Suppression of Membrane-type 1 Matrix Metalloproteinase (MMP)-mediated MMP-2 Activation and Tumor Invasion by Testican 3 and Its Splicing Variant Gene Product, N-Tes

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ABSTRACT

Using expression cloning to screen a human fetal kidney cDNA library for regulator(s) of pro-matrix metalloproteinase (MMP)-2 processing mediated by membrane-type (MT) 1 MMP, we isolated a cDNA whose product interfered with pro-MMP-2 activation. It encodes the NH2-terminal 313-amino acid region of a calcium-binding proteoglycan, testican 3, with a 3-amino acid substitution at the COOH terminus and thus inhibited pro-MMP-2 activation by MT1-MMP with a 3-amino acid substitution at the COOH terminus and thus was named N-Tes. N-Tes comprises a signal peptide, a unique domain, a follistatin-like domain, and a Ca2-binding domain but lacks a COOH-terminal thyroglobulin domain and two putative glycosaminoglycan attachment sites of testican 3. Pro-MMP-2 activation by MT3-MMP was also inhibited by the coexpression of N-Tes. Immunoprecipitation analysis demonstrated direct interaction of N-Tes with either MT1-MMP or MT3-MMP. Expression of testican 1 or testican 3 but not testican 2 also inhibited pro-MMP-2 activation by either MT1-MMP or MT3-MMP. Deletion and substitution of amino acids residues in N-Tes revealed that the unique NH2-terminal domain of N-Tes is responsible for the inhibition of pro-MMP-2 activation by MT-MMPs. Expression of N-Tes and testican 3 was detected in normal brain but down-regulated in glioma tissues. Transfection of either the N-Tes or testican 3 gene into U251 glioma cells or Madin-Darby canine kidney cells transformed by erbB2 suppressed their invasive growth in collagen gel. These results suggest that both N-Tes and testican 3 would interfere with tumor invasion by inhibiting MT-MMPs.

INTRODUCTION

MMPs are a family of Zn2+-dependent enzymes essential for ECM turnover under normal and pathological conditions (1–3). MMPs are overexpressed in various human malignancies (1, 3). The expression of MMPs is specifically regulated at the level of transcription. However, the catalytic activity of these proteinases is further controlled at additional levels, including activation of the proenzyme and inhibition of the active enzyme by specific inhibitors, e.g., TIMPs and α-macroglobulins (4–6). Because MMPs including MMP-2, gelatinase A, and 72-kDa type IV collagenase are secreted as an inactive zymogen, activation is another key regulatory step for MMP function in vivo. The molecular environments in tumors appear conducive to MMP activation. Activated MMP-2 was specifically observed in a variety of tumor tissues, suggesting the presence of pro-MMP-2 activator(s) in tumor tissues (2, 3, 7, 8). This led to the identification of the first MT-MMP, MT1-MMP, as an activator of pro-MMP-2 expressed on the surface of tumor cells (9). To date, six MT-MMPs have been identified by cDNA cloning, and the proteolytic activation of pro-MMP-2 is a common functional role proposed for all MT-MMPs (9–13). Expression of MT1-MMP most closely correlates with the invasive phenotype of human tumors (14–18). Moreover, MT1-MMP-null mice lack activation of pro-MMP-2 and have severe defects in skeletal development and angiogenesis, suggesting an essential role for MT1-MMP in the process of angiogenesis and bone growth (19, 20). This phenotype is much more severe than that seen in the MMP-2-deficient mouse (21), suggesting that MT1-MMP functionality significantly exceeds MMP-2 activation.

The MT1-MMP mechanism of pro-MMP-2 activation has been well studied and partially defined (22–24). It is known to function in concert with the natural endogenous inhibitor TIMP-2; however, many aspects of the regulation of MT1-MMP functionality by substances such as concanavalin A, collagen, and phorbol ester are poorly defined. In this study, we have screened a human fetal kidney cDNA library for the genes that regulate pro-MMP-2 activation by MT1-MMP. We report here that pro-MMP-2 activation by MT1-MMP was inhibited by the cotransfection of a cDNA derived from alternative splicing of the proteoglycan testican 3 gene that encodes the NH2-terminal 313 amino acids of testican 3 followed by a 3-amino acid substitution at the COOH terminus.

