Identification and Characterization of Versican/PG-M Aggregates in Cartilage

Received for publication, September 20, 2005, and in revised form, April 26, 2006 Published, JBC Papers in Press, April 28, 2006, DOI 10.1074/jbc.M510330200

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Versican/PG-M is a large chondroitin sulfate proteoglycan of the extracellular matrix with a common domain structure to aggrecan and is present in cartilage at low levels. Here, we characterized cartilage versican during development and growth. Immunostaining showed that versican was mainly localized in the interterritorial zone of the articular surface at 2 weeks in mice, whereas aggrecan was in the pericellular zone of prehypertrophic and hypertrophic cells of the growth plate. Although its transcription level rapidly diminished during growth, versican remained in the articular cartilage. Biochemical analysis of normal articular cartilage and aggrecan-null cartilage from cmd (cartilage matrix deficiency)/cmd mice revealed that versican was present as a proteoglycan aggregate with both link protein and hyaluronan. Chondroitin sulfate chains of versican digested with chondroitinase ABC contained 71% nonsulfated and 28% 4-sulfated unsaturated disaccharides, whereas those of aggrecan contained 25% nonsulfated and 70% 4-sulfated. Link protein overexpression in chondrocytic N1511 cells, at the early stage of differentiation, in which versican is expressed, enhanced versican deposition in the matrix and prevented subsequent aggrecan deposition. These results suggest that versican is present as an aggregate distinct from the aggrecan aggregate and may play specific roles in the articular surface.

Versican/PG-M (1, 2) is a large chondroitin sulfate proteoglycan of the extracellular matrix (ECM) that exhibits two distinct expression patterns. In various developing embryonic tissues (3), including the nervous system, versican is transiently expressed and plays important roles in cell adhesion (4), migration (5), proliferation, and differentiation (6). In some adult tissues, such as the heart, blood vessels, and brain, it is constitutively expressed and serves as a structural macromolecule of the ECM.

In developing cartilage, versican is transiently expressed at a high level in the mesenchymal condensation area and rapidly disappears during cartilage development (7, 8). Recent immunohistochemical studies on developing limb bud cartilage revealed that an area positive for versican gradually shifts out of the diaphysis and is replaced by an area positive for aggrecan. Differentiating chondrocytic N1511 and ATDC5 cells showed similar expression patterns of these molecules (9). These reciprocal patterns of versican and aggrecan expression suggest that versican serves as a temporary framework in developing cartilage matrix. Although the aggrecan aggregate (10) is the major component of cartilage ECM and versican has not been detected by immunohistochemical studies (3), constitutive low level transcription of the versican gene is observed in cartilage (11) and chondrocytes (11, 12). In addition, extracts of human adult articular cartilage contain versican (13), suggesting its distinct role there.

The core protein of versican consists of N- and C-terminal globular domains (G1 and G3) and two chondroitin sulfate (CS) domains (CS-α and CS-β). The N-terminal G1 comprises A, B, and B’ loops and binds to both hyaluronan (HA) (14) and link protein (LP). The C-terminal G3 domain binds fibulin-1 and -2 (15, 16), tenascins (17, 18), and heparan sulfate proteoglycans (19). As aggrecan in cartilage forms a proteoglycan aggregate with both HA and LP, versican is believed to form similar stable aggregates in the presence of both HA and LP. Indeed, versican aggregates have been isolated from dental pulp (8), and versican secreted from cultured vascular smooth muscle cells forms aggregates with HA and LP (20).

We recently demonstrated that the versican G1 domain binds to both LP and HA in a different manner than aggrecan G1 (21); the B-B segments of LP and versican G1 bind each other, whereas the A loops of LP and aggrecan G1 interact. These results suggest that versican is present as an aggregate in articular cartilage, with HA and LP, and plays a unique role distinct from the aggrecan aggregate. However, the versican aggregate has not been identified in cartilage, and the function of versican has not been determined.

