Determinants of Versican-V1 Proteoglycan Processing by the Metalloproteinase ADAMTS5*

Simon J. Foulcer†, Courtney M. Nelson‡, Maritza V. Quintero§, Balagurunathan Kuberan*, Jonathan Larkin*, Maria T. Dours-Zimmermann‖, Dieter R. Zimmermann‖, and Suneel S. Apte††

From the †Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195, the ‡Departments of Medicinal Chemistry and Bioengineering, University of Utah Health Sciences Center, Salt Lake City, Utah 84112, the §Experimental Medicine Unit, GlaxoSmithKline, King of Prussia, Pennsylvania 19406, and the ‖Institute of Surgical Pathology, University Hospital of Zurich, 8091 Zurich, Switzerland

Background: The mechanisms of versican proteolysis by ADAMTS proteases are unknown.

Results: The ADAMTS5 ancillary domain and specific chondroitin sulfate chains of versican are required for proteolysis.

Conclusion: Docking between the ADAMTS5 ancillary domain and CS chains is a major mechanism underlying versican proteolysis. Proteolysis by ADAMTS5 has a similar requirement for GAG chains.

Significance: The findings suggest strategies for blocking versican cleavage.

Proteolysis of the Glu441-Ala442 bond in the glycosaminoglycan (GAG) β domain of the versican-V1 variant by a disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif (ADAMTS) proteases is required for proper embryo morphogenesis. However, the processing mechanism and the possibility of additional ADAMTS-cleaved processing sites are unknown. We demonstrate here that if Glu441 is mutated, ADAMTS5 cleaves inefficiently at a proximate upstream site but normally does not cleave elsewhere within the GAGβ domain. Chondroitin sulfate (CS) modification of versican is a prerequisite for cleavage at the Glu441-Ala442 site, as demonstrated by reduced processing of CS-deficient or chondroitinase ABC-treated versican-V1. Site-directed mutagenesis identified the N-terminal CS attachment sites Ser907 and Ser925 as essential for processing of the Glu441-Ala442 bond by ADAMTS5. A construct including only these two GAG chains, but not downstream GAG attachment sites, was cleaved efficiently. Therefore, CS chain attachment to Ser907 and Ser925 is necessary and sufficient for versican proteolysis by ADAMTS5. Mutagenesis of Glu441 and an antibody to a peptide spanning Thr432-Gly445 (i.e., containing the scissile bond) reduced versican-V1 processing. ADAMTS5 lacking the C-terminal ancillary domain did not cleave versican, and an ADAMTS5 ancillary domain construct bound versican-V1 via the CS chains. We conclude that docking of ADAMTS5 with two N-terminal GAG chains of versican-V1 via its ancillary domain is required for versican processing at Glu441-Ala442. V1 proteolysis by ADAMTS1 demonstrated a similar requirement for the N-terminal GAG chains and Glu441. Therefore, versican cleavage can be inhibited substantially by mutation of Glu441, Ser907, and Ser925 or by an antibody to the region of the scissile bond.

Versican is a member of a family of large aggregating proteoglycans (termed lecticans) present in the extracellular matrix that includes aggrecan, brevican, and neurocan (1). These proteoglycans have a similar structure, comprising a globular N-terminal domain (G1), a central glycosaminoglycan (GAG)2 domain containing attachment sites for GAG chains, and a globular C-terminal domain (G3) (2). Aggrecan alone has an additional globular domain (G2) that lies between the G1 and G3 domains on the N-terminal side of its GAG domain. The predominant GAG present in these proteoglycans is chondroitin sulfate (CS). Through their G1 domains, these proteoglycans bind to hyaluronan, an interaction that is stabilized by link protein, forming large multimeric complexes that interact with cell surface hyaluronan receptors such as CD44 (3–5). The versican G3 domain binds to fibrulin 1 and 2, fibrillin 1, and tenascin C and R (6–8). Versican is a component of the pericellular matrix as well as the interstitial extracellular matrix networks (9–11). Versican is widely expressed in embryonic and extraembryonic tissues (12–14), whereas aggrecan is specific for cartilage and the central nervous system, and brevican and neurocan are only found in the central nervous system (1). As a component of embryonic cardiac jelly, the interdigit mesenchyme, and the prechondrogenic mesenchyme of the limb, hyaluronan-versican complexes have crucial roles in embryogenesis (3, 15–22) and in regulating cell migration, proliferation, and apoptosis (9, 20, 23–25). Versican is present in adult vasculature, skin, and the central nervous system, as well as in solid tumors of several organs (26–29). Versican has several splice isoforms arising from alternative splicing of two large exons encoding CS attachment domains, termed GAGα and GAGβ (30–32). These variants are V0 (containing GAGα and GAGβ), V1 (containing GAGβ only), V2 (containing GAGα only), and V3, lacking either GAG domain. V2 is primarily found in the central nervous system and V0 in the nervous and...

* This work was supported, in whole or in part, by National Institutes of Health Program of Excellence in Glycoscience Awards P01 HL-107147 (to S. S. A.) and P01 HL-107152 (to B. K.) and by funding from Sabrina’s Foundation (to S. S. A.).
† To whom correspondence should be addressed: Dept. of Biomedical Engineering, ND20, Cleveland Clinic, 9500 Euclid Ave., Cleveland, OH 44195. Tel.: 216-445-3278; Fax: 216-444-9198; E-mail: aptes@ccf.org.
‡ The abbreviations used are: ADAMTS, a disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif; GAG, glycosaminoglycan; CS, chondroitin sulfate.
cardiovascular systems, but V1 is widely expressed, especially in the embryo (12, 13, 26, 33–36). V3 expression and localization are poorly defined. Recently, a new variant, V4, generated by splicing at a cryptic site within the GAGβ domain, has been identified in cancer cells (28).

ADAMTS proteases cleave aggrecan at multiple sites, an activity named aggrecanase, which is a major contributor to cartilage destruction in osteoarthritis (37–39). Among these sites, one within the aggrecan G1-G2 interglobular domain (Glu−374-Ala−375) has been deemed critical because it released the entire GAG domain (40). Additional ADAMTS cleavage sites have been identified within the GAG-bearing domain (39). Although aggrecanase sites lack a sequence consensus, Sandy et al. (35) noticed a preference for ADAMTS cleavage after glutamate residues and predicted a cleavage site in versican-V1 corresponding to the aggrecan interglobular domain by comparison of versican and aggrecan core protein sequences. They generated a neoepitope antibody recognizing the predicted new C terminus generated after ADAMTS cleavage, i.e. the sequence DPEAAE441 (corresponding to DPEAAE1428 in V0) (35). The predicted scissile bond Glu441-Ala442 was cleaved by ADAMTS1 and ADAMTS4, and this versicanase activity has been detected in the aortic intima (35). Subsequently, ADAMTS5, ADAMTS9, ADAMTS15, and ADAMTS20 have been found to cleave this site (41–43). Analysis of mice lacking Adams1, Adams5, Adams9, and Adams20 identified anomalies in ovulation, interdigital web regression, skin pigmentation, cardiac development, and palate formation that were associated with reduced versican processing (16, 17, 19, 33–44). The N-terminal V1 fragment extending to DPEEA441 and now termed versikine (19) induced apoptosis in Adams5+Adams20-deficient interdigital webs, which failed to undergo regression because of reduced extracellular matrix breakdown and apoptosis (17). Therefore, a major physiological role that has emerged for several ADAMTS proteases is processing of versican during embryogenesis, although it remains unclear whether versican is the only substrate that explains developmental defects in ADAMTS gene mutants.

