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Roles of lumican and keratocan on corneal transparency

Winston W.-Y. Kao and Chia-Yang Liu

Department of Ophthalmology, University of Cincinnati, Cincinnati, OH 45267-0527, USA

Lumican and keratocan are members of the small leucine-rich proteoglycan (SLRP) family, and are the major keratan sulfate (KS) proteoglycans in corneal stroma. Both lumican and keratocan are essential for normal cornea morphogenesis during embryonic development and maintenance of corneal topography in adults. This is attributed to their bi-functional characteristic (protein moiety binding collagen fibrils to regulate collagen fibril diameters, and highly charged glycosaminoglycan (GAG) chains extending out to regulate interfibrillar spacings) that contributes to their regulatory role in extracellular matrix assembly. The absence of lumican leads to formation of cloudy corneas in homozygous knockout mice due to altered collagenous matrix characterized by larger fibril diameters and disorganized fibril spacing. In contrast, keratocan knockout mice exhibit thin but clear cornea with insignificant alteration of stromal collaegenous matrix. Mutations of keratocan cause cornea plana in human, which is often associated with glaucoma. These observations suggest that lumican and keratocan have different roles in regulating formation of stromal extracellular matrix. Experimental evidence indicates that lumican may have additional biological functions, such as modulation of cell migration and epithelium-mesenchyme transition in wound healing and tumorgenesis, besides regulating collagen fibrillogenesis.
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Introduction

Corneal strength and transparency depend upon the development and maintenance of an organized stromal extracellular matrix, including uniformly small collagen fibril diameter and consistent interfibrillar spacing. This is regulated by a proper ratio of matrix components and appropriate stromal hydration. In corneal stroma, these regularly packed fibrils are further organized into lamellae with adjacent layers approximately perpendicular to one another [1,2]. The mechanisms that govern the assembly of the different levels of stromal architecture are not well understood; however, proteoglycan-collagen and collagen-collagen interactions have been implicated. It has been suggested that the stoichiometry and interactions of different collagen types play important roles in modulating collagen fibril diameter [1,3–5]. The small leucine-rich proteoglycans (SLRPs) are thought to regulate the collagenous matrix assembly in connective tissues via their bi-functional character: the protein moiety binds collagen fibrils at strategic loci and the highly charged hydrophilic glycosaminoglycans (GAGs) regulate interfibrillar spacings [1,2,6–10].

The major proteoglycans in the corneal stroma are lumican, keratocan, mimecan and decorin with core proteins around 50 kDa [7,11], much smaller than most other proteoglycans. These highly charged compounds constitute the second most abundant biological materials in stroma, after collagen, and contribute to hydrophilic properties of the stroma [11–16]. Three distinct KS-containing proteoglycan core proteins have been identified in cornea. Molecular cloning data have established that the previously identified as 37B and 37A are core proteins of lumican and keratocan, respectively [11,12,14,17–19], uniquely abundant in the cornea. The nature of a 25 kDa core protein was recently shown to be osteoglycin, which had earlier been identified in bone, and was renamed mimecan [20]. Sequence analysis indicated that keratocan, lumican and mimecan are related to three other known small proteoglycans: decorin, fibromodulin, and biglycan [11,17–22].

Our laboratory has cloned and characterized the genes of lumican and keratocan, and has created knockout mice of both genes. In the present review, we will summarize our recent studies examining the functions of lumican and keratocan in

To whom correspondence should be addressed: Winston W.-Y. Kao, Ph.D., Department of Ophthalmology, University of Cincinnati, 3223 Eden Ave, Cincinnati, OH 45267-0527, USA. Tel.: 1-513-558-2802; Fax: 1-513-558-3108; E-mail: Winston.Kao@UC.Edu

the formation and maintenance of transparent cornea during embryonic development and in adults.

Biochemistry and molecular biology of lumican and keratocan

Core protein

The core proteins consist of four major domains (1) signal peptide, (2) negatively charged N-terminal peptide, (3) tandem leucine-rich repeats and (4) the carboxyl terminal peptide. Rotary shadowing-electron microscopy and molecular modeling have shown that these leucine-rich-repeat core proteins are horseshoe shaped [6,7,23], similar to the shape of ribonuclease inhibitor, another leucine rich repeat protein [24,25], presumably mediating protein-protein interactions [6,7,22,26]. It has been suggested that the protein moiety binds collagen fibrils to regulate collagen fibril diameters by means of highly charged GAG chains that extend out to regulate interfibrillar spacings [7,11], thus playing a role in regulating extracellular matrix assembly. This proposition is substantiated by the observation that the absence of lumican and keratocan causes the alteration of collagen fibril spacing and fibril diameter in corneal stroma of lumican and keratocan knockout mice [26,27]. Variations in the properties of individual proteoglycans derive from amino acid substitution in less conserved N- and C-terminal domains, from surface residues and changes in number and length of leucine rich repeats, and from variations in glycosylation.

