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Review

# Small leucine-rich repeat proteoglycans in corneal inflammation and wound healing

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ABSTRACT

The small leucine rich repeat proteoglycans are major components of the cornea. Lumican, keratocan, decorin, biglycan and osteoglycin are present throughout the adult corneal stroma, and fibromodulin in the peripheral limbal area. In the cornea literature these proteoglycan have been reviewed as structural, collagen fibril-regulating proteins of the cornea. However, these proteoglycans are members of the leucine-rich-repeat superfamily, and share structural similarities with pathogen recognition toll-like receptors. Emerging studies are showing that these have a range of interactions with cell surface receptors, chemokines, growth factors and pathogen associated molecular patterns and are able to regulate host immune response, inflammation and wound healing. This review discusses what is known about their innate immune-related role directly in the cornea, and studies outside the field that find interesting links with innate immune and wound healing responses that are likely to be relevant to the ocular surface. In addition, the review discusses phenotypes of mice with targeted deletion of proteoglycan genes and genetic variants associated with human pathologies.



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## 1. Introduction

The cornea, comprised of a stratified epithelium, basement membrane, Bowman's layer, stroma, Descemet's membrane and a single cell layered endothelium, is the outermost, avascular,

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refractive and protective barrier of the eye. Approximately 500 μm thick in humans, the stroma makes up 90% of the cornea. The stroma contains collagen fibrils of uniform diameter that are organized into orthogonal lamellae. The stroma is also rich in proteoglycans that interact with collagens to regulate fibril thickness, interfibrillar spacing and hydration, required to maintain the optical qualities of the cornea necessary for vision (Hassell and Birk, 2010; Meek and Knupp, 2015). This review discusses the role of the stromal proteoglycans in corneal inflammation and wound healing responses.

Proteoglycans are proteins, covalently conjugated to one or more glycosaminoglycan (GAG) side chains, chondroitin sulfate, keratan sulfate or heparan sulfate. The stromal proteoglycans belong to a group known as the small leucine-rich repeat proteoglycans (SLRPs) carrying characteristic tandem repeats of leucine-rich repeat motifs in their core proteins. Of the ~17 known SLRPs, lumican (LUM), keratocan (KERA), mimecan/osteoglycin (OGN), decorin (DCN) and biglycan (BGN) are major components of the corneal stroma. Fibromodulin (FMOD), abundant in the sclera, is also present in the peripheral limbal region of the cornea. Therefore, the review covers these six SLRPs and their known and speculated functions in the cornea.

Historically, proteoglycans were purified by dissociative extraction, ion-exchange and molecular sieve chromatography, and sedimentation-equilibrium centrifugation. The proteoglycans were characterized by gel electrophoresis before or after cleavage of the GAG side chains to visualize the core proteins (Hassell et al., 1986; Heinegard and Sommarin, 1987). The undigested samples had high "polydispersity" appearing as smears in polyacrylamide gels while the digested "monodisperse" core proteins shifted to a faster migrating sharp band - a characteristic behavior of all proteoglycans. The earliest study of SLRPs described a low buoyant density proteoglycan in cesium chloride density centrifugations of extracts from nasal cartilage that carried chondroitin sulfate side chains (Heinegard et al., 1981) and a protein with high leucine content by amino acid analysis. Later, sequencing of cDNA clones prepared from a fibroblast cell line led to the identification of decorin (Krusius and Ruoslahti, 1986). Within a decade genes encoding biglycan (Fisher et al., 1989, 1991), decorin (Santra et al., 1994; Scholzen et al., 1994), lumican (Blochberger et al., 1992a; Chakravarti and Magnuson, 1995; Chakravarti et al., 1995), fibromodulin (Antonsson et al., 1993; Sztrolovics et al., 1994), keratocan (Funderburgh et al., 1998; Tasheva et al., 1999), mimecan/osteoglycin (Funderburgh et al., 1997) and other members were sequenced and localized to specific chromosomes. An explosion of molecular biological studies and development of gene-targeted mice now present an exciting and evolving picture of the breadth of functions and molecular interactions of the core proteins (Chakravarti, 2001; Chakravarti et al., 1998; Danielson et al., 1997; Liu et al., 2003; Svensson et al., 1999; Tasheva et al., 2002; Xu et al., 1998).

