Rapid activation of matrix metalloproteinase-2 by glioma cells occurs through a posttranslational MT1-MMP-dependent mechanism

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Abstract

Matrix metalloproteinase-2 (MMP-2) has been suggested to play a crucial role in tumor invasion and angiogenesis. In order to understand the mechanisms underlying proMMP-2 activation, we compared the biochemical and cellular events triggered by two potent MMP-2 activators, the lectin concanavalin A (ConA) and the cytoskeleton disrupting agent cytochalasin D (CytoD). Incubation of U87 human glioma cells for 24 h in the presence of ConA or CytoD induced a marked activation of proMMP-2 and this activation was correlated in both cases with an increase in the mRNA levels of MT1-MMP. At the protein level, proMMP-2 activation induced by CytoD or ConA strongly correlated with the appearance of a 43-kDa MT1-MMP proteolytic breakdown product in cell lysates. Interestingly, CytoD also induced a very rapid (2 h) activation of proMMP-2 that was independent of protein synthesis. Under these conditions, CytoD also promoted the rapid proteolytic breakdown of the 63 kDa pro form of MT1-MMP, resulting in the appearance of the 43 kDa MT1-MMP processed form. Overexpression of a recombinant full-length MT1-MMP protein in glioma cells resulted in the activation of proMMP-2 that was correlated with the generation of the 43 kDa fragment of the protein. By contrast, overexpression of the protein in COS-7 cells promoted proMMP-2 activation without inducing the production of the 43 kDa fragment. These results thus suggest that activation of proMMP-2 occurs through both translational and post-translational mechanisms, both involving proteolytic processing of membrane-associated MT1-MMP. This processing of MT1-MMP is, however, not essential to proMMP-2 activation but may represent a regulatory mechanism to control the activity of MT1-MMP. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Matrix metalloproteinases (MMPs) represent a growing family of zinc-dependent proteases involved in matrix turnover occurring during normal and pathological processes [1]. MMPs are secreted by cells as proenzymes that must be cleaved in order to become functional. This latency of the MMP zymogens is due to the presence of the N-terminal pro domain that shields the catalytic cleft through coordination of the catalytic zinc molecule [2]. Cleavage of the pro domains of MMPs is mediated in most
cases by soluble MMPs or by proteases of the serine families such as plasmin, plasma kallikrein and neutrophil elastase [1,3]. By contrast to most MMPs, proMMP-2 possesses a propeptide that is not susceptible to proteolytic cleavage by serine proteinases [4] and recent evidence have suggested that its activation is distinct and involves another member of the MMP family, the MT1-MMP [5]. MT-MMPs are unique among the members of the MMP family in that they contain a transmembrane domain that allows their localization to the cell surface [5,6]. Five members of this family have been described to date but most of the knowledge of this subclass has been obtained from the study of MT1-MMP function [5,7–9]. This protein acts as a membrane receptor for proMMP-2, a binding that requires TIMP-2 [7]. Cross-linking and binding experiments have revealed that the N-terminal domain of TIMP-2 binds with high affinity to the catalytic domain of MT1-MMP, followed by the binding of the C-terminal domain of proMMP-2 to the C-terminal domain of TIMP-2, resulting in the formation of a trimolecular complex [7,8]. This results in the processing of proMMP-2 by cleavage of the propeptide region of the enzyme by an adjacent MT1-MMP, followed by autoproteolysis to generate the fully active enzyme [2,9].