Domain I: Signal peptides

All members of SLRPs have 16–20 residues of signal peptides which target the nascent polypeptides to the endoplasmic recticulum. These peptides are presumably cleaved co-translationally providing highly diverse N-termini of individual SLRPs. For example, following the signal peptides there are propeptides of 14 and 21 amino acid residues which are absent in mature decorin and biglycan and are presumably cleaved during maturation of the molecules [28,29]. It has been proposed that the propeptides may function as the recognition/attachment sites for xylosyltransferase, the first enzyme in GAG biosynthesis [30]. However, this feature of propeptide is not conserved in lumican, keratocan and fibromodulin [11,22,31].

Domain II: A negatively charged domain carrying sulfated GAG and sulfotyrosine

In contrast to decorin, biglycan and epiphycan, which have GAG attachment sites at the N-terminal end proximal to the cysteine-rich region [22,28,29], lumican, keratocan and fibromodulin have sulfotyrosine residues at the N-terminal end of the mature core proteins [11,31–33] (Figure 1). This sulfated tyrosine may affect interactions with cationic domains of extracellular matrix or cell surface proteins. Antonsson et al. [34] have demonstrated that the deletion of 50 amino acid residues from the consensus sequences of tyrosine sulfation of fibromodulin abolishes the formation of keratan, measured by incorporation of [^{35}S]sulfate into the truncated fibromodulin molecule. However it is not known whether it is the removal of tyrosine sulfation or the deletion of the 50 amino-acids that results in the abrogation of sulfated KS substitution in fibromodulin, and possibly lumican. It also is not known, whether the deletion of sulfotyrosine will prevent the interactions between lumican and other extracellular matrix components and cells.

Domain II contains a cluster of highly conserved cysteine residues with a general consensus $CX_{2\text{-}3}CXCX_{6\text{-}9}C$, where X is any amino acid and the subscripts denote the number of intervening residues. These cysteine residues are involved in the disulfide bond formation at the N-terminal region of the core proteins. Two additional well conserved cysteine residues are located at C-terminal end of the core protein (for detail see following sections). The disulfide bonds at N- and C-termini of the core proteins are essential for the molecules binding collagen fibrils [5].

We have recently examined the role of a highly conserved cysteine-containing domain proximal to the N-terminus of lumican in collagen fibrillogenesis using site-specific mutagenesis to prepare plasmid DNA encoding for wild type ($^{37}CX_3^{41}CXCX_9C$) and a mutant (designated C/S) replacing a cysteine with a serine residue, $^{37}CX_3^{41}SXCX_9C$. cDNAs were cloned into pSecTag2A vector (Invitrogen). Cultured MK/T-1 cells, an immortalized cell line from mouse keratocytes [35], were transfected with the cDNAs. Stable transformants were selected and cloned in the presence of Zeocin [36]. The stable transformants maintain a dendritic morphology and are capable of synthesizing a 3-dimensional extracellular collagenous matrix in the presence of transforming growth factor (TGF-β1) and ascorbic acid. Ultrastructural analyses by transmission electron microscopy showed that both cell lines form a multilayered stroma *ex vivo*, but the matrices assembled by the two cell lines differed. Compared to the mutant cell line, the wild type cells assembled a more organized matrix with regions containing orthogonal collagen fibrils. In addition, the fibrils in the extracellular matrix formed by the mutant cell line exhibited alterations in fibril packing and structure. Immunonstaining analyzed with confocal microscopy showed a further difference in this matrix due to the strong presence of lumican and type I collagen co-localization from the lumican wild-type cells, but a lack thereof from the mutant C/S lumican cells (Figure 2). The results indicate that the cysteine-rich domain of lumican is important in collagen fibrillogenesis and in stromal matrix assembly. To examine the role of domain II in collagen fibrillogenesis further, we have recently created transgenic mice that over-express wild type and C/S mutant recombinant lumican in corneal stroma using the keratocyte-specific keratocan promoter. Analysis of the recombinant lumican proteins in lumican null mice ($Lum^{-/-}$) will yield useful information about whether the disulfide bond formation within Domain II is essential for

