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MINI REVIEW

Keratan sulfate: structure, biosynthesis, and function

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The last 5 years have seen a marked increase in research on keratan sulfate (KS) and a concomitant increase in our understanding of the range of molecules that carry this adaptable polysaccharide. More than 15 KS-linked proteins have been identified and many of the genes encoding these have been cloned. KS-containing molecules have been identified in numerous epithelial and neural tissues in which KS expression responds to embryonic development, physiological variations, and to wound healing. A corneal cell culture system has been developed in which long-term KS biosynthesis is maintained. Progress has been made toward identification of the glycosyl- and sulfotransferases responsible for KS biosynthesis. A mouse knockout of a corneal KS-proteoglycan has provided the first experimental support for the role of KS in corneal transparency. Evidence has also been presented supporting functional roles of KS in cellular recognition of protein ligands, axonal guidance, cell motility, and in embryo implantation. These findings have served to expand the concept of what keratan sulfate is and the potential roles it may play in the cellular biology of diverse tissues.

Key words: keratan sulfate/proteoglycan/cornea/cartilage/minireview

Six decades of keratan sulfate

Keratan sulfate was identified in 1939 by Suzuki in extracts of cornea (Suzuki, 1939), and by the 1950s the efforts of Karl Meyer (who coined the name keratosulfate) and others had characterized this material as a linear polymer of lactosamine, 3Galβ1–4GlcNAcβ1, sulfated at the C6 of both hexose moieties (Meyer *et al.*, 1953). Over the decades since its initial characterization interest in keratan sulfate has never waned. In fact, from the 1970s until the last half of the 1990s, the annual publication of articles with keratan sulfate as a major topic has increased about 5-fold. Several previous reviews have examined structure and biosynthesis of KS through 1994 (Hascall, 1982; Nieduszynski *et al.*, 1990a; Funderburgh *et al.*, 1991a; Greiling, 1994). This Mini Review, therefore, will focus primarily on the most recent findings of KS structure, the proteins that carry KS, the enzymatic mechanisms of KS synthesis, and emerging functional roles for KS.

Keratan sulfate structure

KS classes

Originally the designations KSI and KSII were based on differences between KS from cornea and that of cartilage. Corneal KS is N-linked to Asn residues in the core protein, whereas cartilage KS is O-linked to Ser or Thr residues. These linkage structures, however, are not tissue-specific in their localization. The class designations currently are employed with respect to the linkage structure, not tissue localization. Thus, the term KSI includes all Asn-linked KS molecules, and KSII is used to refer to all KS linked to protein through GalNAc-O-Ser/Thr. A third type of KS linkage (Man-O-Ser) has been identified that might be considered KSIII (Krusius *et al.*, 1986).

Corneal KSI

Corneal KS is the prototype for KSI and is the most extensively characterized. The amount of KS in cornea is more than 10-fold that in cartilage and is 2–4 orders of magnitude greater than KS found in other tissues (Funderburgh *et al.*, 1987). Linkage of corneal KS to protein involves a complex-type biantennary oligosaccharide N-linked to asparagine in the core protein (illustrated in Figure 1A). Analyses of a highly purified subfraction of porcine corneal KS found the KS chain to extend only the C-6 branch of the linkage oligosaccharide with the C-3 branch terminating with a single lactosamine capped by sialic acid (Oeben *et al.*, 1987). This model is illustrated in Figure 1A.