Since MMP-2 is constitutively secreted by many cancer cells, expression of MT1-MMP at the cell surface may thus represent a key regulatory step for MMP-2 activation and tumor invasion [5]. For this reason, many studies have focused on the pathways that are involved in the expression of MT1-MMP. Numerous studies have shown that agents that promote proMMP-2 activation such as the lectin concanavalin A [10], phorbol esters [11] or the cytoskeleton disrupting agent cytochalasin D [12,13], induce a concomitant increase in MT1-MMP mRNA, suggesting that this upregulation is necessary for proMMP-2 activation. However, the increase in MT1-MMP observed at the mRNA level is not always correlated with the presence of the protein in cell lysates and, in many cases, cells expressing high levels of MT1-MMP do not show any detectable proMMP-2 activation [14]. At the protein level, the strongest correlation existing between MT1-MMP and proMMP-2 activation is the presence of an immunoreactive 43 kDa protein that reacts with anti-MT1-MMP antibodies [15–18]. This intermediate represents a truncated form of the protein cleaved within the catalytic site and that is presumably devoid of enzymatic site [16]. It is, however, not clear how this fragment is involved in the proMMP-2 activation process and in the overall molecular events linking MT1-MMP processing to proMMP-2 activation.

In this study, we have compared the activation of proMMP-2 induced by two different agents, the lectin concanavalin A (ConA) and the cytoskeleton disrupting agent cytochalasin D (CytoD), and examined the effect of these agents on the expression and processing of the MT1-MMP protein. We show that in a glioma cell line, CytoD rapidly induces activation of proMMP-2 in a protein synthesis-independent manner and that this activation involves the processing of MT1-MMP from its 63 kDa pro form to a 43 kDa fragment. This processing of the protein is however not essential to proMMP-2 activation but may represent a regulatory mechanism to control the activity of MT1-MMP. These results suggest that changes of the actin cytoskeleton structure of cancer cells may represent a rapid stimulus triggering the degradation of extracellular matrix proteins by MMP-2.

2. Material and methods

2.1. Materials

The human glioblastoma cell line U87 and COS-7 cells were purchased from the American Tissue Culture Collection and were maintained in modified Eagle’s medium (MEM) containing 10% fetal bovine serum (HyClone Laboratories), 100 units/ml penicillin and 100 μg/ml streptomycin. Concanavalin A, cytochalasin D and APMA were purchased from Sigma. The inhibitors of signal transduction tested in this study were provided by BioMol. The anti-MT1-MMP polyclonal antibodies (raised against the hinge region) and monoclonal antibodies against human MMP-2 and TIMP-2 were from Chemicon. The TriZOL reagent was from Gibco and the Titan one-tube RT–PCR kit was from Roche Molecular Biochemicals. Reagents for electrophoresis were purchased from BioRad.
2.2. Activation of proMMP-2

For experimental purposes, cells were plated in Nunc 33-mm 6-well plastic dishes at 10^5 cells/dish, and grown in a humidified atmosphere containing 5% CO₂ and 90% air at 37°C. The extent of proMMP-2 activation was evaluated by the appearance of a 62 kDa gelatinolytic band on gelatin zymograms following stimulation of the cells. Briefly, 80% confluent U87 cells were serum-starved by overnight incubation in serum-free MEM. Under these conditions, the conditioned media contained large amounts of proMMP-2 with no detectable levels of active enzyme. To induce activation, ConA (10 μg/ml) or CytoD (1 μM) were added to the conditioned media and the cells were further incubated for different periods of time. For the inhibition studies, the cells were preincubated for 1 h in the presence of the tested inhibitors prior to the addition of CytoD or ConA.

Gelatin zymography was performed with a Mini-Protean II apparatus according to standard procedures. Briefly, the samples (20 μl of conditioned medium) were suspended in a buffer containing 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 2% sodium dodecyl sulfate (SDS) and 0.00625% bromophenol blue and loaded without heating on a 9% SDS-polyacrylamide gel containing 0.1% gelatin. Electrophoresis (SDS-PAGE) was carried out at a constant voltage of 100 V. After electrophoresis, the gels were soaked in 2.5% Triton X-100 (2×30 min) at room temperature and rinsed five times in Nanopure water. The gels were incubated 20 h at 37°C in a buffer containing 50 mM Tris-HCl (pH 7.6), 20 mM NaCl, 5 mM CaCl₂ and 0.02% Brij-58. The gels were then stained in 0.1% (w/v) Coomassie blue R-250 in 30% methanol and 10% acetic acid, and destained in the same solution without the dye.