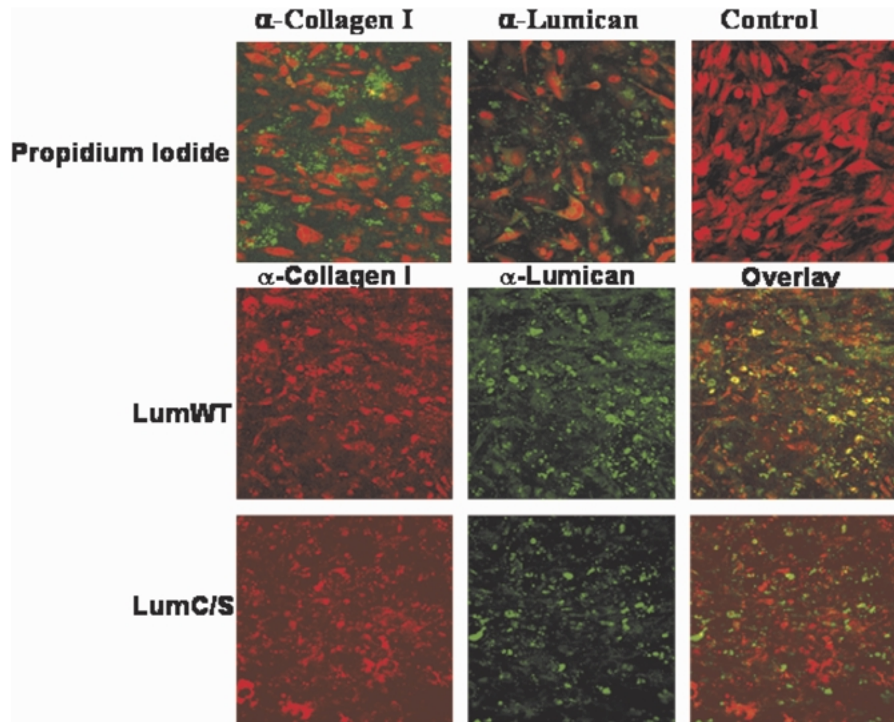
Figure 1. Comparison of N-terminal and C-terminal domains of lumican and keratocan from various species. The deduced amino acid sequence is given as single *uppercase letters.* The * indicates identical residue among species. All the sequences are retrievable from the NCBI/Genbank ClustalW Multiple Alignment program which is available at website http://decypher2.stanford.edu/algo-cw/CW_ax.html-ssi.

lumican to bind to collagen molecules and thus to modulate collagen fibrillogenesis *in situ*.

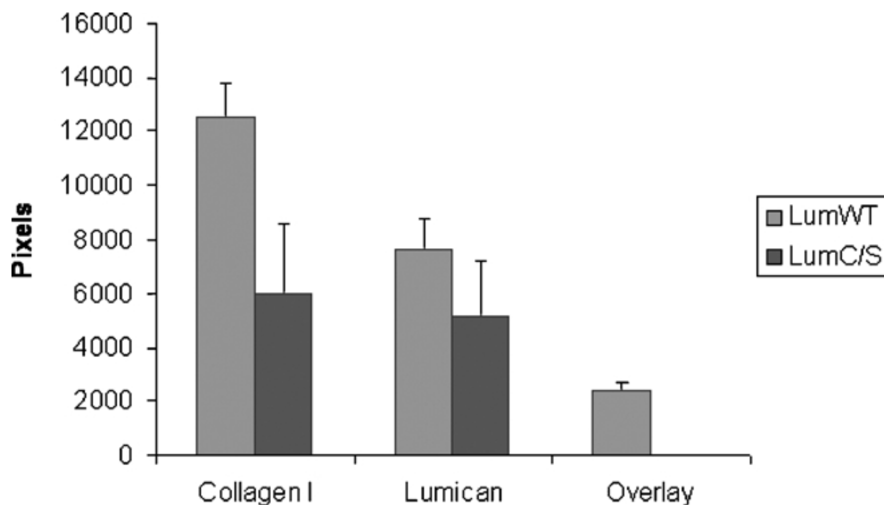
Domain III: Tandem leucine-rich repeats

The central domain of most SLRPs, which can account for 60 to 80% of the total amino acids, is comprised of ten tandem leucine repeats, with the exception of epiphycan and mimecan, which contain only six repeats [7,22,28]. The leucine rich repeat domain is flanked by the two well conserved cysteine-rich N- and C-terminal peptides. Most of the leucine residues are in conserved positions with a general consensus sequence of LXXLXLXXNXLSXL [25,37]. This domain contains the consensus sequences for N-linked glycosylations, e.g., three sites in decorin substituted with chondroitin/dermatan sulfate and four sites substituted with KS in lumican and keratocan [11,32,38]. It has been postulated that the leucine rich repeat domains and GAG chains of SLRPs play an important role in modulating collagen fibrillogenesis. For example, fibromodulin as well as decorin bind to types I and II collagen, and inhibit collagen fibrillogenesis [5,31,39–41].

