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# Testican-1, an inhibitor of pro-MMP-2 activation, is expressed in cartilage[1]

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## Summary

*Objective*: Recently, testican-1 has been described to be an inhibitor of MT1-MMP and MT3-MMP mediated pro-MMP-2 activation. As MT1-MMP mediated pro-MMP-2 activation is of significance for cartilage destruction in osteoarthritis, we studied the expression and localization of testican-1 in human articular cartilage.

*Methods*: Cartilage samples from the medial and lateral tibia plateau were obtained from osteoarthritic patients who underwent joint replacements, and were graded histomorphologically by Mankin score. Testican-1 expression was assessed in RNA isolated directly from cartilage as well as in freshly isolated chondrocytes by reverse transcriptase-polymerase chain reaction (RT-PCR) and quantified by real-time RT-PCR. Testican-1 protein was localized by immunohistochemistry in human osteoarthritic cartilage samples, in human fetal knee joint, and in knees from mice.

*Results*: Testican-1 mRNA could be detected in cartilage and in freshly isolated chondrocytes both from moderately and from severely damaged osteoarthritic cartilage. In the same donor, expression in chondrocytes from more severely affected regions was decreased compared with chondrocytes from less affected regions. By immunolocalization, testican-1 protein could be detected in chondrocytes predominantly of the superficial and transitional zones. Matrix staining in these zones was greatly reduced in samples from more severely affected osteoarthritic cartilage. A similar distribution was found in the articular cartilage of knees from 7-week-old mice. In addition to articular cartilage, testican-1 was also present in growth plate cartilage.

*Conclusions*: Testican-1 is a component of cartilage, both of the joint and of the growth plate. Given its activity as an inhibitor of MT1-MMP mediated pro-MMP-2 activation, it is reasonable to speculate that it participates in the regulation of matrix turnover in cartilage.


*Key words*: Testican, Cartilage, Chondrocytes, Osteoarthritis.

## Introduction

Testican-1 is a highly conserved chimeric proteoglycan carrying both chondroitin sulfate and heparan sulfate chains, initially characterized as the precursor of a seminal plasma glycosaminoglycan-bearing peptide[1,2]. Due to the modular organization of its core protein, encompassing four osteonectin/SPARC-like domains, a Kazal-like motif and a CWCV domain present in thyropin-type cysteine protease-inhibitors, it also has been called SPOCK[3]. Testican-1 is differentially expressed in tissues, with highest expression occurring in specific regions of the brain[4,5]. Due to its expression by some, but not all, endothelial cells it also can be found in several other tissues[6,7]. During the past years, two structurally related proteins have been identified, defining a new family of modular proteoglycans[8,9].

Recently, a regulatory activity of testican family members on protease activities has been demonstrated. Whereas both testican-1 and testican-3 inhibit activation of pro-MMP-2 by either MT1-MMP or MT3-MMP, testican-2 abrogates this inhibition of MT-MMPs[9,10]. Furthermore, testican-1 has been shown to inhibit the cysteine protease cathepsin L[11], and the heparan sulfate chains present on testican may contribute to the regulation of the collagenolytic activity of cathepsin K[27]. Therefore, it has been suggested that testicans may regulate the degradation of extracellular matrices[10].

As progressive destruction of the cartilagenous matrix is the morphological hallmark of osteoarthritis, we investigated the expression of testicans by human chondrocytes.

## Materials and methods

### MATERIALS

Rabbit antiserum raised against the recombinantly expressed extracellular calcium-binding (EC) module of human testican-1 was kindly provided by Dr Rupert Timpl, Munich, who died unexpectedly last year. This antiserum has been shown to stain a distinct band corresponding to the core protein of testican-1 in immunoblots of several human tissue extracts and shows no cross-reactivity with human BM-40/osteonectin/SPARC[12]. Due to a 95% overall amino acid identity between human and mouse testican-1, cross-reactivity of this antiserum with mouse testican-1 could be anticipated[3]. The LSAB kit and 3-amino-9-ethylcarbazol were purchased from DAKO (Hamburg, Germany).

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Pronase (protease type XIV) and collagenase (from *Clostridium histolyticum*, EC 3.4.24.3) were from Sigma (Taufkirchen, Germany).

RNeasy Mini Kit, RNase-free DNase, Omniscript reverse transcriptase and HotStarTaq DNA polymerase were from Qiagen (Hilden, Germany), SYBR Green PCR Master Mix was from Applied Biosystems (Darmstadt, Germany), the pCR4-TOPO cloning vector was from Invitrogen (Karlsruhe, Germany) and all primers were synthesized by MWG Biotech (Munich, Germany) except for the random hexanucleotide primers which were from Roche Diagnostics (Mannheim, Germany). All other chemicals used were of analytical grade.

### TISSUE SAMPLING

Human articular cartilage was obtained from the tibial plateaus from 11 donors (aged from 47 to 79 years) receiving a total knee replacement. Written, informed consent was obtained from all the patients according to the guidelines of the local ethics commission. Samples of eight donors exhibiting differences in Mankin score of at least 4 between cartilage from the lateral and medial tibia plateau, respectively, were included in this study. After a horizontal cut of the most proximal tibial bone, the tibial plateau with cartilage and subchondral bone was removed and split into a medial and a lateral part. Growth plate cartilage from fetal knees were procured at autopsy from fetuses from the 18th to the 25th gestational week that died from diseases unrelated to the skeleton. These samples were kindly provided by the Department of Pathology, Augsburg, Germany.

