Developmentally Regulated Appearance of Spliced Variants of Type XII Collagen in the Cornea

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PURPOSE. To determine whether temporal and spatial changes in the distribution of the long and short alternatively spliced variants of type XII collagen are associated with any specific morphogenetic events in pre- and postnatal development of the cornea and surrounding tissues.

METHODS. The distribution of alternatively spliced variants of type XII collagen in fetal and newborn rabbit tissues was analyzed immunohistochemically using monoclonal antibodies that recognize either only the long form or both the short and the long forms of type XII collagen.

RESULTS. During early fetal development of the cornea in rabbit (days 14–17), the short form of type XII collagen was detected in the corneal stroma, the sclera, and the stroma in the rudimentary eyelid folds, whereas the long form was present only in the sclera. The long form was first evident in the cornea at day 24 but only in the posterior stroma. At later stages of prenatal development, the distribution of the long variant gradually extended toward the anterior stroma and in the newborn rabbit, the long variant was distributed throughout the entire stroma. However, in the eyelid, although the short form was present along the entire subepidermal regions both during fetal and neonatal development, the long form was transiently expressed between days 21 and 24 and was restricted to the subepidermal regions at the junction of the opposing eyelids. The long form of type XII collagen was first detectable in the basal epithelial cells and in its basement membrane (BM) at day 12 after birth, just before the opening of the eyelids. It continued to be present in the corneal BM zone in the adult rabbit but was not present in the limbal or conjunctival BM zone.

CONCLUSIONS. The expression and distribution of the alternatively spliced forms of type XII collagen are developmentally and differentially regulated in the cornea, the sclera, and the eyelid. Although the short form is expressed in the stromal matrices of the cornea and surrounding tissues from early stages of corneal development, the appearance and distribution of the long variant form of type XII collagen coincide with the pattern of stromal condensation. Its first appearance in the corneal epithelial BM precedes the eyelid opening by 1 to 2 days, possibly suggesting that it may be involved in the tighter anchoring of the corneal epithelium to the underlying tissue or in promoting stromal condensation to assist in the separation of the corneal epithelium from the juxtaposed palpebral conjunctival epithelium of the eyelid.

Development of a normal transparent cornea results from the temporal and spatial regulation of cellular migration, proliferation, differentiation, and synthesis and organization of extracellular matrix (ECM) macromolecules. The cornea is composed of three tissue layers: the epithelium, the stroma, and the endothelium, each with their own unique ECMs, which are continuous with the ECMs of the surrounding tissues of the limbus. The ECMs in the cornea and the surrounding tissues comprise unique combinations of genetically distinct collagens, proteoglycans, and other glycoproteins. Although the heterotypic fibrils of type I and type V collagens form the lamellar framework of the corneal stroma, other collagens and proteoglycans occupy the interfibrillar space and some of these interfibrillar components are likely to be involved in regulating the thickness and spacing of the collagen fibrils. Type XII collagen is one of the more recently discovered components of the corneal ECMs and its role in the development and maintenance of the cornea currently remains speculative. Type XII collagen belongs to the FACIT group (fibril-associated collagen with interrupted triple helices) and it is a homotrimer, containing three noncollagenous domains (NC1, NC2, and NC3) that are spaced apart by two triple helical collagenous domains.