The presence of irregular collagen fibril diameter and fibril spacings observed in the stroma of $Lum^{-/-}$ mice strongly suggests that lumican is important in collagen fibrillogenesis [26]. However, there is no direct evidence to show that KS proteoglycans associate with collagen fibril *in situ*, or to explain how they modulate collagen fibrillogenesis *in vivo*. The addition of KS proteoglycans, which were extracted with 4 M guanidine/HCl and presumably contained a mixture of lumican, keratocan and mimecan [17], resulted in the formation of smaller type I collagen fibrils *in vitro* [5]. Surprisingly, cultured corneal fibroblasts that do not synthesize KS proteoglycans,



(A)



(B)

Figure 2. Immunolocalization of collagen and lumican secreted by MK/T-1 cells. (A) Confocal images of 3D cell cultures (top panels) immunostained (green) for collagen (top right panel) and lumican (top middle panel) along with propidium iodide staining (red) show secretion of both these proteins into the extracellular matrix. The top left panel is the non-immune control with propidium iodide staining. Confocal images of 3D cell cultures immunostained for collagen (red) and lumican (green) are shown (middle and bottom panels). Cells from wild type lumican mice (LumWT—middle panels) and lumican-modified mice (LumC/S—bottom panels) both show a rich collagen extracellular matrix (left panels) with recombinant lumican secreted into this matrix (middle panels). An overlay (right panels) of LumWT immunostaining shows the strong presence of a lumican and collagen co-localization as seen in yellow. LumC/S cells show very little co-localization of lumican and collagen as compared to LumWT cells. The two bottom panels are confocal micrograph overlays of collagen or lumican immunostaining with propidium iodide staining to show secretion of these two components into the extracellular matrix. (B) Histogram of analyzed confocal micrographs depicting collagen, lumican, and co-localization (overlay) pixels. LumWT (gray bar) and LumC/S (black bar) confocal images were computer analyzed to determine the number of green (lumican), red (collagen) or yellow (co-localization) pixels. LumWT clones secreted recombinant protein which co-localized with collagen significantly more than LumC/S clones.

but synthesize and deposit small-diameter fibrils with a collagen composition and structure similar to those seen in the corneal stroma [3]. Available data indicate that de-glycosylated chicken lumican core protein is capable of regulating collagen fibrillogenesis *in vitro* [5]. In addition, recombinant human decorin isolated from a Baculovirus expression system can also modulate collagen fibrillogenesis *in vitro* [42]. These observations are consistent with the notion that core proteins of SLRPs that are not substituted with keratan sulfate may have a role in modulating collagen fibrillogenesis. This is consistent with the previously stated suggestion that core protein binding to collagen may regulate fibril diameter, while the interaction of GAG chains may be involved in regulating interfibrillar spacings [5,43]. Nevertheless, there is no direct evidence to support this in regard to KS modulation of interfibrillar spacings *in vivo*. It is possible that KS proteoglycans may be co-localized with the beaded filaments of type VI collagen in a fashion similar to that of decorin in corneal stroma [44]. Thus, lumican and/or other KS proteoglycans may exert an effect on the formation of collagen lamellae in stroma by interaction with type VI collagen.

We have recently developed a technique for expressing recombinant lumican protein by stromal injection of plasmid DNA into corneas of $Lum^{-/-}$ mice. Our preliminary results indicate that expression of wild type recombinant lumican restores the thickness of stroma of the $Lum^{-/-}$ mice (unpublished observation). This makes it feasible to examine the functions of KS on collagen fibrillogenesis by expressing mutant lumican cDNA in which the asparagine residues involved in N-glycosylation can be substituted with threonine using site-specific mutagenesis.

Domain IV: The carboxyl terminal domain

The carboxyl domain of SLRPs is the least characterized so far, comprising a ∼50 amino acid domain with several stretches of residues differing among SLRP family members (Figure 1). Domain IV contains two cysteine residues that are positioned 32 residues apart in all cases, except for keratocan and PRELP (proline arginine-rich end leucine-rich repeat protein) where an insertion of an additional seven and eight amino acids, respectively occurs [45,46]. The resulting disulfide bond, such as has been demonstrated for bovine biglycan [47], provides formation of a 41 or 42 residue loop respectively at this end of the molecule.