MATERIALS AND METHODS

Reagents. DMEM was from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Primers were synthesized by Genset (Kyoto, Japan). A human fetal kidney cDNA library was obtained from EdgeBio Systems (Gaithersburg, MD).

Cell Culture. Human embryonic kidney 293T cells, U251 glioma cells, and MDCK epithelial cells were obtained from Health Science Resources Bank (Osaka, Japan) and cultured in DMEM supplemented with 5% FCS.

Expression Cloning. A human fetal kidney cDNA library constructed in pEAK8 expression vector was screened for the genes associated with regulation of MT1-MMP-mediated pro-MMP-2 processing as described previously (25).

Cloning of the Testican 1, Testican 2, Testican 3, and BM-40 cDNAs. Expression plasmids for testican 1, testican 2, testican 3, and BM-40 tagged with FLAG epitope at the COOH terminus were constructed as described previously (25). The following primers were used to obtain human testican 2, human testican 3, and mouse BM-40 cDNAs (GenBank accession numbers CAA19999, CAA40774, NM_016950, and NM_009242, respectively): (α) testican 1 5′-primer starting at nucleotide 392, GGAGATCT-AGCTCGAGCAACTCGGACTAG and 3′-primer starting at nucleotide 1751, GGAGATCT-CCATATGTACCCGACCTCATC; (β) testican 2 5′-primer starting at nucleotide 279, CAGGTCGAATTCGAGACATCGAGTGAAG and 3′-primer starting at nucleotide 1558, GGAGATCTAGATGTCGTC; (ε) testican 3 5′- primer starting at nucleotide 50, AAAGCAGCG-GTCGTC; and 3′- primer starting at nucleotide 1429, CATGAA-TTCAATGTATACATCATGCATG.

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3 The abbreviations used are: MMP, matrix metalloproteinase; ECM, extracellular matrix; TIMP, tissue inhibitor of metalloproteinase; MT, membrane-type; MDCK, Madin-Darby canine kidney; RT-PCR, reverse transcription-PCR; FS, follistatin-like; EC, extracellular Ca2+-binding; TV, thyroglobulin.

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nucleotide 83, GAG GGA TCC CAG CAT GAG GCC and 3′-primer starting at nucleotide 995, GCA GGA TCC GTG AAC TTA GAT CAC.

The cDNA fragment for each gene was PCR-amplified using mouse brain cDNA as a template with primers starting at the indicated nucleotide. Note that pro-MMP-2 processing was inhibited in 96-well microplates as described previously (25).

Materials and Methods

Fig. 1. Expression cloning. A, plasmid DNA from the human fetal kidney cDNA library was cotransfected with MMP-9, MMP-2, and MT1-MMP into 293T cells cultured in 96-well microplates as described in "Materials and Methods," and cell lysates were subjected to gelatin zymography at 48 h after transfection. Note that processing of pro-MMP-2 to the active form was inhibited in Lane 9 in the first screening. B, in the second screening, single clones of plasmid DNA were extracted and analyzed as described above. Note that pro-MMP-2 processing was inhibited in Lanes 3, 5, and 6.

Construction of Expression Plasmids for N-Tes Mutants.

The cDNA fragment for each gene was PCR-amplified using human or mouse brain cDNA as a template with primers starting at the indicated nucleotide with an extra BamHI or BglII site (underlined) as described previously (25). The amplified fragment was digested with BamHI or BglII, and inserted at BglII sites of pSG-FLAG plasmid as described previously (25), except that testican 3 cDNA was inserted between Smal and BglII sites. Nucleotide sequences of cDNA fragments were confirmed by using a LI-COR DNA sequencer.

Immunoprecipitation.

293T cells cultured in a 60-mm dish were transfected with 1 µg of N-Tes-FLAG and 3 µg of MT1-MMP expression plasmids, and 48 h after transfection, cells were labeled with 100 µCi/ml [3H]leucine at the indicated time.

Concentration and purification of the immunoprecipitated material was carried out as described previously (25).

Antigen-antibody complex was collected with 20 µl of antibody and was incubated overnight at 4 °C.

Fig. 2. Nucleotide sequence and the deduced amino acid sequence of clone 5. A, Signal sequence (U Domain) and domain structure (EC domain). The amino acid sequence of clone 5 is shown. The predicted signal sequence, unique domain, extracellular calcium-binding domain (EC domain), and COOH-terminal region with two putative glycosaminoglycan attachment sites are shown.