2.3. Isolation of cell lysates and crude membrane fractions

Cells were grown in 75-cm² flasks to 80% confluence, serum-starved overnight and incubated in the absence or in the presence of CytoD or ConA as described above. After removal of the conditioned media, cells were washed two times in phosphate-buffered saline and collected by scraping. For the preparation of whole cell lysates, cells were resuspended in a buffer containing 1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl and 20 mM Tris-HCl (pH 7.4). The mixture was incubated on ice for 1 h and insoluble matter was removed by centrifugation. For the isolation of crude membrane fractions, the suspended cells were homogenized using a Polytron instrument (3×20-s bursts at 50% maximal power), centrifuged at low speed to remove cell debris and unbroken cells, and ultracentrifuged to collect the particulate fraction. The resulting membranes were resuspended in 20 mM Tris-HCl (pH 7.4) and stored at −80°C until use.

2.4. Immunoblotting procedures

Equal amounts of proteins from control and treated cells were resuspended in sample buffer and separated by SDS-PAGE, using 9% acrylamide gels. After electrophoresis, proteins were electrotransferred to a 0.45-μm pore size polyvinylidene difluoride (PVDF) membrane using a Milliblot graphite electrobetter (Millipore) in the presence of a transfer buffer containing 96 mM glycine, 10 mM Tris-HCl and 10% methanol. Hydrophobic or non-specific sites were blocked overnight at 4°C with 5% non-fat dry milk in Tris-buffere saline (150 mM Tris, 20 mM Tris-HCl, pH 7.5) containing 0.3% Tween-20 (TBST). Membranes were washed three times in TBST and incubated with the primary antibodies (1/1000 dilution) in TBST containing 3% bovine serum albumin and 0.02% sodium azide, followed by a 1-h incubation at room temperature with horseradish peroxidase-conjugated anti-rabbit IgG (1/10000 dilution) or anti-mouse (1/2500 dilution) in TBST containing 5% non-fat dry milk. PVDF membranes were washed three times in TBST and immunoreactive material was visualized by enhanced chemiluminescence (ECL, Amersham-Pharmacia Biotech).

2.5. Total RNA isolation and RT–PCR analysis

Total RNA was extracted from cultured U87 cells using the TriZOL Reagent (Life Technologies) a mono-phasic solution of phenol and guanidine isothiocyanate. RNA was quantified using absorbance at 260 nm and subjected to RT–PCR using the Titan One Tube RT–PCR kit (Roche Molecular Biochem-
icals). Primers used for the PCR reactions were custom synthesized (Sheldon Biotechnology Centre, Montreal, Quebec, Canada) and were all derived from human sequences [13]. MMP-2 and MT1-MMP primers respectively amplify a 227 bp and a 182 bp fragment. The human β-actin primers (Boehringer Mannheim), that amplify a 587 bp fragment from reverse-transcribed preparations, were used as the standard. Typically, first strand cDNA was synthesized by RT using 1 μg of total RNA, 5 units of RNase Inhibitor and 1 μl of Titan Enzyme Mix (AMV and Expand High Fidelity) which were added to a 50-μl reaction mixture containing 1.5 mM MgCl₂, 5 mM DTT, dNTP (0.2 mM each), and primers (0.4 μM each). Samples were placed for 20 min in a Perkin-Elmer GeneAmp 9700 thermocycler equilibrated at 60°C. The RT-PCR mixture was then heated to 94°C for 5 min and run for 25 (β-actin) or 30 (MMP-2 and MT1-MMP) cycles at 94°C for 30 s, 55°C for 1 min and 72°C for 1 min, followed by 10 min elongation at 72°C. Under these conditions, the three gene products were found to be at the exponential phase of the amplification [13].