Despite the exceptional biological relevance of versican processing by ADAMTS proteases, it is a poorly understood process. Among the questions that have not been addressed are whether versican is cleaved at additional sites in the core protein and which molecular determinants in versican or ADAMTS proteases are crucial for the enzyme-substrate interaction and proteolysis. This knowledge would offer potential means to block versican processing as a way of further investigating the biological relevance of versican processing. Collectively, these unresolved questions motivated this analysis of versican-V1 processing by ADAMTS5.

**EXPERIMENTAL PROCEDURES**

**ADAMTS and Versican Expression Plasmids and Site-directed Mutagenesis—**Mammalian expression plasmids for ADAMTS1 and ADAMTS5 expression have been described previously (41, 49). A versican-V1 plasmid in vector pSecTagA (Invitrogen), the versican V4 expression plasmid, and the G1-DPEAAE plasmid made by inserting a stop codon after Glu441 have been published previously (17, 28, 50). The V1 expression plasmid had an intervening 3′-untranslated sequence between the stop codon and the epitope tags. Therefore, an XhoI restriction site was inserted to disrupt the stop codon using the QuikChange mutagenesis kit (Stratagene, Santa Clara, CA), the 3′-untranslated sequence was excised, and the plasmid was religated to render the versican ORF continuous with the nyc and His6 tags. To generate the constructs V-5GAG-myc, V-2GAG-myc, and DPEAAE-myc (Fig. 1A), a second XhoI site was placed at the appropriate location within the versican ORF. Mutagenized plasmids were digested with XhoI, and the region between the two XhoI sites was eliminated by agarose electrophoresis followed by religation of the plasmids. Specific glycine or serine residues within four N-terminal GAG attachment sites (i.e. Ser-Gly or Gly-Ser motifs within an acidic sequence consensus) (2) in the V-5GAG construct were mutated by site-directed mutagenesis (Ser507 to Ala, Ser525 to Gly, Gly645 to Val, and Ser655 to Ala). Residues around the Glu441-Ala442 scissile bond were mutated using the QuikChange mutagenesis kit (Stratagene). All introduced mutations were verified by nucleotide sequencing.

**Cell Culture, Transfections, and Enzymatic Deglycosylation—**HEK293F cells (ATCC) were cultured in DMEM supplemented with 10% FBS and antibiotics. CHO-K1 and pgsA-745 cells (ATCC) (51) were cultured in 1:1 Ham’s F12 and DMEM supplemented with 10% FBS and antibiotics. ADAMTS and versican expression plasmids were transiently transfected or cotransfected using FuGENE6 (Roche Diagnostics). Conditioned medium from empty vector (pcDNA3.1 MycHis, Invitrogen)-transfected cells was used as the control in versican digests. Serum-free medium was collected from transfected cells after 48 h. Cells were lysed in 1% (w/v) Triton X-100, 10 mM Tris HCl (pH 7.6) containing complete protease inhibitor mixture (Roche Diagnostics) to obtain a cell lysate. To detect N-glycosylation of the versikine-myc construct, it was reduced by addition of 2% mercaptoethanol and boiling for 5 min prior to incubation with peptide N-glycanase F (New England Biolabs, Ipswich, MA) for 2 h at 37 °C. Unless specified otherwise, reagents were from Sigma-Aldrich (St. Louis, MO).

**Generation of Anti-VC, a Cleavage-blocking Versican Polyclonal Antibody—**Anti-VC was generated in rabbits against the peptide sequence NH2-(C)T432VPKDKPEAAEARRG445-COOH spanning the ADAMTS cleavage site (in *italics*) in the versican-V1 core protein. The N-terminal Cys residue was added for conjugation to keyhole limpet hemocyanin, and the keyhole limpet hemocyanin-peptide conjugate was injected into rabbits (YenZym Antibodies, LLC, South San Francisco, CA). Immune sera were affinity-purified against the immobilized peptide antigen. To block ADAMTS5 cleavage of versican V5-GAG, anti-VC was incubated with V-5GAG at increasing concentrations for 30 min at 37 °C. These V-5GAG-anti-VC complexes were then used in subsequent versican digestion (versicanase) assays.

**Characterization of Anti-DPEAAE Specificity—**NH2-DPEAAE-COOH peptide or variations of it were synthesized by the Lerner Research Institute Molecular Biotechnology Core. Versikine-containing conditioned medium was diluted 1:2 in coating buffer (40 mM Na2CO3 (pH 9.6)), and 200 μl was used to
coat F96 Maxisorb plates (Nunc, Rochester, NY) by overnight incubation at room temperature. The wells were washed with 50 mM HEPES, 100 mM NaCl, 0.05% (v/v) Tween 20 (pH 7.4), blocked by incubating with 200 µl of 1% (w/v) BSA (2 h, 37 °C), and the washing steps were repeated. Anti-DPEAAE (Affinity Bioreagents, Golden, CO) was preincubated with increasing concentrations of the peptides for 30 min at 37 °C. These were incubated with the versikine-coated wells (4 h, 37 °C) and washed. Alkaline phosphatase-conjugated rabbit antibody (Bio-Rad) was added to each well (2 h, 37 °C) and detected using p-nitrophenyl phosphate tablets (Sigma) and detection of the product at A$_{405}$.

Quantification of ADAMTS5 Concentration—ADAMTS5 concentration in the single batch of HEK293F conditioned medium used for this study was determined using a solid phase binding assay. Purified, recombinant ADAMTS5 Pro-Cat-Dis (provided by Dr. David Buttle, Sheffield University, UK) was coated overnight (in coating buffer) on F96 Maxisorb plates at increasing concentrations alongside multiple dilutions of ADAMTS5 conditioned medium. The wells were washed and blocked as described above, and ADAMTS5 was detected using 12F4, a monoclonal antibody with a conformational epitope spanning the catalytic and disintegrin-like domains (Glaxo-SmithKline, King of Prussia, PA). Anti-mouse alkaline phosphatase-conjugated antibody (Bio-Rad) was added, and the bound antibody was detected using p-nitrophenyl phosphate tablets. Non-specific antibody interactions were accounted for by subtracting the absorbance of wells coated with empty vector control conditioned medium. The concentration of ADAMTS5 was deduced from the monoclonal antibody-binding curve generated from the absorbance at 405 nm of the recombinant protein. This gave a value of ~3 µg/ml. For all versican digests, 100 µl of conditioned medium was used (~300 ng of ADAMTS5).

Versican Antibodies, Versican Digestion, Western Blotting, and Densitometry—To detect versicanase activity, conditioned medium from ADAMTS transfections was combined with versican conditioned medium in a 1:1 ratio, incubated for 16 h at 37 °C, and analyzed using 10% SDS-PAGE. Alternatively, HEK293F cells were cotransfected with 1 µg each of ADAMTS5 and versican plasmids, and the conditioned medium was analyzed as above. Western blotting was done under reducing conditions using anti-VC or anti-DPEAAE; anti-GAG A (against amino acids 357–567) (14), anti-GAG B (against amino acids 654–847),$^4$ anti-GAG B (against amino acids 1028–1274) (Dours-Zimmermann, M.T. and Zimmermann, D.R., unpublished), and anti-GAG B (against amino acids 1659–2101) (14) and enhanced chemiluminescence (GE Healthcare). Band intensity was quantitated using ImageJ software (National Institutes of Health, Bethesda, MD). To quantify versicanase activity in some assays, the band intensity obtained with anti-DPEAAE was divided by the band intensity of versican (V-5GAG) in the conditioned medium using anti-myc to give a relative intensity ratio. The protein content of V-5GAG, V-2GAG, and versican-V1 within the conditioned medium was determined by treating with chondroitinase ABC (Seikagaku, Tokyo, Japan) at a concentration of 0.1 units/ml for 2 h at 37 °C, and analyzed by 6% SDS-PAGE and Western blotting as described above.