### TISSUE PROCESSING

Specimens for histomorphological examinations were obtained from the immediate vicinity of the cartilage used for the isolation of chondrocytes, and were fixed in 4% buffered formaldehyde for at least 72 h. After rinsing with water, they were decalcified in 10% EDTA solution, pH 7.4, for up to 6 weeks. 3.5 μm sections were cut from paraffin-embedded tissue, placed on glass slides pretreated with silane, and routinely stained with hematoxylin/eosin and safranin-O. All samples were classified histomorphologically using the grading system of Mankin[13]. To visualize the distribution of sulfated glycosaminoglycans, occasionally sections were stained with alcian blue at pH 1.0.

### ISOLATION OF CHONDROCYTES

Cartilage samples were obtained from differently affected regions of the lateral and medial tibial plateau, cut into small pieces and washed with phosphate-buffered saline. After sequential digestion with 0.2% pronase for 1 h and 0.025% collagenase overnight, both in DMEM without addition of fetal calf serum, the released chondrocytes were immediately used for RNA isolation.

Alternatively, RNA was isolated directly from cartilage specimens as described by Gehrsitz *et al.*[14].

### REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION

Total RNA was extracted either directly from cartilage specimens or from the chondrocytes immediately upon release from the cartilage samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and digested with RNase-free DNase (Qiagen) to remove any contaminating genomic DNA. cDNA synthesis from total RNA was performed with Omniscript reverse transcriptase (Qiagen) using $(dT)_{15}$ (1 μM) and random hexanucleotide primers (5 μM; Roche Diagnostics, Mannheim, Germany) simultaneously. Aliquots of the cDNAs were incubated with HotStarTaq DNA polymerase (Qiagen) and the following primers (each at a final concentration of 1 pM): for testican-1 (accession XM_004038) 5′-AGA GTC ATC AAG CCC ACC AG-3′ and 5′-AGA AAG CTT GCC ATC CTT GA-3′ (product size 225 bp), for testican-2 (accession NM_014767) 5′-CGA TGG CAA ACC AGA GAC TT-3′ and 5′-TCC AGG AAG AGG TCA GCA CT-3′ (product size 222 bp), for testican-3 (accession NM_016950) 5′-GGA CTC ACT TGG CTG GAT GT-3′ and 5′-CAA GGT GGG TCT TGC TGT CT-3′ (product size 201 bp), for GAPDH (accession NM_002046) 5′-GAG TCC ACT GGC GTC TTC AC-3′ and 5′-GGT GCT AAG CAG TTG GTG GT-3′ (product size 188 bp), and for β-actin (accession NM_001101) 5′-TGG GAC GAC ATG GAG AAA AT-3′ and 5′-CAG AGG CGT ACA GGG ATA GC-3′ (product size 202 bp). The primer pairs for testican-1, GAPDH and β-actin were designed to encompass at least one intron in the genomic sequence to allow for discrimination of any sequences amplified from contaminating genomic DNA. All primers were synthesized by MWG Biotech (Munich, Germany) and were of high purity salt free (HPSF)-quality. The polymerase was activated (15 min at 96°C) and then up to 40 cycles (45 s at 94°C, 45 s at 60°C, 45 s at 72°C) were performed on a RoboCycler Gradient 96 (Stratagene, Amsterdam, The Netherlands). The amplification products had the expected size and were verified by cloning into the pCR4-TOPO vector (Invitrogen, Karlsruhe, Germany) and subsequent sequencing. Amplification products were visualized by agarose gel electrophoresis after staining with ethidium bromide.

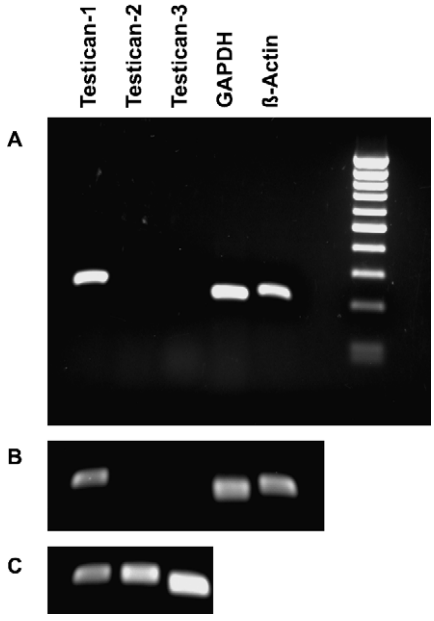


Fig. 1. Representative RT-PCR analysis of testican expression using RNA obtained from freshly isolated chondrocytes (A) and RNA directly isolated from cartilage specimens (B). Amplification products were separated on agarose gels and visualized after staining with ethidium bromide. For control, amplification reactions were performed with 1 pg of the respective plasmid DNA (C).

