ADAMTS4 (Aggrecanase-1) Activation on the Cell Surface Involves C-terminal Cleavage by Glycosylphosphatidyl Inositol-anchored Membrane Type 4-Matrix Metalloproteinase and Binding of the Activated Proteinase to Chondroitin Sulfate and Heparan Sulfate on Syndecan-1*

Gui Gao‡, Anna Plaas§, Vivian P. Thompson‡, Sue Jin¶, Fengrong Zuo**, and John D. Sandy**‡***

From the ‡Center For Research in Skeletal Development and Pediatric Orthopaedics, Shriners Hospital for Children, Tampa, Florida 33612, the Departments of §Internal Medicine and ¶Pharmacology and Therapeutics, University of South Florida, Tampa, Florida 33620, and ¶Arthritis Department, Roche Biosciences, Palo Alto, California 94304

Received for publication, November 4, 2003, and in revised form, December 19, 2003
Published, JBC Papers in Press, December 29, 2003, DOI 10.1074/jbc.M312100200

C-terminal truncation of ADAMTS-4 from the p68 form to the p53 form is required for activation of its capacity to cleave the Glu373-Ala374 interglobular domain bond of aggrecan. In transfected human chondrosarcoma cells, this process is not autoproteolytic because the same products form with an inactive mutant of ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin-like motif 4) and truncation is completely blocked by tissue inhibitor of metalloproteinase-1. Instead, activation can be mediated by glycosylphosphatidyl inositol-anchored membrane type 4-matrix metalloproteinase (MT4-MMP, MMP-17) because co-transfection with the active form of MT4-MMP markedly enhanced activation, whereas an inactive mutant of MT4-MMP was ineffective. Treatment of co-transfected cells with phosphatidylinositol-specific phospholipase C liberated the complex of MT4-MMP and p68 ADAMTS4 from the cell membrane, but the p53 ADAMTS4 remained associated. Specific glycosaminoglycan lyase digestions, followed by product analyses using fluorescence-assisted carbohydrate electrophoresis and immunoprecipitation experiments, showed that the p53 form is associated with syndecan-1 through both chondroitin sulfate and heparan sulfate. We conclude that ADAMTS-4 activation in this cell system involves the coordinated activity of both glycosylphosphatidylinositol-anchored MT4-MMP and the proteoglycan form of syndecan-1 on the cell surface.

The proteolytic processing of extracellular matrix (ECM) is widely studied in many tissues and cell types in relation to normal progression of fertilization, embryogenesis, development, growth, and aging (1). Uncontrolled proteolysis of ECM has also been implicated in many disease states, such as those characterized by tumor invasion (2), chronic inflammation (3), non-healing wounds (4), or excessive tissue destruction (5).

Recently, it has become clear that proteolysis of the major constituents of the mammalian ECM, i.e. collagens and proteoglycans, is achieved not only by the classical matrix metalloproteinase (MMP) family (such as MMP-1, -2, -3, -9, -13) but also by a relatively new group of metalloproteinases called the ADAMTSs (a disintegrin and metalloproteinase with thrombospondin-like motif) family. This group of about 20 related species (6, 7) appears to exhibit a more restricted substrate specificity than MMPs. For example, ADAMTS-2, -3, and -14 appear to be specific procollagen-N-proteinases (8); ADAMTS-13 is the von Willebrand factor-cleaving protease (9); and ADAMTS-1, -4, -5, and -9 exhibit a degree of specificity for degradation of the aggregating proteoglycans of ECM (aggrecan, brevican, and versican) (6, 10, 11).

Control of both MMP and ADAMTS activity is exerted at multiple points including transcription, translation, post-translational processing (including zymogen activation), substrate accessibility, and inhibition by naturally occurring inhibitors such as tissue inhibitors of metalloproteinase (TIMPs). Zymogen activation of both MMPs and ADAMTSs requires, minimally, the removal of the N-terminal prodomain; this can be achieved by intracellular furin-mediated cleavage, such as occurs with the MT-MMPs (12) and the ADAMTSs (7). Prodomain removal can also be achieved by the action of other MMPs, such as for the MT1-MMP-mediated activation of pro-MMP-2 (13), or through activation cascades involving co-activators such as plasmin (14). In this regard, we have been studying the activation of ADAMTS4 in a human chondrosarcoma cell line stably transfected to express the full-length protein and have found (15) that proteolytic activity (evaluated by cleavage at Glu373-Ala374 in aggrecan or cleavage at Glu141, Ala142 in versican V1) requires not only furin-mediated removal of the prodomain but also truncation of the C-terminal region of the proteinase.

Moreover, in this cell system, the C-terminal truncation is inhibited by TIMP-1 and a hydroxamate-based MMP inhibitor, suggesting a requirement for a TIMP-1-sensitive MMP in the activation process, an idea first suggested by the finding of TIMP-1 and n-carboxyalkyl peptide inhibition of IL-1-mediated aggrecanolysis in bovine cartilage explants (16).

Moreover, in previous work on the induction of aggrecanase-mediated aggrecan degradation in rat chondrosarcoma cells...
and bovine cartilage explants (17, 18), we found that both IL-1 and retinoic acid-induced aggrecanase was markedly inhibited by agents (such as mannosamine, 2-deoxyxylose, and phosphatidylinoositol-specific phospholipase C (PIPLC)), which are known to interfere with the synthesis or function of glycosylphatidyl inositol (GPI)-anchored proteins. Furthermore, these agents were effective inhibitors of ADAMTS4 truncation and activation in the human chondrosarcoma cell system (15).

When taken together, these results suggest that the control of ADAMTS4 (aggrecanase-1) activity in many cell-dependent systems is controlled by activation through a TIMP-1-inhibitable, GPI-anchored MMP. In the present report, we provide data that indicate that MT4-MMP (MMP-17) (12) is the protease responsible for ADAMTS4 activation and that moreover, the activated enzyme form can be bound to the cell surface through the glycosaminoglycan chains of membrane-associated syndecan-1.