Synthesis of Click-xyloside and Xyloside Treatment of Cells—Click-xyloside synthesis was as described previously (52). Briefly, copper-catalyzed click chemistry was performed at room temperature in 1:1 acetone:water. 1.2 molar equivalents of pentyne (catalog no. Wako 322-49451, Wako Chemicals, Richmond, VA), 0.2 mol equivalents of aqueous CuSO$_4$, and 0.4 molar equivalents of β-sodium ascorbate were added to 1 molar equivalent peracetylated β-xylosyl azide. The reaction product, click-xyloside, was purified on a flash silica column using an ethyl acetate-hexane gradient. The purified product was subsequently deprotected in dry MeOH/sodium methoxide at pH 10. The deprotected compound was purified on a reverse phase silica column to obtain the final product, MQ-1-31, which was characterized by $^1$H NMR and negative mode LC-MS.

HEK-293F cells stably expressing V-5GAG were seeded to 30% confluence and incubated for 16 h in medium supplemented with 10% FBS and antibiotics. The cells were washed with PBS, and the medium was replaced with serum-free medium containing click-xyloside (dissolved in dimethyl sulfoxide) at a stock concentration of 10 mM) at the appropriate concentration. For controls, the appropriate amount of dimethyl sulfoxide was added to the medium. After 48 h of further culture, the conditioned medium was collected, and the effect of increasing click-xyloside concentration on V-5GAG cleavage by ADAMTS5 was determined.

V-5GAG Pulldown by the ADAMTS5 Ancillary Domain—Anti-FLAG-agarose beads (50 µl) were washed three times with TBS, added to conditioned medium containing the ADAMTS5 ancillary domain, and incubated at room temperature for 2 h. The resin was washed five times with TBS (150 mM NaCl and 50 mM Tris-HCl (pH 7.6)), added to V-5GAG conditioned medium, and incubated at room temperature for a further 2 h. The resin was washed five times with TBS and resuspended to a volume of 100 µl with TBS prior to digestion with chondroitinase ABC (0.1 units/ml) at 37 °C for 2 h. The supernatant was analyzed by Western blotting for V-5GAG using anti-VC. A control pulldown assay was performed using the medium of cells transfected with an empty vector (p3XFLAG-CMV9) instead of the ADAMTS5 ancillary domain conditioned medium. The purpose of this control was to show that V-5GAG was not binding nonspecifically to the anti-FLAG-agarose beads.

Particle Exclusion Assay—The RBC exclusion assay was used to visualize the pericellular matrix and was carried out essentially as described previously (10). Briefly, formalin-fixed sheep RBCs were washed with PBS and resuspended to a final concentration of 1.0 × 10$^8$ RBCs/ml. Dermal fibroblasts obtained

$^3$ J. Larkin, T. A. Lohr, L. Elefante, J. Shearin, R. Matico, J.-L. Su, Y. Xue, F. Liu, C. Genell, R. E. Miller, P. B. Tran, A.-M. Malfait, C. C. Maier, and C. J. Matheny, manuscript submitted.

$^4$ M. T. Dours-Zimmermann and D. R. Zimmermann, unpublished data.
from wild-type C57Bl/6J mice were plated at ~30% confluence in 6-well plates and incubated in serum-free medium with anti-VC or control rabbit isotype-matched IgG antibody for 24 h. The RBC suspension (200 μl) was added to each well along with calcein (final concentration 1 μg/ml) for cell visualization and incubated for 20 min to allow the RBCs to settle around the cells. Images of the cells were taken with an inverted wide-field Leica microscope (DR IRB, Heidelberg, Germany) using a ×20 objective lens in fluorescent and phase-contrast modes. Pericellular matrix exclusion zones were quantified using ImageJ (Media Cybernetics, Silver Spring, MD) by subtracting the area of the fluorescent image (i.e. the cell) from the total area of the cell plus the exclusion zone as observed in phase-contrast mode.

Collagen Gel Contraction Assay—The collagen gel contraction assay was performed as described previously (10). Melted 4% agarose (Amresco, Solon, OH) was allowed to gel in 24-well plates around 10-mm cloning rings to form 10-mm diameter molds for the collagen gels. Rat tail collagen (3.2 mg/ml, catalog no. 354236, BD Biosciences) was diluted to a final concentration of 1.6 mg/ml with DMEM containing 10% FBS, antibiotics, and dermal fibroblasts (2 × 10⁵ cells). Antibody (either anti-VC or rabbit IgG isotype-matched control) was added at the appropriate concentration, and the gels were allowed to polymerize at 37 °C for 1 h. The gels were overlaid with 1 ml DMEM supplemented with 10% FBS, antibiotics, and either anti-VC or the control antibody at the appropriate concentration. The gels were detached from the agarose mold and allowed to contract overnight (16 h) at 37 °C as suspended gels. The gels were visualized under a stereomicroscope, and the area was quantified using ImageJ.

Statistical Analysis—Data represent the mean ± S.D. of at least three independent experiments. Statistical analysis was performed using the unpaired Student’s t test.

RESULTS

The Glu441-Ala442 Bond Is a Major Site of Versican Proteolysis in the Versican GAGβ Domain—New human versican-V1 constructs (Fig. 1A) and a new versican antibody to a peptide straddling the Glu⁴⁴¹-Ala⁴⁴² bond, named anti-VC, were gen-
erated to facilitate this analysis. Western blotting of full-length versican (V1) and V-5GAG transfected into HEK293F cells demonstrated that they were modified appropriately with CS chains because a high molecular weight smear arising from each was resolved into a sharper band of stronger intensity following chondroitinase ABC digestion (Fig. 1, B and C). Anti-VC detected versican-V1 and V-5GAG specifically after digestion with chondroitinase ABC, with a reactivity similar to commercial anti-GAG/H9252 and anti-myc antibody on Western blot analyses (Fig. 1, B and C). The specificity of anti-VC was validated by blockade of its reactivity against V-5GAG on Western blot analyses after incubation with the peptide immunogen (data not shown). Without chondroitinase ABC digestion, as expected, versican-V1 and V-5GAG migrated poorly into the gel or not at all (Fig. 1, B and C). V-5GAG, which contains fewer CS chains than V1 and is smaller, was detectable using anti-myc but not anti-VC (Fig. 1C). The observed difference likely results from differences between the affinities and optimal concentrations of the two antibodies in these Western blot analyses. V-5GAG, but not V1, was detectable by anti-myc on Western blot analyses (Fig. 1C and data not shown for V1), despite the cloning of the V1-ORF in-frame with the myc-His6 tag, possibly because of proteolytic loss of the tag in the latter construct. In subsequent experiments, we used anti-GAG/β for detection of V1, anti-myc for detection of V-5GAG, and anti-VC for detection of either construct. When either versican-V1 or V-5GAG were digested with ADAMTS5-containing medium, but not the medium of vector-transfected cells, and the digests were

**FIGURE 2. Characterization of anti-DPEAAE and recombinant human versikine.** A, competitive solid-phase binding assay examining the reactivity of anti-DPEAAE. Versikine was coated in each well and detected by anti-DPEAAE antibody preincubated with the indicated peptides at increasing concentrations (data points are shown as mean ± S.E., n = 6). Peptides other than DPEAAE do not block anti-DPEAAE effectively. $B$, representative Western blot analyses contrasting versikine and versikine-myc immunoreactivity to anti-VC (left panel) and anti-DPEAAE (right panel). Anti-DPEAAE fails to detect versikine-myc as a consequence of epitope masking by the myc tag. $IB$, immunoblot. $C$, representative Western blot analysis showing the change in migration of versikine-myc following peptide N-glycanase F (PNGase F) treatment. $D$, representative Western blot analysis demonstrating that ADAMTS5 does not cleave versikine. Versikine (left panel) and versikine-myc (right panel) were incubated with ADAMTS5 or empty vector control conditioned medium and probed with anti-VC. No change in molecular species was observed following ADAMTS5 treatment.
treated with chondroitinase ABC, a 70-kDa band corresponding to versikine was obtained (Fig. 1D). ADAMTS5 cleaved versican upon coincubation of a versican construct with ADAMTS5 conditioned medium as well as after cotransfection of ADAMTS5 and versican constructs.