PCR products were resolved on 2% agarose gels containing 1 μg/ml ethidium bromide.

2.6. Cloning and transfection of MT1-MMP

The cDNA encoding MT1-MMP was isolated by nested PCR using total cellular RNA extracted from U-87 glioblastoma cells as the template. The first amplification was performed for 30 cycles at 55°C using the oligonucleotide primers derived from nucleotides 40 to 64 (5’-TGGCGGTGCGACCCCAAGGGCTGGG-3’, sense) and 1937 to 1913 (5’-ACCCACCACCCTGCTGCCACTGG-3’, antisense) of the human MT1-MMP cDNA (GenBank accession number D26512). The resulting 1.9 kb cDNA fragment was reamplified with the oligonucleotides (5’-CAGCTGCAGGAATTCGTGGTCTCGGACCATGTCTCCCG-3’, sense) and (5’-CA-GCTGCAGATGGGCAGTCCACCTTGGTCCACGC-3’, antisense) for 40 cycles at 60°C, and the resulting 1.4 kb cDNA was subcloned into pCR-2.1 vector using the TOPO-TA cloning kit (Invitrogen). The HindIII and XbaI restriction sites present in the pCR-2.1 vector were used for subcloning into a pcDNA (3.1+) expression vector (Invitrogen). Cells were transiently transfected with the resulting plasmids using the non-liposomal formulation FUGENE-6 transfection reagent (Roche Molecular Biochemicals) and cell lysates were prepared 36 h post-transfection. Mock transfections of cultures with the pcDNA (3.1+) expression vector alone were used as controls.

3. Results

3.1. Activation of proMMP-2 by human glioblastoma cells

The U87 human glioblastoma cell line constitutively secretes large amounts of proMMP-2 into the conditioned medium and was used as a model for the study of proMMP-2 activation. We first examined the potential activation of this process by a variety of agents or culture conditions that have been used to activate proMMP-2 in other cell systems. As shown in Fig. 1A, the addition of the lectin ConA promoted a strong activation of proMMP-2, as reflected by the appearance of a 62 kDa band in gelatin zymograms. A similar activation was observed after treatment of the cells with CytoD, a well-characterized cytoskeleton-disrupting agent (Fig. 1A). The effect of CytoD was not related to a non-specific effect of the agent on cell shape since colchicine, a microtubule destabilizing drug that also induce cell rounding, had no effect on proMMP-2 activation. Other agents reported to promote activation of proMMP-2 such as PMA [11], elastin [19], and orthovanadate [20] were also examined but failed to induce proMMP-2 activation at all tested concentrations. Cells grown on fibronectin-coated dishes [17] also failed to promote detectable activation of proMMP-2. Based on these results, the activation of proMMP-2 induced by ConA and CytoD was further investigated in this cell line.

We first compared the kinetics of proMMP-2 activation induced by ConA and CytoD (Fig. 1B). In order to visualize the activation of proMMP-2 at short incubation times, the cells were serum-starved overnight to allow the production of proMMP-2 in the conditioned medium, after which CytoD and ConA were added. Under these conditions, we observed that addition of CytoD (1 μM) rapidly in-
duced the activation of proMMP-2, the active 62 kDa form of the protein being detected as early as 1 h after CytoD addition. This response of U87 cells to CytoD was distinct from that to ConA since activa-

We next examined the effect of a wide variety of inhibitors of signal transduction pathways on the rapid induction of proMMP-2 activation by CytoD. The tested inhibitors included inhibitors of protein kinases A (H-89), C (calphostin C, and GF109203) and tyrosine kinases (genistein), inhibitors of intracellular signaling intermediates such as the mitogen-activated protein kinase (PD98052), phospholipase C (U73122), phosphatidylinositol 3-kinase (LY294002), and also inhibitors of calcium channels (thapsigargin), myosin phosphatase (tautomycin) and protein synthesis (cycloheximide). Cells were serum-starved for 18 h and preincubated for 1 h with the indicated inhibitors prior to the addition of 1 μM of CytoD for 2 h. As shown in Fig. 1C, incubation of cells in the presence of CytoD for 2 h resulted in a detectable activation of proMMP-2 that was insensitive to all tested inhibitors. These results suggest that the rapid activation of proMMP-2 by CytoD occurs through a posttranslational mechanism that does not involve the activation of well-characterized signaling cascades.