For quantification, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was performed on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems) and the following primers (each at a final concentration of 0.32 pM): for testican-1 5′-GTA AAA TGC AGC CCT CAC AAA GT-3′ and 5′-GCT TGC GGC TGA CAC ACA-3′ (product size 70 bp) and for GAPDH (accession NM_002046) 5′-TGG TAT CGT GGA AGG ACT CAT G-3′ and 5′-TCT TCT GGG TGG CAG TGA TG-3′ (product size 56 bp). The amplification products obtained with these primers had the expected size and were verified by cloning into the pCR4-TOPO vector and subsequent sequencing. For real-time PCR, the

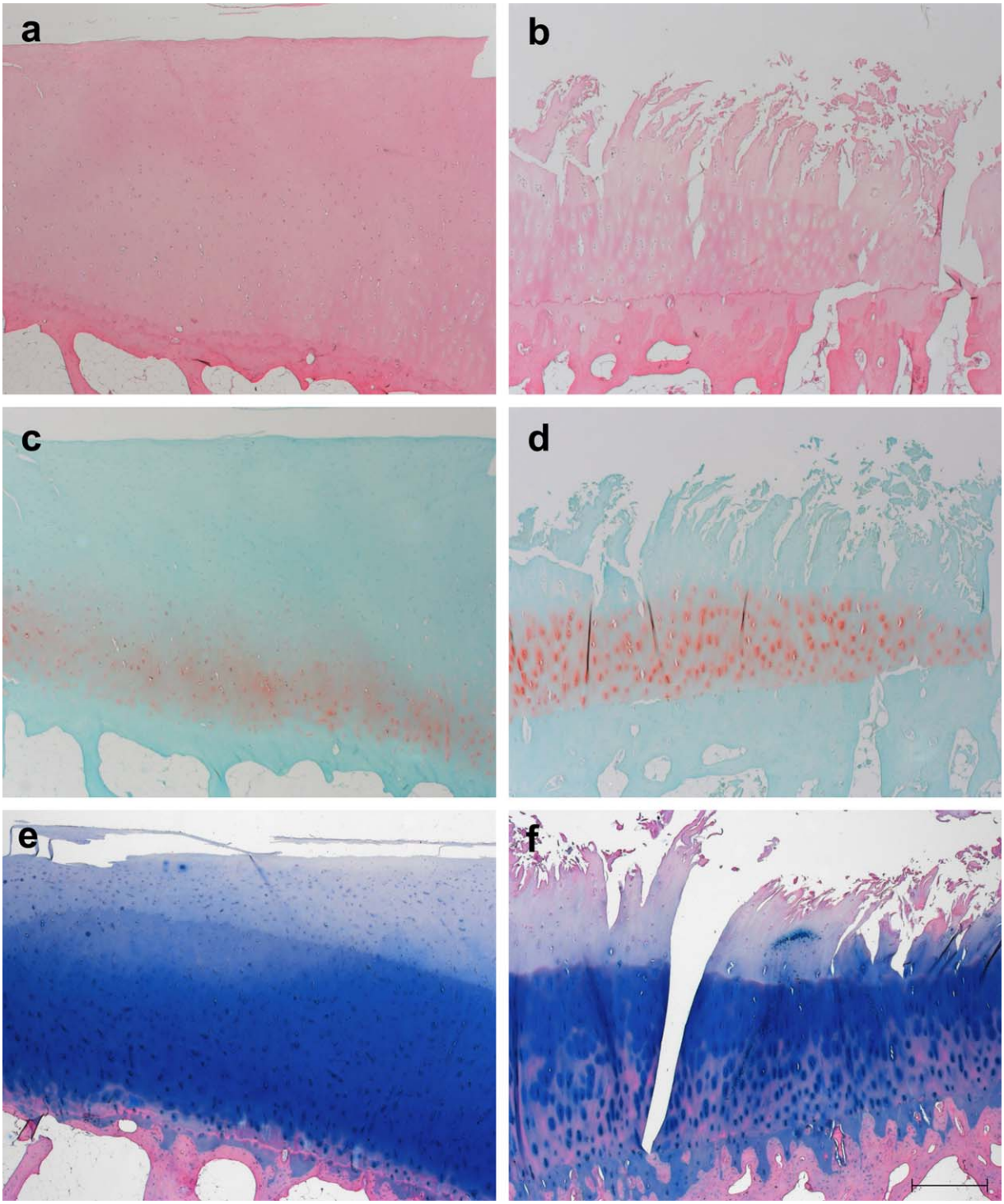


Fig. 2. Histochemical characterization of moderately (a,c,e) and severely (b,d,f) damaged osteoarthritic cartilage samples (bar = 500 μm). Sections were stained with hematoxylin/eosin (a,b), safranin-O/fast green (c,d), and alcian blue at pH 1.0 (e,f), respectively. In the severely damaged cartilage, deterioration of the surface and clustering of chondrocytes as well as loss of sulfated glycosaminoglycans is clearly visible.

polymerase was activated (10 min at 95°C) and then up to 45 cycles (15 s at 95°C and 1 min at 60°C) were performed. In each case, the correctness of the amplification products was controlled by determination of the dissociation curves and by agarose gel electrophoreses. Relative expressions were calculated using GAPDH as reference gene.

### IMMUNOHISTOCHEMISTRY

Deparaffinized samples were incubated for 4 min at ambient temperature with 1 mg/ml pronase in 9 mM $CaCl_2$, pH 7.8, to improve immunoreactivity, rinsed with phosphate-buffered saline, and incubated for 15 min at ambient temperature with 1% sodium azide to inactivate endogenous peroxidase activity. After blocking nonspecific protein binding sites with protein block solution (DAKO, Hamburg) and with 10% normal goat serum, samples were incubated with a rabbit antiserum (diluted 1:1000) raised against testican-1 (kindly provided by Dr Rupert Timpl) for 2 h at 37°C or overnight at 4°C. Bound antibody was visualized using the LSAB kit (DAKO) and 3-amino-9-ethylcarbazol (DAKO) as chromogen according to the instructions of the manufacturer. As a negative control, sections were processed for each sample in the absence of primary antibody.