It is of interest to note that reduction and alkylation of bovine corneal lumican [5] as well as bovine skin decorin [38] results in total abrogation of their functional properties, exemplified by a loss of collagen fibrillogenesis-controlling activity. Thus, the presence of disulfide bridges in lumican at amino and/or carboxyl ends of the molecules may play a fundamental role in the ability of these two proteoglycans to interact with collagen fibrils. We can presume that a similar concept would also apply to other SLRPs, e.g., fibromodulin, which also interacts with fibrillar collagens [48,49].

Spatio-temporal expression patterns of lumican and keratocan

Lumican is ubiquitously expressed by many interstitial connective tissue mesenchymal cells of developing embryos and adult animals. Northern hybridization and *in situ* hybridization indicate that in early stages of embryonic development (day 7 post coitus), embryos express little or no lumican. Thereafter, different levels of lumican mRNA can be detected in various organ systems, such as cornea stroma, dermis, cartilage, heart, lung, and kidney [50], with cornea and heart having the highest expression. Immunoblotting studies found that core proteins for KS became abundant in the cornea and sclera by postnatal day 10, but formation of the KS could not be detected until after the eyes open. These results indicate that lumican core protein is widely distributed in most interstitial connective tissues, and that substitution with KS in cornea is concurrent with eye opening and may contribute to corneal transparency [11].

We have also examined keratocan expression during embryonic development. Northern blotting and *in situ* hybridization were employed to examine keratocan gene expression during mouse development. Unlike the expression of the lumican gene in many tissues other than cornea, keratocan mRNA is more selectively expressed in the corneal tissue of the adult mouse [45]. During embryonic development, keratocan mRNA was first detected in periocular mesenchymal cells migrating toward developing corneas on embryonic day 13.5 (E13.5). Its expression was gradually restricted to corneal stromal cells on E14.5 through E18.5. Interestingly, keratocan mRNA can be detected in scleral cells of E15.5 embryos, but not in E18.5 embryos. In adult eyes, keratocan mRNA can be detected in corneal keratocytes, but not in scleral cells.

Structure and promotors of *Lum* and *Kera*

The genomic DNA of *Lum* and *Kera* have been cloned from various species e.g., human, mouse, chicken and quail [45,50–56]. Like other members of SLRP, these genes span about 7–9 kb in mouse and human genomes (Figure 3). Both genes are located in human chromosome 12 and mouse chromosome 10. Their mRNAs are spliced into threes exons. Exon 1 has about 74 bp and 173 bp of untranslated region of lumican and keratocan mRNA, respectively [45,50]. Exon 2 contains most of the coding sequence including all leucine-rich repeat domains, whereas exon 3 encodes the rest of coding sequence of the C-terminal domain (Figure 3A).

Human *Lum* was shown to possess one major transcription start site, resulting in exon 1 of the gene, giving rise to the first 74 base pairs (bp) of the 5′-untranslated region. About 1.6 kilobase pairs of the upstream promoter sequence were sequenced and analyzed to identify elements responsible for gene expression. Both human and mouse *Lum* and *Kera* do not have a typical TATAA sequence in the vicinity of the transcription start site [57,58]. But an atypical TATCA sequence residing 41 bp upstream of human *Lum* was shown to be necessary