Type XII collagen is expressed in at least two different alternatively spliced variant forms consisting of 340-kDa and 220-kDa α-chains, respectively. These forms differ in the NC3 domains; the short form lacks a part of the amino terminal region present in the NC3 domain of the long form. The short variant form of type XII collagen is widely distributed in the adult dense connective tissues. Either one or both forms are expressed during embryonic development of a variety of tissues; however, the long form gradually diminishes from most tissues, and the short form persists in many of the dense connective tissues. The long form is also expressed in cells in culture. In the human adult cornea, unlike other...
tissues, the long form is the predominant one and is uniformly distributed along the surface of the collagen fibrils in the human corneal stroma.\textsuperscript{8} It is also a component of the corneal epithelial and endothelial basement membranes (BM) but is absent in the limbal BM zone.\textsuperscript{31} Earlier studies have indicated different distribution patterns of type XII collagen in embryonic and young chick corneas\textsuperscript{6,29} and of its mRNA in the rabbit cornea.\textsuperscript{7} Based on its distribution in chick cornea, which was restricted to the matrix interfaces between the Bowman’s membrane and the stroma and between the Descemet’s membrane and stroma, Gordon et al.\textsuperscript{6} speculated that type XII collagen may have a functional role in the stabilization of the matrix interfacial regions. A recently reported observation that type XII collagen can promote contraction of collagen gels mediated by dermal fibroblasts in vitro suggests that type XII collagen may have a role in tissue compaction during tissue morphogenesis.\textsuperscript{30} The purpose of the present study was to determine whether the expression and distribution of the long and/or short variant forms of type XII collagen are differentially regulated during corneal development and whether chronological changes in their distribution are associated with any specific morphogenetic events, specifically those involving tissue condensation. Because the long variant form of type XII collagen is a component of the corneal epithelial BM zone and is absent in the limbus, where the corneal epithelial stem cells reside,\textsuperscript{31,32} we wanted to determine whether the expression of type XII collagen in corneal epithelial cells is associated with corneal epithelial differentiation or with any specific morphogenetic event during the fetal development of the cornea.

**METHODS**

**Tissues and Immunohistochemical Staining**

All the procedures involving rabbits were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Pregnant New Zealand white rabbits at various stages of gestation (11–28 days) were killed by an intravenous injection of sodium pentobarbital (Euthasol; Delmarvia Laboratories, Midlothian, VA). The corneas from the adult rabbits with limbus and 1 to 2 mm of surrounding sclera were excised, cut in halves, and immediately frozen in Tissue-Tek II OCT compound (Miles Laboratories, Elkhart, IN). The fetuses were removed and killed by an intracardiac injection of Euthasol, and either the whole head or the eyes were excised and immediately frozen in OCT compound.\textsuperscript{33} Similarly, the eyes and corneas were dissected from 2, 5, 9, 12, and 16-day-old postnatal rabbits, frozen in OCT compound, and stored at −70°C until cryostat sectioning. Cryostat sections (7.0-μm thick) of all these tissues were transferred to gelatin-coated slides and immunoreacted using an indirect immunofluorescence technique, as described previously.\textsuperscript{34} The stained sections were viewed and photographed using an Olympus Vanox-S photomicroscope equipped for fluorescence microscopy. All the pictures, unless indicated otherwise, were taken with the same exposure time. For double immunostaining, the same procedure was used except that the primary antibodies consisted of a mixture of rat anti-laminin (ICN Biomedicals, Costa Mesa, CA) and a mouse anti-type XII collagen monoclonal antibodies (MAbs), and the secondary antibodies were a mixture of goat fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Organon Teknika, Durham, NC) and rhodamine-conjugated anti-rat IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) antibodies. The stained sections were analyzed using an Olympus IX70 inverted microscope with Bio-Rad RadiancePLUS confocal system with Laser sharp acquisition software (Bio-Rad Laboratories, Hercules, CA). The data for the red and green fluorescence were collected sequentially with the optical sections set at 0.5 μm.

**Development and Selection of Monoclonal Antibodies**

A panel of MAbs to type XII collagen was developed using α1(XII) collagen chains of immunoaffinity-purified human type XII collagen as the immunogen.\textsuperscript{35} These antibodies were tested for their cross-reactivity with rabbit type XII collagen by western blot analysis as well as immunohistochemical analysis.

**Extraction of Type XII Collagen from Rabbit Cornea and Sclera**

Corneal epithelium from frozen rabbit eyes (Pel Freez, Rogers, AR) was removed by scraping, and the de-epithelialized corneas or pieces of scleral rims were frozen in liquid nitrogen and pulverized, suspended in a buffer (NET) consisting of 1 M NaCl in 100 mM Tris–HCl (pH 7.8), containing protease inhibitors (10 mM EDTA, 1.0 mM N-ethyl maleimide, 1.0 mM phenylmethylsulfonyl fluoride; 5 ml/cornea or 10 ml/scleral rim), stirred for 48 hours at 4°C, and the homogenate centrifuged at 27,000g for 2 hours at 4°C. The supernatants were dialyzed against 0.25 M NaCl NET buffer, the dialysates were centrifuged at 12,000g for 30 minutes, and the supernatants containing type XII collagen were either used immediately or stored at −20°C for further use.

**Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblot Analysis**

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed in 5% polyacrylamide-SDS slab gels, according to Laemmli.\textsuperscript{35} The high molecular weight standards (47–202 kDa from Bio-Rad, Hercules, CA; and nonreduced α2-macroglobulin, 340 kDa; Boehringer Mannheim, Indianapolis, IN) were used to estimate the molecular weights of the polypeptides recognized by the MAbs. The samples were reduced with 0.6% dithiothreitol before electrophoresis. For western blot analysis, the proteins in the gel were electrophoretically transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA), and the blots immunoreacted with the hybridoma culture supernatants containing specific antibodies, followed by horseradish peroxidase-conjugated rabbit anti-mouse IgG secondary antibody.\textsuperscript{36} To detect horseradish peroxidase, 4-chloro-1-naphthol (Bio-Rad) or ECL reagent (Amersham Life Science, Arlington Heights, IL) was used.

**Enzyme Digestions**

For bacterial collagenase digestion,\textsuperscript{37} 0.25 M NET extracts were dialyzed against a buffer containing 50 mM Tris–HCl, 5 mM CaCl\textsubscript{2}, and 25 μM N-ethyl maleimide (pH 7.6), and equal aliquots of the dialyzed extracts were incubated with or without bacterial collagenase (Advanced Biofactures, Lynnbrook, NY).
RESULTS

MAbs to Rabbit Type XII Collagen and the Distribution of the Long and Short Variant Forms in the Adult Rabbit Cornea

A library of MAbs, developed using the \( \alpha \)-chains of the human long variant form of type XII collagen as the immunogen, was tested against rabbit corneal protein extracted in 0.25 M NaCl-containing buffer (NET). Western blot analysis indicated that MAb 2E4, which recognizes an epitope located in the NC3 domain of the long variant form of human type XII collagen, cross-reacted with a polypeptide with a \( M_r \) of approximately 340 kDa (Fig. 1). After bacterial collagenase digestion of the NET extract, the size of the reactive band was reduced by 40 kDa, as expected. Another antibody, MAb 3C7, which recognizes an epitope located in the NC3 domain of both the short and the long forms of human type XII collagen, reacted with the 340- and 220-kDa polypeptides in the NET extract and with 300- and 180-kDa polypeptides in the collagenase-treated NET extracts (Fig. 1). These results indicated that both the short and long variant forms of type XII collagen were present in the rabbit cornea. Based on the relative intensities of the 340- and 220-kDa polypeptides in the western blot analysis and the corresponding bands stained with Coomassie blue, the long variant form was more predominant in the adult cornea.

Immunohistochemical Analysis

Cryostat sections of the frozen normal adult rabbit tissue when immunoreacted with MAb 2E4, revealed the distribution of the long variant form of type XII collagen. Serial sections of the same tissues were reacted with MAb 3C7 to detect the presence of the short and/or long variant forms. An absence of staining with MAb 2E4 in the regions that reacted with MAb 3C7 indicated the presence of only the short form. Immunofluorescence analysis of the tissues from five different rabbits showed a linear intense staining in the corneal epithelial BM zone. Staining in the corneal BM zone terminated at the limbus. Figure 2 shows staining of a corneal section with MAb 2E4. In addition to a uniform fluorescence in the stromal matrix, a punctate periodic distribution was evident in the collagen lamellae (Fig. 2). The staining intensity in the corneal stroma tapered in the peripheral cornea toward the limbus and increased significantly in the limbal stroma, which forms the limbal-scleral junction. Although the staining intensity in the sclera was comparable to that in the corneal stroma, the punctate staining was sporadic in the sclera. Subepithelial loose connective tissue in the limbus and conjunctiva did not react with MAb 2E4 or 3C7.

The staining pattern with MAb 3C7 was very similar to that with MAB 2E4 except that the uniform fluorescence seen in the stromal matrix was significantly brighter (not shown). Both antibodies also reacted with the Descemet's membrane. The stronger staining with MAb 3C7 was probably due to its
reactivity with both the short and long forms of type XII collagen present in the cornea.

