IL-1B is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [131].

Several other candidate genes have been proposed in KC, including SOD1 (superoxide dismutase 1 gene) (MIM 147450, locus 21q22.11), TGβI, DOCK9 (dedicator of cytokinesis 9 gene), which is located at 13q32, ZEB1, FLG [123, 132–136], LOX (lysyl oxidase) at 5q23.2, VSX1 (visual system homeobox-1 gene) (KTCN1, MIM605020, locus 20p11.2), TGF-β1, IL-1A (interleukin 1A gene), IL-1B (interleukin

Table 5 Up-regulated or down-regulated proteins and their genes (if identified) in keratoconus

Up-regulated proteins	Gene [reference]	Down-regulated proteins	Gene [reference]
Collagen type XV	COL15 [28]	Collagen	12, 26
Inflammatory mediators	47, 51, 52	Collagen type I, II, IV	COL1A1, COL1A2, COL2A1 [26]
Proinflammatory cytokines	47, 51, 52	COL4A1	COL4A1 [35]
Cell adhesion molecules	47, 51, 52	a1-PI (a1 proteinase inhibitor)	14, 28
MMPs	47, 51, 52	a2 Macroglobulin	A2 M [14, 50]
Degradative enzymes	14	Inhibitors of cellular proteases	14
Acid phosphatases	14	Aldehyde dehydrogenase	ALDH [14]
Acid esterases	14	Superoxide dismutase	SOD [14]
Acid lipases	14	Decorin	DCN [58, 84]
Cathepsin B	CTSB [14, 50]	Keratocan	KERA [58, 84]
Cathepsin G	CTSG [14, 50]	Lumican (LUM)	LUM [58, 84]
Cellular proteases	14	Biglycan	BGN [58, 84]
Nitrotyrosine malonaldehyde	14	KS (Keratan sulfate) antigenicity- central corneal	KERA [59]
Glutathione S transferase (GSTs)	GST [14]	Collagen type IV	COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, COL4A6 [28]
iNOS (inducible nitric oxide synthase)	NOS1, NOS2, NOS3 [14]	IGF-2 transcripts	IGF2 [94]
GAG (glycosaminoglycan) polyanions	84	IGKC (immunoglobulin kappa chain)	IGKC [44]
(AZGP1) zinc-alpha-2-glycoprotein	AZGP1 [69]	Lactoferrin (LF)	LTF [44]
GCDFP15 (gross cystic disease fluid protein 15)-prolactin-inducible protein (PIP)	PIP [64, 69]		
SP1 (specificity protein 1)	SP1 [73]		
Amyloid deposits	72		
BMP4 (bone morphogenic protein 4)	BMP4 [21, 86, 87]		

Table 5 continued

Up-regulated proteins	Gene [reference]	Down-regulated proteins	Gene [reference]
TGF	TGFBI [21, 86, 87]		
HSPGs (heparan sulfate proteoglycans)	HSPG2 [83, 85]		
IL-1	IL1α, IL1β [87, 89]		
IGFBP (IGF binding proteins) 3 and 5	IGFBP3, IGFBP5 [94]		
DUSPs (dual-specificity phosphatases)	(DUSP) [95]		
MKPs (mitogen-activated protein kinase phosphatases)	(MAPK) [95, 96]		
TNF-a	TNF [44, 47, 51, 52]		
ANGPTL7 (angiopoietin-related protein 7)	ANGPTL7 [84]		
TIMP3	TIMP3 [81, 142]		
MMP2	MMP2 [14]		
MMP9	MMP9 [55]		

Table 6 Proteins (and their genes) associated with keratoconus but uncertain as to their role

Protein	Gene [reference]
HLA-A26	HLA-A*26 [82]
HLA-B40	HLA-B*40 [82]
HLA-DR9	HLA-DR*9 [82]
Gelsolin	GSN [44, 97]
Cytokeratins	KRT [44, 97]

Table 7 Proteins (and their genes) found in keratoconus tears but uncertain as to their role

Protein	Gene [reference]
Lipocalin	LCN [44]
Lysozyme C	LYZ [44]
Immunoglobulin alpha (IgA)	IgA [44]
Immunoglobulin kappa (IGKC)	IGKC [44]
Precursors to prolactin	44

1B gene) at 2q14, IPO5, STK24 and HGF (hepatocyte growth factor) [89, 112, 120, 133, 134, 137, 138]. It is of interest that the protein encoded by FLG is an intermediate filament-associated protein that aggregates keratin intermediate filaments in the mammalian epidermis [139]. It is also of interest that the SOD1 gene, along with the CRB1 gene, have been implicated in Leber congenital amaurosis and Down syndrome, both of which are associated with KC [133, 140].

Genes VSX1, SOD1, IL-1B, COL4A3, COL4A4 and LOX are the most probable genetic substrates of KC [28, 112, 120, 133, 141], although the role of VSX1 in the pathogenesis of KC remains controversial [142]. The LOX gene encodes an enzyme that initiates the crosslinking of collagens and elastin by catalyzing

oxidative deamination of the epsilon amino group in lysine residues of elastin and lysine and hydroxylisine residues of collagen [81]. Other genes that have been implicated in KC pathogenesis are the microRNA 184 (miR-184) gene which is positioned at 15q22–q25 [143], and the SPARC, MMP-2, MMP-9, COL6A1, COL8A1, and TIMP-3 [81, 112]. The TIMP-3 gene belongs to the tissue inhibitor of metalloproteinase gene family that are involved in ECM degradation [144]; mutations in these genes have been linked to Sorsby's fundus dystrophy, an autosomal dominant disorder [144]. In addition, the MPDZ-NF1B (rs132183), FOXO1 (rs2721051), RXRA-COL5A1 (rs1536482), COL5A1 (rs7044529), FNDC3B (rs4894535) and BANP-ZNF469 [112, 145, 146] genes have been implicated in the pathogenesis of KC.

Finally, in KC patients from Poland, two SNPs in the RAD51 [147] gene have been genotyped [148]. The protein encoded by the RAD51 gene interacts with BRCA1 and BRCA2, which are important elements of the cellular response to DNA damage. BRCA2 regulates both the intracellular localization and the DNA-binding ability of this protein, and its inactivation is thought to lead to genomic instability and tumorigenesis [147].

CONCLUSION

The pathophysiology of keratoconus is multifactorial and still elusive. Differential expression of several corneal proteins in KC [14, 49] results in changes in the structural integrity of the cornea, its collagen content and its morphology [14, 49]. The biochemical abnormalities observed in corneal epithelium and stroma in KC include increased activity of degradative enzymes and reduced activity of protease inhibitors [50]. Corneal thinning, therefore, is probably caused by the up-regulation of cellular proteases and the down-regulation of their inhibitors [14]. The increase of proteinase activity [50] results in the induction of degradative process in the cornea [50]. Moreover, oxidative damage and keratocyte apoptosis seem to play an important role in the etiology of KC.

Exploring the proteomic changes in KC and analyzing its complex genetics will increase our understanding of its pathophysiology, and, most importantly, will potentially enable targeted genetic treatments in the future.

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