In this study, we investigated the expression, localization, and aggregate formation of versican in cartilage to gain insights into its function. Versican was mainly localized in the interterritorial zone of the articular surface, whereas aggrecan was rather diffused, especially with dense staining in the territorial zone of prehypertrophic chondrocytes. The versican aggregate was isolated by cesium chloride density gradient ultracentrifugation from normal articular and aggrecan-null cartilage. Although transcription of the versican gene dramatically decreased after birth, versican remained in the articular cartilage in the form of the proteoglycan aggregate. LP overexpression in chondrocytic N1511 cells, which synthesize versican at the early stage of differentiation, significantly enhanced versican deposition and inhibited subsequent aggrecan deposition. These results suggest that the versican aggregate is present in the articular surface and may provide ECM properties distinct from deeper zones where aggrecan aggregates are abundant.

* This work was supported by a grant-in-aid for scientific research on priority areas (Kakenhi to H. W.) and a grant-in-aid for scientific research (Kakenhi to H. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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2 The abbreviations used are: ECM, extracellular matrix; HA, hyaluronan; LP, link protein; CS, chondroitin sulfate; HABP, hyaluronan-binding protein; ELISA, enzyme-linked immunosorbent assay; cmd, cartilage matrix deficiency; TBS, Tris-buffered saline.
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**EXPERIMENTAL PROCEDURES**

*Immunostaining and Detection of HA by HABP in Tissues—Aggrecan-null cartilage matrix deficiency (cmd) lines (22) were maintained in our animal facility. Mouse tissues were fixed in 10% buffered formalin, embedded in paraffin, and sliced into 4-μm sections. When necessary, hard tissues were decalcified with 0.5 M EDTA for 14 days. For immunostaining, tissue sections were pretreated with 1 unit/ml of protease-free chondroitinase ABC (Seikagaku Corp.) at 37°C for 30 min. For aggrecan and versican, rabbit polyclonal anti-mouse aggrecan (a gift from Dr. T. Yada, ×500) and anti-mouse versican (anti-CS-α and anti-CS-β, gifts from Dr. T. Shimomura, ×500) were used, respectively. To recognize all three variant forms (V0, V1, and V2) of versican except for V3, which was not expressed as a protein, we used a mixture of anti-CS-α and anti-CS-β antibodies. Biotinylated HABP, (Zymed Laboratory Inc.), was used for HA detection. LP immunostaining was performed with mouse monoclonal anti-LP, which reacts with mouse LP (8A4, ×100; Developmental Studies Hybridoma Bank) using HistoMouse™ SP kit (Zymed Laboratory Inc.).*

**Identification of Proteoglycan Aggregates—**NATIVE versican was prepared from mouse brain as described previously (23). In a pilot test of density gradient ultracentrifugation, native versican, biotinylated LP, and HA were best separated at a density of 1.42 mg/ml of CsCl, when versican was fractionated into A1-A3. 220 mg (wet weight) of cmd/cmd cartilage, 2-week-old mouse femoral articular cartilage up to 1 mM in 0.5 M guanidine hydrochloride (GdnHCl), 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM N-ethylmaleimide, and 0.36 mM pepstatin A. The homogenate was stirred over-night (0.4 M GdnHCl) in polyallomer tubes at 110,000 g for 24 h. The second extraction was collected and ultracentrifuged under an associative condition (0.4 M GdnHCl, Tris-HCl, pH 8.0, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM N-ethylmaleimide, and 0.36 mM EDTA) in 0.36 mM pepstatin A. The extract was applied to dissociative ultracentrifugation at a density of 1.61 g/ml (first centrifugation). The solution (0.4 M GdnHCl, Tris-HCl, pH 8.0, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM N-ethylmaleimide, and 0.36 mM EDTA) in 0.36 mM pepstatin A was applied to Elisa as below. The precipitate was dissolved in distilled water and then digested with actinase E (Kaken Pharmaceutical) in 50 mM Tris-HCl, pH 8.0, for 24 h at 37°C. The digest was analyzed for HA content by bioassay using 0.2% bovine serum albumin (BSA). An anti-aggrecan antibody (×250) or an anti-versican antibody (×250) in 50 ml of BSA containing 0.2% bovine serum albumin was mixed with the same volume of peroxidase-conjugated goat anti-rabbit antibody (×10,000; Cappel) and incubated for 30 min at 37°C. After washing, antibody-blotted membranes were dried and exposed to autoradiography medium. After autoradiography, the membranes were stained with 0.5% Alcian blue (pH 2.0) overnight. Versican was immunostained as above. The area stained with Alcian blue was measured using NIH Image version 1.63.