We evaluated the specific immunoreactivity of anti-DPEAAE to versikine by preincubating the antibody with a variety of peptides, such as those that deleted Glu441 (DPEAA), replaced it with Ala (DPEAAA), or added one or two C-terminal Ala residues (DPEAAEA, DPEAAEAA). Neither of these peptides blocked anti-DPEAAE reactivity against versikine as effectively as peptide DPEAAE (Fig. 2A). Anti-DPEAAE failed to react with versikine when a C-terminal myc-His6 tag was present (Fig. 2B). In contrast, anti-VC could detect versikine or versikine-myc-His6 with similar reactivity on Western blot analyses. Therefore, anti-DPEAAE is a true neoepitope antibody to versikine that is absolutely dependent on Glu441 for its reactivity, whereas anti-VC detects versikine because it contains 10 of the 14 immunogen peptide residues (Fig. 1A). As shown in Fig. 1, anti-VC can detect versikine in samples electrophoresed without prior chondroitinase ABC digestion as well as intact versican substrate if the sample is digested prior to electrophoresis (Fig. 1, B–D). In subsequent experiments, we used anti-DPEAAE or anti-VC to detect versikine but did not use anti-DPEAAE for analysis of cleavage site mutants because of its stringent specificity.

Consistent with previous reports, versikine migrated electrophoretically with an observed molecular mass of ~70 kDa (Figs. 1D and 2, B–D), which was inconsistent with its predicted mass of 48.9 kDa. Because versikine lacks CS chains, we digested it with peptide N-glycanase F to determine whether the discrepancy could be explained by modification at three potential sites for N-glycosylation, i.e. Asn57, Asn330, and Asn411. When treated with peptide N-glycanase F, the observed molecular mass was reduced by ~5 kDa (Fig. 2C). The presence of a highly negatively charged region (amino acids 361–408) in versikine likely leads to local intrinsic disorder that can manifest as aberrant migration in SDS-PAGE. Indeed, analysis of the sequence of versikine using online prediction tools (IUPreD and FoldIndex) predicted a strong tendency to local disorder in residues 360–441. When versikine or myc-tagged versikine were digested with ADAMTS5 and the digests were immunoblotted with anti-VC, versikine migration was unchanged, suggesting that, when released from versican, versikine was not cleaved further by ADAMTS5 (Fig. 2D).

To investigate whether cleavage occurred at additional sites within the GAGβ domain, we analyzed ADAMTS5-digested versican-V1 by Western blotting with four polyclonal antibodies spanning the GAGβ-domain (Fig. 3A). Antibody A, the most N-terminal and adjacent to the G1 domain, detected a 70-kDa band similar to anti-VC when either V1 or V-5GAG were incubated with ADAMTS5 (Fig. 3B). This species likely corresponds
to versikine because the peptide used to generate antibody A, like the VC peptide, spans the Glu\(^{441}\)-Ala\(^{442}\) processing site. However, antibodies B-D did not identify fragments resulting specifically from digestion with ADAMTS5 (Fig. 3B). We conclude that ADAMTS5 did not process the versican GAG\(\beta\) core protein at sites other than Glu\(^{441}\)-Ala\(^{442}\). However, fragments not resulting from ADAMTS5 digestion (i.e., observed in both the experimental and control lanes) were seen, suggesting that versican may be cleaved by other proteases expressed by HEK293F cells (Fig. 3B).

The ADAMTS5 Ancillary Domain Binds to Versican-V1 and Is Essential for Proteolysis—To determine which region of ADAMTS5 bound to versican, we utilized a construct containing the propeptide, catalytic domain, and disintegrin-like domain (ADAMTS5 Pro-Cat-Dis) or the entire ancillary domain (Fig. 4A). In contrast to full-length ADAMTS5, ADAMTS5 Pro-Cat-Dis did not cleave versican V5-GAG, suggesting a requirement of the ancillary domain for versican binding (Fig. 4B). To investigate whether the ancillary domain promoted versican cleavage by localizing ADAMTS5 to the versican core protein, coimmuno-precipitation was performed. The FLAG epitope-tagged ADAMTS5 ancillary domain was first successfully pulled down using anti-FLAG-agarose beads (Fig. 4C). The FLAG resin + ancillary domain complex was incubated with V-5GAG conditioned medium and washed extensively. The FLAG resin + ancillary domain complexes were incubated with chondroitinase ABC, and the supernatant was analyzed by Western blotting using anti-VC. A coprecipitating band of \(~150\) kDa corresponding to V-5GAG was observed (Fig. 4D). This band was not seen when immunoprecipitation was performed using empty vector-transfected conditioned medium as a control. Therefore, the ancillary domain of ADAMTS5 interacted specifically with V-5GAG via the CS chains.

Versican Chondroitin Sulfate Chains Are Required for Proteolysis by ADAMTS5—When either versican-V1 or V-5GAG were digested with chondroitinase ABC prior to incubation with ADAMTS5, there was a substantial reduction in band intensity of versikine (Fig. 5A), suggesting that CS chains play a central role in mediating ADAMTS5 proteolysis at the Glu\(^{441}\)-Ala\(^{442}\) site. Because V-5GAG digestion by ADAMTS5 gave a comparable versikine product, as did a digest of versican-V1 (Figs. 1D and 5A), we considered it likely that V-5GAG contained the determinant(s) necessary for processing at Glu\(^{441}\)-Ala\(^{442}\). We compared proteolysis of V-5GAG expressed in

---

**FIGURE 4.** The ADAMTS5 ancillary domain binds versican-V1. A, schematic of the ADAMTS5 constructs used. B, Western blot analysis obtained under reducing conditions showing that, in contrast to full-length ADAMTS5, ADAMTS5-Pro-Cat-Dis does not cleave versican V-5GAG. IB, immunoblot. C, Western blot analysis showing pulldown of the ADAMTS5 ancillary domain using anti-FLAG beads. D, V-5GAG is pulled down along with the ADAMTS5 ancillary domain. The V-5GAG input protein is electrophoresed at the left to provide a size standard for the coeluting V-5GAG seen in the far right lane.
CHO-K1 cells and the mutant derivative cell line, CHO-K1 pgs-745A, which lacks xylosyltransferase and is, therefore, unable to add GAG chains to core proteins. ADAMTS5 did not efficiently cleave V-5GAG expressed from CHO-K1 pgs745A cells, despite comparable levels of core protein secreted from CHO-K1 or CHO-K1 pgs745A cells (Fig. 5B). In addition, digests of V-5GAG from CHO cells cultured in the presence of a click-xyloside, which reduces GAG-attachment to core proteins, demonstrated a dose-dependent reduction of versikine production relative to the amount of V5-GAG secreted by the cells (Fig. 5C). Together, these results clearly indicate a key role for the CS chains in mediating ADAMTS5 cleavage of versican.