Additionally, to demonstrate the specificity of the immunoreaction, antiserum was incubated with recombinantly expressed EC module of testican-1 (kindly provided by Dr Takako Sasaki) immobilized to polyvinylidene difluoride membrane (2 $\mu g/cm^2$) prior to incubation of the samples.

## Results

### TESTICAN-1 IS EXPRESSED BY CHONDROCYTES FROM HUMAN ARTICULAR CARTILAGE

In order to investigate whether human articular cartilage chondrocytes express testican-1, mRNA was prepared either from chondrocytes immediately after their isolation or directly from the cartilage specimens and subjected to RT-PCR using sequence specific primers. As can be seen

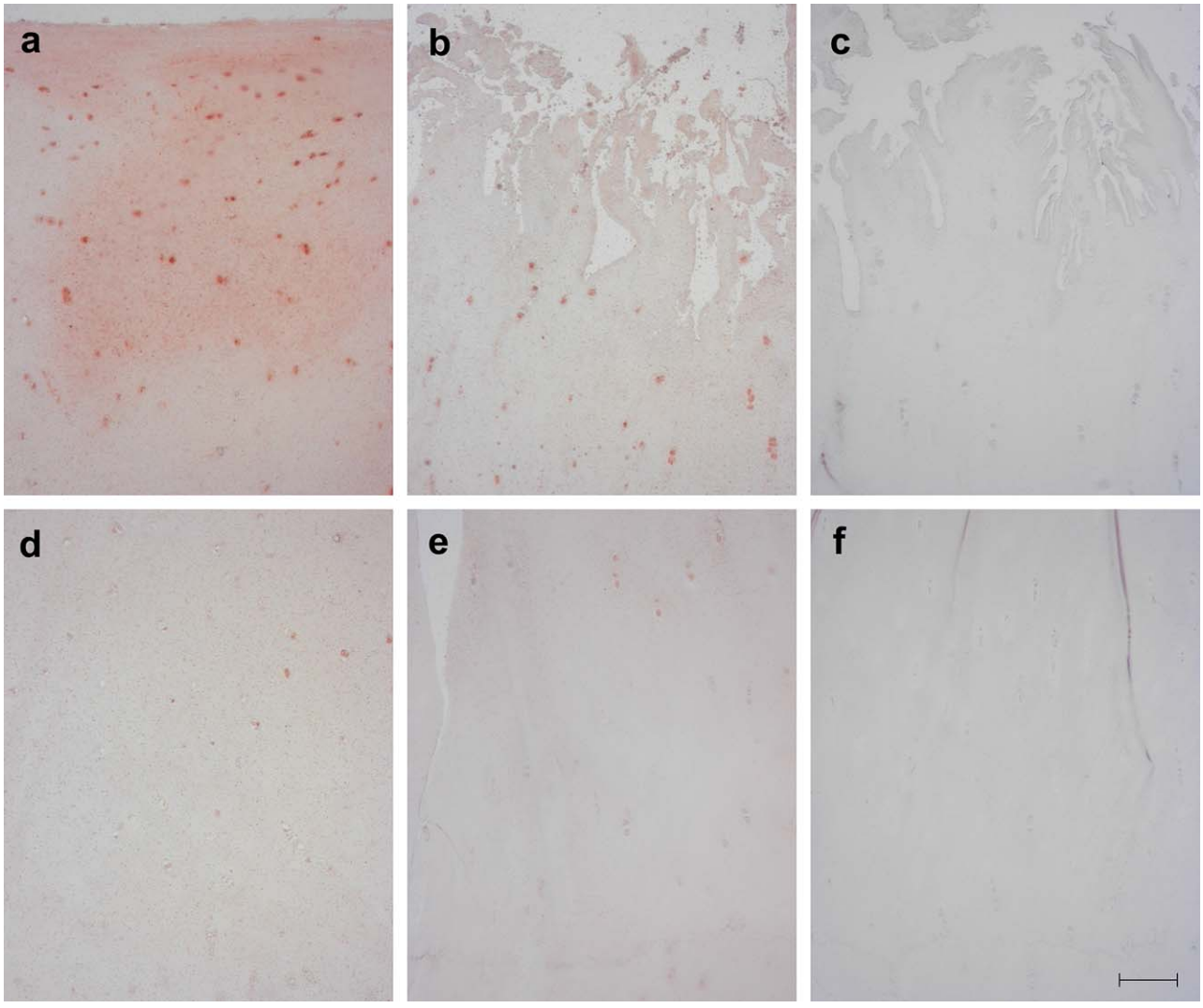


Fig. 3. Immunohistochemical localization of testican-1 in moderately (a,d) and severely (b,e) damaged osteoarthritic cartilage samples at the surface (a–c) and near the tide mark (d–f). Primary antibody was omitted in the negative controls (c,f). Chondrocytes of the superficial and transitional zones stain positive for testican-1, both in moderately and severely affected cartilage. In moderately damaged cartilage there is strong matrix staining in the superficial and transitional zone that is greatly reduced in the severely damaged cartilage. Bar = 100 μm.