Fig. 3. Chromosome #4 Genomic DNA. A, N-Tes mRNA. B, Testican3 mRNA.

To create the N-Tes/testican 2 chimeric cDNA fragment, the COOH-terminal testican 2 cDNA fragment starting at nucleotide 550 was PCR-amplified with the testican 2 3′ primer and primer AAGGATCCGTGCCA-GAAGATGAAGTGCGAC, which has an extra BamHI site (underlined). The COOH-terminal fragment (nucleotides 359-1046) of N-Tes cDNA was removed by digestion with BamHI and BglII, and the amplified fragment described above was inserted to generate N-Tes/testican 2 chimeric cDNA. To generate the testican 2/N-Tes chimeric cDNA fragment, the NH2-terminal fragment of testican 2 (nucleotides 279–550) was amplified by PCR with testican 2 5′ primer and primer AGGAGATCTTCTGATATGGATTTAGGCCTTCAT-CAT, which starts from nucleotide 550 with an extra BamHI site (underlined). The amplified fragment was exchanged with NH2-terminal fragment of N-Tes cDNA (nucleotides 1–550) to generate testican 2/N-Tes chimeric cDNA.

Clinical Samples and Histology.

Fig. 4. Immunohistochemical staining of testican 3 in glioblastoma tissue sections. A, testican 3 staining in glioblastoma tissue sections. B, testican 3 staining in glioblastoma tissue sections. C, testican 3 staining in glioblastoma tissue sections.
tumors used in this study is based on the revised WHO criteria for tumors of the central nervous system (26). All of the tumor tissues were obtained at primary resection, and none of the patients had been subjected to chemotherapy or radiation therapy before resection.

**Semiquantitative RT-PCR Analysis of N-Tes and Testican 3 mRNA.** Expression of N-Tes, testican 3, and glyceraldehyde-3-phosphate dehydrogenase mRNA was examined by semiquantitative RT-PCR as described previously (17) using the following PCR primers: (a) RT-N-Tes, GAGTTGGTGCTACTGCTTCCA (5′; 1008) and GTGCTTTCTGTGGCTCATA (3′; 1289); and (b) RT-Tes-3, TGCTATGAGGACCTGAGCTC (5′; 918) and T CCACAAGCGCATGACACTGTG (3′; 1192).

**Establishment of Cell Lines Expressing N-Tes-FLAG and Testican 3-FLAG.** N-Tes-FLAG or testican 3-FLAG cDNAs were cloned in pCEP4 vector (Invitrogen) and transfected into MDCK cells transformed with erbB2 (27) or into U251 glioma cells, and transfected cells were selected under 400 μg/ml hygromycin B.

**Collagen Gel Culture.** Type I collagen gel was prepared according to the manufacturer’s instructions (Nitta Gelatin, Tokyo, Japan). A 6-μl aliquot of collagen gel containing 3 × 10^3 U251 cells was polymerized on 1 ml of collagen gel in a 35-mm dish and then sandwiched with another 1 ml of collagen gel. Gels were covered with 2 ml of culture medium and incubated for 5 days. For the MDCK-erbB2 cell invasion assay (27), 2 ml of collagen gel containing approximately 1.0 × 10^5 cells was polymerized in a 35-mm plastic dish, covered with 2 ml of culture medium, and incubated for 7 days.