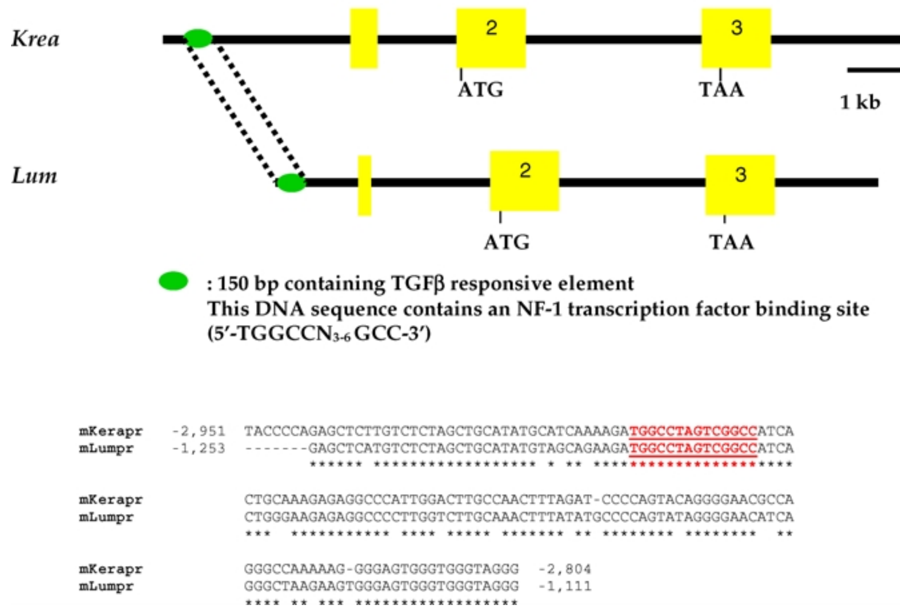


Figure 3. Structure of lumican (*Lum*) and keratocan (*Kera*) genes. Both *Lum* and *Kera* genes contain three exons represented as *boxes* and indicated by *numbers* The translation initiation codon ATG and termination codon TAA are in *bold.* The sequences between −2944 and −2804 (bold uppercase letters) of the mouse *Kera* promoter (m*Kera*pr) share 89% homology with the mouse *Lum* promoter (m*Lum*pr). The * indicates identical residue between m*Kera*pr and m*Lum*pr. A putative NF-1 binding consensus sequence, 5′-TGGCCN$_{3\text{-}6}$GCC-3′**,** which may be a response to TGF-β2 signaling, is in red and underlined.

for transcription, although it was incapable of supporting transcription by itself. A GC box residing 74 bp upstream of the transcription start site also was essential for the initiation of transcription. Sp3 was identified as the transcriptional activator binding to the GC box. No additional elements that significantly modulated transcription were noted in the promoter sequence analyzed, when using human adult chondrocytes as the cell source for transfection in reporter assays. In contrast, reporter assays carried out in human fetal lung fibroblasts, where *Lum* expression is depleted, revealed the presence of a repressor element located between 384 and 598 bp upstream of the transcription start site. A GATA-binding site located between bp −386 and −391 was identified as being necessary for repression of transcription. The mouse *Lum* promoter does not possess an equivalent site, and this may explain why the *Lum* is expressed in fetal murine cartilage but not in fetal human cartilage [57].

Analysis of the nucleotide sequence of 5′-flanking *Kera* reveals that there is no TATAA consensus sequence or GC-rich regions within 100 bp up stream of the transcription initiation site. One CCAAT sequence was found from −74 to −70. Many consensus transcription factor binding sites are found in the −3.2 kb 5′ to *Kera*; however, their function in the regulation of expression remains unknown [58]. Since both *Kera* and *Lum* are highly expressed in corneal keratocytes, it is reasonable to expect that common regulatory *cis*-elements may exist in the promoter region of both genes. Sequence alignment revealed little similarity between the mouse *Kera* promoter (3.2 kb) and the *Lum* promoter (1.2 kb, GenBank accession no. AF186467). However, a stretch of 139 bp (from −2943 to −2804) in the distal region of the 3.2 kb *Kera* promoter shares 89% homology to the *Lum* promoter (−1253 to −1111) in which it contains an NF-1 transcription factor binding site (Figure 3B). This NF-1 binding site may serve as a potential TGF-β responsive elements similar to what has been found in many TGF-β target genes, e.g., perlecan, mouse type I collagen α2 chain, elastin, type I plasminogen activator inhibitor, and growth hormone genes [59–62]. To test whether TGF-β2 can regulate mouse *Kera* and *Lum* expression, we performed northern blot hybridization to compare the endogenous *Kera* mRNA level in the eye among E18.5 wild-type embryos, heterozygous, and homozygous TGF-β2 knockout embryos [63]. Indeed, the *Kera* and *Lum* mRNA levels are down-regulated in TGF-β2-null embryonic eyes, indicating that the mouse *Kera* and *Lum* genes might be one of the target genes of TGF-β2 signaling *in vivo* [58].