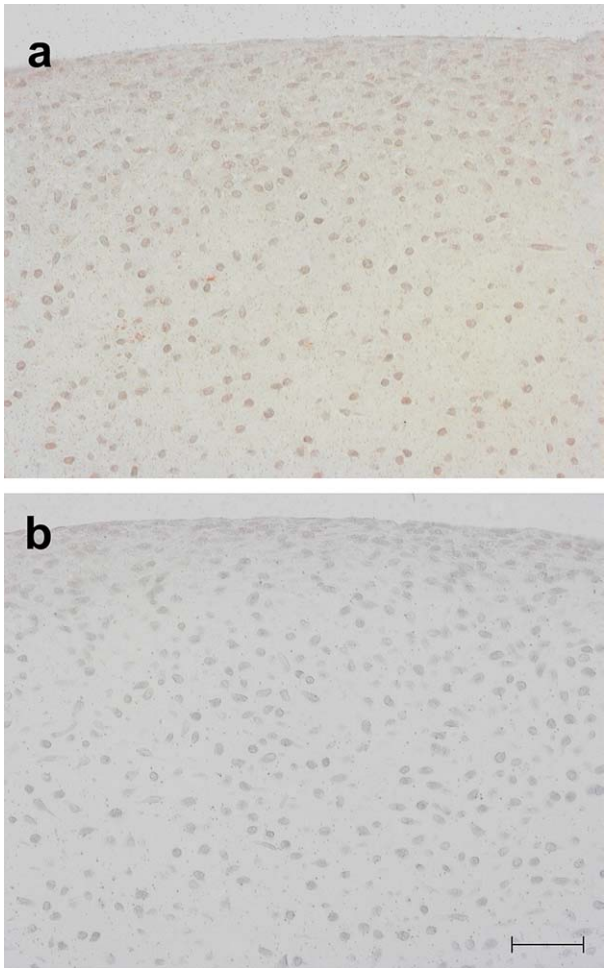


Fig. 4. Immunohistochemical localization of testican-1 (a) in human fetal knee at the articular surface. Primary antibody was omitted in the negative control (b). There is a faint immunostaining of the chondrocytes. Bar = 100 μm.

from Fig. 1, there was strong expression of testican-1 both in the RNA obtained from freshly isolated chondrocytes (A) and in RNA directly isolated from the cartilage specimens (B). However, there appeared to be differences in quantity between different donors and between different sampling sites (not shown). In contrast, expression of testican-2 and testican-3 was only barely, if at all, detectable.

To study whether osteoarthritic changes have an influence on testican-1 expression, we compared by means of real-time PCR expression in chondrocytes from the medial and lateral tibia plateau of each donor individually to compensate for interindividual differences. In five donors with more severe changes on the medial tibia plateau (difference in Mankin score of 5.8 ± 1.5), expression in medial chondrocytes was reduced to 47 ± 29%, in three donors with more severe changes on the lateral tibia plateau (difference in Mankin score of 6.7 ± 1.5), expression in lateral chondrocytes was reduced to 26 ± 23% (results given in mean ± S.D.).

### IMMUNOLOCALIZATION OF TESTICAN-1 IN HUMAN ARTICULAR CARTILAGE

In order to localize testican-1 protein in human osteoarthritic cartilage, cartilage samples exhibiting moderate [Fig. 2(a,c,e)] and severe [Fig. 2(b,d,f)] alterations, respectively, were immunostained using an antiserum raised against the recombinantly expressed EC module of human testican-1[12]. In moderately affected cartilage, there was prominent cellular and matrix staining in the superficial and transitional zones [Fig. 3(a,d)]. In severely affected cartilage, clusters of chondrocytes in these superficial zones were positive for testican-1 too, but matrix staining was strongly reduced [Fig. 3(b,e)].

In the fetal knee samples, there was a faint staining of chondrocytes of the articular cartilage [Fig. 4(a)], but testican-1 could be detected throughout the matrix down to the growth plate [Fig. 5(a,c)], where proliferating cells exhibited the strongest immunoreactivity. Staining intensity of hypertrophic chondrocytes appeared to be reduced.

### IMMUNOLOCALIZATION OF TESTICAN-1 IN CARTILAGE FROM MICE

In order to get a more general idea of the significance of the occurrence of testican-1 in cartilage and to establish whether mice could be used as a model for testican-1 deficiency and/or overexpression, we prepared sections of knee joints from 7-week-old mice and immunostained them for testican-1. As in the human joint, most intensive staining of chondrocytes could be seen in the superficial layer [Fig. 6(a)]. In the mouse, too, testican-1 could also be detected in growth plate cartilage where the proliferating columnar chondrocytes exhibited the strongest immunoreactivity [Fig. 6(b)]. Additionally, there was also staining in the cartilagenous matrix of the growth plate. Particularly, the staining intensity of the cells was greatly reduced upon preadsorption of the antiserum on immobilized testican-1 EC module.

## Discussion

To the best of our knowledge we here describe for the first time that testican-1 is expressed by chondrocytes and occurs in cartilage. So far this proteoglycan, that has initially been identified as the progenitor of a seminal heparan/chondroitin sulfate-bearing peptide[1,2], has been described to be expressed in specific regions of the brain[4,5] as well as by endothelial cells[6], apart from its occurrence in testis. In articular cartilage, we could detect testican-1 both in individual chondrocytes and in clustering chondrocytes present in more severely damaged osteoarthritic cartilage. As can be expected for a secreted molecule, its distribution was not restricted to the cells, but it was also a constituent of the cartilagenous matrix, most prominently in the superficial and transitional zone. Both matrix staining and expression were reduced in more severely damaged osteoarthritic cartilage.