Other reports suggest that C-3 branches of the linkage oligosaccharides can be extended with KS chains in addition to the C-6 branches. Structural analysis of monkey corneal KS linkage region oligosaccharides found no evidence that the C-3 was terminated with sialic acid (Nilsson *et al.*, 1983). Evidence for two-chain (biantennary) extension of the linkage oligosaccharide was also provided from the comparison of the molecular weight of bovine KS released by N-glycanase (i.e., with an intact linkage oligosaccharide) to the KS chain length (Tai *et al.*, 1996). In addition, synthesis of neither corneal KS nor KS on fibromodulin, an N-linked type I KS proteoglycan from cartilage, is fully blocked by swainsonine, an inhibitor of processing of the C-6 arm of the precursor oligosaccharide (Ziegler and Mersmann, 1984; Plaas and

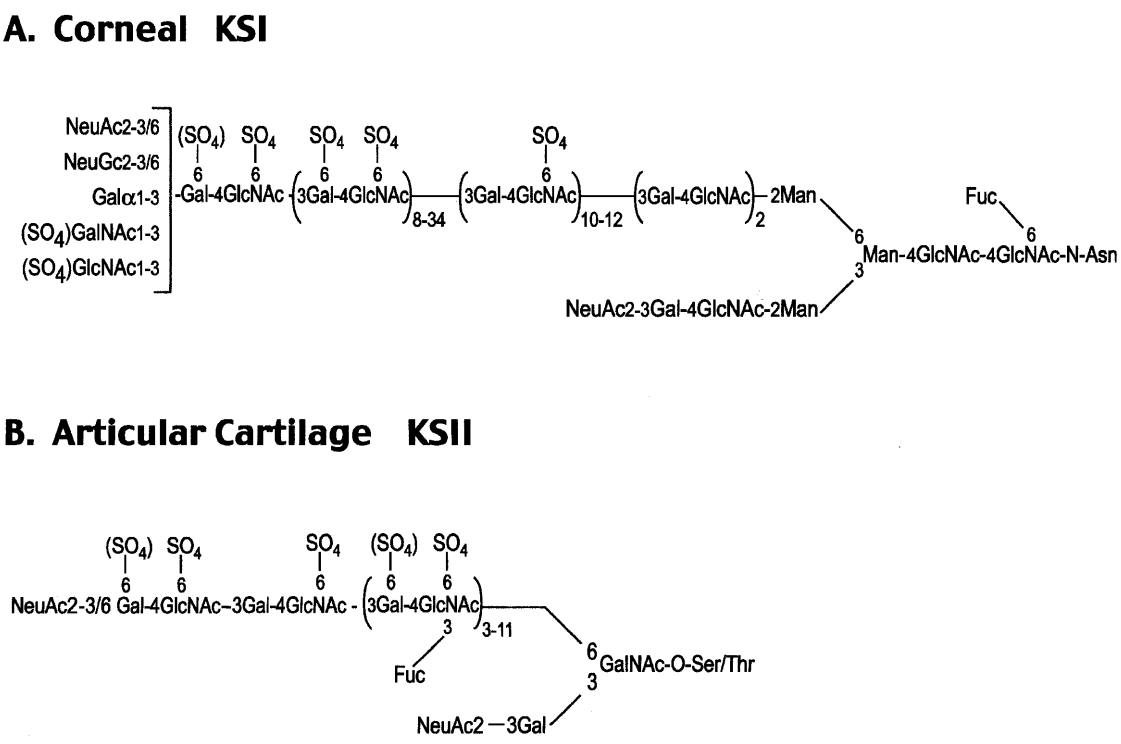

Fig. 1. Summary of structural features of keratan sulfate molecules. Numbers to the lower right of the large parenthesis show the approximate number of N-acetyllactosamine monomers in each domain. Numbers separated by a forward slash present optional attachment locations. Sulfates in parentheses indicates partial or incomplete sulfation of monomers at this site. Sources of these structural assignments are given in the text.

Wong-Palms, 1993), implying that KS must be extended on the C-3 arm of the mannose-containing core.

