Caveolin-1 inhibits membrane-type 1 matrix metalloproteinase activity

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Membrane-type 1 matrix metalloproteinase (MT1-MMP) is a zinc-dependent proteinase found in cholesterol-rich lipid rafts on the plasma membrane. MT1-MMP hydrolyzes extracellular matrix (ECM) proteins, activates pro-matrix metalloproteinase-2 (proMMP-2) and plays an important role in ECM remodeling, cancer cell migration and metastasis. The role of caveolin-1, an integral protein of caveolae, in the activation of MT1-MMP remains largely unknown. Here, we show that the expression of caveolin-1 attenuates the activation of proMMP-2, reduces proteolytic cleavage of ECM and inhibits cell migration. We utilized the cytoplasmic tail domain deletion (ΔCT) or the E240A mutant of MT1-MMP. Co-expression of caveolin-1 with the wild-type or the ΔCT MT1-MMP decreased the proMMP-2 activation and inhibited collagen degradation and cell migration. Caveolin-1 had no effect on the catalytically inert E240A MT1-MMP. Our findings suggest that caveolin-1 is essential in the down-regulation of MT1-MMP activity by promoting internalization from the cell surface. [BMB reports 2008; 41(12): 858-862]

INTRODUCTION

Matrix metalloproteinase (MMP) belongs to a family of zinc-dependent proteinases that are capable of digesting components of the extracellular matrix (ECM) (1). The membrane type 1 matrix metalloproteinase (MT1-MMP) is involved in the remodeling of ECM in normal processes such as embryonic development, reproduction, and angiogenesis. In addition, it plays important roles in tumor invasion and metastasis by degrading ECM components, including collagen (2,3). MT1-MMP is synthesized as a proprotein which is activated intracellularly by furin, a proprotein convertase. The complete protein is then transported to the plasma membrane (4). The cytoplasmic tail domain of MT1-MMP interacts with intracellular regulatory proteins and modulates its translocations across the cell surface (5). The hemopexin-like domain and a nonenzymatic component of the catalytic domain of MT1-MMP are essential for MT1-MMP-mediated cell migration (6). MT1-MMP is also involved in the cleavage of cell surface receptors (1), as well as the cleavage of the proMMP-2 to an active form in a ternary complex with TIMP-2 (tissue inhibitor of metalloproteinase-2) (2,7). MMP-2 cleaves gelatin and collagen in ECM and promotes tumor progression and metastasis in invasive cancers by degrading basement membrane and interstitial connective tissue of the ECM (8).

Recently it was reported that the MT1-MMP is localized in the cholesterol-rich lipid rafts on the plasma membrane, known as caveolae (9). Caveolae are flask-shaped, 50-100 nm invaginations of the plasma membrane. They contain caveolin proteins which play a crucial role in caveolae formation. In addition, they facilitate interactions with a number of signaling molecules as a scaffolding protein, such as G protein-coupled receptors, Src-like kinases, G proteins, and nitric oxide synthase (13). Labrecque et al. showed that, following stimulation of endothelial cells with VEGF, caveolin-1 phosphorylated at Tyr14 is associated with MT1-MMP in caveolae (14). Atkinson et al. showed that cholesterol depletion in HT1080 cells accumulated MT1-MMP on the cell surface and lead to the activation of MMP-2 (15). However, the role of caveolin-1 in the regulation of MT1-MMP activity remains largely unknown.

In this study we sought to elucidate whether the MT-MMP activity in COS-7 cells is regulated by caveolin-1 by using the cytoplasmic tail domain deletion (ΔCT) or the E240A mutant of MT1-MMP. We show that caveolin-1 attenuates the activity of MT1-MMP by promoting internalization from the cell surface.
RESULTS

Expression of MT1-MMP and activation of proMMP-2

To determine the molecular mechanism of caveolin-1 in the activation of MT1-MMP and MMP-2, we employed two MT1-MMP mutants with different characteristics: ΔCT lacking the cytoplasmic tail domain and the E240A (Ala substitution for Glu240) (Fig. 1A). The E240A MT1-MMP mutant is catalytically inactive (16). The two mutants were constructed as described in Materials and Methods and the expression in COS-7 cells were determined by immunoblotting, as shown in Fig. 1B. We co-expressed caveolin-1 with each of the two MT1-MMP mutants to investigate the effect on proMMP-2 activation. ProMMP-2 is a cellular substrate for MT1-MMP. It is activated upon cleavage by MT1-MMP and it is secreted out of cells as an active protease (17). We incubated COS-7 cells expressing MT1-MMP and/or caveolin-1 with a conditioned medium of HT1080 cells known to contain large quantities of proMMP-2 (8). The activity of MMP-2 was analyzed using gelatin zymography 24 hours after transfer of conditioned medium (Fig. 1C). In the presence of MT1-MMP, proMMP-2 was cleaved into both the intermediate and mature forms of MMP-2, whereas in mock transfection, there was no cleavage of proMMP-2. Our data suggest that proMMP-2 is activated by MT1-MMP expressed on COS-7 cells, which is consistent with the observations of Sato et al. (2). GM6001, an inhibitor of MMPs, inhibited the activity of MT1-MMP completely. However, the ΔCT MT1-MMP increased the activity of MMP-2 2-fold. The deletion may have extended the time that the mutant MT1-MMP stayed on the cell surface, thus increasing its proteolytic activity. However, co-expression of caveolin-1 with the wild-type or the ΔCT MT1-MMP decreased the intensity of proMMP-2 zymogram by ~30%, suggesting that caveolin-1 promotes the internalization of MT1-MMP from the cell surface. The E240A MT1-MMP prevented the activation of MMP-2, which is consistent with earlier observations which determined that the catalytic domain is essential in promoting MT1-MMP-induced MMP-2 activity (16). Our data demonstrate that caveolin-1 negatively regulates the activity of MT1-MMP, leading to inhibition of proMMP-2 cleavage.