To determine whether specific CS chains of V-5GAG mediated ADAMTS5 cleavage, we mutated four of the CS attachment sites (Fig. 6A). Loss of individual GAG attachment sites did not affect the secretion efficiency of the respective mutants, as evident from comparable levels of each mutant in the medium of transfected cells (Fig. 6B, bottom panel). However, loss of two CS chains nearest the Glu^{441}-Ala^{442} scissile bond (i.e. mutagenesis of

**FIGURE 5. CS modification of the versican-V1 core protein is essential for processing at the Glu^{441}-Ala^{442} site.** A, representative Western blot analyses showing the effect of the CS modification on the susceptibility of versican-V1 and V-5GAG to proteolysis by ADAMTS5. A strong versikine band (asterisks) is seen only when the versican substrate was not treated with chondroitinase ABC (C’ABC) prior to digestion with ADAMTS5. IB, immunoblot. B, V-5GAG was expressed in CHO-K1 cells or the pgs745A CHO-K1 mutant lacking xylosyltransferase and incubated with conditioned medium containing ADAMTS5. Top panel, reduced versikine generation from substrate obtained from pgs745A cells, which is also visualized better than that which is from CHO-K1 cells without chondroitinase ABC treatment because it lacks CS chains. Bottom panel, obtained after chondroitinase ABC treatment and showing comparable levels of V-5GAG secreted by each cell line. C, click-xyloside addition reduces versican cleavage dose-dependently. Left panel, top left, representative Western blot analysis of ADAMTS5 digests of V-5GAG expressed in the presence of increasing concentrations of click-xyloside without subsequent chondroitinase ABC digestion. Versikine is indicated by the asterisk. Note that V-5GAG was poorly visualized until cells were treated with 200 μM xyloside. Left panel, bottom, Western blot analysis of chondroitinase ABC-treated conditioned media demonstrating that V-5GAG expression was unaffected by addition of click-xyloside. Right panel, the decrease in versikine intensity was normalized to V-5GAG expression and quantified, confirming the click-xyloside-mediated, dose-dependent decrease in V-5GAG cleavage. All data are mean ± S.E. of three experiments. **, p < 0.005.
Ser\textsuperscript{507} and Ser\textsuperscript{525}) led to a statistically significant reduction in versikine product. Elimination of CS attachment to Ser\textsuperscript{644} or Ser\textsuperscript{646} (by mutating Gly\textsuperscript{645}) and Ser\textsuperscript{655} was without a similar effect (Fig. 6B). Therefore, we conclude that the two most N-terminal CS chains are required for processing by ADAMTS5. To test this possibility, versicanase digests were undertaken using V-2GAG, a construct containing only the two N-terminal CS attachment sites identified as crucial after mutagenesis of V5-GAG, i.e. Ser\textsuperscript{507} and Ser\textsuperscript{525} (Fig. 6C). This construct was cleaved efficiently by ADAMTS5 (Fig. 6C). We conclude that the CS chains attached to Ser\textsuperscript{507} and Ser\textsuperscript{525} are necessary and sufficient for versican-V1 processing by ADAMTS5.
Recently, a novel versican isoform, V4, which arises by use of a cryptic splice site in the GAG/H9252-encoding exon, was described. V4 contains G1, G3, and the five N-terminal CS-attachment sites. It is essentially similar to the V-5GAG construct other than having a C-terminal G3 domain. A V4 construct was processed by ADAMTS5 comparably with V-5GAG (data not shown).

Glu441 Is Required for Versican Proteolysis by ADAMTS5—In view of the prevalence of Glu as the P1 residue in peptide bonds cleaved by ADAMTS proteases in aggrecan and versican (35, 39), we asked whether proteolysis of V-5GAG was affected when Glu441 was mutated (to Ala) (Fig. 7A). This mutant was secreted into medium of transfected cells at comparable levels as V-5GAG (Fig. 7B), but its digestion by ADAMTS5 was reduced substantially (Fig. 7C). Instead, an anti-VC reactive fragment was observed that migrated slightly more rapidly, suggestive of proteolysis at a site immediately upstream, i.e., following Glu 438. When both Glu438 and Glu441 were mutated, however, no digestion of V-5GAG occurred, as detected by both anti-VC and an antibody to the versican G1 domain (Fig. 7C). Notably, these mutations did not abolish versican recognition by anti-VC (Fig. 7B, compare top and bottom panels).

Anti-VC Blocks Versican Processing—Because the anti-VC immunogen peptide straddles the Glu441-Ala442 cleavage site, we asked whether anti-VC antibody binding to versican could sterically hinder its proteolysis. Incubation of V-5GAG with anti-VC demonstrated a dose-dependent accumulation of undigested V-5GAG (Fig. 8A) and reduced the versikine product in the digests (Fig. 8B), indicative of inhibition of versican proteolysis. Previously, loss of ADAMTS5 activity in skin fibroblasts has been shown to lead to an accumulation of a versican-rich pericellular matrix and a fibroblast-to-myofibroblast transition (10). When wild-type mouse skin fibroblasts were treated with anti-VC, there was an accumulation of pericellular matrix (Fig. 9A) and enhanced contractility of dermal fibroblasts in collagen gels (Fig. 9B). This effect of anti-VC, similar to that demonstrated previously upon inactivation of ADAMTS5 or overexpression of versican-V1 (10), suggests that anti-VC can be used to block versican proteolysis by ADAMTS5.

ADAMTS1 Has Similar Requirements for Versican Processing as ADAMTS5—ADAMTS1 proteolysis of versican is required for ovulation (53, 54) and for compaction of the developing myocardium (55). Here we extend the major findings of our investigation to ask whether ADAMTS1 employed similar mechanisms as ADAMTS5 for versican processing. Like ADAMTS5, ADAMTS1 could generate versikine when V-5GAG was generated in CHO-K1 cells but not in xylosyl-
transferase-deficient pgs-745A cells (Fig. 10A). Furthermore, mutation of Glu 441 and Glu438 abrogated versican processing by ADAMTS1 (Fig. 10B), and prevention of GAG attachment at Ser507 and Ser525 by mutagenesis of these residues also prevented or reduced proteolysis by ADAMTS1 (Fig. 10C).

**DISCUSSION**

Because of the great interest in characterizing ADAMTS4 and ADAMTS5 activity in osteoarthritis, proteolysis of aggrecan, the major cartilage proteoglycan, has been investigated extensively (39, 40). However, the mechanisms of versican cleavage have not been investigated previously. This analysis, focusing on ADAMTS5 and ADAMTS1, which are major versicanases during embryogenesis, demonstrates similarities and distinctions between ADAMTS proteolysis of versican and aggrecan. These studies show that, unlike aggrecan, which is cleaved by ADAMTS proteases, including ADAMTS5, at multiple sites within the CS-bearing domain (39, 40), cleavage of the versican-V1 core protein primarily occurred at the Glu441-Ala442 site or, in its absence, at a putative upstream site but not elsewhere within the GAG-domain.

We have shown here that ADAMTS5 relies on specific determinants in versican for interaction and proteolysis respectively, *i.e.* two specific CS chains and Glu441. These findings constitute the first understanding of how versican is cleaved by an ADAMTS protease. Initially, the analysis showed that enzymatically eliminating CS modification of the core protein led to reduced proteolysis. Because digestion by chondroitinase ABC leaves residual core oligosaccharide stubs, we sought additional evidence using CHO-pgs745A cells, which lack the ability to attach xylose (51), the first residue of the nascent CS chain. Reduced ADAMTS5 and ADAMTS1 proteolysis of V-5GAG expressed by these cells or by cells cultured in the presence of a click-xyloside that acts as a decoy for CS attachment (52) supported the requirement of CS chains in V-5GAG for proteolysis. We conclude that the two N-terminal-most CS chains are likely binding sites for ADAMTS5 exosites and provide it with access to the Glu441-Ala442 site. ADAMTS5 binding to the CS chains could lead to a conformational change in the versican core protein that renders the Glu441-Ala442 site accessible or contributes to opening of the ADAMTS5 catalytic site, previously shown to exist in both open and closed conformations (56).