The presence of testican-1 was not limited to osteoarthritic articular cartilage. We could also detect testican-1 in articular cartilage and growth plate cartilage of knees from human fetuses and mice. In articular cartilage, chondrocytes positive for testican-1 were predominantly localized in the superficial and transitional zones. In growth plate cartilage, there was prominent staining of the cartilagenous matrix, and cellular staining appeared to be reduced in hypertrophic chondrocytes.

At present, only speculations can be made with regard to the functions of testican-1 in cartilage. One interesting feature of this molecule is that it can carry heparan sulfate chains. Though sulfated glycosaminoglycans are major constituents of cartilage, these are predominantly of the

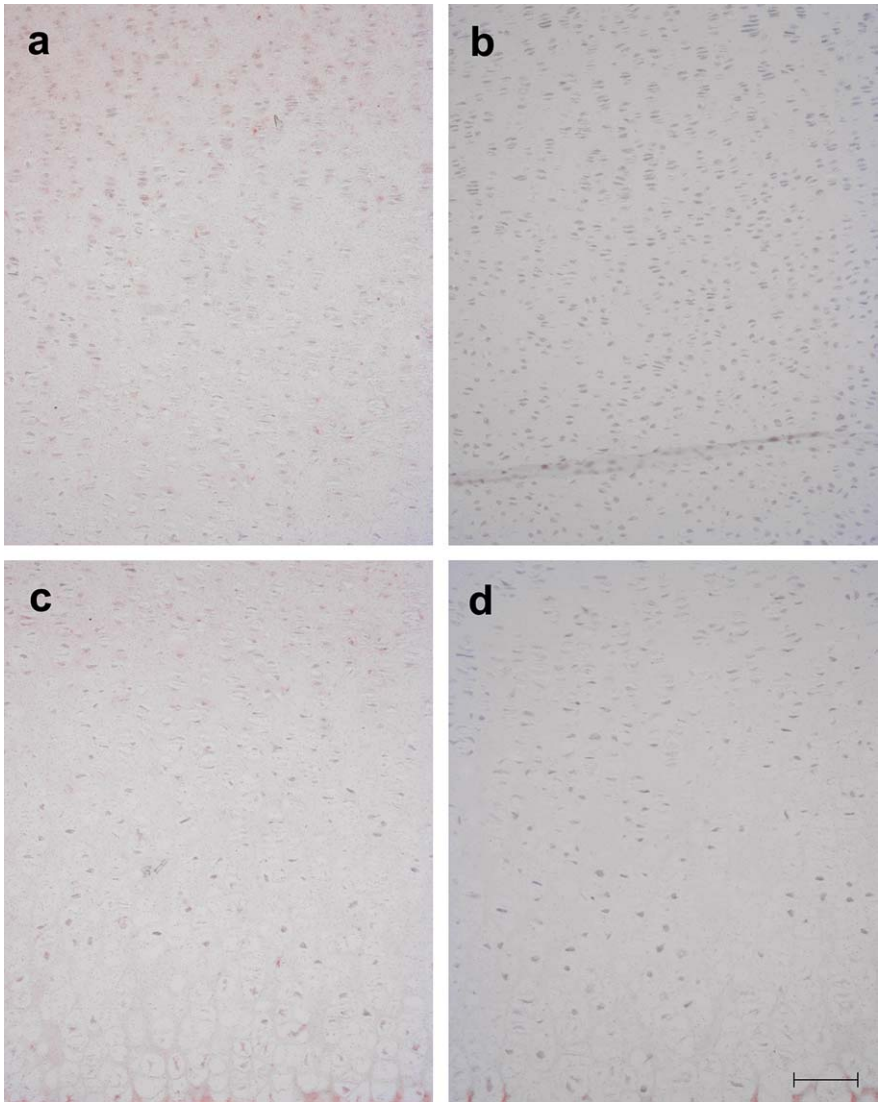


Fig. 5. Immunohistochemical localization of testican-1 (a,c) in human fetal knee near the growth plate. Primary antibody was omitted in the negative controls (b,d). There is a faint staining of chondrocytes and a diffuse matrix staining, particularly in the zone of proliferating chondrocytes. Bar = 100 μm.

chondroitin sulfate and keratan sulfate type present on aggrecan. Heparan sulfate chains, on the other hand, are of particular interest due to their ability to bind to and modulate the activities of numerous proteins, among them many of the growth factors important for chondrocyte regulation[15,16]. Indeed, perlecan, another heparan sulfate proteoglycan, has recently been shown to be of great importance for normal cartilage development[17]. Not only the activities of growth factors but also of other proteins can be modulated by interaction with glycosaminoglycans. An interesting example with regard to cartilage is cathepsin K, that contributes to the degradation of the superficial gliding surfaces of articular hyaline cartilage in osteoarthritis[18]. Whereas glycosaminoglycans predominantly occurring in cartilage, such as chondroitin sulfate and keratan sulfate, enhance the collagenolytic activity of this degradative enzyme, heparan sulfate selectively inhibits its activity[19]. Given its preferential localization in the superficial zones of articular cartilage, testican-1 could well contribute to the regulation of cathepsin K activity.