EXPERIMENTAL PROCEDURES

Materials—Mannosamine, 2-deoxyxylose, gelatin (porcine skin), anti-MT4-MMP (M3684), heparinase II (H 6512), and heparan sulfate (Sigma), protease-free. PIPLC (Pierce). PIP/PS (1:1000) was from Boehringer. Goat anti-rabbit IgG and protein A/G-agarose were from Calbiochem. Dulbecco’s modified Eagle’s medium (product number 12800 for cell growth and 23800 for serum-free culture) and culture-tested distilled water were from Invitrogen. Fetal bovine serum was from HyClone (Logan, UT). The ECL detection kit was from Amersham Life Science, and TIMP1 was supplied from Merck. JSCYNH was prepared and described previously as anti-YNHR (15). JSCNT, JSCVMA, JSCFRK, and JSCALT were raised in rabbits against the ovalbumin-conjugated peptides CGGNITEGE, CVMHVDPEEP, CCGSOSFRK, and CCGTGSALT, respectively (by Invitrogen). The antibodies were affinity purified against the relevant immunizing peptide on Sulfolink (Pierce) and used at 0.2–0.9 μg of Ig per instruction.

Chondroitinase ABC (protease-free), keratanase II, and endo-β-galactosidase were from Seikagaku Corporation (Cape Cod Associates). Purified recombinant ADAMTS4 was a kind gift from Wyeth Inc. (Boston, MA). Anti-MMP2 (rabbit Ab 45) was kindly provided by Dr. William Stetler-Stevenson (National Cancer Institute, Bethesda, MD). Antibodies mSLED and BB-4 to syndecan-1 were kindly provided by Dr. Alan Rapraeger (University of Wisconsin), and antibodies to biglycan were from Dr. Peter Roughley (19). The expression vectors to murine MT4-MMP (HA) in pDNA3.1 Zeo+ and the inactive mutant (human MT4-MMP/E/A) in PG66 were kindly provided by Dr. Motoharu Seiki (University of Tokyo). The expression vector for mouse furin (pCMV-fur) was kindly provided by Dr. Kazuhisa Nakayama of the University of Tsukuba.

Cell Culture, Co-transfection, and Inhibitor Studies—Preparation and culture of the JJO12-TS4 (human chondrosarcoma cell line stably transfected with ADAMTS4) and VA13-TS4 (human fibroblast cell line of Tsukuba). MMP (E/A) in PSG6) were kindly provided by Dr. Motoharu Seiki from Dr. Peter Roughley (19). The expression vectors to murine MT4-MMP and Syndecan-1 were inserted into viral vectors in which an inactive mutant (human MT4-MMP/E/A) and the inactive mutant (human MT4-MMP/E/A) were expressed in the absence or presence of 1.5 international units of PIPLC. After incubation for 16 h at 4 °C, 15 μl of protein A/G agarose suspension was added, and incubation was continued for 1 h on a rocker. Protein A/G agarose was bound by brief centrifugation, the supernatant was removed, and 50% was used for gelatin zymography under non-reducing conditions on 8% SDS-PAGE gels prepared to include 0.1% (w/v) gelatin. After electrophoresis, gels were washed twice for 15 min with 2.5% Triton X-100 in water, rinsed with water, and added to activating buffer (1% Triton X-100, 10 mM CaCl2, 50 mM Tris-HCl, and 100 mM NaCl, pH 7.5) at 37 °C for 16 h with rocking. After removal of buffer, the gels were stained for 30 min with Brilliant Blue R-250 (0.1% (w/v) in 50% (v/v) methanol, 1% (v/v) acetic acid) and destained in 40% (v/v) methanol, 10% (v/v) acetic acid.

Co-immunoprecipitation Protocols—For immunoprecipitation experiments, JJO12-TS4 cells were cotransfected with MT4-MMP and after 3 days were cultured in serum-free medium for 3 days as above, except that the enzyme digestions were for 2 h each, and the proteins were resuspended in gel loading buffer, and heated at 100 °C for 5 min. Finally, the beads were pelleted, and the supernatants were taken for Western analysis (15).

The effect of Ca2+ and Immunoprecipitates with Glycosaminoglycan Lyases and Analysis of HS and CS—Replicate cultures of JJO12-TS4 cells were co-transfected with MT4-MMP and switched to serum-free medium for 3 days as above. Cells (106 per treatment) were recovered from dishes by gentle agitation and pelleted for digestion with PIPLC (see above). Cells were pelleted, washed, and then digested by Chase ABC (protease-free) (0.16 unit/ml in DMEM medium, 50 mM sodium acetate, and 10 mM EDTA, pH 7.5) alone, heparinase II (2.5 units/ml in DMEM medium and 5 mM CaCl2, pH 7.5) alone, or the enzymes sequentially, in which case the cells after Chase ABC digestion were pelleted, washed and then digested by heparinase II as above. After each treatment (30 min at 37 °C), cells and supernatants were separated by brief centrifugation. In experiments in which the aggrecanase was immunoprecipitated in the proteoglycan form, the protein A/G agarose beads were treated with Chase ABC and heparinase II sequentially as described above, except that the enzyme digestions were for 2 h each, and after the Chase digestion, the CaCl2 concentration was adjusted to 20 mM before addition of the heparinase II. The total sample was added to an equal volume of 2× gel loading buffer and heated at 100 °C for 5 min. Finally, the beads were pelleted, and the supernatants were taken for Western analysis.

FACE Analysis—Cell layers were digested with proteinase K, and the solubilized glycosaminoglycan (GAG) was desalted by G50 chromatography and concentrated by lyophilization as described (21). These GAG were further purified with a column (10 μl of 0.1 ammonium acetate, pH 7.3). Buffer salts were removed by centrifugation. Chondroitinase ABC (proteinase-free) (0.16 unit/ml in DMEM medium, 50 mM sodium acetate, and 10 mM EDTA, pH 7.5) alone, heparinase II (2.5 units/ml in DMEM medium and 5 mM CaCl2, pH 7.5) alone, or the enzymes sequentially, in which case the cells after Chase ABC digestion were pelleted, washed and then digested by heparinase II as above. After each treatment (30 min at 37 °C), cells and supernatants were separated by brief centrifugation. In experiments in which the aggrecanase was immunoprecipitated in the proteoglycan form, the protein A/G agarose beads were treated with Chase ABC and heparinase II sequentially as described above, except that the enzyme digestions were for 2 h each, and after the Chase digestion, the CaCl2 concentration was adjusted to 20 mM before addition of the heparinase II. The total sample was added to an equal volume of 2× gel loading buffer and heated at 100 °C for 5 min. Finally, the beads were pelleted, and the supernatants were taken for Western analysis.
and then overlaid with water. After polymerization (5–10 min at room temperature), the water was discarded, and the cassette was filled with 2 ml of the degassed stacking gel solution (8% acrylamide, N-methylbisacrylamide (38.5:1.5), 5% (v/v) glycerol, 0.1 M Tris borate (pH 8.3), 0.25% (w/v) ammonium persulfate, and 0.5% (v/v) TEMED), and an 8–10 cm gel was inserted during polymerization. Before electrophoresis, gels were removed from the storage pouch, plates were washed with water, and combs were released from the stacking gel. The resulting sample wells were rinsed and then filled with electrophoresis buffer (0.1 M Tris, 0.09 M boric acid, and 5 mM EDTA, pH 8.3), and cassettes were placed in the Glyco electrophoresis gel tank (Prozyme, San Leandro, CA) filled with pre-cooled electrophoresis buffer. Aliquots (5 µl) of the fluorotagged samples were loaded, and products were separated by electrophoresis at 500 V (~40 mA/gel) for 80 min at 4 °C. For image analyses, glass plates were removed, and the gels were placed directly onto a UV light box. Images were captured and recorded, and fluorescent bands were quantitated using the Kodak EDAS Imaging System and corresponding software.