![Fig. 1. proMMP-2 activation to the 62 kDa form occurs upon incubation of U87 glioma cells with ConA and CytoD. (A) Effect of various agents or culture conditions on the activation of proMMP-2 by U87 cells. U87 cells (80% confluent) were serum-starved for 18 h, followed by a 24-h incubation in the absence (lane 1) or in the presence of the following agents: 10 μg/ml concanavalin A (lane 2), 1 μM cytochalasin D (lane 3), 5 μM colchicine (lane 4), 100 nM PMA (lane 5), 100 μM orthovanadate (lane 6), 50 μg/ml elastin (lane 7). In lane 8, cells were seeded on a fibronectin matrix and serum-starved as described above. Conditioned media were collected, centrifuged to eliminate cell debris and 20 μl of the resulting media were subjected to gelatin zymography. The electrophoretic migration of APMA-activated MMP-2 is shown on the right. (B) Time course of proMMP-2 activation induced by ConA and cytochalasin D in U87 cells. Cells were serum-starved as described in A. after which 1 μM CytoD (upper panel) or 10 μg/ml of ConA (lower panel) were added. The cells were incubated for the indicated times and the extent of proMMP-2 activation was monitored by gelatin zymography. (C) Inhibition of the rapid CytoD-induced activation of proMMP-2. Cells were starved in serum-free medium for 18 h and then preincubated for 2 h in the absence (lanes 1 and 2) or in the presence of the following inhibitors: 100 nM calphostin C (lane 3), 100 nM H-89 (lane 4), 50 μM genistein (lane 5), 5 μM GF109203 (lane 6), 10 μM PD98059 (lane 7), 10 μM U73122 (lane 8), 10 μM LY294002 (lane 9), 10 nM tautomycin (lane 10), 30 nM thapsigargin (lane 11) and 5 μg/ml cycloheximide (lane 12). The extent of proMMP-2 activation induced by 2 h incubation with 1 μM CytoD was monitored as described above. Lane 1 represents conditioned media from unstimulated cells.

![Fig. 2. RT-PCR analysis of MT1-MMP and MMP-2 mRNA levels following treatment of U87 cells with ConA or CytoD. Total RNA was extracted from U87 glioblastoma cells either untreated (lanes 1) or treated for 24 h with ConA (lanes 2), or CytoD (lanes 3) or 24 h with cytochalasin D (lanes 4) and subjected to RT-PCR using primers specific for MT1-MMP, MMP-2 or β-actin as a control. The resulting PCR products (10 μl) were resolved on 2% agarose gels containing 1 μg/ml ethidium bromide. Lanes 5 represent control reactions in which reverse transcriptase was omitted.]

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Numerous studies have suggested that both ConA- and CytoD-induced activation of proMMP-2 occur through an upregulation of the MT1-MMP mRNA levels [10,12,13]. We thus examined if a similar mechanism was involved in the activation of proMMP-2 in U87 cells. Total RNA was isolated from cells treated either for 2 h or 24 h with CytoD and for 24 h with ConA, and RT–PCR was performed using specific sense and anti-sense primers for MT1-MMP, MMP-2, or β-actin as a control. As shown in Fig. 2, treatment of the cells for 2 h with CytoD had no effect on either MT1-MMP or MMP-2 mRNA levels. By contrast, 24-h incubation with CytoD or ConA induced a 2-fold increase in the mRNA levels of these proteins whereas the levels of β-actin were unchanged. These results suggest that the activation of proMMP-2 induced by 24 h incubation with CytoD and ConA is related to an upregulation of the MT1-MMP levels. However, these results support the notion that the rapid activation of proMMP-2 induced by 2 h incubation with CytoD does not involve an increase in the synthesis of the MT1-MMP protein.