This evidence for biantennary extension of the KSI linkage is also supported by experiments in which endo-β-galactosidase-treated bovine corneal KS proteoglycans were labeled on the GlcNAc "stub" from which the KS chain had been cleaved and also labeled on sialic acids oxidized by mild periodate (Funderburgh *et al.*, 1991b). Tryptic peptides from the labeled proteins were identified that contained label for both KS and sialic acids, as would be predicted from a single-arm extension of KS as shown in Figure 1A. Additionally, some peptides were labeled only for KS attachment indicating a lack of sialic acid in the linkage and a possible biantennary KS extension at these sites. These studies suggest that both mono- and biantennary extensions of the linkage may occur in KSI and that the location of the site on the core protein may influence the type of extension. Heterogeneity is also seen in modification of the linkage region of ZP3, a zona pellucida protein substituted with N-linked KS. In this protein KS can modify either the C-3, the C-6, or both arms of the biantennary linkage oligosaccharide (Noguchi and Nakano, 1992).

Oeben *et al.* (1987) found sulfation of a purified fraction of porcine corneal KS to be distributed in a distinct, non-random pattern. Disaccharides nearest the reducing end were found to be nonsulfated (Figure 1A), and distal to this nonsulfated domain follows a series of disaccharides sulfated only on the GlcNAc moiety. The nonreducing end of the porcine corneal KS chains consists of a domain of variable length (8–34) of only disulfated disaccharides. This highly sulfated domain is responsible for the heterogeneity in the charge and size characteristic of corneal KS. There is also a suggestion that N-sulfation of GlcN may occur in the highly sulfated domain of corneal KS (Tang *et al.*, 1986). The nonreducing terminus of each chain is capped with a variety of structures. In bovine corneal KS about 70% of corneal chains terminate with neuraminic acid, the remainder with βGalNAc or α-Gal (Tai *et al.*, 1997, 1996).

Non-corneal KSI

Fibromodulin, PRELP, and osteoadherin are proteins in cartilage and bone modified with N-linked KS chains (Antonsson *et al.*, 1991; Bengtsson *et al.*, 1995; Sommarin *et al.*, 1998). Several proteins of the ovarian zona pellucida carry carbohydrates considered to be KS (Noguchi and Nakano, 1992). The cartilage proteoglycan aggrecan also contains 2–3 N-linked KS chains in addition to 20 or more that are O-linked (Barry *et al.*, 1995). N-linked KS has also been isolated from the dermis of the pacific mackerel (Ito *et al.*, 1984). In addition to these well-defined examples of non-corneal KSI, sulfated lactosamine appears to be a common component of cell surface and extracellular glycoproteins. Some of this sulfation involves O-linked Lewis x structures, that differ from KS in that they are sulfated only at the non-reducing terminus and are fucosylated at the terminal GlcNAc (Hemmerich *et al.*, 1995; Capon *et al.*, 1997). Structures of other sulfated lactosaminoglycans are yet to be characterized, and it seems likely that some will be found to be KS.

Although the KSI linkage is not tissue specific, other characteristics of KS in non-corneal tissues diverge from the corneal model. KS chains in fibromodulin and osteoadherin are relatively short (8–9 disaccharides) and are more highly sulfated than KS in cornea (Lauder *et al.*, 1997). KS in fibromodulin lacks the clear domain structure of corneal KS, but like corneal KS it displays reduced Gal sulfation near the reducing terminus. Groups capping the non-reducing terminus of fibromodulin KS are more typical of those on cartilage KS (Figure 1B) than of those on corneal KS (Lauder *et al.*, 1997). KS structure, therefore, may be dictated primarily by tissue-specific factors, such as glycosyltransferases, rather than the type of linkage to core protein.