**Generation of the Inactive Mutant (E362Q) of ADAMTS-4**—Full-length ADAMTS-4 cDNA was obtained by reverse transcription-PCR strategy based upon the published sequence (22). Briefly, total RNA (1 µg) was isolated from human primary synoviocytes and used for cDNA synthesis with SMART kit (Clontech Laboratories, Inc., Palo Alto, CA) according to the manufacturer’s protocol. The full-length ADAMTS4 cDNA was amplified from the synoviocyte cDNA by PCR using primers designed to engineer a NotI site (5’-GGCCGGCCG-3’) at the 5’-end and an XbaI site (5’-AGATCT-3’) at the 3’-end of the gene for the convenience of subcloning it into the pcDNA 3.1(+) mammalian expression vector (Invitrogen). The primers were designed to engineer a NotI site (5’-GGCCGGCCG-3’) at the 5’-end and an EcoRV site (5’-GCGGCCGC-3’) at the 3’-end of the gene for the convenience of subcloning it into the mammalian expression vector pcDNA 3.1(+) (Invitrogen, San Diego). The pairs of primers for amplifying ADAMTS-4 were 5’-ATGCCGCGCGCCT-CAATCTGCAGAACAGAT-3’ and 5’-CCGATATCTGGCAAGGTCAC- CACTGGTCAC-3’). The cycling conditions for PCR reactions were 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 3 min. The PCR products were digested and ligated into NotI/EcoRV-digested pcDNA3.1(+) vector. The sequence of the construct was verified by DNA sequencing.

**RESULTS**

**C-terminal Truncation of ADAMTS-4 in Cell Culture Is Not Autoproteolytic but the Same Products Can Be Generated by Autoproteolysis**—Structural details of the molecular forms of ADAMTS-4, the epitope locations, and the proposed cell surface complexes described in this report are provided in a schematic (see Fig. 12). Because the C-terminal truncation and activation of ADAMTS4 in the JJ012 cell system was completely blocked by 125 nM TIMP-1 (15) and because ADAMTS4 is not inhibited by TIMP-1 at these concentrations (23), it appeared to be unlikely that the cell-dependent process was autoproteolytic. However, it was shown recently (20) that a very similar C-terminal truncation process occurs on autoproteolytic digestion of recombinant ADAMTS4, and that this also results in marked increases in the aggrecanase activity of the recombinant enzyme (24). Therefore, to determine the relationship between the truncated forms generated in the JJ012 cell culture and by autoproteolysis, we prepared neo-epitope antibodies (JSCFRK and JSCALT) (see Fig. 12) to the C-terminals of the autoproteolytic forms (20) and compared the products from the two systems by Western analysis (Fig. 1) with the neo-epitope antibodies (Fig. 1, middle and right panels) as well as with combined antibodies to two internal peptide epitopes (JSCVMA and JSCYNH; Fig. 1, left panel). In keeping with previous reports (20, 23), the purified recombinant preparation (rTS4) was largely the p68 form, although it did contain some of the p53 form (see left panel), and the p53 form was, as expected, highly reactive with JSCFRK (center panel) but not with JSCALT (right panel). The autoproteolyzed sample (rTS4 auto) contained all three forms (left panel), and again, as expected, the p53 form was detected with JSCFRK (center panel), and the p40 form was detected with JSCALT only (right panel). The cell culture products (JJ012) migrated in the same positions as the autoproteolytic forms (left panel), and the species migrating at the p53 and p40 positions reacted with the JSCFRK (middle panel) and JSCALT (right panel) antibodies, respectively. It is clear that the neo-epitope antibody to the p53 form (middle panel) cross-reacts with the p40 form but not the p68 form, whereas the antibody to the p40 form (right panel) appears to be highly specific for that species. These data are consistent with the idea that the products generated by JJ012 cell-dependent proteolysis (JJ012, Fig. 1) result largely, if not entirely, from cleavage at the same sites (Lys584-Phe585 for p53 and Thr581-Phe582 for p40), as are cleaved autoproteolytically. We will therefore adopt the p68, p53, and p40 notation (20) for these three species in this paper (they were referred to previously as p75, p60, and p50, respectively (15)), and we will continue to refer to the full-length ADAMTS4 (containing the prodomain) as p100 (Fig. 1, left panel; see also Fig. 12).

The conclusion from the TIMP-1 data (15) that the cell-derived p53 and p40 forms are not autoproteolytic was further supported by the finding that the same species were also generated by JJ012 cells transiently expressing an inactive mutant (E362Q) of ADAMTS4 (Fig. 2, lane 2), which was shown previously (20) not to generate the p53 and p40 species by...
autoproteolysis. In addition, we found (data not shown) that the p53 and p40 species formed by cells expressing the inactive mutant reacted with the appropriate neo-epitope antibodies (JSCFRK and JSCALT, respectively) and also confirmed that the C-terminally truncated active site mutant product was indeed inactive as an aggrecanase (Fig. 2, lane 4), under conditions where the wild-type product (see Fig. 2, lane 1) was active (Fig. 2, lane 3).