3.2. Activation of proMMP-2 by glioma cells is correlated with the processing of MT1-MMP to a 43 kDa fragment

We next examined whether the increase in the mRNA levels of MT1-MMP was correlated with a similar increase in its protein levels within the cells. Immunoblotting of the control cell lysate detects a 55 kDa form of the MT1-MMP protein, reported to represent the active form of the enzyme (Fig. 3). Incubation of the cells with either ConA or CytoD induced a major activation of proMMP-2 but did not promote a significant increase in the amount of this protein, rather inducing the appearance of the MT1-MMP 43 kDa inactive form (p43) (Fig. 3A). Interestingly, the presence of the MT1-MMP 43 kDa (p43) inactive form is strongly correlated with the extent of proMMP-2 activation. For example, addition of increasing concentrations of ConA resulted in the gradual appearance of this protein that was concomitant with the presence of the active form of MMP-2 (62 kDa) in the conditioned medium (Fig. 3B). In fact, we observed throughout this study that the extent of proMMP-2 activation by glioma cells following stimulation with ConA and Cyto D (A) Cells were incubated for 24 h in the presence of 10 μg/ml of ConA or 1 μM Cyto D and proMMP-2 activation in the CM was monitored by gelatin zymography while the processing of MT1-MMP in the corresponding cell lysates was analyzed by immunoblotting with anti-MT1-MMP polyclonal antibodies. (B) Conditioned media (upper panel) and whole cell lysates (lower panel) were isolated from cells stimulated with the indicated concentrations of ConA, as described in Section 2. The extent of proMMP-2 activation was examined by immunoblotting of the CM with an anti-MMP-2 monoclonal antibody and the presence of MT1-MMP in the corresponding cell lysates was examined by immunoblotting with polyclonal antibodies raised against the hinge region of the human MT1-MMP protein. (C) Zymograms and corresponding cell lysates obtained throughout this study (n = 46) were scanned by laser densitometry. proMMP-2 activation was quantified as the ratio of the active to the inactive form whereas processing of MT1-MMP was expressed as the ratio of the 43 kDa form to the 55 kDa form.
cells was always correlated with the presence of the p43 proteolytic fragment of MT1-MMP. A large number of zymograms with various levels of activated MMP-2 were analyzed as a function of the levels of the MT1-MMP 43 kDa fragment in the corresponding lysates. To allow comparison of data from one experiment to another, we expressed the activation of proMMP-2 as the ratio of active/pro forms, whereas the levels of p43 were expressed as the ratio of p43/p55. As shown in Fig. 3C, we observed a very strong correlation between p43 levels and the extent of proMMP-2 activation.

We next examine whether the rapid (2 h) activation of proMMP-2 by CytoD also involves the processing of MT1-MMP at the cell membrane. U87 cells were treated with Cyto D or ConA as described in the legend to Fig. 1. After collecting the CM, cells were washed, homogenized and used for the isolation of a crude membrane fraction. Equal amounts of membrane proteins (10 µg per lane) were resolved on a 9% acrylamide gel and electrotransferred to PVDF membranes. The resulting membranes were immunoblotted with either anti-MT1-MMP polyclonal antibodies (upper panel), with an anti-MMP-2 monoclonal antibody (middle panel) or with an anti-TIMP-2 monoclonal antibody (lower panel).