Previously, an *Escherichia coli*-expressed, GAG-free versican polypeptide spanning residues Gly357 to Asp567 has been used to demonstrate proteolytic processing of versican by ADAMTS1 and ADAMTS4 (35). In contrast, our work suggests that CS-modified versican is the preferred ADAMTS5 and ADAMTS1 substrate. We speculate that the CS-chains provide an anchorage site near the scissile bond that may be otherwise elusive in a polypeptide that is predicted to be unstructured. At the scissile bond, Glu441 was an essential determinant, and its elimi-
FIGURE 9. Anti-VC induces the accumulation of pericellular matrix and myofibroblast transition in mouse dermal fibroblasts. A, erythrocyte exclusion assay demonstrating increased pericellular matrix around dermal fibroblasts in the presence of anti-VC. Arrows indicate the pericellular matrix border around calcine-labeled fibroblasts (green). Representative images are shown for both control IgG and anti-VC. Quantification of pericellular matrix area demonstrates a significant increase in anti-VC treated cells ($p < 0.005$), as shown in the whisker plot (bottom panel), attributed to reduction in versican cleavage. Each dot in the plot is a single cell measurement. B, collagen contraction assay to evaluate fibroblast contractility in the presence of anti-VC. Representative images are shown in the top panel. Quantification of the gel area demonstrates a significant reduction in the presence of anti-VC ($p < 0.005$), suggestive of increased contraction of the collagen gel by embedded fibroblasts (bottom panel). Each dot in the plot represents a single gel.

FIGURE 10. ADAMTS1 cleavage of V-5GAG uses similar molecular determinants as ADAMTS5. A, versican and V-5GAG were expressed in CHO-K1 cells or the pgs745A CHO-K1 mutant (as shown in Fig. 5) and incubated with conditioned medium containing ADAMTS1. A reduction in versikine generation was observed using V-5GAG expressed in pgs745A CHO-K1 cells. C'ABC, chondroitinase ABC; IB, immunoblot. B, Western blot analysis showing the effect of the cleavage site mutants (described in Fig. 7) on digestion by ADAMTS1. Cleavage, speculated to occur after Glu$^{438}$, is observed in mutant A (indicated by the asterisk). Cleavage at this site is eliminated following mutation of Glu$^{438}$ (V-5GAGB). C, representative Western blot analysis of V-5GAG CS chain attachment mutants (described in Fig. 6) after digestion with ADAMTS1 followed by chondroitinase ABC treatment. The two CS chain attachment mutants that had reduced cleavage by ADAMTS5 also had reduced ADAMTS1-mediated versican cleavage (versikine is indicated by the asterisk). A chondroitinase ABC digest of the V-5GAG conditioned medium shows comparable expression levels of all constructs.
Previous work examining ADAMTS4 cleavage of aggrecan had not identified specific CS chains as crucial determinants of proteolysis within the CS2 region (60, 61). However, the requirement for the ancillary domain has been established for ADAMTS4 and ADAMTS5 cleavage of aggrecan (62, 63) and appears to be similar for cleavage of versican. Therefore, the ADAMTS5 ancillary domain, on the basis of its binding to the CS chains, likely contains one or more exosites for ADAMTS5 activity against aggrecan and versican. Such exosites are necessary because the catalytic domains of ADAMTS proteases typically have little activity against native substrates, and most binding attributes are located in the ancillary domain. For example, ADAMTS1 binding to the extracellular matrix was dependent on its ancillary domain (64), TSR1 of ADAMTS4 has been shown to be essential for aggrecanase activity through binding to the CS chains (61), and truncation experiments as well as chimeric proteins of ADAMTS4 and ADAMTS5 have shown altered activity against aggrecan after manipulation of the ancillary domains (63, 65). Furthermore, the crystal structure of the ADAMTS13 ancillary domain in conjunction with mutagenesis suggested the presence of several discontinuous exosites for its substrate von Willebrand factor (66). The importance of exosites in substrate recognition is particularly evident in thrombotic thrombocytopenic purpura, where autoantibodies are commonly directed to ADAMTS13 exosites, inhibiting substrate recognition and reducing von Willebrand factor processing (67, 68).

Anti-DPEAAE antibody is a widely used and valuable tool for versican analysis (10, 16, 17, 33, 35, 42, 44–46, 53, 56–58), but the determinants of its reactivity have not been mapped previously. Our finding that Glu441 was absolutely required for anti-DPEAAE reactivity necessitated the development of an antibody for this work whose reactivity was not dependent on the presence of Glu441. As shown here, anti-VC retains its reactivity and specificity after mutagenesis of both Glu441 and Glu438. Furthermore, we have shown that anti-VC is function-blocking for proteolysis by ADAMTS5 and can be used to manipulate versican processing in vitro and, potentially, in vivo. Applied to skin fibroblasts, anti-VC effects similar to those elicited previously by genetic inactivation of ADAMTS5, i.e. accumulation of pericellular matrix and a fibroblast-to-myofibroblast phenotype switch demonstrated by enhanced contraction of a collagen gel (10).

**REFERENCES**

1. Yamauchi, Y. (2000) Lecticans: organizers of the brain extracellular matrix. *Cell Mol. Life Sci.* 57, 276–289
2. Zimmermann, D. R., and Ruoslahti, E. (1989) Multiple domains of the large fibroblast proteoglycan, versican. *EMBO J.* 8, 2975–2981
3. LeBaron, R. G., Zimmermann, D. R., and Ruoslahti, E. (1992) Hyaluronate binding properties of versican. *J. Biol. Chem.* 267, 10003–10010
4. Matsumoto, K., Shionyu, M., Go, M., Shimizu, K., Shinomura, T., Kimata, K., and Watanabe, H. (2003) Distinct interaction of versican/PG-M with hyaluronan and link protein. *J. Biol. Chem.* 278, 41205–41212
5. Shi, S., Grothe, S., Zhang, Y., O’Connor-McCourt, M. D., Poole, A. R., Roughley, P. I., and Mort, J. S. (2004) Link protein has greater affinity for versican than aggrecan. *J. Biol. Chem.* 279, 12060–12066
6. Aspberg, A., Adam, S., Kostka, G., Timpl, R., and Heinigard, D. (1999) Fibulin-1 is a ligand for the C-type lectin domains of aggrecan and versican. *J. Biol. Chem.* 274, 20444–20449
7. Aspberg, A., Binkert, C., and Ruoslahti, E. (1995) The versican C-type lectin domain recognizes the adhesion protein tenascin-R. *Proc. Natl. Acad. Sci. U.S.A.* 92, 10590–10594
8. Isogai, Z., Aspberg, A., Keene, D. R., Ono, R. N., Reinhardt, D. P., and Sakai, L. Y. (2002) Versican interacts with fibrillin-1 and links extracellular microfibrils to other connective tissue networks. *J. Biol. Chem.* 277, 4565–4572
9. Evanko, S. P., Angello, J. C., and Wight, T. N. (1999) Formation of hyaluronan- and versican-rich pericellular matrix is required for proliferation and migration of vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 19, 1004–1013
10. Hattori, N., Carrino, D. A., Lauer, M. E., Vasanj, A., Wylie, J. D., Nelson, C. M., and Apte, S. S. (2011) Pericellular versican regulates the fibroblast-
Versican Processing by ADAMTS5