**RESULTS**

**Screening of Human Fetal Kidney cDNA Library.** Plasmid DNA from a human fetal kidney cDNA library was cotransfected with MMP-2, MMP-9, and MT1-MMP cDNA into 293T cells, and cell lysates were analyzed by gelatin zymography 48 h after transfection (Fig. 1). A latent form of pro-MMP-9, a latent form of pro-MMP-2, and an activated intermediate form of MMP-2 were detected as 92-, 68-, and 64-kDa bands, respectively (Fig. 1, top panel). Processing of pro-MMP-2 to generate the activated intermediate form was significantly less efficient in Lane 9 as compared with that in other lanes. Single clones of plasmid DNA were prepared from the pool of plasmid DNA transfected into cells for Lane 9, and cells transfected with each clone of plasmid DNA were examined by gelatin zymography (Fig. 1, bottom panel). The activated intermediate form of MMP-2 was not observed in Lanes 3, 5, and 6. Each of the plasmid DNAs transfected into cells for Lanes 3, 5, and 6 contained an insert fragment of 1.4 kb, and their nucleotide sequence was determined (DNA Data Bank of Japan accession number AB056866). The nucleotide sequence of the first 1038 5′ bases was identical to that of the testican 3 gene, except that nucleotide residues 311–319 of the testican 3 gene (GenBank accession number NM_016950) were missing in clone 5, and the nucleotide sequence from nucleotide 1039 to the 3′ end (nucleotide 1510) of all three clones was unique (Fig. 2). The open reading frame was mapped between nucleotides 108 and 1046 and encoded 313 amino acids of the testican 3 NH2-terminal fragment, except that amino acid residues 64–66 of testican 3 are missing, and the COOH-terminal 3 amino acid residues were substituted. However, a testican 3 cDNA that we obtained by PCR amplification of a human brain cDNA library as a template also did not contain the 9 nucleotide residues from 311 to 319, suggesting that their deletion is not specific to clone 5. Because clone 5 encodes the NH2-terminal 316 amino acid residues of testican 3, the gene product was designated as N-Tes. The 3′ half of N-Tes and the testican 3 cDNA sequences were found in the chromosome 4 genomic sequence database (BAC clone RP11-440L30, accession number AC020599), which indicated that the N-Tes gene is a splicing variant of the testican 3 gene. The domain structure of N-Tes was compared with that of testican 3 (Fig. 2C). Both N-Tes and testican 3 contain a hydrophobic stretch of 21 amino acids ending with a consensus signal peptidase cleavage site (28). The NH2-terminal region (residues 22–85 of N-Tes; residues 22–88 of testican 3) does not show homology to any known proteins (Fig. 2B; U, unique domain) except testican 1 and testican 2 proteins (29–31). It is followed by a cysteine-rich FS domain (residues 86–191 of N-Tes) and an EC domain (residues 87–313 of N-Tes) and, as such, is a member of a small family of FS-EC proteins. BM-40/SPARC
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The secretion and deposit of N-Tes are mediated by proteolytic enzyme activity, and testican 3 proteins into the ECM were compared (Fig. 3). N-Tes-FLAG (37 kDa) was coprecipitated with MT1-MMP by the antibody against MT1-MMP from cells cotransfected with both genes; however, N-Tes-FLAG was not precipitated by the antibody from transfected cells cultured with the N-Tes-FLAG gene alone. MT1-MMP coprecipitated with N-Tes-FLAG or TMP-2 contained 50- and 47-kDa species; however, the 47-kDa band of MT1-MMP was faint in cells transfected with the MT1-MMP gene alone. N-Tes-FLAG (35 kDa) was precipitated with a monoclonal antibody against a FLAG epitope from cells transfected with the N-Tes-FLAG gene. The antibody against the FLAG epitope failed to precipitate MT1-MMP from cells transfected with the MT1-MMP gene alone; however, MT1-MMP was coprecipitated with N-Tes-FLAG from cells transfected with both genes by the antibody against the FLAG epitope. MT3-MMP (54 kDa) was also coprecipitated with N-Tes-FLAG by the antibody against the FLAG epitope from cells cotransfected with the MT3-MMP and N-Tes-FLAG genes, but not from cells transfected with the MT3-MMP gene alone. These results indicate that both MT1-MMP and MT3-MMP form stable complexes with N-Tes in cotransfected cells.

Mapping of the Domain of N-Tes Required for Inhibition of MT-MMPs. The effect of N-Tes expression on pro-MMP-2 processing by MT1-MMP or MT3-MMP was compared with that of testican 1, testican 2, testican 3, and BM-40 (Fig. 5). Pro-MMP-2 processing by MT1-MMP or MT3-MMP was also inhibited by the expression of testican 1 and testican 3, but expression of testican 2 or BM-40 did not show an apparent inhibitory effect.