KSII

A structure of bovine articular cartilage KS is illustrated in Figure 1B. The chains are shorter than KS of cornea (5–11 disaccharides) and lack the characteristic domain structure. Cartilage KSII is highly sulfated, consisting almost completely of disulfated monomers interrupted occasionally by single monosulfated lactosamine monomers (Nieduszynski *et al.*, 1990b). Linkage to the protein is via serine or threonine and involves a mucin type "core 2" oligosaccharide. Sialylation of Gal linked to the C-3 of the linkage GalNAc is only partial. The KSII chains are capped by sialic acid at the C-3 or C-6 of the terminal GlcNAc. α-Linked fucose is also present on the C-3 of sulfated GlcNAc throughout the chain but not within four hexose moieties of the nonreducing terminus (Brown *et al.*, 1996). This feature distinguishes KS molecules from the Lewis x (Le^x) antigens which are fucosylated on the GlcNAc penultimate to the nonreducing terminus. These KS-like glycoforms are present at endothelial cell surfaces and serve as selectin ligands (Tangemann *et al.*, 1999). KS from tracheal cartilage does not exhibit fucosylation of the internal GlcNAc, and carries only (2→3) linked sialic acids at the chain terminus (Nieduszynski *et al.*, 1990b; Dickenson *et al.*, 1991). Thus, tissue-specific glycosyltransferase activities and not primary protein sequence appear to major determinants of much of KSII chain structure and capping.

Man-O-linked KS (KSIII)

A third type of linkage between KS and protein has been described in proteoglycans from brain (Krusius *et al.*, 1986). These KS chains are linked to Ser/Thr in the core protein via mannose, i.e., KS-Man-O-Ser. This linkage appears to be present in phosphocan-KS (I. Nieduszynski, personal communication), however, full characterization of the proteins in which the Man-O-Ser KS linkage occurs is pending.

KS biosynthesis

Biosynthetic enzymes

KS is elongated via the action of glycosyltransferases that alternately add Gal and GlcNAc to the growing polymer. The Gal-transferase activity in corneal cells resembles the β-1,4-galactosyltransferase enzyme (β4Gal-T1) abundant in serum and milk (Christner *et al.*, 1979). In recent years a family of at least seven β4Gal-T genes has been identified, and roles for each are currently being defined (Amado *et al.*, 1999). During chick development a corneal β4Gal-T transcript was found to increase during development in concert with corneal KS synthesis. Activity of this enzyme was maintained at an unusually high level in adult corneal cells (Cai *et al.*, 1996). Comparison with known cDNA sequences indicates that the upregulated corneal β4Gal-T is identical to β4Gal-T1. Interestingly, β4Gal-T1 activity continues at elevated levels in cultured corneal cells that have lost KS synthesis (Christner *et al.*, 1979). Although β4Gal-T1 appears to be the enzyme involved in corneal KS synthesis, this specific enzyme has not been linked to KS biosynthesis in other tissues.

The N-acetylglucosaminyltransferase (GnT) enzyme responsible for KS synthesis has not been identified. A number of GnT enzymes are known, of which two seem potential candidates. A widely distributed enzyme (iGnT) has been shown to participate in synthesis of linear polylactosamine (known as "i" antigens) (Sasaki *et al.*, 1997). RNA transcripts for this enzyme are enriched in brain, a tissue in which KS is actively synthesized. Recently a second GnT enzyme (β3GnT) active in synthesis of linear polylactosamine has been identified and cloned (Zhou *et al.*, 1999). At the current time, however, no evidence has been presented linking a specific GnT to KS synthesis.

Sulfation of KS in cornea is carried out by at least two sulfotransferase enzymes (Ruter and Kresse, 1984). Two enzymes have been identified and cloned that add sulfate to KS (Habuchi *et al.*, 1996; Fukuta *et al.*, 1997). One of these adds sulfate to GalNAc moieties of chondroitin sulfate and also to Gal in KS. The second enzyme also transfers sulfate to Gal of KS but does not act on chondroitin sulfate. Messenger RNA for the KS-specific sulfotransferase shows enhanced expression in brain and cornea. It would therefore appear likely that this sulfotransferase represents an enzyme involved in KS biosynthesis.