Mouse *Kera* expression tracks the corneal morphogenesis during eye development and becomes restricted to keratocytes of the adult, implicating a cornea-specific gene regulation [45]. To examine the functionality of the mouse *Kera* promoter, we have cloned and sequenced a 3.2 kb genomic DNA fragment 5′ of the mouse gene. This was used to prepare a reporter gene construct that contained the 3.2 kb 5′ flanking sequence, exon

1 and 0.4 kb of intron 1 of *Kera*, and β-geo hybrid reporter gene [58]. The β-galactosidase activity was assayed in tissues of two of five transgenic mouse lines obtained via microinjection. In adult transgenic mice, β-galactosidase activity was detected in cornea, but not in other tissues such as lens, retina, sclera, lung, heart, liver, diaphragm, kidney, and brain. During ocular development, the spatial–temporal expression patterns of the β-galactosidase recapitulated that of endogenous *Kera* in transgenic mice. Using X-Gal (5-bromo-4-chloro-3-indolyl-β-galactopyronoside) staining, strong β-galactosidase activity was first detected in periocular tissues of E13.5 embryos, and restricted to corneal keratocytes at E14.5 and thereafter. Interestingly, in addition to the cornea, β-galactosidase activity was transiently found in some non-ocular tissues, such as ears, snout, and limbs of embryos of E13.5 and E14.5, but was no longer detected in those tissues of E16.5 embryos. The transient expression of endogenous keratocan in non-ocular tissues during embryonic development was confirmed by *in situ* hybridization. Taken together, our results suggest that the 3.2 kb *Kera* promoter contains sufficient *cis*-regulatory elements to drive heterologous minigene expression in cells expressing keratocan. The identification of keratocyte-specific expression of β-galactosidase reporter gene in the adult transgenic mice is an important first step in characterizing the *Kera* promoter in order to use it to drive a foreign gene expression in corneal stroma.

Clinical manifestations of *Lum*$^{-/-}$ and *Kera*$^{-/-}$ mice

To elucidate the role of lumican and keratocan in maintaining corneal transparency, we have ablated the genes in mice via gene targeting techniques.

Lum$^{-/-}$ mice

The homozygous *Lum*$^{-/-}$ mutant mice were born alive in the expected Mendelian ratio and were fertile [26,27]. The skin of the adults was fragile as evidenced by the disruption of skin when experimental animals were sacrificed by cervical dislocation, and the back skin hairs were disarranged. It is of interest to note that the male *Lum*$^{-/-}$ mice appear to produce a lower smaller number of offspring than the female *Lum*$^{-/-}$ mice when they are mated with wild type mice. The reason for this phenomenon is not known; but could be secondary to the fragile skin phenotype [27]. Corneal haze (recognized with a stereomicroscope, slit lamp and/or *in vivo* confocal microscope) and cloudiness were noted in *Lum*$^{-/-}$ mice at 3 months and older. Electron microscopic examination revealed abnormal thick collagen fibers seen in the posterior corneal stroma of 4-month old mice, whereas the anterior stromal collagen fibers appeared normal. These larger collagen fibrils were not observed in the stroma of younger *Lum*$^{-/-}$ mice. The corneal haze was concurrent with the presence of a disorganized collagenous matrix [26,64–66].

It is of interest to note that the absence or reduction of lumican in sclera also caused alteration of fibril diameter. The wild-type mouse sclera consisted of irregularly arranged lamellae of collagen fibrils, with an average diameter of 47.37 ± 0.648 nm in the anterior sclera and 54.68 ± 0.342 nm the posterior sclera. Collagen fibrils in the sclera of *Lum*$^{+/-}$ and *Lum*$^{-/-}$ mice were significantly larger in diameter in anterior (72.61 ± 0.445 and 84.47 ± 0.394 nm, respectively) and posterior (75.92 ± 0.361 and 80.90 ± 0.490 nm, respectively) scleral regions compared with wild-type mice ($P < 0.001$). The results indicate that null mutations in one or both alleles of the lumican gene result in significant defects in scleral collagen fibril formation that could lead to alterations in ocular shape and size with severely affected vision [67].

Kera$^{-/-}$ mice

We have generated *Kera*$^{-/-}$ mice which are viable and fertile. At the age of 6 months, the animals have clear corneas, in contrast to the cloudy cornea of *Lum*$^{-/-}$ mouse. However, they have a thin corneal stroma. At age 20 weeks, *Kera*$^{-/-}$ mice have abnormal collagen fibril spacing in corneal stroma in comparison to that of wild-type mice (unpublished observations). Interestingly, Pellegata et al. have recently reported that human mutations in *Kera*, causes cornea plana a condition which consists of forward convex curvature flattening of the cornea, leading to a decrease in refraction. Two alleles, CNA1 and CNA2, are associated with this hereditary disorder [68]. CNA2 has been mapped to human chromosome12q in which the *Kera* gene is located. Mutations in *Kera* have been identified in 47 cornea plana (CNA2) patients. Forty six Finish patients have been found to be homozygous for a founder of missense mutation (A<u>A</u>C to A<u>G</u>C at codon 247), leading to a highly conserved amino acid change from Asn to Ser in a leucine repeat of keratocan, and one American patient has been found to be homozygous for a mutation leading to a premature stop codon that truncates the KERA protein. More recently, Lehmann et al. reported that a novel keratocan mutation of single nucleotide substitution at codon 215 (threonine to lysine) in the highly conserved leucine-rich repeat motif may result in altered keratocan tertiary structure, and thereby function in regulation of corneal collagen fibrillogenesis [69].