**Identification by Immunodepletion of MT4-MMP as the Major Gelatinase of ADAMTS4-transfected Human Chondrosarcoma Cells**—Because the C-terminal truncation and activation of ADAMTS4 in the JJ012 cells was not autoproteolytic but required a TIMP-1-inhibitable, GPI-anchored MMP, we next examined the possible role of MT4-MMP in this process. This was motivated by the finding (25) that MT4-MMP (MMP-17) is quite widely expressed in cell lines, whereas the only other known GPI-anchored MMP (MT6-MMP) appears to be very restricted in expression to neutrophils and some brain tumors (26). We examined both cell lysates and ECM preparations for MT4-MMP by Western analysis and also for gelatinase activity. This revealed major immunoreactive bands at about 64 and 105 kDa, and somewhat surprisingly, the major gelatinolytic activity in these samples also ran at 64 kDa (data not shown), consistent with its identification as MT4-MMP (27). To confirm this, portions of ECM were immunodepleted with antisera to MT4-MMP and MMP2, and the gelatin zymography clearly indicated that the 64-kDa species represented MT4-MMP (data not shown).

**C-terminal Truncation of ADAMTS4 Is Enhanced by Cotransfection with MT4-MMP and Requires Active MT4-MMP**—Having detected MT4-MMP in the JJ012-TS4 cells, consistent with it having a role in ADAMTS-4 processing (15), we next examined the effect of transient transfection with MT4-MMP on the extent of ADAMTS4 processing. Addition of 0, 0.1, 1.0, and 5.0 μg of MT4-MMP cDNA generated a dose-dependent increase in the abundance of the immunoreactive 64-kDa MT4-MMP in the cell lysates (Fig. 3, lower panel) and a parallel increase in the C-terminally truncated p53 form of ADAMTS4 in the medium (Fig. 3, upper panel, lanes 1–4), consistent with the role of MT4-MMP in this process. In a parallel experiment, cells were transiently transfected with 1 μg of cDNA for either wild-type MT4-MMP (lane 6) or the active site mutant of MT4-MMP (lane 7). Although the abundance of 64-kDa MT4-MMP was similarly increased over control in both cultures (bottom panel), an increase in p53 ADAMTS4 was only observed with the wild-type MT4-MMP (lane 6), showing that MT4-MMP is effective in this system only if it is proteolytically active.

**C-terminal Truncation of ADAMTS-4 Is Enhanced by MT4-MMP in Fibroblasts and Is Entirely Dependent on Furin-mediated Prodomain Removal**—To examine whether the requirement for MT4-MMP in C-terminal truncation of ADAMTS4 is a feature of processing in other cell types, we next examined the effect of co-transfection with furin and MT4-MMP on a human fibroblast cell line (VA-13) stably transfected with ADAMTS4 (Fig. 4). Because this cell line appears to be furin-deficient (15), transfection with ADAMTS4 alone (Fig. 4, lane 1) generated only the p100 full-length protein, whereas co-transfection with furin (Fig. 4, lane 2) generated mainly the furin-cleaved form (p68), and cotransfection with both furin and MT4-MMP (Fig. 4, lane 4) resulted in increased conversion of the p68 form to the p53 form (lane 4 versus lane 2). Interestingly, co-transfection with MT4-MMP alone (lane 3) did not alter the processing seen in control (lane 1), showing that furin-mediated cleavage to generate the p68 form (lane 2) is a prerequisite for MT4-MMP-mediated C-terminal truncation. Analysis of the media from these cultures for aggrecanase activity confirmed that only the medium containing high levels of the p53 form (Fig. 4, lane 4) was active (not shown). It is therefore clear that the combination of furin cleavage, followed by MT4-MMP cleavage processing, is a feature of fibroblast-like cells as well as chondrocytic cells.
Inhibitors of GPI Anchor Synthesis Prevent Enhanced C-terminal Truncation by Co-transfection with MT4-MMP—We had shown previously (15) that inhibitors of GPI anchor synthesis, such as 2-DFG and mannosamine, were capable of preventing ADAMTS4 C-terminal processing in JJ012-TS4 cells. To confirm that the identical pathway is operating in cells with high levels of processing attributable to co-transfection with MT4-MMP, we repeated this experiment at the same concentration of inhibitors (Fig. 5). The expected marked increase in p53 product was observed with MT4-MMP cotransfection (Fig. 5, lane 2 versus lane 1) and either 2-DFG (lane 3) or mannosamine (lane 4) were completely effective in blocking this increase. Indeed, the 2-DFG blocked even the control level of p53 formation. Analysis of portions of these media for aggrecanase activity (Fig. 5, lower panel) showed, as expected, that the Glu373-Ala374 cleaving activity was well correlated with the abundance of the p53 form.

Kinetics of Processing of ADAMTS-4 by Association with GPI-anchored MT4-MMP—We showed previously that 150 nm TIMP-1 or a hydroxamate-based MMP inhibitor could completely block the formation of the p53 and p40 species but, unlike the GPI anchor inhibitors, allowed for appearance of the p68 species in the ECM fraction (see Fig. 5, Ref. 15). This is consistent with a processing pathway in which the transfer of the p68 form to the extracellular compartment is dependent on its association with intracellular MT4-MMP, followed by presentation of the complex on the cell surface for MT4-MMP-mediated C-terminal truncation (see the schematic in Fig. 12). To further investigate features of the secretion and activation process, we next examined the effect of increasing concentrations of TIMP-1 on the abundance of the different forms in the cell-associated, ECM, and medium compartments (Fig. 6).

It can be seen that whereas 30 nm TIMP-1 was completely ineffective as an inhibitor of processing (Fig. 6, compare lanes 2, 5, and 8 to control lanes 1, 4, and 7, respectively), 270 nm TIMP-1 completely blocked p53 formation and caused an accumulation of the p68 form in the medium and ECM and also an accumulation of the p100 form in the cells. This is consistent with a model in which the p100 form is processed intracellularly by furin cleavage to the p68 form, which in turn is secreted in association with MT4-MMP for cleavage, in a TIMP-1-sensitive step, on the cell surface.

