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Involvement of syntaxin 4 in the transport of membrane-type 1 matrix metalloproteinase to the plasma membrane in human gastric epithelial cells☆

Tomohiko Miyata[a], Hirohide Ohnishi[a,*], Junko Suzuki[b], Yukako Yoshikumi[b], Hideki Ohno[b], Hirosato Mashima[b], Hiroshi Yasuda[c], Takako Ishijima[a], Hiroyuki Osawa[a], Kiichi Satoh[a], Keijiro Sunada[a], Hiroto Kita[a], Hironori Yamamoto[a], Kentaro Sugano[a]

[a] *Department of Gastroenterology, Jichi Medical School, Tochigi 329-0498, Japan*
[b] *Department of Gastroenterology, University of Tokyo School of Medicine, Tokyo 113-8655, Japan*
[c] *Division of Gastroenterology, Showa University Fujigaoka Hospital, Kanagawa 227-8501, Japan*

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Abstract

Membrane-type 1 matrix metalloproteinase (MT1-MMP) localized on the plasma membrane plays a central role in various normal biological responses including tissue remodeling, wound heeling, and angiogenesis and in cancer cell invasion and metastasis, by functioning as a collagenase and activating other matrix metalloproteinases. In order to elucidate the molecular mechanism of the MT1-MMP targeted localization on the plasma membrane, we examined the participation of syntaxin proteins in MT1-MMP intracellular transport to the plasma membrane in human gastric epithelial AGS cells. Western blotting showed that syntaxin 3 and 4 proteins, which are known to function in intracellular transport towards the plasma membrane, were expressed in AGS cells. Immunocytochemistry revealed that transient transfection of AGS cells with dominant-negative mutant syntaxin 4 decreased plasma membrane MT1-MMP expression. In contrast, transient transfection with either dominant-negative mutant syntaxin 3 or 7 did not affect MT1-MMP localization on the plasma membrane. Cell surface biotinylation assay and Matrigel chamber assay demonstrated that stable transfection with dominant-negative mutant syntaxin 4 decreased the amount of MT1-MMP on the plasma membranes and inhibited the cell invasiveness. We suggest that syntaxin 4 is involved in the intracellular transport of MT1-MMP toward the plasma membrane.

Keywords: Syntaxin; Matrix metalloproteinase; AGS cell; Intracellular transport

Matrix metalloproteinases (MMPs) are a family of zinc-binding endopeptidases that hydrolyze components of the extracellular matrix (ECM). MMPs play important roles in normal biological processes, such as embryogenesis, tissue remodeling, and wound healing, by regulating ECM organization [1]. MMPs also play promoting roles in cancer invasion and metastasis [2]. For example, MMPs regulate the degradation of ECM surrounding the tumor surface [3] and enhance neovascularization during cancer invasion and metastasis [2,4]. MMPs are either secreted from the cell (secreted type MMPs) or anchored to the plasma membrane as integral proteins (membrane type MMPs) [1]. Of these,

☆ *Abbreviations:* MT1-MMP; membrane-type 1 matrix metalloproteinase, GFP; green fluorescence protein, PBS; phosphate-buffered saline, SNARE; soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors, TIMPs; tissue inhibitors of metalloproteinases.

* Corresponding author. Fax: +81 285 44 8297.
E-mail address: hohnishi@jichi.ac.jp (H. Ohnishi).

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much attention has been recently directed to membrane type1-MMP (MT1-MMP), since MT1-MMP plays central roles in MMP regulation of ECM organization. For instances, MT1-MMP cleaves multiple ECM components, including collagen types I and III, fibronectin, vitronectin, laminin, cartilage, and proteoglycan [3]. Furthermore, MT1-MMP activates secreted-type MMPs, such as MMP2, MMP9, and MMP13. Although MT1-MMP exerts its important biological effects on the plasma membrane, the mechanism whereby MT1-MMP localizes to the plasma membranes remains unknown. The current study is designed to elucidate the molecular mechanism of MT1-MMP intracellular transport to the plasma membranes.