The most important mediators of normal matrix turnover and pathological matrix destruction in cartilage, however, are the matrix metalloproteinases (MMPs)[20,21]. These zinc-dependent neutral endopeptidases are synthesized as inactive pro-enzymes, and both synthesis and activation are strictly regulated. Once activated, MMP activity is further regulated by the presence of tissue inhibitors of metalloproteinases. One of the particularly interesting enzymes in the context of cartilage matrix remodelling and destruction is MMP-2 due to its wide substrate specificity against cartilage matrix constituents, its expression in cartilage, and its upregulation in osteoarthritis[22]. Furthermore, MMP-2 is an activator of pro-MMP-13[23], the enzyme responsible for most of the collagen degradation in cartilage[24]. Activation of pro-MMP-2 in cartilage is achieved by membrane-type 1 MMP (MT1-MMP)[20], and this activation is sensitive to inhibition by testican-1 and testican-3[9]. Like testican-1, MT1-MMP predominantly localizes to chondrocytes in the superficial and transitional zones[22]. It thus appears possible that one of the functions of testican-1

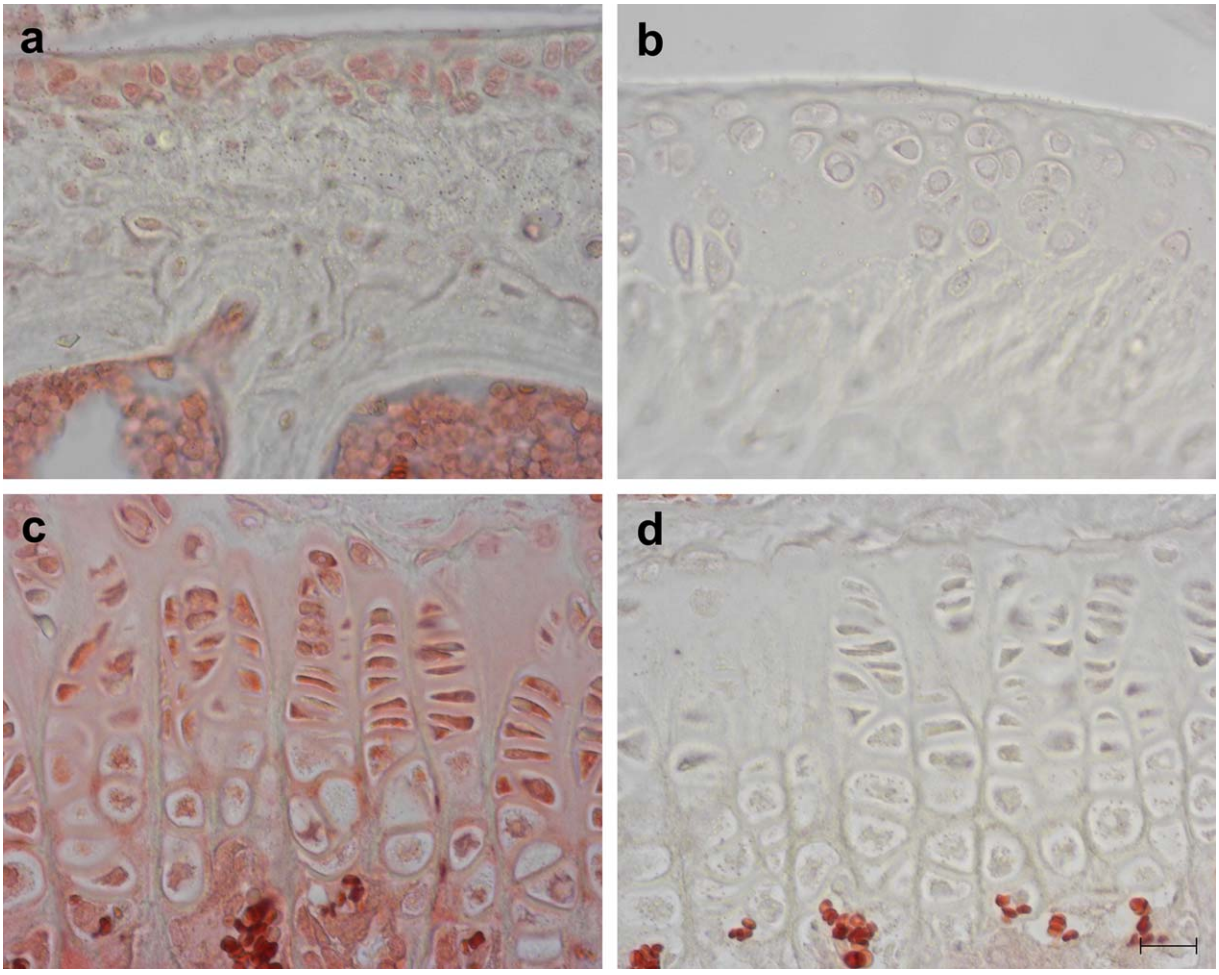


Fig. 6. Immunohistochemical localization of testican-1 (a,c) at the articular surface (a,b) and in the tibial growth plate (c,d) in the knee joint of 7-week-old mice. Primary antibody was omitted in the negative controls (b,d). At the articular surface, staining of the chondrocytes of the superficial zone can clearly be seen. In the growth plate, proliferating chondrocytes exhibit the strongest immunoreactivity. Due to incomplete inhibition of endogenous peroxidase activity by the azide treatment, there is also unspecific staining of erythrocytes. Bar = 20 μm.

in cartilage is being an inhibitor of an intricately regulated activation cascade of degradative enzymes. Its apparent reduction in hypertrophic chondrocytes of growth plate cartilage would fit with such a hypothesis, as it has been shown recently that MT1-MMP deficiency is associated with impaired endochondral ossification[25,26]. Undoubtedly, the functional significance of testican-1 in cartilage deserves further investigation.