To directly test the idea that the p68 associates with GPI-anchored MT4-MMP on the cell surface, we next examined the capacity of PIPLC treatment of isolated cells to solubilize the two proteins. As expected, cleavage of GPI anchors by PIPLC treatment released a proportion (Fig. 7A, lane 4), but not all, of the 64-kDa MT4-MMP from the cells (panel A, lane 2), whereas there was essentially no release without PIPLC (lane 3); the same pattern was observed in the release of the p68 form of ADAMTS4 (Fig. 7B, lanes 6 and 8), showing that it is released by PIPLC treatment in a manner consistent with its association with GPI-anchored cell surface MT4-MMP. Interestingly, a proportion of the p100 form was released from the cells by incubation in medium alone (Fig. 7B, lane 7), and this process was enhanced by PIPLC treatment, as seen in the disappearance from the cell pellet (lane 6 versus lane 5) and the increase in the medium (lane 8 versus lane 7). This suggests that a proportion (~50%) of the p100 form is loosely associated with
MT4-MMP and p68 ADAMTS4. Cultures of JJ012-TS4 cells were grown to ~80% confluence, and the medium was changed to growth medium containing GenePORTER and 1 μg of cDNA for MT4-MMP. After 48 h, the medium was changed to serum-free medium, and after an additional 3 days, the culture reactions were terminated by separation of the medium, ECM, and cell pellet fractions. Cell pellets were treated in serum-free medium without and with PIPLC (see “Experimental Procedures” for detail), and the released products were separated from the cells for Western analysis of both preparations with anti-MT4-MMP (panel A, lanes 1–4) and combined antibodies JSCVMA/JSCYNH (panel B, lanes 5–8).

The cells, whereas the remainder is associated with GPI-anchored MT4-MMP, indicating that in these cells, it can appear on the cell surface without removal of the prodomain. A repeat of this experiment with ADAMTS-4-transfected VA-13 fibroblast-like cells also showed that treatment with PIPLC released all of the p68 form, and again the p100 form was ~50% released in buffer alone and 50% released by PIPLC (data not shown).

To further examine the association of p68 and MT4-MMP, we next took a PIPLC-released supernatant containing both p68 and MT4-MMP and carried out bidirectional immunoprecipitation. Precipitates were prepared with (and without) anti-MT4-MMP and anti-TS4 (JSCYNH), and the resulting products were analyzed with JSCVMA/JSCYNH and anti-MT4-MMP, respectively. The results (Fig. 8) showed that the p68 and MT4-MMP were indeed associated in the PIPLC-released material and that this association was sufficiently robust to allow for immunoprecipitation with antibodies to either component. In the absence of precipitating antibodies, neither the p68 nor MT4-MMP was detected on blots (not shown), and neither species was detected in immunoprecipitates with antibodies to an irrelevant protein, human biglycan (Fig. 8, lanes 3 and 6).

Most interestingly, in contrast to the p100 and p68 species, the PIPLC treatments released little if any of the cell-associated p53 form (Fig. 7B, lane 6), suggesting that p53 is associated with a cell surface component other than GPI-anchored MT4-MMP.

The p53 Form Is Associated with CS and HS on Cells—The studies with PIPLC suggested that during or after formation of p53 by MT4-MMP cleavage, it is transferred to a different cell surface “receptor.” Because p53 has been shown to require 0.4 M NaCl for elution from heparin-Sepharose (20), we examined the possibility that it is bound to the cell surface by association with sulfated glycosaminoglycans. For this purpose, we prepared PIPLC-treated cells (as in Fig. 7, lane 6) and digested them with chondroitinase ABC alone, heparitinase II alone, or both enzymes sequentially. Western analysis (Fig. 9) showed that ~50% of p53 was released from the cells and recovered in the supernatant after treatment with chondroitinase ABC alone (Fig. 9, lanes 2 and 6) or heparitinase II alone (Fig. 9, lanes 3 and 7), and it was completely released after sequential treatment with the two enzymes (Fig. 9, lanes 4 and 8). This suggests that the p53 is associated with these cells through an interaction with both CS and HS (see Fig. 12).

To examine whether these GAG-lyase treatments digested all of the GAG on these cells (prepared as in Fig. 9, lane 1), we incubated them with chondroitinase ABC or heparitinase II or buffer alone (see “Experimental Procedures” for details) and subsequently assayed for CS or HS contents by fluorescence-assisted carbohydrate analysis. The data (Fig. 10) showed that JJ012 cells, enriched in the p53 form, contained both cell-associated HS (unsulfated, mono- and disulfated disaccharides in lane 1) and CS (predominantly 4-sulfated disaccharides in lane 3). Moreover, these cell-associated GAGs were each essentially completely removed by digestion with heparitinase II (lane 2) or chondroitinase ABC (lane 4).

The p53 Form Is Associated Only with GAGs on Syndecan-1—The only transmembrane proteoglycan shown clearly to be substituted with both CS and HS is syndecan-1 on normal mouse mammary epithelial cells (28–30). Because JJ012 cells were found to contain mRNA transcripts for this syndecan-1
A Model for ADAMTS4 Processing and Activation—The data presented (summarized in Fig. 12) suggest that ADAMTS4, and therefore perhaps other aggrecanases (ADAMTS-1, -4, -5, -9), are subject to a complex but discrete set of interactions with other cellular products during the process of secretion and cell surface activation. After furin-mediated prodomain removal, it appears that the p68 intermediate form associates with GPI-anchored MT4-MMP. Whether this association occurs intracellularly or only on the cell surface is not known, although in most experiments, PIPLC treatment eliminated >80% of the p68 from the cells (see Fig. 7, lanes 5 and 6), suggesting that there is no large pool of intracellular complex. The association appears to be quite stable, and it does not mask antibody binding sites because it can be trapped by immunoprecipitation with either anti-TS4 or anti-MT4-MMP. In addition, similar experiments with a fibroblast cell line, VA13, and primary bovine synovial cells (unpublished data) confirmed that, in these cells, the p68 form is also generated and associates with a PIPLC-releasable component on the cell surface.

During maintenance of the JJ012 cells in serum-free medium, there follows an MT4-MMP-mediated proteolysis of the p68 to generate the p53 form, which can be detected both on the cell surface (in association with the GAG chains of syndecan-1) and in the medium. By analysis with C-terminal specific neoepitope antibodies (Figs. 1 and 12), we have found that this proteolysis occurs at the Lys<sup>604</sup>-Phe<sup>605</sup> site, identified previously as an autocatalytic cleavage site (20), suggesting that this bond is particularly susceptible to proteolysis by both MT4-MMP and ADAMTS4 and perhaps other metalloproteinases. Such MT4-MMP-mediated proteolysis at Thr<sup>581</sup>-Phe<sup>582</sup> also generates the p40 form, which is generated in variable yield in this system and is not found on the cell surface but appears to be released directly to the medium, consistent with the reduction in GAG-binding affinity observed with the identical autocatalytic product (20). Proteolysis of the p68 to the p53 form was totally blocked by TIMP-1 at 270 nM (Fig. 6), and this was accompanied by accumulation of p68 in the medium and ECM but not in the cell-associated fraction, suggesting that the TIMP-1 was interacting with the MT4-MMP and resulting in a dissociation of the ADAMTS4 p68 from the complex without proteolysis. This mode of inhibition was apparently different from that provided by the GPI anchor synthesis inhibitors (Fig. 5), which resulted instead in the non-appearance of the p68, as would be expected if furin-mediated cleavage of the p100 to p68 and/or p68 translocation to the cell surface were dependent on the continued presence of GPI-anchored MT4-MMP. This conclusion provides a simple explanation for the findings (17, 18, 31) that inhibitors of GPI-anchor synthesis can block IL-1-induced aggrecanolysis in cartilage explants.