KS is also sulfated on the GlcNAc moieties and presumably a specific enzyme is responsible for this activity. Nakazawa *et al.* (1998) have demonstrated that GlcNAc-6-sulfotransferase (Gn6ST) activity in keratocyte extracts specifically sulfates nonreducing terminal GlcNAc(β1–3)Gal-R. Partially desulfated KS received sulfation only on Gal moieties by these extracts. The cDNAs for two Gn6ST enzymes with specificity for nonreducing terminal GlcNAc have been identified and cloned (Uchimura *et al.*, 1998; Lee *et al.*, 1999); however, there remains a question if either of these represent the enzymes involved in KS synthesis. Patients with macular corneal dystrophy, a disease in which KS lacks GlcNAc sulfation throughout the body, had unaltered levels of this enzyme activity in their serum (Hasegawa *et al.*, 1999). The implication of these findings is that an enzyme of similar specificity with restricted tissue localization may be responsible for KS synthesis.

The specificity of the Gn6ST enzyme suggests that KS GlcNAc sulfation may occur simultaneously with elongation and only on the terminus of the growing chain (Degroote *et al.*, 1997; Uchimura *et al.*, 1998). The idea of coordinated elongation and sulfation of KS is supported by biosynthetic studies with cell-free corneal extracts that showed a coordinate change in the Vmax of both elongation and sulfation activities with respect to KS chain length (Keller *et al.*, 1989).

KS-linked proteins

KS can be identified in a wide range of tissues, indicating that the enzymatic machinery to produce this polymer is not severely restricted in its tissue localization. Conversely, only a limited number of proteins are known to carry KS as a post-translational modification. The logical conclusion to be drawn from these two observations is that expression of these core proteins is an important determinant of KS biosynthesis. Understanding the genes and gene expression patterns for KS proteoglycan core proteins, therefore, is essential to complete understanding of KS biosynthesis. Over the past decade a number of KS-modified proteins and the genes that code for them have been identified. These are summarized in Table I. Immunolocalization studies of KS suggest that there are likely to be more KS-linked proteins not yet characterized.

Small leucine-rich proteoglycans (SLRPs)

This is an extensive group of related proteins that are typically components of interstitial matrix. Members of this family share a series of 24-amino acid leucine-rich repeats (LRRs) that make up the central portion of each protein. Modeling studies and direct observation suggest that the LRRs fold the protein into a series of β-sheets, creating an arch-shaped three dimensional structure with KS attachment sites positioned so that the KS chains extend from the convex side of the arch (Scott, 1996; Iozzo, 1997). In cornea, KS is present on three SLRP proteins, lumican, keratocan, and mimecan (Funderburgh *et al.*, 1993, 1997a; Corpuz *et al.*, 1996). Fibromodulin and PRELP in cartilage and osteoadherin in bone are also KS-modified SLRP-family proteins (Antonsson *et al.*, 1991; Bengtsson *et al.*, 1995; Sommarin *et al.*, 1998). KS-linked tryptic peptides of fibromodulin contain all of the four potential N-glycosylation sites in the protein, however the size of the intact molecule suggests that only one site on each protein molecule is extended with KS (Plaas *et al.*, 1990). A similar approach identified KS attachment sites in chicken corneal lumican and keratocan (Dunlevy *et al.*, 1998). The KS attachment site in each case was N-terminal to the first leucine of an LRR indicating a surface location for each chain. Most of the sites were characterized by three aromatic amino acids N-terminal to the KS linkage site.

Aggrecan

The major proteoglycan of cartilage is the very large protein aggrecan in which KS is found in two separate domains. The majority of KS is O-linked to aggrecan between the G2 and G3 regions in a domain characterized by a repeated six-amino acid motif (Flannery *et al.*, 1998). The sequence of the motif is highly conserved in different vertebrate species, but the numbers of repeated units varies (Barry *et al.*, 1994; Flannery *et al.*, 1998). This variation may account for differences in KS content of aggrecan of different species. In rodents the motif sequence is not conserved, and in these species skeletal KS is greatly reduced or absent. KS is also linked to aggrecan near the N-terminus of the protein in the HA-binding domain in both O- and N-linked forms (Barry *et al.*, 1995). KS chains in the HA-binding region may have different length and sulfation compared to the chains from the GAG-binding region of the molecule. This suggests that protein conformation in this huge molecule may control access to the glycosyl- or sulfotransferases during passage through the Golgi.