### 1.1. Core protein structure and leucine rich repeat types

The acronym SLRP coined in the 1990s diffuses their connection to the leucine-rich repeat (LRR) superfamily of ~370 proteins which includes pathogen recognition receptors and other regulators of innate immunity. Almost the entire core proteins in SLRPs consist of tandem repeats of LRR motifs in which the minimum conserved residues are LXXLXLXXNXL with varying lengths of 20–27 amino acids (Bella et al., 2008; McEwan et al., 2006). All of the SLRPs discussed here have 12 such motifs numbered LRR 1-12. The crystal structures of decorin and biglycan core proteins have been resolved (Scott et al., 2004, 2006); similar to ribonuclease inhibitor (RNI), the first LRR protein to be crystallized, they have a curved solenoid shape, where each LRR motif forms a β strand and the inner concave surface forms a β sheet (Fig. 1). The 4 cysteine residues at the N-terminus are disulfide bonded at alternate residues to form the N-terminal cap, while the C-terminal two-cysteine residues form disulfide bonds with each other. The difference in the spacing of the N-terminal cysteine residues is used to group the SLRPs into five classes; biglycan and decorin (Fisher et al., 1989) are Class I, lumican (Blochberger et al., 1992b), fibromodulin (Oldberg et al., 1989) and keratocan (Corpuz et al., 1996) are Class II and osteoglycin (Funderburgh et al., 1997) belongs to Class III.

The penultimate LRR motif, with 30–39 residues, is atypical, forms an extended loop or "ear" and found in the SLRP subfamily only. Sequence alignment studies of the "ear" motif suggest evolution of the SLRPs from an ancestral gene by large-scale genome duplication and loss of genes (Huxley-Jones et al., 2009; Park et al., 2008). A recent study used hidden Markov modeling that uses a statistical Bayesian network-based approach to identify patterns, on all 375 LRR superfamily members to identify seven signature LRR motifs (Ng et al., 2011). Not surprisingly, the closely related SLRPs, lumican, fibromodulin and keratocan have similar distributions of 5/7 signature LRR motifs. In a hierarchical clustering of all the LRR proteins, the SLRPs form a tight cluster, and interestingly, several TLR members are placed close to the SLRPs.

### 1.2. Glycosaminoglycan side chains

The Class II SLRPs lumican, keratocan, fibromodulin and Class III osteoglycin, are post-translationally modified with keratan sulfate (KS) side chains covalently linked to an asparagine residue. Lumican and fibromodulin have five potential KS substitution sites, keratocan four and osteoglycin has one (Kalamajski and Oldberg, 2010). The cornea contains the type I KS form which has a long (~50) linear poly- N-acetyl lactosamine consisting of repeating units of the disaccharide [→3Galβ(1→4)GlcNAcβ(1→], linked to an asparagine residue via a mannose containing branched oligosaccharide (Funderburgh, 2002; Quantock et al., 2010). Treatment

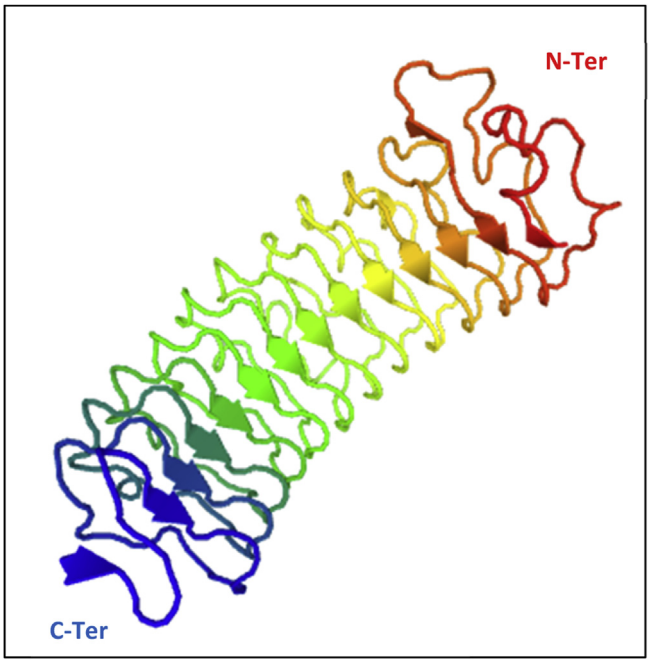


**Fig. 1.** Model structure for decorin provided by SwissModel (Kiefer et al., 2009). Decorin has a curved solenoid shape, where each LRR motif forms a β-strand and the inner concave surface forms a β-sheet (arrows).