In cultures where the cell surface p68 had been depleted by proteolysis or release over 3 days in serum-free medium, the p53 form was recovered largely in the medium (see Fig. 6, lanes 1, 4, and 7); however, in some cultures, it was found that abundant p68 was still present on the cell surface, and in this case there was a similarly abundant cell surface pool of p53 (Fig. 7, lane 5), suggesting that processing was still occurring. In these cultures, the p53 was found to be associated with both CS and HS on syndecan-1 (Figs. 9–11). Although we have not shown that the CS and HS chains are present on the same core protein (as suggested in Fig. 12), the composition of the GAGs (Fig. 10) is consistent with that described for CS and HS on a single syndecan-1 core protein produced by normal epithelial cells (28, 29). It is intriguing that the p53 is found associated with both types of GAG, because this would imply that the binding of the proteinase to the GAG chains is not attributable.
to a unique structural motif found in either CS or HS but rather a more general binding property of all GAG chains on syndecan-1 for ADAMTS4. Indeed, it has been postulated (29) that the more distal HS chains on syndecan-1 may transfer bound protein to the membrane-proximal CS chains for further processing. Significantly, the finding that the p53 alone binds to syndecan-1, despite the presence of both p68 and p40 in the system, suggests that the syndecan GAG-binding activity on ADAMTS4 is masked by the spacer domain (on p68) and that both the thrombospondin-1-like and cysteine-rich regions are required for binding of p53 to GAG (see Fig. 12 for domain compositions of p68, p53, and p40). Indeed, a requirement of the thrombospondin-1 motif for binding of ADAMTS-4 to aggrecan has been described previously (32).

Whereas the synthesis of GPI-anchored MT4-MMP appears to be required for ADAMTS4 secretion and activation in this cell system, the influence of syndecan-1 on the process is not clear. Although the highly active p40 form is apparently released to the medium without association with syndecan-1, the finding of p53 bound to the CS and HS chains of syndecan-1 suggests that the MT4-MMP and syndecan-1 (both with membrane associations) may themselves be closely associated in an activation complex. Thus, it seems unlikely that the MT4-MMP-generated p53 would become freely diffusible before binding to the GAG components of syndecan-1. Whether the syndecan-1 is required as a cofactor for MT4-MMP activity in this complex is not known; however, such a requirement might explain why we have not been able to reproducibly generate the p53 and p40 forms of ADAMTS4 by incubation of the p68 form with a commercially available recombinant catalytic domain of MT4-MMP. Moreover, whether the p68 and p53 forms are active when present in these associations is unknown. Further work will establish whether this or a related activation complex is also required for processing of the other aggrecanases, ADAMTS-1, -4, -5, and -9.

Control of Aggrecanase Activity by the Activation Complex—The discovery in cartilage explants (33, 34) and human synovial fluids (35, 36) of products of the “aggrecanase”-dependent cleavage of aggrecan at the Glu373-Ala374 bond has been followed by the description of at least seven proteinases (atrolysin C (37), MMP-8 (38), MT1-MMP (39)), ADAMTS-1 (40, 41), ADAMTS-4 (42), ADAMTS-5 (43), and ADAMTS-9 (44) with the capacity to cleave at this “aggrecanase” site. Although some studies have indicated a predominant role for ADAMTS4 and/or ADAMTS5 in human osteoarthritic cartilage (44), the lack of highly specific inhibitors or assay methods and a poor understanding of the activation states of these proteinases means that the relative importance of these enzymes in aggreganolyis in different normal tissues and pathologies remains unclear. Treatment of most cartilages with IL-1, tumor necrosis factor-α, retinoic acid, fibronectin fragments, or neprilysin (16, 18, 45–52) markedly enhances aggrecanolysis in the tissue; however, there is a lack of data on the effect of these treatments on mRNA, protein, or activity levels for the different aggrecanases. Indeed, earlier work, which showed that TIMP-1 and MMP inhibitors (16, 53) or inhibitors of GPI anchor synthesis

**Fig. 11.** Demonstration of the association of ADAMTS-4 (p53) and the GAG chains of syndecan-1 by immunoprecipitation of JJ012 cell lysates. JJ012 cells were prepared (as in Fig. 7, lane 6), and a portion was digested sequentially with Chase ABC and Hepase II. Undigested and digested cell lysates were taken for immunoprecipitation with anti-syndecan-1 (B-B4), anti-ADAMTS4 (JSCYNH), anti-biglycan. Western analysis of immunoprecipitates for ADAMTS-4 with JSCVMA (lanes 1–3) and for syndecan-1 with mS1ED (lanes 4–6) is shown. See text for details. *, IgG heavy chain.

**Fig. 12.** Schematic describing the proposed processing of ADAMTS-4 on human chondrosarcoma cells. The schematic describes a process in which ADAMTS-4 (p100) and GPI-anchored MT4-MMP are processed intracellularly by furin-mediated removal of the prodomain (P) before association in the secretory pathway. The GPI-anchored complex (MT4-MMP:ADAMTS4 (p68)) moves to the cell surface, where the MT4-MMP-mediated removal of the spacer domain generates the p53 form, which can be found in association with both CS and HS on syndecan-1. The ADAMTS (p40) is generated by removal of the spacer and cysteine-rich domains, and it appears in the medium. Also shown (boxed) are the cysteine-rich domains, and it appears in the medium. Also shown (boxed) are the cysteine-rich domains, and it appears in the medium. Also shown (boxed) are the cysteine-rich domains, and it appears in the medium. Also shown (boxed) are the cysteine-rich domains, and it appears in the medium. Also shown (boxed) are the cysteine-rich domains, and it appears in the medium.