**Distribution of Type XII Collagen Variant Forms in the Developing Cornea and the Surrounding Tissue**

**Early Stages of Corneal Development.** A schematic presentation of the main stages of the fetal development of the rabbit cornea and eyelid is shown in Figure 3. The changes in the relative thickness of the cornea during the development are shown (Figs. 3D through 3H). The early stage of development (Fig. 3A) is marked by the separation of the lens cup from the surface ectoderm and the migration of the mesenchymal cells from the regions lateral to the lip of the optic cup into the presumptive corneal region to form the endothelial layer and stroma. In the rabbit, this occurs during days 13 to 14 of gestation (gestation period is approximately 30 days). At this stage, MAb 2E4 reacted weakly in the scleral region. However, MAb 3C7 reacted with the corneal stromal region as well as the scleral region (not shown). At day 17 (B), rudimentary eyelid folds (Li fold) are developed, corneal endothelial layer (C End) is formed, and corneal stromal cells of neural crest origin, which migrate into the cornea, start laying down the stromal matrix in the cornea (C). By day 21 (C), the eyelid folds have come together and fused. The differentiated epithelia seen at this stage include corneal (CE), palpebral conjunctival (PCjE), bulbar conjunctival (BCjE), and epidermal (Epd) epithelia. CJs, conjunctival stroma. (D) through (F) show the relative thickness of the cornea and pattern of more closely packed denser matrix formation which proceeds from the posterior to anterior stroma (Str) as the development progresses: day 17 (D), day 21 (E), day 24 (F), day 29 (G), and postnatal day 15 (H).

**Mid Stages of Corneal Development.** By day 21, the growing eyelids had come together and the surface epithelial layers of the opposing epidermis had fused (Fig. 3C). Histologically, the subepidermal regions under the fused epithelium of the eyelids appeared to be denser than the rest of the subepidermal matrix (not shown). Interestingly, the long form of type XII collagen was detectable in this very restricted subepidermal region where the eyelid folds had come together and fused (Fig. 4D). The corneal thickness had further increased. The long variant form was still not detectable in the cornea, but its increased expression was evident in the sclera. However, the short form was detectable in the entire corneal stroma, in the sclera, and in a narrow band in the subepidermis of the entire eyelid (Fig. 4C).

Between days 21 and 24, the thickness of the corneal stroma had further increased and the stromal condensation had begun in the posterior region. At day 24, the long form was expressed in the posterior stroma in the cornea (Fig. 4F). It was no longer present in the subepidermal regions at the junction of the opposing eyelids. It continued to be present in the sclera. The short form was evident in the eyelid, both in the connective tissue of the palpebral conjunctiva and in the dermal region of the eyelid (Fig. 4E). It was also detectable in the anterior region of the cornea. Because the long form was...
present in the sclera and posterior cornea, it was not possible to definitively conclude whether the short form was present in these regions. Based on the intensities of immunofluorescence with MAb 2E4, the distribution patterns and the concentrations of the long variant form were significantly different in different regions of the sclera (Fig. 5). The relative immunofluorescence was highest in the posterior sclera (Fig. 5C) followed by the anterior sclera, close to the cornea (Fig. 5A), and then the equatorial regions (Fig. 5B). In the cornea and posterior sclera, punctate periodic intense staining was evident along the collagen lamellae. The punctate staining could be better visualized (not shown) by taking the photomicrographs at a higher magnification with a shorter time of exposure, similar to that shown in Figures 2B and 2C for the adult cornea. This punctate staining was sporadic in the other regions of the sclera.

**Late Stages of Corneal Development.** During the later stages of corneal development, stromal condensation continues to progress from the posterior to anterior regions as shown schematically in Figure 3. At day 28, close to the end of the gestation period, staining for the long form had extended further into the anterior stroma (Fig. 6B). The staining for the long variant was not detectable in the eyelid. The staining for the short form was significantly less in the connective tissues of the eyelid and was restricted more to the subepidermal region (papillary layer; not shown).

**Postnatal Development.** Postnatally, the corneas were analyzed at days 2, 5, 9, 12, and 16. The eyelids remained closed until days 14 to 15. Temporal increases in the intensities of staining with MAb 2E4 for the long form were evident in the corneal stroma (days 12 and 16, shown in Figs. 6D and 6F, respectively). The increase in staining progressed from the posterior toward the anterior stroma, and by day 16 appeared to be more uniform throughout the entire thickness of the corneal stroma. In addition to the uniform staining throughout the corneal stromal matrix, intense punctate periodic staining was also evident. The staining pattern in the sclera was not altered significantly (not shown). Negligible staining for the long form was evident in the connective tissue of the eyelid. The pattern of staining for the short form with MAb 3C7 was similar to that with MAb 2E4 in the sclera and cornea, except
that 3C7 reacted more strongly, possibly due to its reactivity with both the short and the long variant forms of type XII collagen (Figs. 6C and 6E). Negligible reactivity with MAb 2E4, but reactivity with MAb 3C7 in the subepidermal regions and subepithelial regions around the hair follicles, indicated the presence of the short form and the disappearance of the long form in those regions (not shown).