Table I. Keratan sulfate-linked proteins

Protein/gene	KS-protein linkage	Tissue/cell type localization	Reference
Lumican	KSI	Cornea	Blochberger *et al.*, 1992
Keratocan	KSI	Cornea	Corpuz *et al.*, 1996
Mimecan	KSI	Cornea	Funderburgh *et al.*, 1997a
Fibromodulin	KSI	Cartilage	Oldberg *et al.*, 1989
Aggrecan	KSI and KSII	Cartilage	Barry *et al.*, 1995
PRELP	KSI	Cartilage	Bengtsson *et al.*, 1995
Osteoadherin	KSI	Bone	Sommarin *et al.*, 1998
PZP3	KSI	Zona pellucida	Noguchi and Nakano, 1992
MUC1	NA[a]	Endometrial epithelium	Aplin *et al.*, 1998
CD44	NA	Carcinoma line KM12L4	Takahashi *et al.*, 1996
		Epidermis	Tuhkanen *et al.*, 1997
Cytokeratins	NA	Epidermal keratinocytes	Schafer and Sorrell, 1993
ABAKAN	NA	Brain	Seo and Geisert, 1995
SV2	NA	Synaptic vesicles	Carlson, 1996
Phosphocan KS	KSIII	Brain	Margolis *et al.*, 1996
Claustrin	NA	Brain	Burg and Cole, 1994
PG-1000	NA	Electric organ	Carlson *et al.*, 1996

[a]Information not available.

Cell-associated proteoglycan

KS has been recently demonstrated to be associated with a number of epithelial tissues. Keratinocytes, uterine endometrial cells, corneal endothelium, sebaceous gland, salivary gland, and sweat gland epithelia exhibit KS immunoreactivity in adult tissues (Sorrell and Caterson, 1990; Shiozawa *et al.*, 1991; Fullwood *et al.*, 1996). KS is also found in a variety of epithelia-derived carcinoma cells (Ito *et al.*, 1996; Leygue *et al.*, 1998). The endometrial protein MUC1 has recently been shown to be modified with KS (Aplin *et al.*, 1998). MUC1 is a common component of the mucin layer associated with the apical surfaces of secretory epithelia. This single molecule might prove to be responsible for the presence of KS in many of the numerous glandular surfaces in which it has been reported. Another cell-surface molecule CD44 contains KS (Takahashi *et al.*, 1996; Tuhkanen *et al.*, 1997). This protein occurs as a number of alternately spliced forms, some of which contain heparan sulfate. CD44 and SV2 (discussed below) are the first known integral membrane proteins to be identified with KS. A third type of cell-associated KS was described as the modification of intracellular keratin molecules with KS in keratinocytes (Schafer and Sorrell, 1993). These examples of cell-associated KS demonstrate that, like chondroitin and heparan sulfates, KS molecules are not restricted to interstitial connective tissues but modify a variety of proteins with considerable variety in localization.

Brain proteoglycans

One of the most active areas of recent KS research concerns proteoglycans of the central nervous system. After cornea and skeletal tissues, brain appears to exhibit the most abundant KS and is one of the tissues most rich in enzymes of KS biosynthesis. The major cartilage proteoglycan aggrecan is present in neural tissues, but aggrecan in the CNS may not contain KS (Domowicz *et al.*, 1995). Several proteoglycans that appear to be unique to nervous tissue have been described, including ABAKAN (Seo and Geisert, 1995), SV2 (Carlson, 1996), PG-1000 (Carlson *et al.*, 1996), claustrin (Burg and Cole, 1994), and phosphocan-KS (Margolis *et al.*, 1996). Each appears to be a unique KS-linked protein with highly specific localization, produced by a limited population of cells. Other KS-linked proteins also occur in neural tissue but are yet to be fully characterized (Miller *et al.*, 1997).