## Acknowledgments

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## References

1. Bonnet F, Perin J-P, Maillet P, Jolles P, Alliel PM. Characterization of a human seminal plasma glycosaminoglycan-bearing polypeptide. Biochem J 1992; 288:565–9.
2. Alliel PM, Perin J-P, Jolles P, Bonnet F. Testican, a multidomain testicular proteoglycan resembling modulators of cell social behaviour. Eur J Biochem 1993;214:347–50.
3. Charbonnier F, Perin J-P, Roussel G, Nussbaum J-L, Alliel PM. Clonage de la testicane/SPOCK chez l'homme et la souris. Expression au niveau neuromusculaire et perspectives en pathologie. C R Seances Soc Biol Fil 1996;191:127–33.
4. Bonnet F, Perin JP, Charbonnier F, Camuzat A, Roussel G, Nussbaum JL, *et al*. Structure and cellular distribution of mouse brain testican. Association with the postsynaptic area of hippocampus pyramidal cells. J Biol Chem 1996;271: 4373–80.
5. Marr HS, Basalamah MA, Bouldin TW, Duncan AW, Edgell CJ. Distribution of testican expression in human brain. Cell Tissue Res 2000;302:139–44.
6. Marr HS, Basalamah MA, Edgell CJ. Endothelial cell expression of testican mRNA. Endothelium 1997;5: 209–19.
7. Basalamah MA, Marr HS, Duncan AW, Edgell CJ. Testican in human blood. Biochem Biophys Res Commun 2001;283:1083–90.

8. Vannahme C, Schubel S, Herud M, Gosling S, Hulsmann H, Paulsson M, *et al*. Molecular cloning of testican-2: defining a novel calcium-binding proteoglycan family expressed in brain. J Neurochem 1999;73: 12–20.
9. Nakada M, Yamada A, Takino T, Miyamori H, Takahashi T, Yamashita J, *et al*. Suppression of membrane-type 1 matrix metalloproteinase (MMP)-mediated MMP-2 activation and tumor invasion by testican 3 and its splicing variant gene product, N-Tes. Cancer Res 2001;61:8896–902.
10. Nakada M, Miyamori H, Yamashita J, Sato H. Testican 2 abrogates inhibition of membrane-type matrix metalloproteinases by other testican family proteins. Cancer Res 2003;63:3364–9.
11. Bocock JP, Edgell CJ, Marr HS, Erickson AH. Human proteoglycan testican-1 inhibits the lysosomal cysteine protease cathepsin L. Eur J Biochem 2003;270: 4008–15.
12. Kohfeldt E, Maurer P, Vannahme C, Timpl R. Properties of the extracellular calcium binding module of the proteoglycan testican. FEBS Lett 1997;414:557–61.
13. Mankin HJ, Dorfman H, Lipiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. J Bone Joint Surg 1971;53A:523–37.
14. Gehrsitz A, McKenna LA, Söder S, Kirchner T, Aigner T. Isolation of RNA from small human articular cartilage specimens allows quantification of mRNA expression levels in local articular cartilage defects. J Orthop Res 2001;19:478–81.
15. Selleck SB. Proteoglycans and pattern formation. Sugar biochemistry meets developmental genetics. Trends Genet 2000;16:206–12.
16. Canalis E, Ed. Skeletal Growth Factors. Philadelphia, PA: Lippincott Williams & Wilkins.
17. Costell M, Gustafsson E, Aszodi A, Morgelin M, Bloch W, Hunziker E, *et al*. Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol 1999;147:1109–22.
18. Konttinen YT, Mandelin J, Li TF, Salo J, Lassus J, Liljestrom M, *et al*. Acidic cysteine endoproteinase cathepsin K in the degeneration of the superficial articular hyaline cartilage in osteoarthritis. Arthritis Rheum 2002;46:953–60.
19. Li Z, Yasuda Y, Li W, Bogyo M, Katz N, Gordon RE, *et al*. Regulation of collagenase activities of human cathepsins by glycosaminoglycans. J Biol Chem 2003 Nov 26 [Epub ahead of print].
20. Smith LR. Degradative enzymes in osteoarthritis. Front Biosci 1999;4:D704–12.
21. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res 2001;3:107–13.
22. Imai K, Ohta S, Matsumoto T, Fujimoto N, Sato H, Seiki M, *et al*. Expression of membrane-type 1 matrix metalloproteinase and activation of progelatinase A in human osteoarthritic cartilage. Am J Pathol 1997; 151:245–56.
23. Knäuper V, Will H, Lopez-Otin C, Smith B, Atkinson SJ, Stanton H, *et al*. Cellular mechanisms for human procollagenase-3 (MMP-13) activation. Evidence that MT1-MMP (MMP-14) and gelatinase A (MMP-2) are able to generate active enzyme. J Biol Chem 1996; 271:17124–31.
24. Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, *et al*. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 1997; 99:1534–45.
25. Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, *et al*. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 1999;99:81–92.
26. Zhou Z, Apte SS, Soininen R, Cao R, Baaklini GY, Rauser RW, *et al*. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc Natl Acad Sci USA 2000;97:4045–57.
27. Li Z, Yasuda Y, Li W, Bogyo M, Katz N, Gordon RE, *et al*. Regulation of collagenase activity of human cathepsins by glycosaminoglycans. J Biol Chem 2004; 279:5470–9.