The long form of type XII collagen was first evident in the BM zone and in the basal corneal epithelial layer (Fig. 6D, inset) at postnatal day 12. The intensity of staining in the BM zone was increased by day 16 when the eyelids had already opened. In addition to the linear continuous staining, an intense punctate staining was evident in the BM zone (Figs. 7A and 7B, respectively). This periodic punctate staining in the BM zone was better visualized by taking the photomicrograph with a shorter exposure time (Fig. 7A) than that used in Figure 7B. To determine whether type XII collagen staining was localized to the BM, double immunofluorescence analysis of laminin (a component of the BM) and type XII collagen was performed. The confocal microscopic analysis indicated that the fluorescent red staining for laminin (Fig. 8A) colocalized with the fluorescent green linear staining for type XII collagen in the BM zone (Fig. 8B) as judged from the yellowish–orange band in the merged image (Fig. 8C). Therefore, the linear staining for type XII collagen was localized in the BM. The punctate staining in the BM zone for type XII collagen (Fig. 8B, arrows) was fluorescent yellow in the merged image (Fig. 8C), indicating that the punctate staining was also colocalized with laminin in the BM zone.

**DISCUSSION**

Type XII collagen, a member of the FACIT group, has been shown to be present in chick, mouse, and human corneas and in several other dense connective tissues in the adult. Differential splicing of its transcript gives rise to the short and long variant forms. The two forms have identical short signal peptides at the N-terminus followed by the alternatively spliced NC3 domain and then identical COL2, NC2, COL1, and NC1 domains. The NC3 domain of the long variant has several subdomains, including 18 fibronectin type III repeats (FNIII), 4 von Willebrand factor A (vWFA) complete repeats, and a thrombospondin-terminal-like-domain. The NC3 domain of the short form is shorter by approximately 18 weeks. 

**FIGURE 5.** Immunofluorescence analysis of the regional distribution of long variant form of type XII collagen in day 24 fetal sclera. Cryostat sections of the rabbit tissues were immunoreacted with MAb 2E4. Schematic representation of the cross section of the eye at day 24 of gestation shows the regions A, B, and C (boxed), which correspond to the stained tissues. Scale bar, 50 μm.

**FIGURE 6.** Immunofluorescence analysis of the type XII collagen distribution in corneas during late prenatal and postnatal development. Cryostat sections of the tissues from fetal rabbits at day 28 of gestation (A and B) and newborn rabbits at day 12 (C and D) and day 16 (E and F) immunoreacted with MAb 3C7 (A, C, and E), which reacts with both the long and short variant forms of type XII collagen, or with MAb 2E4 (B, D, and F), which reacts with only the long variant form of type XII collagen. Inset in (D) shows the epithelial (Epi) BM zone and intracellular staining at a higher magnification. Scale bar, 50 μm.

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1150 amino acids and lacks 8 FNIII and 2 vWFa subdomains. Both forms have wider tissue distribution during development, and they become more restricted in certain adult tissues, such as the dense connective tissues. Although the short form is the more predominant one in most tissues in the adult, we have shown that the long form is the predominant form in the adult human cornea. The present study indicated that the same is the case in the adult rabbit cornea. The significance of the differential expression of these two forms during fetal development and in adult tissues is not known. Based on the observation that type XII collagen can promote collagen I gel compaction mediated by dermal fibroblasts, Nishiyama et al. has suggested that type XII collagen may be involved in tissue compaction. Corneal stromal condensation begins at the mid stages (between days 21 and 24) of corneal development in the rabbit and proceeds from posterior to anterior stroma. The condensation pattern of the stromal tissue coincided with the temporal and spatial distribution of the long variant form of type XII collagen in the developing cornea. However, the short form was expressed throughout the entire depth of the stroma from very early stages of corneal development and was also expressed in the sclera and the dermis of the eyelid. Thus, it may have a more widespread function, such as the spacing of collagen fibrils and prevention of fibril fusion in different tissues during development. The type I collagen binding region is located at the carboxyl terminal end of the molecule and is, thus, present in both the short and long forms. Recently, the short form has been shown to interact with two proteoglycans, decorin and fibromodulin. The short form of type XII collagen may act as a linking molecule between the collagen fibrils and decorin in the interfibrillar space and, thus, may assist in regulating interfibrillar matrix organization. Decorin is present not only in the cornea but also in several other connective tissues, and, therefore, the type I collagen–type XII collagen–decorin interaction may be a widespread phenomenon in different tissues.