Control of KS synthesis

KS biosynthesis is often markedly altered in response to metabolic, pathologic, or developmental changes in tissues. One characteristic pattern of KS expression is a developmentally correlated increase in KS abundance and sulfation. This phenomenon was first described in the cornea. In chick embryos, sulfated KS is not detected until ~12 h after invasion of the migrating neural crest cells into the primary stroma (Funderburgh *et al.*, 1986). After that time, KS accumulates rapidly in the stroma. This accumulation continues after hatching and in the adult as well. Similarly, in neonatal mouse cornea, lumican is found primarily as a non-sulfated glycoprotein but begins to be modified with KS at 10–14 days, about the time of eye opening (Ying *et al.*, 1997). The size and charge of this murine corneal KS increases for at least 1 year. Studies of KS abundance in human corneas suggest a similar increase of KS throughout life (Praus and Brettschneider, 1975).

Cartilage KS appears to exhibit developmental changes similar to those of cornea. The KS content of aggrecan in cartilage undergoes an age-related increase in KS chain length and sulfation (Brown *et al.*, 1998; Lauder *et al.*, 1998). Rat brain, as well, shows little embryonic KS, developing the majority of KS activity after birth (Meyer-Puttlitz *et al.*, 1995). On the other hand, much of the KS in the brain exhibits unique, complex, developmentally-regulated patterns (Miller *et al.*, 1997). This level of fine modulation suggests a specialized developmental function for KS-linked molecules of the CNS.

A second widely observed property of KS biosynthesis is its volatility in wound healing and *in vitro*. Cell types that secrete KS (neural cells, chondrocytes, and keratocytes) are quiescent *in vivo*, but when maintained *in vitro*, chondrocytes and keratocytes, depending on culture conditions, often assume a fibroblastic morphology and lose KS synthesis. In corneal wounds keratocytes are activated to divide, adopt a fibroblastic phenotype similar to cultured corneal fibroblasts, and synthesize little KS. Sub-acute or chronic pathological conditions affecting the cornea also frequently lead to loss of KS in the stroma (Funderburgh *et al.*, 1990; Rodrigues *et al.*, 1992). In the brain, microglial KS is reduced during inflammation (Jander *et al.*, 2000) and cerebral KS is reduced as a result of Alzheimer's disease (Lindahl *et al.*, 1996). Reduction of KS in both brain and cornea appears in association with inflammation, suggesting a role for proinflammatory cytokines in the down-regulation of KS biosynthesis.

Numerous studies have documented the disappearance of KS in cultured corneal fibroblasts. A detailed analysis of the products synthesized by these cultures demonstrated expression of all three of the KS-linked proteins but found them to be modified with truncated oligolactosamine with little or no sulfation (Funderburgh *et al.*, 1996). These results suggest that downregulation of KS biosynthesis *in vitro* (and by implication in healing wounds) stems from downregulation of KS-specific glycosyl- and/or sulfotransferases. A study using freshly isolated chicken keratocytes showed a marked reduction in sulfation of KS GlcNAc residues after short-term culture of these cells in serum (Nakazawa *et al.*, 1998). Specific enzymes required for polymerization and sulfation of KS may, therefore, be key regulators of KS biosynthesis *in vitro* and possibly *in vivo* as well.

Recent development of a culture method for keratocytes that maintains biosynthesis of macromolecular, fully sulfated KS for extended periods *in vitro* provides an important tool for identification of KS-specific biosynthetic enzymes (Beales *et al.*, 1999). KS biosynthesis by keratocytes cultured using this method was downregulated by fetal bovine serum and by transforming growth factor β (TGFβ), but fibroblast growth factor 2 (FGF) acted to maintain KS synthesis *in vitro* (Long *et al.*, 2000). This experimental system provides the opportunity to examine the effect of various stimuli (e.g., nutrients, inflammatory cytokines, cell-matrix interactions) on KS assembly.