Effect of caveolin-1 on collagen degradation by MT1-MMP

MMPs cleave ECM components, such as collagen. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis and other connective tissue disease due to ablation of a collagenolytic activity (18). Thus, we examined the effect of caveolin-1 on collagen degradation by MT1-MMP. As shown in Fig. 2, when expressed alone in COS-7 cells in culture plates coated with collagen, the ΔCT mutant degraded 2-fold more collagen than the wild-type MT1-MMP. The E240A mutant did not degrade collagen. However, when caveolin-1 was co-expressed with the wild-type or the ΔCT mutant of MT1-MMP, collagen degradation was attenuated. These data suggest that caveolin-1 promotes the internalization of MT1-MMP from the cell surface.

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Attenuation of MT1-MMP activity by caveolin-1
Hye-Nan Kim and Hye-Shin Chung

Fig. 2. Effect of caveolin-1 on collagen degradation by MT1-MMP. COS-7 cells plated in the collagen-coated 12-well plates were transiently transfected with MT1-MMP and/or caveolin-1 cDNA. Four days post-transfection, cells were trypsinized and collagen-coated plates stained with Coomassie Brilliant Blue solution. The degradation of collagen in each well was quantified by transmittance densitometry using Gel Doc XR system (Bio-Rad). Cav-1, caveolin-1.

resulting in the decrease of collagen degradation.

Effect of caveolin-1 on cell migration
Because migration of mammalian cells is dependent on the expression of MT1-MMP and other matrix metalloproteinases, we examined whether caveolin-1 expression has an effect on the migration capabilities of COS-7 cells expressing the ΔCT or E240A mutant. Cells were transfected to collagen-coated trans-wells for 2 hours. Cells were transfected with MT1-MMP and/or caveolin-1, 5 days post-transfection, cells were soaked in methanol for 1 hour, then stained with Hematoxylin and Eosin Y. Stained cells were counted. Cav-1, caveolin-1.

DISCUSSION
The most important finding in our study is that caveolin-1, an integral protein of cholesterol-rich lipid rafts on the cell surface, inhibits the activity of MT1-MMP. MT1-MMP is crucial for extracellular matrix remodeling because it reduces collagen degradation and inhibits cell migration, thereby promoting the internalization of MT1-MMP from the cell surface. The attenuation of MT1-MMP activity by caveolin-1 also reduced the activation of proMMP-2.

Caveolae, which are cholesterol-rich lipid rafts on the cell surface, were revealed to be important in both clathrin-dependent and -independent endocytosis and signaling (10). Several signaling molecules, including GPCRs, G proteins, Src-like kinases, and nitric oxide synthase, are localized in caveolae and interact with caveolins (10). The specific localization of matrix metalloproteinases in caveolae, including MT1-MMP and MMP-2, strictly controls the activation and deactivation of the protease action in cell migration across the cell surface, as shown in cancer cell invasion and metastasis. Evidence shows that MT1-MMP is associated with caveolin-1, and that mutations of the Cys572 and Val582 residues in the cytoplasmic tail domain of MT1-MMP reduced the association of MT1-MMP and caveolin-1 (14). However, since the expected caveolin-1-binding motifs are localized in the extracellular hemopexin domain of MT1-MMP, it was suggested that these residues do not represent known caveolin-1 binding sites and that additional proteins might be involved in the regulation of phospho-caveolin-1 and MT1-MMP association (14). In our study, collagen degradation of the ΔCT MT1-MMP mutant was attenuated by co-expression of caveolin-1. Our findings also suggest the possibility of yet undefined proteins involved in the interaction of caveolin-1. The role of caveolin-1 in the association and activation of MT1-MMP in cell migration needs to be further elucidated.

Our data demonstrate that MT1-MMP is crucial for the activation of proMMP-2. This finding is consistent with the observations of Sato et al. (2). MT1-MMP-induced activation of proMMP-2 is thought to involve two molecules of MT1-MMP as a dimeric unit (19). One of the two MT1-MMP molecules forms a trimeric activation complex with TIMP-2 and proMMP-2 (19). The proMMP-2 in the trimeric complex is then activated by the other MT1-MMP molecule, which is free of TIMP-2 (20). MT1-MMP plays an important role in cancer cell migration and metastasis, and the signaling pathways leading to activation of MT1-MMP are under intense investigations for phar-
maceutical applications. The findings of this study could be utilized to learn how to inhibit the migration of cancer cells by regulating the expression of caveolin-1. However, since the inhibition of caveolin-1 also affects the movement of normal cells, this therapeutic approach will require the development of a sophisticated tool to selectively inhibit the ECM remodeling of tumor cells.