Additional functions of lumican (other than serving as modulator of collagen fibrillogenesis)

The wide tissue distribution of non-sulfated lumican implies that it may have functions other than regulation of collagen fibrillogenesis. During corneal wound healing, newly synthesized lumican, keratocan and mimecan proteoglycans are undersulfated compared to these same proteoglycans in normal adult stroma [23,70]. Our recent observations that epithelial cells transiently express lumican during the healing of epithelium debridement are intriguing, since they imply that lumican may have a role in modulating wound healing. In normal unwounded corneas, lumican is expressed by stromal keratocytes. Our data show that injured mouse corneal epithelium ectopically and

transiently expresses lumican during the early phase of healing, suggesting a potential lumican functionality unrelated to regulation of collagen fibrillogenesis; e.g., modulation of epithelial cell adhesion or migration [27]. An anti-lumican antibody was found to retard corneal epithelial wound healing in organ cultured mouse eyes, and healing of a corneal epithelial injury in $Lum^{-/-}$ mice was significantly delayed compared to $Lum^{+/-}$ mice. These observations are consistent with the hypothesis that lumican may modulate cell behaviors such as adhesion and migration, thus contributing to corneal epithelial wound healing. It should be noted that macrophages bind to non-sulfated lumican core protein, but not that of keratocan. It was found that the highly sulfated KS proteoglycans of cornea do not promote macrophage adhesion; but low-sulfated KS lumican that is present in pathologic corneas may act to localize macrophages in regions of inflammation. The lumican receptor differs from macrophage scavenger receptors and from receptors for several other extracellular matrix molecules [71]. It is not known, however, whether other cell types, such as corneal epithelial cells or keratocytes can also bind to lumican.

The speculation that lumican may have a role in modulating cell behavior is further supported by the observation that following cataract surgery undesirable opaque scar tissues consisting of interstitial extracellular matrix components, such as fibrous collagen types and lumican, are often formed by lens epithelial cells that undergo proliferation and epithelium-mesenchyme transition [78]. This transition is characterized by the expression of α-smooth muscle actin (α-SMA) by lens eptithelial cells. Interestingly, the expression of lumican by injured lens epithelial cells precedes the expression of type I collagen and α-SMA. Following a capsular injury in mouse, lens epithelial cells began to express lumican protein in 8 hrs following injury and reached a peak in 24–48 hrs, whereas type I collagen and α-SMA were detected at Day 3 and 5 of injury, respectively. Thus, it is plausible to hypothesize that lumican may modulate lens epithelium-mesenchyme transition in response to injury. To examine this possibility, we further examined the expression of α-SMA by lens epithelium of $Lum^{+/-}$ and $Lum^{-/-}$ mice. Histology showed the accumulation of epithelial-shaped lens cells around the injury in $Lum^{-/-}$ mice and elongated fibroblast-like cells in $Lum^{+/-}$ mice. Up-regulation of α-SMA expression by healing lens cells was significantly delayed in $Lum^{-/-}$ mice (unpublished observations). These observations lend support to the hypothesis that lumican modulates epithelium-mesenchyme transition of mouse lens epithelial cells.

Many studies have shown that lumican may also be involved in tumorgenesis. For example, it has been shown that lumican accumulated in stroma of several tumors and cancerous tissues, e.g., breast, colorectal and pancreatic tumor, and benign prostatic hyperplasia [72–76]. The expression of lumican by cervical carcinoma squamous epithelial cells has also been reported [77]. However, the role of lumican in tumorgenesis remains unknown. It has recently been suggested that lumican may suppress induction of transformation by v-src and v-K-ras [77]. The authors showed that suppressive factors for transformation by viral oncogenes are expressed in primary rat embryo fibroblasts. One of the suppressive factors isolated with subtracted cDNA library by using rat embryonic fibroblasts and a rat normal fibroblast cell line, F2408, was identified as lumican. Over expression of lumican inhibited colony formation of v-K-ras and v-ras transformed rat fibroblasts in soft agar. Tumorigenicity in nude mice induced by these oncogenes was also suppressed in the lumican-expressing clones. These results indicate that lumican has the ability to suppress transformation by v-src and v-K-ras.

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