myofibroblast transition: a role for ADAMTS5 protease-mediated proteolysis, J. Biol. Chem. 286, 34298–34310
11. Matsumoto, K., Kamiya, N., Suwan, K., Atsumi, F., Shimizu, K., Shinomura, T., Yamada, Y., Kimata, K., and Watanabe, H. (2006) Identification and characterization of versican/PG-M aggregates in cartilage. J. Biol. Chem. 281, 18257–18263
12. Cattaruzza, S., Schiappacassi, M., Ljungberg-Rose, A., Spessotto, P., Perisinnotto, D., Mörgelin, M., Mucignat, M. T., Colombatti, A., and Perris, R. (2002) Distribution of PG-M/versican variants in human tissues and de novo expression of isoform V3 upon endothelial cell activation, migration, and neoangiogenesis in vitro. J. Biol. Chem. 277, 47626–47635
13. Henderson, D. J., and Copp, A. J. (1998) Versican expression is associated with chamber specification, septation, and valvulogenesis in the developing mouse heart. Circ. Res. 83, 523–532
14. Zimmermann, D. R., Dours-Zimmermann, M. T., Schubert, M., and Bruckner-Tuderman, L. (1994) Versican is expressed in the proliferating zone in the epidermis and in association with the elastic network of the dermis. J. Cell Biol. 124, 817–825
15. Chochock, K., Hatano, S., Takagi, H., Watanabe, H., Kimata, K., and Kogantewel, P. (2010) Versican facilitates chondrocyte differentiation and regulates joint morphogenesis. J. Biol. Chem. 285, 21114–21125
16. Enomoto, H., Nelson, C. M., Somerville, R. P., Mielke, K., Dixon, L. J., Powell, K., and Apte, S. S. (2010) Cooperation of two ADAMTS metalloproteases in closure of the mouse palate identifies a requirement for versican proteolysis in regulating palatal mesenchyme proliferation. Development 137, 4029–4038
17. McCulloch, D. R., Nelson, C. M., Dixon, L. J., Silver, D. L., Wylie, J. D., Lindner, V., Sasaki, T., Cooley, M. A., Argraves, W. S., and Apte, S. S. (2009) ADAMTS metalloproteases generate active versican fragments that regulate interdigital web regression. Dev. Cell 17, 687–698
18. Mjaatvedt, C. H., Yamamura, H., Capehart, A. A., Turner, D., and Markwald, R. R. (1998) The Cspg2 gene, disrupted in the hdf mutant, is required for right cardiac chamber and endocardial cushion formation. Dev. Biol. 202, 56–66
19. Nandadasa, S., Foulcer, S., and Apte, S. S. (2014) The multiple, complex roles of versican and its proteolytic turnover by ADAMTS proteases during embryogenesis. Matrix Biol. 35, 34–41
20. Perisinnotto, D., Iacobacci, P., Bellina, I., Doliana, R., Colombatti, A., Pettway, Z., Bronner-Fraser, M., Shinomura, T., Kimata, K., Mörgelin, M., Löfberg, J., and Perris, R. (2000) Avian neural crest cell migration is disrupted in a novel versican-cleaving proteoglycanase. J. Biol. Chem. 275, 501–508
21. Williams, D. R., Jr., Presar, A. R., Richmond, A. T., Mjaatvedt, C. H., Hoffmann, S., and Capehart, A. A. (2005) Versican expression during skeletal/joint morphogenesis and patterning of muscle and nerve in the embryonic mouse limb. Anat. Rec. A Discov. Mol. Cell Evol. Biol. 282, 95–105
22. Snow, H. E., Riccio, L. M., Mjaatvedt, C. H., Hoffman, S., and Capehart, D. R. (2010) ADAMTS metalloproteases activate versican fragments in human cartilage: evidence for both matrix metalloproteinase and aggrecanase activity. J. Biol. Chem. 285, 13372–13378
23. Schmalfeldt, M., Bandlow, C. E., Dours-Zimmermann, M. T., Winterhalter, K. H., and Zimmermann, D. R. (2000) Brain derived versican V2 is a potent inhibitor of axonal growth. J. Cell. Sci. 113, 807–816
24. Rodriguez-Mazaneque, J. C., Zimmermann, D. R., Lemire, J. M., Fischer, J. W., Wight, T. N., and Clowes, A. W. (2001) Versican V1 proteolysis in human aorta in vivo occurs at the Glu441-Ala442 bond, a site that is cleaved by recombinant ADAMTS-1 and ADAMTS-4. J. Biol. Chem. 276, 2321–2324
25. Fosang, A. J., and Little, C. B. (2008) Drug insight: aggrecanases as therapeutic targets for osteoarthritis. Nat. Clin. Pract. Rheumatol. 4, 420–427
26. Tortorella, M. D., Pratta, M., Liu, R. Q., Austin, J., Ross, O. H., Abbaszade, H., Murphy, G., Sandy, J. D., and Iruela-Arispe, M. L. (2002) ADAMTS1 cleaves aggrecan at multiple sites and is differentially inhibited by metalloproteinase inhibitors. Biochim. Biophys. Res. Commun. 293, 501–508
27. Kongtawelert, P. (2010) Versican facilitates chondrocyte differentiation in the zone in the epidermis and in association with the elastic network of the dermis. J. Cell Biol. 124, 817–825
28. Yang, B. L., Yang, B. B., Erwin, M., Ang, L. C., Finkelstein, J., and Yee, A. J. (2003) Versican G3 domain enhances cellular adhesion and proliferation of bovine intervertebral disc cells cultured in vitro. Life Sci. 73, 3399–3413
29. Yee, A. J., Akens, M., Yang, B. L., Finkelstein, J., Zheng, P. S., Deng, Z., and Yang, B. (2007) The effect of versican G3 domain on local breast cancer invasiveness and bone metastasis. Breast Cancer Res. 9, R47
30. Dunn, S., Kleber, M., Matasci, P., Sommer, L., and Zimmermann, D. R. (2006) Versican V0 and V1 guide migratory neural crest cells. J. Biol. Chem. 281, 12123–12131
31. Bode-Lesniewska, B., Dours-Zimmermann, M. T., Odermatt, B. F., Briner, J., Heitz, P. U., and Zimmermann, D. R. (1996) Distribution of the large aggregating proteoglycan versican in adult human tissues. J. Histochem. Cytochem. 44, 303–312
32. Gao, D., Joshi, N., Choi, H., Ryu, S., Hahn, M., Catena, R., Sadik, H., Argani, P., Wagner, P., Vahdat, L. T., Port, J. L., Stiles, B., Sukumar, S., Altorki, N. K., Rafii, S., and Mittal, V. (2012) Myeloid progenitor cells in the premetastatic lung promote metastases by inducing mesenchymal to epithelial transition. Cancer Res. 72, 1384–1394
33. Kischel, P., Wirtzregny, D., Dumont, B., Turtoiy, A., Greffy, Y., Kirsch, S., De Pauw, E., and Castronovo, V. (2010) Versican overexpression in human breast cancer lesions known and new isoforms for stromal tumor targeting. Int. J. Cancer 126, 640–650
34. Ricciardelli, C., Sakko, A. J., Ween, M. P., Russell, D. L., and Horsfall, D. J. (2009) The biological role and regulation of versican levels in cancer. Cancer Metastasis Rev. 28, 233–245
35. Dours-Zimmermann, M. T., and Zimmermann, D. R. (1994) A novel glycosaminoglycan attachment domain identified in two alternative splice variants of human versican. J. Biol. Chem. 269, 32992–32998
36. Lemire, J. M., Braun, K. R., Maurel, P., Kaplan, E. D., Schwartz, S. M., and Wight, T. N. (1999) Versican/PG-M isoforms in vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 19, 1630–1639
37. Shinomura, T., Nishida, Y., Ito, K., and Kimata, K. (1993) cDNA cloning of PG-M, a large chondroitin sulfate proteoglycan expressed during chondrogenesis in chick limb buds. Alternative spliced multiforms of PG-M and their relationships to versican. J. Biol. Chem. 268, 14461–14469
38. Schmalfeldt, M., Bandlow, C. E., Dours-Zimmermann, M. T., Winterhalter, K. H., and Zimmermann, D. R. (2000) Brain derived versican V2 is a potent inhibitor of axonal growth. J. Cell. Sci. 113, 807–816
39. Rodriguez-Mazaneque, J. C., Westling, J., Thai, S. N., Luque, A., Knauper, V., Murphy, G., Sandy, J. D., and Iruela-Arispe, M. L. (2002) ADAMTS1 cleaves aggrecan at multiple sites and is differentially inhibited by metalloproteinase inhibitors. Biochim. Biophys. Res. Commun. 293, 501–508
40. Fosang, A. J., and Little, C. B. (2008) Drug insight: aggrecanases as therapeutic targets for osteoarthritis. Nat. Clin. Pract. Rheumatol. 4, 420–427
46. Kern, C. B., Wessels, A., McGarity, J., Dixon, L. J., Alston, E., Argraves, W. S., Geeting, D., Nelson, C. M., Menick, D. R., and Apte, S. S. (2010) Reduced versican cleavage due to ADAMts9 haploinsufficiency is associated with cardiac and aortic anomalies. Matrix Biol. 29, 304–316