Around day 21 of gestation, the growing eyelids come in contact with each other and fuse. Interestingly, the transient expression of the long variant form of type XII collagen in the eyelids was observed during this morphogenetic event and was restricted to the subepidermis of the fused eyelids. Histologically, the subepidermal matrix appears denser in this region. The reason for this may be that there is a condensation of the matrix in these regions to accommodate the sudden decrease in the rate of the further expansion of the eyelids after the epithelia have come together. Therefore, the transient synthesis of type XII collagen may be associated with the possible condensation of the matrix, which forms the scaffolding for the overlying epithelium. After fusion of the eyelids, the rates of growth of the eyelids have to be well coordinated with the ongoing increases in the surface area of the cornea. During these stages, the absence of the long variant form of collagen XII suggested that it is not involved in the later stages of eyelid morphogenesis.

In the adult rabbit cornea, the long variant form of type XII collagen is present in the corneal epithelial BM but not in the limbal BM. We had speculated that this distribution may be associated with corneal epithelial differentiation. Based on the expression of a corneal epithelial keratin, K3, the differentiated corneal epithelial cells are evident by day 21 of fetal development. However, the long form of type XII collagen did not appear in the corneal epithelial BM zone during fetal development, indicating that its expression was not associated with corneal epithelial differentiation. The long variant form of type XII collagen was first detected in the basal epithelial cells and in the BM at day 12 of postnatal development of the cornea. Its temporal expression closely preceded the opening of the eyelids. It is possible that the deposition of type XII collagen in the BM zone may promote subepithelial tissue contraction and, thus, assist in the retraction of corneal epithelial cells from the palpebral conjunctival epithelium. Another possibility may be that it promotes a tighter adhesion of the corneal epithelial cells to the underlying matrix during and after the process of eyelid opening. The colocalization of laminin with the punctate staining, which was significantly brighter than the linear staining, indicated that type XII collagen has two distinctly different organizational patterns in the BM of the corneal epithelial cells. The brighter punctate staining is likely to be due to clustering of type XII collagen molecules. This clustering may be associated with the adhesion or anchoring complexes.
involving collagen fibril formation and organization, and tissue variant form may be involved in the morphogenetic events postnatal development. Changes in the distribution of the long and short variant forms is temporally and spatially regulated. The absence of the punctate periodic organization of type XII collagen in the cornea-scleral junction may be related to thicker variable diameter and less organized collagen fibrils in this region. It is clear from the present study that the expression of type XII collagen, both short and long variant forms, is temporally and spatially regulated in the cornea and the surrounding tissues during fetal and postnatal development. Changes in the distribution of the long variant form may be involved in the morphogenetic events involving collagen fibril formation and organization, and tissue condensation. Several subdomains and motif structures in type XII collagen have been identified, some of which may be important in the interaction of these two variant forms of type XII collagen with other matrix molecules or with the cells. In vitro studies have shown that two heparin-binding domains are present in the long variant form and only one in the short form. Thus, these two forms may interact with heparan sulfate proteoglycans in the matrix or on the cell surface in different manners. The collagen-binding domains are present in both the short and the long forms. Possible glycosylation sites, which are likely to be the attachment sites of the chondroitin sulfate chains, are absent in the short form. This explains why only the long form has been shown to exist as a proteoglycan in tissues. This structural difference in these two molecules may be important and responsible for the possible differences in the functions of the two variant forms. Further studies are essential to determine the role of type XII collagen in corneal development, morphogenesis, and maintenance.

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References

Type XII Collagen in Corneal Development