To map the domain of N-Tes responsible for the inhibition of MT1-MMP or MT3-MMP, serial deletion mutants of N-Tes were compared for the inhibitory effect. Expression of a deletion mutant of N-Tes that consists of the NH2-terminal 122 amino acid residues (Δ122) still inhibited pro-MMP-2 activation as effectively as that of wild-type N-Tes. Expression of N-Tes deletion mutants consisting of the NH2-terminal 97 or 85 amino acid residues (Δ97 and Δ85, respectively) inhibited MT1-MMP or MT3-MMP less effectively than did wild-type N-Tes. Mutation of four cysteine residues in N-Tes mutant Δ122 to serine had no effect. However, deletion of amino acid residues 33–84 from N-Tes abolished the inhibitory effect. These results suggest that the unique NH2-terminal domain of N-Tes is required for inhibition of MT1-MMP or MT3-MMP.
To confirm the inhibitory domain of N-Tes, chimeric proteins of N-Tes and testican 2 were created and tested for their effect on pro-MMP-2 processing mediated by MT1-MMP or MT3-MMP. The chimeric protein, which consists of a signal peptide and the unique domain of N-Tes and FS, EC, TY and COOH-terminal domains of testican 2 (Fig. 5C, N-Tes/Tes-2), showed an efficient inhibitory effect. However, substitution of the signal peptide and the unique NH₂-terminal domain of N-Tes with those from testican 2 (Fig. 5C, Tes-2/N-Tes) abolished inhibition. These results confirmed that the unique NH₂-terminal domain of N-Tes is responsible for the inhibition of MT1-MMP and MT3-MMP.

**Down-Regulation of N-Tes and Testican 3 mRNA Expression in Glioma Tissues.** Expression of N-Tes and testican 3 mRNA in normal brain and glioma tissues was examined by semiquantitative RT-PCR (Fig. 6). Both N-Tes mRNA and testican 3 mRNA were detected in RNA from normal brain; however, expression of both mRNAs was down-regulated in glioma tissues. N-Tes mRNA expression in T98 and U87 glioma cell lines was at a level comparable with that in normal brain, but N-Tes mRNA expression was significantly lower in U251 cells. None of glioma cell lines expressed detectable levels of testican 3 mRNA.

**Inhibition of Tumor Invasion by N-Tes or Testican 3.** U251 glioma cells were stably transfected with N-Tes-FLAG or testican 3-FLAG gene, and expression of both genes was examined by Western blotting with antibody against the FLAG epitope (Fig. 7A). N-Tes-FLAG and testican 3-FLAG fusion protein was detected as a 37- and 55-kDa band, respectively. In Fig. 7B, parental U251 cells (Wild), U251 cells transfected with pCEP4 (Mock), N-Tes-FLAG (N-Tes), and testican 3-FLAG (Tes-3) plasmid were compared for their invasion into collagen gel. Transfection of either N-Tes-FLAG or testican 3-FLAG significantly down-regulated invasion of U251 cells.

MDCK cells transformed by erbB2 (MDCK-erbB2), which express MT1-MMP and show an invasive growth in collagen gel (27), were also transfected with N-Tes-FLAG or testican 3-FLAG plasmid, and the invasive character of transfected cells was compared (Fig. 8). Transfection of the N-Tes-FLAG or testican 3-FLAG gene did not induce any morphological change in MDCK-erbB2 cells. Parental or mock-transfected MDCK-erbB2 cells exhibited invasive growth in collagen gel, similar to that seen in our previous studies (27), but this was noticeably reduced after transfection with either the N-Tes-FLAG or testican 3-FLAG gene.

**DISCUSSION**

Testican was first defined as an unnamed chondroitin/heparan sulfate proteoglycan in seminal plasma (29). Corresponding cDNAs were isolated from human testis libraries, and the deduced protein was named testican (30). To date, three members of the testican family have been identified by cDNA cloning, with deduced amino acid homologies of 42%, 51%, and 44% for testican 1/testican 2, testican 1/testican 3, and testican 2/testican 3, respectively (30–32). In this study, we have cloned a cDNA that is derived from alternative splicing of the testican 3 gene and encodes the NH₂-terminal 313 amino acid residues of testican 3 with substitution of 3 amino acid residues at the COOH terminus (N-Tes). Testicans 1–3, and N-Tes...
SUPPRESSION OF MT1-MMP BY TESTICAN 3 AND N-Tes

Fig. 8. Suppression of invasion of MDCK-erbB2 cells. A, morphology of parental MDCK-erbB2 cells (Wild) or MDCK-erbB2 cells stably transfected with control plasmid (Mock), N-Tes-FLAG plasmid (N-Tes), or testican 3-FLAG plasmid (Tes-3). ×200. B, expression of N-Tes or testican 3 tagged with FLAG epitope was examined by Western blotting with antibody against FLAG epitope. C, culture supernatants of each culture were analyzed by gelatin zymography. D, parental U251 glioma cells (−) or cells stably transfected as described above were cultured in collagen gel as described in “Materials and Methods.”