47. Russell, D. L., Doyle, K. M., Ochsner, S. A., Sandy, J. D., and Richards, J. S. (2003) Processing and localization of ADAMTS-1 and proteolytic cleavage of versican during culmus matrix expansion and ovulation. J. Biol. Chem. 278, 42330–42339

48. Silver, D. L., Hou, L., Somerville, R., Young, M. E., Apte, S. S., Pavan, W. J., Vera, M., Sum, P. E., Lavallie, E. R., Stahl, M., and Somers, W. (2008) Identification of prodomain determinants involved in ADAMTS-1 biosynthesis. J. Biol. Chem. 279, 33237–33245

49. Victor, X. V., Nguyen, T. K., Ethirajan, M., Tran, V. M., Nguyen, K. V., and Longpré, J. M., and Leduc, R. (2004) Identification of prodomain determinants involved in ADAMTS-1 biosynthesis. J. Biol. Chem. 279, 33237–33245

50. Dutt, S., Cassoly, E., Dours-Zimmermann, M. T., Matasci, M., Stoeckli, T., Mackie, S., Olland, S., Lin, L., Zhong, X., Kriz, R., Reifenberg, E. L., and Zimmermann, D. R. (2011) Versican V0 and V1 direct the growth of peripheral axons in the developing chick hindlimb. J. Neurosci. 31, 5262–5270

51. Bai, X., Wei, G., Sinha, A., and Esko, J. D. (1999) Chinese hamster ovary cell mutants defective in glycosaminoglycan assembly and glucuronosyltransferase I. J. Biol. Chem. 274, 13017–13024

52. Victor, X. V., Nguyen, T. K., Ethirajan, M., Tran, V. M., Nguyen, K. V., and Kubera, B. (2009) Investigating the elusive mechanism of glycosaminoglycan biosynthesis. J. Biol. Chem. 284, 25842–25853

53. Brown, H. M., Dunning, K. R., Robker, R. L., Boerboom, D., Pritchard, M., Lane, M., and Russell, D. L. (2010) ADAMTS1 cleavage of versican mediates essential structural remodeling of the ovarian follicle and cumulus-oocyte matrix during ovulation in mice. Biol. Reprod. 83, 549–557

54. Brown, H. M., Dunning, K. R., Robker, R. L., Pritchard, M., and Russell, D. L. (2006) Requirement for ADAMTS-1 in extracellular matrix remodeling during ovarian folliculogenesis and lymphpangiogenesis. Dev. Biol. 300, 699–709

55. Stankunas, K., Hang, C. T., Tsun, Z. Y., Chen, H., Lee, N. V., Wu, J. I., Shang, C., Bayle, J. H., Shou, W., Iruela-Arispe, M. L., and Chang, C. P. (2008) Endocardial Br1 represses ADAMTS1 to maintain the microenvironment for myocardial morphogenesis. Dev. Cell 14, 298–311

56. Mosaik, L., Georgiadis, K., Shane, T., Svenson, K., Hebert, T., McDonagh, T., Mackie, S., Olland, S., Lin, L., Zhong, X., Kriz, R., Reifenberg, E. L., Collins-Racie, L. A., Corcoran, C., Freeman, B., Zollner, R., Marvell, T., Vera, M., Sum, P. E., Lavallie, E. R., Stahl, M., and Somers, W. (2008) Crystal structures of the two major aggrecan degrading enzymes, ADAMTS4 and ADAMTS5. Protein Sci. 17, 16–21

57. Ilic, M. Z., Handle, C. J., Robinson, H. C., and Mok, M. T. (1992) Mechanism of catabolism of aggrecan by articular cartilage. Arch. Biochem. Biophys. 294, 115–122

58. Hering, T. M., Kollar, J., and Huynh, T. D. (1997) Complete coding sequence of bovine aggrecan: comparative structural analysis. Arch. Biochem. Biophys. 345, 259–270

59. Manteau, S. E., Ilic, M. Z., and Handley, C. J. (2002) Highly sulfated glycosaminoglycans inhibit aggrecanase degradation of aggrecan by bovine articular cartilage explant cultures. Matrix Biol. 21, 429–440

60. Miwa, H. E., Gerkens, T. A., and Hering, T. M. (2006) Effects of covalently attached chondroitin sulfate on aggrecan cleavage by ADAMTS-4 and MMP-13. Matrix Biol. 25, 534–545

61. Tortorella, M., Pratta, M., Liu, R. Q., Abbaszade, I., Ross, H., Burn, T., and Arner, E. (2000) The thrombospondin motif of aggrecanase-1 (AD-AMTS-4) is critical for aggrecan substrate recognition and cleavage. J. Biol. Chem. 275, 25791–25797

62. Gendron, C., Kashivaghi, M., Lim, N. H., Enghild, J. I., Thegersen, I. B., Hughes, C., Caterson, B., and Nagase, H. (2007) Proteolytic activities of human ADAMTS-5: comparative studies with ADAMTS-4. J. Biol. Chem. 282, 18294–18306

63. Kashivaghi, M., Enghild, J. I., Gendron, C., Hughes, C., Caterson, B., Itoh, Y., and Nagase, H. (2004) Altered proteolytic activities of ADAMTS-4 expressed by C-terminal processing. J. Biol. Chem. 279, 10109–11019

64. Kuno, K., and Matsuhashi, K. (1998) ADAMTS-1 protein anchors at the extracellular matrix through the thrombospondin type I motifs and its spacing region. J. Biol. Chem. 273, 13912–13917

65. Fushiki, K., Troeberg, L., Nakamura, H., Lim, N. H., and Nagase, H. (2008) Functional differences of the catalytic and non-catalytic domains in human ADAMTS-4 and ADAMTS-5 in aggrecanolytic activity. J. Biol. Chem. 283, 6706–6716

66. Akiyama, M., Takeda, S., Kokame, K., Takagi, J., and Miyata, T. (2009) Crystal structures of the noncatalytic domains of ADAMTS13 reveal multiple discontinuous exosites for von Willebrand factor. Proc. Natl. Acad. Sci. U.S.A. 106, 19274–19279

67. Zheng, X. L., Wu, H. M., Shang, D., Falls, E., Skipwith, C. G., Cataland, S. R., Bennett, C. L., and Kwaan, H. C. (2010) Multiple domains of ADAMTS-13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. Haematologica 95, 1555–1562

68. Zheng, X. L. (2013) Structure-function and regulation of ADAMTS-13 protease. J. Thromb. Haemost. 11, 11–23

69. Matthews, R. T., Gary, S. C., Zerillo, C., Pratta, M., Solomon, K., Arner, E. C., and Hockfield, S. (2000) Brain-enriched hyaluronan binding (BE-HAB)/brevican cleavage in a glioma cell line is mediated by a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family member. J. Biol. Chem. 275, 22695–22703

70. Viapiano, M. S., Hockfield, S., and Matthews, R. T. (2008) BEHAB/brevican requires ADAMTS-mediated proteolytic cleavage to promote glioma invasion. J. Neurooncol. 88, 261–272