MATERIALS AND METHODS

Reagents and antibodies
Dulbecco’s Modified Eagle’s Medium (DMEM), HEPES, penicillin, and streptomycin were purchased from Gibco-BRL (Gaithersburg, MD). Fetal bovine serum was purchased from Biowhittaker (San Diego, CA). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA). Supersignal West pico chemiluminescent was purchased from Pierce. Monoclonal antibodies to MT1-MMP (Ab-1) and caveolin-1 were purchased from Calbiochem. Triton X-100, methanol, and xylene were purchased from Duksan (Korea). All other reagents, unless stated otherwise, were purchased from Sigma (St. Louis, MO).

Construction of MT1-MMP
The full length hMT1-MMP cDNA subcloned in pcDNA3.1(+) vector was kindly provided by Dr. Stephen J. Weiss (University of Michigan, Ann Arbor, USA). The cDNA for caveolin-1 was kindly provided by Dr. Choong Won Kim (Gyeongsang National University, Jinju, Korea). The cytoplasmic domain deleted ΔCT mutant was generated by PCR using the following primers; forward primer: 5'-GGCTTCCATGGCAGACG-3', reverse primer: 5'-ATGCTCGAGCCCCAGCATG-3'. The E240A MT1-MMP mutant was generated by PCR using the following primers; forward primer: 5'-GGCTTCCATGGCAGACG-3', reverse primer: 5'-ATGCTCGAGCCCCAGCATG-3'. Accuracy of the mutant constructs in the expression vector was confirmed by DNA sequence analysis.

Cell culture and transfection
COS-7 cells were cultured in DMEM supplemented with 0.02 M HEPES, 100 IU/ml penicillin, 100 μg/ml streptomycin, 44 mM NaHCO3, and 10% (v/v) fetal bovine serum. Cultured cells were transfected with Fugene 6 (Roche), according to manufacturer’s instructions. Five days post transfection cells were analyzed by DNA sequence analysis.

Gelatin zymography
Cultured medium was analyzed to examine MMP-2 activation by MT1-MMP for gelatin zymography as described by Stanton et al. (8). Conditioned medium from HT1080 cells was added to transfected COS-7 cells for 24 hours. The cell medium was collected and analyzed on SDS-PAGE with an 8.4% gel in non-reducing condition. The gel was rotated for 30 min in 2.5% Triton X-100, then incubated at 37°C for 17 hours in incubation buffer (50 mM Tris·Cl, pH 7.6, 5 mM CaCl2, 1 μM ZnCl2). The gel was observed after staining with Coomassie Brilliant Blue (Janssen).

Western blotting
Transfected cells were cultured in 12-well plates for 48 hours, then washed with PBS with 1× inhibitor mixture (1 μg/ml peptatin A, 100 μM PMSF, 1 μg/ml E-64, 10 mM EDTA). Cells were scraped in 50 μl of 2× non-reducing sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 10% glycerol, 2% β-mercaptoethanol, bromophenol blue, 1× inhibitor mixture) for 5 min on ice. Samples were separated by SDS-PAGE with a 12% separation gel. Following electrophoresis, the proteins were transferred to nitrocellulose membrane, and then blocked for 1 hour at room temperature in 5% non-fat dry milk and 0.1% Tween-20 in PBS, pH 7.4. MT1-MMP and caveolin-1 were identified using a monoclonal antibody against MT1-MMP (Ab-1) and a monoclonal antibody against caveolin-1 (Calbiochem). The detection was made with Supersignal West pico chemiluminescent (Pierce) and the films were scanned for densitometry analysis.

Collagen degradation
Type I collagen was separated from rat tail and mixed with 0.34 N NaOH and 10× DMEM in the ratio of 8 : 1 : 1. The solution was added to 12-well plates and grown for 2 hours at 37°C. The plate was washed with distilled water 3 times and dried. 1 × 106 COS-7 cells in DMEM were transfected to each well and grown for 5 hours. FBS was added to each well and cells were incubated for 17 hours. Cells were transfected with pCMT1 and/or caveolin-1 using Lipofectamine 2000, then supplemented with 20 μM GM6001. Four days post-transfection, cells were trypsinized and stained with Coomassie Brilliant. The extent of collagen degradation was determined by measurement of light transmittance to Coomassie stained gel using Gel Dox XR system (Bio-Rad).

Migration assay
Cells were cultured overnight in DMEM with 10% FBS, then detached with enzyme-free buffer. 4×105 cells were plated into trans-well coated with 1 mg/ml collagen. 90% to 95% confluent cells were transfected with Fugene 6 (Roche), according to manufacturer’s instructions. Five days post transfection cells were counted after hemotoxilin (Sigma) and Eosin Y (Sigma) treatment.

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REFERENCES