**Quantitative Reverse Transcription PCR—**To analyze aggrecan or versican transcription in cartilage, we obtained cartilage from C57/B16 mice at days 0 and 2 and at 8 weeks. Poly(A) RNA (200–600 ng) was prepared from cartilage using the Micro-Fast Track™ kit (Invitrogen) and reverse transcribed to generate cDNA using the Superscript II First-Strand Synthesis™ system (Invitrogen). Real-time quantitative PCR was performed using TaqMan™ 7700 (PE Applied Biosystems). Sequences for a probe and a set of primers for versican were chosen by the Primer Express™ program as follows: probe, 5’-CATCTAAAC-CCTTTGTCCGAAATGTT-3’; forward primer, 5’-CCAGTGTGAACT-TGATTTTGATGAA-3’; and reverse primer, 5’-AACATAACTTGG-GAGACGACAGACTCT-3’. The sequences of the probe and primers for aggrecan were described previously (25). The probe was labeled with fluorescent reporter dyes 6-carboxyfluorescein and 6-carboxyfluorescein diacetate, which reacts with mouse LP (8A4, ×100; Developmental Studies Hybridoma Bank) using HistoMouse™ SP kit (Zymed Laboratory Inc.).

**Enzyme-linked Immunosorbent Assay (ELISA)—**ELISA plates (MaxiSorp; Nunc) were coated overnight at 4°C with 100 μl of aggrecan (10 μg/ml; Seikagaku) or versican (5 μg/ml; Collaborative Biomedical Products) in 50 mM Tris-HCl, pH 7.5, 0.15 M NaCl (Tris-buffered saline, TBS) containing 0.2% bovine serum albumin. An anti-aggrecan antibody (×250) or an anti-versican antibody (×250) in 50 ml of TBS containing 0.2% bovine serum albumin was mixed with the same volume at different dilutions in TBS containing 0.2% bovine serum albumin and incubated at 37°C for 1 h. After washing wells with TBS containing 0.05% Tween 20 (TBS-T) three times, the mixture was applied to wells and incubated for 1 h at 37°C. After washing wells with TBS-T three times, a peroxidase-conjugated goat anti-rabbit antibody (×10,000; Cappel) was applied and incubated for 30 min at 37°C. After washing, antibody-blotted membranes were dried and exposed to autoradiography medium. After autoradiography, the membranes were stained with 0.5% Alcian blue (pH 2.0) overnight. Versican was immunostained as above. The area stained with Alcian blue was measured using NIH Image version 1.63.