Using this approach, we observed that membranes isolated from control cells contain the 63 kDa pro form of MT1-MMP (p63), in addition to the predominant 55 kDa form (p55) of the protein detected in crude lysates (Fig. 4). The incubation of the cells with CytoD for 2 h resulted in the complete disappearance of p63 and the appearance of the p43 breakdown product. After 24 h incubation with CytoD, p63 returned to its initial levels, while the levels of p55 and p43 remained largely unchanged. A similar profile is observed following a 24 h treatment with ConA. Interestingly, reprobing of the blot with an anti-MMP-2 monoclonal antibody showed the presence of an active membrane-associated form of proMMP-2 which correlated with the ap-
pearance of the 43 kDa form of MT1-MMP. The amount of membrane-associated TIMP-2 were not, however, altered by these treatments. These results indicate that CytoD rapidly induces activation of proMMP-2 and that this process occurs concommitantly with the processing of MT1-MMP to its 43 kDa form.

3.3. Processing of MT1-MMP to the 43 kDa form is not essential for proMMP-2 activation

To investigate whether the processing of MT1-MMP to its 43 kDa fragment represents an essential step in proMMP-2 activation, we examined the effect of overexpressed MT1-MMP in U87 and COS-7 cells. As shown in Fig. 5, the full-length protein overexpressed in both cell lines promote a significant increase in proMMP-2 activation. However, immuno-blotting of the corresponding cell lysates with anti-MT1-MMP antibodies showed very distinct processing of the protein under these conditions. In COS-7 cells, the recombinant protein migrated at its predicted molecular mass value of 63 kDa but the protein was not processed to neither the 55 or the 43 kDa fragments. By contrast, small amounts of the protein overexpressed in U87 cells remained in the 63 kDa form, the majority of the protein being processed to the 55 and 43 kDa forms. These results thus suggest that significant differences in MT1-MMP processing occurs during the activation of proMMP-2 by different cell types and that the degradation of MT1-MMP to the 43 kDa fragment is not essential to proMMP-2 activation.

4. Discussion

Activation of proMMP-2 by MT1-MMP located at the tumor cell surface is thought to represent a crucial step in tumor invasion and metastasis [21]. In gliomas, particularly, the activation of proMMP-2 has been strongly correlated with their invasive potential [22] and MT1-MMP has been suggested to play an important role in glioma invasiveness [23]. These considerations led us to investigate the factors leading to proMMP-2 activation by glioma cells as well as the involvement of the MT1-MMP protein in this process.

As reported for many cell types, the plant lectin ConA and the cytoskeleton disrupting agent CytoD were found to induce a dramatic increase in the activation of proMMP-2 by the glioma cells. The observed increase in proMMP-2 activation induced by prolonged incubation of the cells with these two agents was correlated with an upregulation of the MT1-MMP mRNA levels suggesting that it occurs through translational/transcriptional pathways. However, by contrast to ConA, CytoD was found to rapidly induce proMMP-2 activation, its effects being detectable as early as 1 h after the addition of the toxin. To our knowledge, this represents the fastest induction of proMMP-2 activation reported to date. For example, 48 h incubation with orthovanadate and 72 h incubation with β-amyloid peptide was required to observe a similar activation of proMMP-2 [20,24]. Moreover, this rapid activation was insensitive to a protein synthesis inhibitor and was not associated with an increase in MT1-MMP mRNA levels, suggesting that it occurs through the activation of a posttranslational mechanism.

At the protein level, treatment of the cells for 2 h with CytoD induced the disappearance of the pro form of MT1-MMP (63 kDa) and the concomitant appearance of the 43 kDa fragment, with no significant changes in the levels of the 55 kDa form. This proteolytic processing of MT1-MMP is correlated with the activation of membrane-associated proMMP-2. The observation that incubation of U87 cells for 2 h with CytoD is sufficient to induce the processing of MT1-MMP to its 43 kDa fragment and that under these conditions no 63 kDa is present, clearly indicates that this step is strongly associated with proMMP-2 activation in the absence of protein synthesis. Upon prolonged incubation with either ConA or CytoD, the levels of the 63 kDa form returned to their initial values, while the levels of the 55 and 43 kDa proteins moderately increased. This suggests that the 63 kDa pro form of MT1-MMP is synthesized (from the increased MT1-MMP mRNA levels) but is continuously processed to the 55 and 43 kDa fragments.