**Processing of ADAMTS4 by MT4-MMP and Syndecan-1**

10049

Downloaded from http://www.jbc.org/ by guest on July 26, 2018
therefore appears that the MT4-MMP-mediated activation readily cleaves aggrecan in the CS-attachment region only. It is clear from analysis of a range of tissues including cartilage and perhaps other tissues such as aorta (10) and brain (58) that the C-terminally processed forms appear to predominate in these tissues. In addition, it also seems very likely that MT4-MMP is responsible for ADAMTS4 activation in normal articular cartilages, because in studies with bovine cartilage explants we have shown that IL-1 treatment is accompanied by conversion of p68 to p53 and an increased abundance of extractable 64-kDa MT4-MMP. Furthermore, the inclusion of the GPI anchor synthesis inhibitor, mannosamine, in these cultures blocked the appearance of MT4-MMP and the activation of ADAMTS4 by C-terminal truncation.

If the activity of ADAMTS4 in cartilages, and perhaps in other tissues such as aorta (10) and brain (59), is primarily controlled through cell surface processing on an MT4-MMP/syndecan-1 complex, this has significant implications for the design of therapeutics aimed at controlling ADAMTS4-mediated degradation of aggrecan, versican, and brevican. Agents that could modify the synthesis, furin-mediated activation, and/or GPI anchoring of MT4-MMP might secondarily result in specific control of ADAMTS4 activation and so prevent uncontrolled aggrecanolyis in articular cartilage (60) or promote repair in the injured central nervous system, where intact versican and/or brevican may play a key role in neurite extension and synaptic formation (61). Indeed, agents that could specifically interfere with the association of ADAMTS4, MT4-MMP, and syndecan-1 on the cell surface might be particularly useful.

In summary, the present work suggests that GPI-anchored MT-MMPs and syndecans may collaborate on the surface of cells to maintain normal ECM homeostasis and also to respond to pro-inflammatory signals by increased activation of ADAMTS4 proteinases, which cleave aggregating proteoglycans.

Acknowledgments—We acknowledge the contributions in syndecan analysis made by Rhinnom Smith, who was supported by an Arthritis Foundation (Florida Chapter) Summer Student Fellowship Award.