**Analysis of CS Disaccharide Composition—**Samples fractionated after density gradient ultracentrifugation were precipitated by the addition of 3 volumes of 95% ethanol containing 1.3% potassium acetate. The precipitate was dissolved in distilled water and then treated in 0.2 M NaOH for 24 h at room temperature, neutralized by the addition of 4 μM acetate, and then digested with actinase E (Kaken Pharmaceutical) in 50 mM Tris-HCl, pH 8.0, for 5 h at 50°C. The sample was applied to a DEAE-Sephaloc (Amersham Biosciences) column equilibrated with 50 mM Tris-HCl, pH 7.5. After washing with 10 column volumes of 50 mM
Tris-HCl, pH 7.5, 0.2 M NaCl, GAG-rich fractions were eluted with 50 mM Tris-HCl, pH 7.5, 2 M NaCl. The eluate was ethanol precipitated as above and dissolved in 200 μl of distilled water. The samples were treated with 30 milliunits of chondroitinase ABC in 25 μl of 50 mM Tris-HCl, pH 7.5, 0.04% bovine serum albumin for 4 h at 37°C and filtered with Ultrafree-MC (5,000 molecular weight limit; Millipore). Unsaturated disaccharides in the filtrates were analyzed by reverse phase ion-pair chromatography using Senshu Pak column Docosil with a fluorescence detector according to the method of Toyoda (26) with slight modification of elution conditions. Separately, we treated the above eluate with hyaluronidase from Streptomyces hyalurolyticus (Seikagaku), similarly filtered and analyzed, and confirmed that hyaluronan disaccharide was negligible in the samples.

RESULTS

Distribution of Versican, HA, and LP in Cartilage—Initially, we investigated the presence and localization of versican, aggrecan, LP, and HA in the growth plate and articular cartilage of 2-week-old mice by immunostaining with biotinylated hyaluronan-binding protein (biotinylated HABP). In the growth plate, versican was faintly stained in the interterritorial zone. LP was strongly stained in the pericellular zone of hypertrophic chondrocytes and moderately in the interterritorial zone. HA was diffusely localized with higher deposition in the pericellular zone. In contrast, aggrecan was stained mainly in the territorial zone of chondrocyte columns and the pericellular zone of hypertrophic chondrocytes (Fig. 1A). In the articular surface, versican, LP, HA, and aggrecan were observed in the interterritorial zone. Aggrecan was also localized in the pericellular zone of hypertrophic cells (Fig. 1B). Although the detailed localization of these molecules was different, the colocalization of versican, LP, and HA in the interterritorial zone of the articular surface suggests the presence of their aggregates.

Versican Forms Proteoglycan Aggregates in Vivo—We then examined whether versican is present as a proteoglycan aggregate with HA and LP in cartilage. Because normal cartilage contains a large amount of aggregates, which may inhibit the identification of versican aggregates, we first used cartilage from cmd/cmd mice (22), a natural knock-out of the aggrecan gene. When the localization of the three molecules in cmd/cmd cartilage was examined, both versican and LP were observed mainly on the cartilage margin, whereas HA was diffuse (Fig. 2A), indicating the colocalization of the three molecules at least on the margin of cmd/cmd cartilage. To identify the proteoglycan aggregate, a sample was extracted from cmd/cmd cartilage by 0.5 M GdnHCl and applied to cesium chloride density gradient ultracentrifugation under an associative condition (0.4 M GdnHCl) (27). A major proportion of versican was observed in the A1–3 fractions, and a small proportion, presumably of processed fragments, was found in A6. The major proportion of both LP and HA was seen in A5–6, but some was observed in the A1–3 fractions (Fig. 2B, left). When the sample extracted by 4 M GdnHCl was ultracentrifuged under dissociative conditions (4 M GdnHCl), a major proportion of versican was observed in the D3 fraction. In contrast, the major proportions of both LP and HA were found in the D5–6 fractions (Fig. 2B, right). We confirmed the presence of versican and LP by immunoblot analyses. When the A1–3 and D1–3 fractions were treated with chondroitinase ABC and applied to immunoblot, the versican core protein was observed (Fig. 2C). LP was observed in the A1–3 fractions but not in D1–3. These data clearly indicate that cmd/cmd cartilage contains the proteoglycan aggregate of versican, LP, and HA.

As the presence of versican aggregates in cmd/cmd may not necessarily indicate their presence in normal cartilage, we attempted to detect versican aggregates in normal cartilage. The surface area of articular cartilage at the age of 2 weeks was extracted under associative condi-

FIGURE 1. Localization of versican, LP, and HA. A, 2-week-old mouse growth plate (bar, 50 μm). B, articular cartilage (bar, 50 μm). Versican, LP, and HA are co-localized in the interterritorial zone of the growth plate and the joint surface.