We next evaluated whether this proteolytic processing is essential to proMMP-2 activation, using a recombinant form of MT1-MMP expressed in a homologous (U87) or heterologous (COS-7) cell system. Interestingly, we observed that transfection of
the protein induced proMMP-2 activation in both cell lines but that the overexpressed protein was differentially processed in these cells. In COS-7 cells, MT1-MMP was expressed as a 63 kDa protein with no detectable levels of processed forms. By contrast, overexpression of the protein in U87 cells resulted in moderate amounts of p63 while p55 and p43 increased. These results thus suggest that the pro form of MT1-MMP has the intrinsic ability to induce proMMP-2 activation, as reported recently [25] and that the proteolytic processing of the protein does not participate in this process. Moreover, these results also suggest that active MMP-2 is not responsible for the cleavage of MT1-MMP to its 43 kDa fragment since this cleavage is not observed in COS-7 cells despite equivalent amounts of active MMP-2.

The nature of the MT1-MMP species responsible for the activation of proMMP-2 remains controversial. By analogy to other members of the MMP family, it is postulated that the active form of the protein, lacking the pro domain (55 kDa species) is responsible for the activation of proMMP-2. In this respect, co-expression of MT1-MMP and TIMP-2 in kidney epithelial cells resulted in the binding of TIMP-2 to the active form of MT1-MMP and subsequent activation of proMMP-2 [26]. By contrast, another study reported that the 63 kDa pro form of MT1-MMP was essential for the binding of TIMP-2 and proMMP-2 at the cell surface, also resulting in proMMP-2 activation [25]. However, a soluble form of MT1-MMP lacking the pro domain (55 kDa MT1-MMP) was found to be able to activate proMMP-2 [25]. This suggests that two distinct populations of MT1-MMP may be needed for efficient proMMP-2 activation: an intact 63 kDa form that is involved in the formation of the trimeric complex and a 55 kDa form that would be responsible for the catalytic cleavage of proMMP-2, and possibly for the cleavage of MT1-MMP itself to generate the 43 kDa fragment. Whether these differences in the proposed mechanisms involved in the MT1-MMP-dependent activation of proMMP-2 are related to different experimental approaches or to the complex interplay between the levels of MT1-MMP, TIMP-2 and proMMP-2 in different cell systems remain to be established. However, our results suggest that at least some of these discrepancies may arise from the use of transfected cells overexpressing MT1-MMP compared to native cell systems.

In summary, we show for the first time that tumor cells can promote a rapid and significant activation of proMMP-2 upon changes in the arrangement of their cellular actin cytoskeleton. Importantly, a significant proportion of proMMP-2 was activated at the cell surface and remained associated with the membrane and may thus contribute very actively to tumor invasion [27]. This rapid activation of proMMP-2 is performed through the activation of an endogenous pool of MT1-MMP already located at the cell membrane. Such a transcription-independent mechanism is associated with the consumption of the 63 kDa form of MT1-MMP and the concomitant appearance of the 43 kDa fragment. This is followed by an increase in the MT1-MMP mRNA levels, resulting in the reappearance of the 63 kDa protein at the cell surface, allowing sustained activation of proMMP-2 despite continuous proteolytic processing of MT1-MMP. Since this processing does not appear to be essential for activity, it may serve as a negative autoregulatory mechanism to control the amount of active MT1-MMP at the cell surface. The elucidation of the cytoskeleton-dependent pathways that promote proMMP-2 activation and the concomitant MT1-MMP processing will thus be of considerable interest for our understanding of the mechanisms underlying activation of proMMP-2 by MT1-MMP.

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References