References

1. Sternlicht, M. D., and Werb, Z. (2001) Annu. Rev. Cell Dev. Biol. 17, 463–516
2. Coussens, L. M., Fingleton, B., and Matrisian, L. M. (2002) Science 295, 2387–2392
3. Tolboom, T. C., Pieterman, E., van der Laan, W. H., Toes, R. E., Huijde, A. L., Nelissen, R. G., Breedveld, F. C., and Huizinga, T. W. (2002) Ann. Rheum. Dis. 61, 975–980
4. Schwartz, A. J., Wilson, D. A., Keegan, G. K., Gajjam, V. K., Sun, Y., Weber, T., and Zhang, J. T. (2002) Am. J. Vet. Res. 63, 1564–1579
5. Yamanishi, Y., Boyle, D. L., Clark, M., Maki, R. A., Tortorella, M. D., Arner, E., and Finestone, G. S. (2002) J. Immunol. 168, 1405–1412
6. Somerville, R. P., Longe, J. M., Jungers, K. A., Engl, J. M., Ross, M., Evansko, S., Wight, T. N., Leduc, R., and Apte, S. S. (2003) J. Biol. Chem. 278, 9505–9513
7. Cal, S., Obaya, A. L., Llamazares, M., Garahayu, C., Quesada, V., and Lopez, J. C. (2002) Gene 283, 49–62
8. Colle, A., Vandenberghie, I., Thiir, M., Lambert, C. A., Van Beveren, J., Li, S. W., Prokop, D. J., Lapiere, C. M., and Nusgens, B. V. (2002) J. Biol. Chem. 277, 5756–5766
9. Plaimauer, B., Zimmermann, K., Volkel, D., Antoine, G., Kerschbaumer, R., Jenab, P., Furlan, M., Gerritsen, H., Lammle, B., Schwarz, H. P., and Scheffelin, P. (2002) Endocrinology 143, 8026–8032
10. Sandy, J. D., Westling, J., Kenagy, R. D., Iruela-Arispe, M., Verscharen, C., Rodrigue-Mazaneque, J. C., Zimmermann, D., Lemire, J. M., Fischer, J. W., and Lohmander, L. S. (2001) J. Biol. Chem. 276, 13372–13378
11. Yamada, H., Watanabe, K., Shimomura, K., Yamasaki, M., and Yamaguchi, Y. (1995) Biochem. Biophys. Res. Commun. 216, 956–963
12. Puentes, X. S., Pendas, A. M., Llano, E., Velasco, G., and Lopez, O. (1996) Cancer Res. 56, 844–849
13. Worley, J. R., Thompson, P. B., Lee, M. H., Hutton, M., Soloway, P., Edwards, R. D., Murphy, G., and Knauper, V. (2003) Biochem. J. 372(Pt 3), 799–809
14. Hubar-Dantona, E., Ramos-DeSimone, N., Siple, J., Nagase, H., French, D. L., and Quigley, J. P. (1999) Ann. N. Y. Acad. Sci. 872, 572–587
15. Gao, G., Westling, J., Thompson, P. V., Howell, T. D., Gottschall, P. E., and Sandy, J. D. (2002) J. Biol. Chem. 277, 11034–11041
16. Bonassar, L. J., Sandy, J. D., Lark, M. W., Plaas, A. H., Frank, E. H., and Grodzinsky, A. J. (1997) Arch. Biochem. Biophys. 344, 404–412
17. Sandy, J. D., Thompson, V., Verscharen, C., and Gamett, D. (1999) Arch. Biochem. Biophys. 367, 258–264
18. Patwari, P., Kurz, B., Sandy, J. D., and Grodzinsky, A. J. (2000) Arch. Biochem. Biophys. 374, 79–85
19. Roughley, P. J., White, R. J., Magney, M. C., Liu, J., Pearce, R. H., and Mort, J. S. (1993) Biochem. J. 295(Pt 2), 421–426
20. Flannery, C. R., Zeng, W., Corcoran, C., Collins-Racie, L. A., Chockalingam, P. A., Hebert, T. M., Mackie, S. A., McDonagh, T., Crawford, T. R., Tomkinson, K. N., LaVallie, E. R., and Morris, E. A. (2002) J. Biol. Chem. 277, 42775–42780
21. Plaas, A. H., West, L. A., and Midura, R. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 10050
22. English, W. R., Velasco, G., Stracke, J. O., Knauper, V., and Murphy, G. (2001) FEBS Lett. 491, 137–142
23. English, W. R., Puentes, X. S., Freije, J. M., Knauper, V., Amour, A., Morrey, weather, A., Lopez-Otin, C., and Murphy, G. (2001) J. Biol. Chem. 276, 14046–14055
24. English, W. R., Velasco, G., Stracke, J. O., Knauper, V., and Murphy, G. (2001) FEBS Lett. 491, 137–142
25. Knauper, V., Knauper, V., and Murphy, G. (2001) Arthritis Rheum. 44, 1405–1412
26. English, W. R., Velasco, G., Stracke, J. O., Knauper, V., and Murphy, G. (2001) FEBS Lett. 491, 137–142
37. Tortorella, M. D., Pratta, M. A., Fox, J. W., and Arner, E. C. (1998) J. Biol. Chem. 273, 5846–5850
38. Fosang, A. J., Last, K., Neame, P. J., Murphy, G., Knauper, V., Tschesche, H., Hughes, C. E., Caterson, B., and Hardingham, T. E. (1994) Biochem. J. 304(Pt 2), 347–351
39. Buttner, F. H., Hughes, C. E., Margerie, D., Lichte, A., Tschesche, H., Caterson, B., and Bartnik, E. (1998) Biochem. J. 333(Pt 1), 159–165
40. Kuno, K., Okadab, Y., Kawashimac, H., Nakamurab, H., Miyasakac, M., Ohnoa, H., and Matsushimad, K. (2000) FEBS Lett. 478, 241–245
41. Rodriguez-Manzaneque, J. C., Westling, J., Thai, S. N., Luque, A., Knauper, V., Murphy, G., Sandy, J. D., and Iruela-Arispe, M. L. (2002) Biochem. Biophys. Res. Commun. 293, 501–508
42. Tortorella, M. D., Pratta, M., Liu, R. Q., Austin, J., Ross, O. H., Abbaszade, I., Burn, T., and Arner, E. (2000) J. Biol. Chem. 275, 18566–18573
43. Vankemmelbeke, M. N., Holen, I., Wilson, A. G., Ilic, M. Z., Handley, C. J., Kelner, G. S., Clark, M., Liu, C., Maki, R. A., Burnett, D., and Buttle, D. J. (2001) Eur. J. Biochem. 268, 1259–1268
44. Malfait, A. M., Liu, R. Q., Ijiri, K., Komiya, S., and Tortorella, M. D. (2002) J. Biol. Chem. 277, 22201–22208
45. Plaa, A. H., and Sandy, J. D. (1993) Matrix 13, 135–147
46. Lark, M. W., Gordy, J. T., Weidner, J. R., Ayala, J., Kimura, J. H., Williams, H. R., Mumford, R. A., Flannery, C. R., Carlson, S. S., Iwata, M., et al. (1995) J. Biol. Chem. 270, 2550–2556
47. Arner, E. C., Hughes, C. E., Decicco, C. P., Caterson, B., and Tortorella, M. D. (1998) Osteoarthritis Cartilage 6, 214–228
48. Little, C. B., Flannery, C. R., Hughes, C. E., Mert, J. S., Roughley, P. J., Dent, C., and Caterson, B. (1999) Biochem. J. 344(Pt 1), 61–68
49. Caterson, B., Flannery, C. R., Hughes, C. E., and Little, C. B. (2000) Matrix Biol. 19, 333–344
50. Szetovalics, R., White, R. J., Roughley, P. J., and Mort, J. S. (2002) Biochem. J. 362(Pt 2), 465–472
51. Stanton, H., Ung, L., and Fosang, A. J. (2002) Biochem. J. 364(Pt 1), 181–190
52. Chevrier, A., Mort, J. S., Crine, P., Hoemann, C. D., and Buschmann, M. D. (2001) Arch. Biochem. Biophys. 396, 178–186
53. Tortorella, M. D., Malfait, A. M., Decicco, C., and Arner, E. (2001) Osteoarthritis Cartilage 9, 539–552
54. Pratta, M. A., Scherle, P. A., Yang, G., Liu, R. Q., and Newton, R. C. (2003) Arthritis Rheum. 48, 119–133
55. Buttle, D. J., Saklatvala, J., Tamai, M., and Barrett, A. J. (1992) Biochem. J. 281(Pt 1), 175–177
56. Bryson, R. A., Feltell, R., Kam, C. M., Kerrigan, J., Powe, J. C., and Buttle, D. J. (1998) Arch. Biochem. Biophys. 355, 15–25
57. Meyer, U., Benghezal, M., Imhof, I., and Conzelmann, A. (2000) Biochemistry 39, 3461–3471
58. Westling, J., Gottschall, P. E., Thompson, V. P., Cockburn, A., Perides, G., Zimmermann, D. R., and Sandy, J. D. (2004) Biochem. J. 377(Pt 3), 787–795
59. Yuan, W., Matthews, R. T., Sandy, J. D., and Gottschall, P. E. (2002) Neuroscience 114, 1091–1101
60. Arner, E. C., Pratta, M. A., Decicco, C. P., Xue, C. B., Newton, R. C., Tzakos, J. M., Magolda, R. L., and Tortorella, M. D. (1999) Ann. N. Y. Acad. Sci. 878, 92–107
61. Yamaguchi, Y. (2000) Cell Mol. Life Sci. 57, 276–289
62. Munteanu, S. E., Ilic, M. Z., and Handle, C. J. (2002) Matrix Biol. 21, 429–440
63. Kashiwagi, M., Enghild, J. J., Gendron, C., Hughes, C., Caterson, B., Itoh, Y., and Nagase, H. (December 8, 2003) J. Biol. Chem. 10.1074/jbc.M312122200
64. Sandy, J. D., and Verscharen, C. (2001) Biochem. J. 358 (Pt 3), 615–626
ADAMTS4 (Aggrecanase-1) Activation on the Cell Surface Involves C-terminal Cleavage by Glycosylphosphatidyl Inositol-anchored Membrane Type 4-Matrix Metalloproteinase and Binding of the Activated Proteinase to Chondroitin Sulfate and Heparan Sulfate on Syndecan-1

Gui Gao, Anna Plaas, Vivian P. Thompson, Sue Jin, Fengrong Zuo and John D. Sandy

J. Biol. Chem. 2004, 279:10042-10051.
doi: 10.1074/jbc.M312100200 originally published online December 29, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M312100200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 57 references, 22 of which can be accessed free at http://www.jbc.org/content/279/11/10042.full.html#ref-list-1