FIGURE 2. Presence of the versican aggregate in cmd/cmd cartilage. A, co-localization of versican (Ver), LP, and HA in newborn cmd/cmd cartilage (bar, 100 μm). B, detection of versican (Ver), LP, and HA in fractions of density gradient ultracentrifugation under associative (left) and dissociative (right) conditions. C, immunoblot of A1–3 and D1–3 fractions for versican (Ver) and LP. Nontreated (−) and chondroitinase ABC-treated (+) samples were applied.
tions and processed by cesium chloride density gradient ultracentrifugation at 1.61 g/ml. Although a large amount of aggrecan was present in A1–3 and A7–11, versican was mainly found in A9–11 (Fig. 3A). Repeated ultracentrifugation and collection of versican fractions as described under “Experimental Procedures” successfully removed aggrecan. Subsequent ultracentrifugation at 1.51 g/ml revealed the co-fractionation of both versican and LP mainly in A4 (Fig. 3B), indicating the presence of versican aggregates in normal articular cartilage.

**Versican and Aggrecan Exhibit Different Sulfation Levels of CS Chains**—As cartilage contains two types of proteoglycan aggregates, they may function by harboring different structures of CS chains. We performed disaccharide analysis of CS chains in aggrecan and versican. As both aggrecan-rich (A1–2 in Fig. 3A) and versican-rich (A4 in Fig. 3B) fractions obtained by repeated density gradient ultracentrifugation were confirmed as being >95% pure, respectively (data not shown), we used these fractions for disaccharide analysis. The GAG sample of the aggrecan-rich fraction treated with chondroitinase ABC contained ~25% ΔdiS-0S and 70% ΔdiS-4S. In contrast, that of the similarly treated versican-rich fraction contained ~71% ΔdiS-0S and 28% ΔdiS-4S. All other disaccharide structures such as ΔdiS-6S, ΔdiS-7S, ΔdiS-9S, and ΔdiTriS were negligible in both samples (Table 1). These results indicate that both are only sulfated at the 4-position of GalNAc and that sulfation levels of CS chains are higher in aggrecan than versican.

**Expression Levels of Versican and Aggrecan in Cartilage**—Next, we examined the transcription and protein levels of versican and aggrecan in differently aged mice. Real-time reverse transcription PCR indicated that the levels of versican transcription at 2 and 8 weeks after birth were ~80 and 10%, respectively, that of newborn cartilage, whereas aggrecan transcription gradually increased and at 8 weeks reached 4 times that of the newborn cartilage (Fig. 4A). The amount of these proteoglycans was measured in cartilage at different ages by inhibition ELISA. Versican decreased at 2 weeks to ~60% that of newborn cartilage and remained at 8 weeks, whereas the amount of aggrecan/wet weight gradually increased during growth (Fig. 4B). These results suggest that versican remains in the growing cartilage for at least 2–8 weeks after birth, although its transcription dramatically decreases.

The samples for quantification above included a mixture of articular and growth plate cartilage. In addition, the amount of these proteoglycans may be different between the surface and deep layers of articular cartilage. Thus we measured their amount in the surface layers (up to 1 mm in depth). Repeated extraction in 4 M GdnHCl confirmed almost full extraction. By inhibition ELISA, the surface area contained 41 and 88 µg/mg (wet weight) of versican and aggrecan, respectively. Interestingly, the extraction efficiency using 0.5 M GdnHCl was 23 and 61%, respectively, suggesting that versican with less CS chains is more tightly incorporated in the cartilage matrix of the articular surface layer.