Biological functions of KS

Tissue hydration

In cornea, the high abundance of KS appears to be related to maintenance of a level of tissue hydration critical for corneal transparency. KS- and dermatan sulfate-linked proteoglycans of the cornea have distinct water-binding properties (Bettelheim and Plessy, 1975; Hedbys, 1961). At hydration levels characteristic of normal corneal stroma, dermatan sulfate is fully hydrated whereas keratan sulfate is only partially hydrated, suggesting that KS provides a dynamic buffer for corneal hydration. The importance of KS in the stroma is supported by data from diseases such as macular corneal dystrophy in which the balance of glycosaminoglycans is altered (Hassell *et al.*, 1980). Recently a null mutation for lumican in mice was reported to show a 25% reduction in corneal keratan sulfate and to exhibit a similar loss in stromal hydration (Chakravarti *et al.*, 1998). These mice developed corneal haze after 3–6 months of life, a time course consistent with the accumulation of sulfated KS in the mouse cornea. These results support the long-voiced hypothesis that corneal transparency is dependent on the presence of abundant keratan sulfate.

Cell biology of KS

The role of KS in corneal hydration does not explain the presence of small amounts of KS in so many other tissues. Numerous active biological roles have been documented for hyaluronan, heparan sulfate, and chondroitin sulfate (Iida *et al.*, 1996; Toole, 1997; Lindahl, 1999). Recent studies of KS have presented data suggesting that KS also is an active participant in the cellular biology of the tissues in which it is located. Mouse macrophages express a high-affinity cell surface receptor for lumican and for lumican modified with nonsulfated oligolactosamine. These cells do not bind lumican that carries sulfated KS chains, nor will they attach and spread on plastic surfaces coated with lumican-KS. Removal of KS with endo-β-galactosidase restored attachment and spreading of the cells *in vitro* (Funderburgh *et al.*, 1997b). This anti-adhesive character of KS has been observed in other studies. KS-containing molecules constitute a barrier to neurite growth *in vitro* and appear to direct axon growth patterns during development or regeneration *in vivo* (Burg and Cole, 1994; Olsson *et al.*, 1996). The "barrier" character of KS is also implicated in the findings that KS chains on aggrecan block development of an immune response *in vivo* and *in vitro* to the G1 domain of this protein, suppressing development of antigen-induced osteoarthritis (Guerassimov *et al.*, 1999).

Abundance of the cell-associated KS in the endometrial uterine lining varies markedly during the menstrual cycle, reaching a peak at the time at which embryo implantation occurs (Graham *et al.*, 1994). At this time keratanase-sensitive molecules block access of antibodies to MUC1 ectodomain epitopes, normally accessible at other times in the cycle (DeLoia *et al.*, 1998). These findings suggest a potential role for KS in the implantation process. KS has also been implicated in motility of corneal endothelial cells, a single layer epithelium that lines the posterior surface of the cornea. These cells normally display a mosaic distribution of KS at their apical surface, but after wounding the KS is reduced or absent on migrating cells. KS returns in abundance to the cell surface when the cells cease migration (Davies *et al.*, 1999). Anti-adhesive molecules function in complex and sometimes paradoxical roles during cell attachment and motility (Greenwood and Murphy-Ullrich, 1998). The anti-adhesive properties of KS may well play significant roles in implantation and endothelial cell migration as well as in other biological processes. Correlative studies such as these suggest potential experimental systems in which molecular mechanism of these biological effects can be determined.

Conclusion

The first six decades of research on KS has produced a wealth of information on the structure, localization, and biosynthesis of this complex family of glycocongugates. The next few years are likely to witness the identification of the genes involved in KS biosynthesis and the determination of how expression of these genes is controlled by signals both outside and inside the cell. The sensitive and subtle patterns of KS expression in various tissues during development and wound healing speaks for sensitive and subtle biological roles for these molecules. Unraveling these mysteries may not take another 60 years, but it seems highly likely that at least half of the story of KS is yet to be told.

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