belong to a small family of FS/EC proteins including BM-40/SPARC/ osteonectin, SC-1, and hevin, in which considerable variation exists in the NH2-terminal unique domain (reviewed in Ref. 32). N-Tes shares a similar domain structure with BM-40, which consists of a signal peptide, unique domain, FS domain, and EC domain (32). BM-40 is a matricellular protein that modulates cell adhesion and proliferation and is thought to function in tissue remodeling and angiogenesis (33–38). N-Tes was identified as an inhibitor of MT1-MMP and MT3-MMP, and the NH2-terminal unique domain of N-Tes is responsible for the inhibition of MT1-MMP and MT3-MMP. Thus, testican 3 also interfered with pro-MMP-2 activation by MT1-MMP or MT3-MMP. The unique NH2-terminal domain of N-Tes/testican 3 shares 62% homology with that of testican 1, and testican 1 also inhibited MT1-MMP or MT3-MMP. In contrast, the unique domain of testican 2 has only 54% homology with that of N-Tes/testican 3, and testican 2 failed to inhibit MT1-MMP or MT3-MMP. Although N-Tes shares the same domain organization as BM-40, the amino acid sequence of N-Tes shares only 21% homology with that of BM-40 and even less homology in the unique domain, and BM-40 did not have any inhibitory effect on pro-MMP-2 activation by MT1-MMP or MT3-MMP. In contrast, BM-40 has been reported to induce the production of MMP-3/stromelysin-1, MMP-1/collagenase, and MMP-9/gelatinase B in fibroblasts and monocytes (38) and stimulate pro-MMP-2 activation in breast cancer cell lines (39). Its stimulation of MMP-2 activation was reported to occur by modulation of MT1-MMP activity rather than transcriptional up-regulation of MT1-MMP (39); however, we did not see any increased activation when BM-40 was transfected into our cells. BM-40 is cleaved by several MMPs including MMP-2, MMP-9, MMP-7/matrilysin, and MMP-3 (40), and this cleavage causes an up to 10-fold increase in collagen binding by the EC domain (40). BM-40 positively regulates tumorigenicity and migration of tumor cells (41, 42); however, it shows tumor-suppressing activity in ovarian epithelial cells (43, 44). Such a discrepancy could be cell type dependent because the responses induced by BM-40 differ from one cell type to another.

Although we have cloned N-Tes cDNA from a human fetal kidney cDNA library, the expression level of N-Tes and testican 3 in kidney was not as high as that in rat brain.3 Expression of both N-Tes and testican 3 mRNA was detected in human brain, as was reported previously for testican 1 and testican 2 (32, 45). The fact that testican 1 and 2 mRNA expression levels are highest in the central nervous system, along with other cysteine-rich proteoglycans found in brain such as neurocan, brevican, and versican, suggests that testican may be a part of the specialized ECM of the brain and may participate in diverse steps of neurogenesis (31, 45). Although the biological function of testican family proteins has not yet been studied, they may have a similar function. The function of N-Tes is unknown at this stage. Unlike testican 3, N-Tes was not deposited in ECM in an in vitro experiment; however, its possible incorporation into ECM may depend on the ECM composition. Expression of either N-Tes or testican 3 inhibited invasion into collagen gel of U251 glioma and MDCK-erbB2 cells. Because MT1-MMP plays an important role in the invasive growth of these tumor cells in collagen gel (27), suppression of invasion by N-Tes or testican 3 may be due to inhibition of MT1-MMP. In glioma tissues, mRNA levels of MT1-MMP are enhanced, and this correlates with the invasiveness of tumors, suggesting an important role of MT1-MMP in glioma invasion (17, 18). The mRNA levels of both N-Tes and testican 3 in glioma tissues were significantly lower than those seen in normal brain. These results suggest that down-regulation of N-Tes and testican 3 expression in glioma tissues may contribute at least in part to the invasion of glioma cells.

In conclusion, we have identified N-Tes as an inhibitor of MT1-MMP and MT3-MMP and demonstrated that testican 1 and testican 3 share the same activity. Suppression of tumor invasion by the expression of either N-Tes or testican 3 may provide potential novel approaches to therapy.

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4 Unpublished data.
REFERENCES


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