**Deposition of Versican in the ECM Depends on the Expression of LP**—Versican is transiently expressed at a high level in the mesenchymal condensation area. After the transcription level rapidly decreases, versican remains in the interterritorial zone of developing cartilage as proteoglycan aggregates. The sustained deposition of versican in the ECM may be dependent on the presence of LP. We tested this hypothesis by overexpressing LP in N1511 chondrogenic cells. Chondrocytic differentiation is induced in these cells by combined treatment with dexamethasone and parathyroid hormone at the confluence (24). After the induction, these cells express versican, peaking at 48 h, and decreasing to 40 and 30% at days 4 and 13, respectively. The expression of aggrecan and LP appears at day 4 with a peak at day 13 (9). Forty-eight hours after the induction, versican and HA deposition in the ECM was observed (Fig. 5A) when endogenous LP and aggrecan were not detected (data not shown). Then we transfected subconfluent N1511 cells with an expression vector of human recombinant FLAG-LP. After 24 h, the cells reached confluence and were treated with parathyroid hormone and dexamethasone to induce differentiation. FLAG-LP was overexpressed and incorporated in the matrix and enhanced versican deposition (Fig. 5A). When samples of the extracellular matrix were extracted after cell lysis, treated with chondroitinase ABC, and immunoblotted, a significantly increased amount of versican core protein was observed in the matrix where FLAG-LP was overexpressed (Fig. 5B). In contrast to versican, HA deposition was not increased when LP was overexpressed (Fig. 5A). These results indicate that LP increases versican deposition by forming the proteoglycan aggregate.

**Versican Aggregates Inhibit Aggrecan Deposition**—Both aggrecan and versican can form aggregates with LP and HA. We examined whether these proteoglycans form a composite aggregate or form their own aggregates in a mutually exclusive manner. We overexpressed FLAG-LP and induced differentiation in N1511 cells. At day 13, a large amount of versican remained in the ECM of LP-expressing cells. The mock-transfected cells showed a round, chondrocyte-like shape in Alcian blue-stained cartilaginous nodules, indicating chondrocyte differentiation with aggrecan deposition. In contrast, LP-expressing cell cultures showed much less Alcian blue-stained nodules (Fig. 6A). When the levels of Alcian blue staining were measured, aggrecan deposition was decreased to ~25% in LP-expressing cells (Fig. 6B). By immunoblot, the
levels of aggrecan in the conditioned medium of LP-expressing cells were significantly decreased (Fig. 6C). These results indicate that sustained deposition of versican aggregates inhibits subsequent aggrecan deposition.

DISCUSSION

In this study, we have shown the presence of versican in cartilage. By immunostaining, versican is localized in the interterritorial zone of the articular surface, whereas aggrecan is mainly in the territorial zone of hypertrophic cells. Biochemical analysis of normal articular and cmd/cmd cartilage revealed that versican is present as a proteoglycan aggregate with both LP and HA. Disaccharide analysis has demonstrated that versican CS is less sulfated than aggrecan CS. In vitro studies using chondrocytic N1511 cells demonstrated that LP overexpression at an early stage of differentiation enhanced versican deposition and inhibited subsequent aggrecan deposition. Taken together, these results suggest that cartilage contains both aggrecan aggregates and versican aggregates, which may play their own roles in articular cartilage.

Versican is transiently expressed in the mesenchymal condensation area and is still observed at the epiphyseal end at E15 (8). Although previous immunohistochemical studies failed to detect versican in human adult cartilage (3), biochemical studies demonstrated the presence of versican in human articular cartilage from the fetal stage to mature adult (13). An age-associated decrease in versican transcription of the human articular cartilage has also been reported (11). We have shown that versican is present on the articular surface of mice at 2 weeks after birth. Its transcription in cartilage rapidly decreases from 2- to 8-week-old mice as assessed by real-time quantitative PCR, which may partly be due to a decreased ratio of articular chondrocytes in the total cell population in cartilage. In contrast, deposition remained at 8 weeks. These observations, together with previous reports, suggest that cells on the margin of developing cartilage continuously synthesize versican and may eventually reside as articular chondrocytes and that versican secreted at early stages of life remains in the interterritorial zone of articular cartilage.

We have shown for the first time that versican is present in the form of aggregates with HA and LP in normal articular and cmd/cmd carti-
lage, using associative and dissociative CsCl ultracentrifugation methods (28). Extraction under associative conditions followed by ultracentrifugation excludes the possibility of reconstitution of the aggregate during these biochemical procedures. Versican was separated at a density slightly lower than the condition for aggrecan, indicating that this method is useful for the purification of versican in various tissues. Repeated CsCl density gradient ultracentrifugation successfully removed most aggrecan aggregates, and versican was co-fractionated with both HA and LP, suggesting that these proteoglycans are unlikely to form a composite aggregate.

The versican G1 domain interacts with both LP and HA at the B-B' segment (21) in contrast to aggrecan G1, which binds HA at B-B' and binds LP at the A subdomain. This different manner of interaction may give rise to the possibility that versican aggregates are replaced with aggrecan aggregates, by exchanging HA and LP. However, our result showing that the sustained deposition of versican aggregates inhibited subsequent aggrecan deposition in differentiating N1511 cells does not support this hypothesis. The versican aggregate is as stable as the aggrecan aggregate, and the interaction of versican with either LP or HA is as strong as that of aggrecan with these molecules (21). Thus, the versican aggregate, once formed, may remain and inhibit further deposition of aggrecan aggregates.

Both aggrecan and versican contain globular domains at both N and C termini. Interaction of these globular domains with other molecules may be necessary to form the framework of the matrix structure, and the amount and structure of CS chains may determine the nature of the matrix. Whereas aggrecan contains >100 CS chains, versican contains up to ~20. In addition, CS chains of versican are less sulfated than those of aggrecan, as shown in this study. Indeed, extraction efficiency of versican by 0.5 M GdnHCl was lower than that of aggrecan. These differences may endow versican with both biochemically and physically specific roles. Versican binds to a greater variety of molecules, including fibronectin (29) and midkine (30), than aggrecan. Recently, transforming growth factor β (TGF-β)/Smad-3 signals have been suggested as essential for repressing articular chondrocyte differentiation. Without this, chondrocytes break the quiescent state and undergo abnormal terminal differentiation (31). As versican is known to bind fibrillin-1 (32), which interacts with latent TGF-β-binding protein-1 (LTBP-1) (33), it may regulate TGF-β-mediated signal transduction through the interaction of fibrillin-1 and LTBP-1. Articular cartilage requires a physical property that resists shear force in the surface area and compression in the deeper zone. Together with compact collagen fibers, versican aggregates, with CS chains of decreased number and sulfation levels compared with aggrecan, may provide an appropriate ECM structure of the articular surface.

The patterns and levels of CS sulfation depend on the activity of chondroitin sulfotransferases in individual cells. Versican from mouse embryos at E13.5 contains 6-sulfated and 4-sulfated disaccharides, comparable with ~90% nonsulfated disaccharides (30). Embryonic chondrocytes may have much less C6-sulfotransferase activity than embryonic fibroblasts. In the same context, versican-expressing articular chondrocytes may have less C4-sulfotransferase activity than aggrecan-expressing chondrocytes.

Versican aggregates in the articular cartilage may provide another important role in destructive joint diseases such as osteoarthritis (34). Because versican is degraded by aggrecanase-1 (a disintegrin and met-
alloproteinase with thrombospondin motif-4, ADAMTS-4) (35), a versican fragment cleaved by the enzyme may serve as a marker for the early stage of arthritis. Further studies are required to understand the in vivo roles of versican in cartilage development and homeostasis and in joint destructive diseases.

Acknowledgments—We thank Dr. T. Yada for anti-aggrecan antibody and Dr. Z. D. Zhang, H. Fuwa, and S. Hara for technical assistance.

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