Newly synthesized proteins are transported from the *trans*-Golgi network in vesicles which subsequently dock and fuse with the organelle to which the protein is targeted [5]. The SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) hypothesis provides a model for the fusion events [6]. SNARE proteins present on membranous organelles, including transport vesicles, function to bridge between two membranes about to undergo membrane fusion [7]. They form cytoplasmic coiled-coil bundles with other SNARE proteins via their cytoplasmic amphipathic helices and facilitate docking and fusion of the two organelles. SNARE proteins are classified into Q- and R-SNARE, according to whether they contain a glutamine or an arginine in the central region of their helical bundles, respectively [7].

Syntaxin proteins are Q-SNARE proteins. Several syntaxins have been identified with respect to their specific localization and function, e.g., syntaxins 1, 2, 3, and 4 function in the transport of vesicles toward the plasma membrane. Syntaxin 1 plays a role in the exocytosis of synaptic vesicles, syntaxin 2 in the exocytosis of platelet dense core granules, syntaxin 3 in transport to the apical surface of intestinal epithelial cells, and syntaxin 4 in the translocation of the glucose transporter 4 [8–10]. We examined the participation of syntaxin in MT1-MMP transport in an effort to elucidate the molecular mechanism of MT1-MMP intracellular transport to the plasma membrane. Our data suggest that syntaxin 4 is involved in MT1-MMP transport to the plasma membrane.

Methods

Cell culture. AGS cells, a human gastric epithelial cancer cell line, were obtained from the American Type Culture Collection (Manassas, VA), and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 5% CO_2 atmosphere at 37 °C.

Antibodies. The following commercial antibodies were used: anti-MT1-MMP rabbit polyclonal antibody and anti-syntaxin 3 rabbit polyclonal antibody (Chemicon, Temecula, CA), anti-syntaxin 1 mouse monoclonal antibody and anti-syntaxin 7 goat polyclonal antibody (Santa Cruz, Santa Cruz, CA), anti-syntaxin 2 rabbit polyclonal antibody (Stressgen, Victoria, Canada), and anti-syntaxin 4 mouse monoclonal antibody (BD Biosciences San Jose, CA). Horseradish peroxidase-conjugated donkey anti-mouse, anti-goat, or anti-rabbit IgG antibodies and Cy3-conjugated anti-rabbit IgG antibody were obtained from Jackson ImmunoResearch (West Grove, PA).

Western blotting. Proteins were separated by electrophoresis; 10 μg of protein from each sample was loaded onto 10% SDS–polyacrylamide mini gels, run at 200 V, and subsequently transferred to nitrocellulose membranes (30 V for 3 h). Western blotting was performed as previously described [11], using the enhanced chemiluminescence reagent to visualize the secondary antibody.

Immunofluorescence microscopy. Immunofluorescence microscopy was performed as previously described [12] using a confocal laser microscopy system with an Olympus BX51 microscope (Fluoview, Olympus, Tokyo, Japan). Briefly, cells were fixed with 2% formaldehyde in phosphate-buffered saline (PBS), and incubated sequentially with Blocking Ace (Snow Bland Milk Products, Tokyo, Japan), anti-MT1-MMP antibody followed by Cy3-conjugated anti-rabbit IgG antibody. Membrane permeabilization after fixation was omitted for staining MT1-MMP on the plasma membrane. The images were digitized and then processed using Photoshop 5.0 software (Adobe Systems, Mountain View, CA, USA).

DNA and plasmid constructions. Dominant-negative mutant cDNAs of syntaxins 4 (3–273 aa) and 7 (2–234 aa) lacking their carboxyl-terminal hydrophobic regions were amplified by PCR from human liver and brain cDNA libraries (courtesy of Dr. Yukio Horikawa, Gunma University, Gunma, Japan). Dominant-negative mutant cDNA of syntaxin 3 (2–262 aa) was amplified using full-length rat syntaxin 3 cDNA as a template (courtesy of Dr. Hiromichi Shirataki, Dokkyo University School of Medicine). There is a single residue difference in the amino acid sequence (2–262) between the human and rat syntaxin 3 proteins. PCR was performed using the following primers: (a) syntaxin 3 mutant: sense 5′-AA-GGACCGACTGGAGC AGCTG-3′, anti-sense 5′-TCACTTTCGAGCCTGAC-CCTGAT ACT-3′; (b) syntaxin 4 mutant: sense 5′-GACAGGACCCACGAG-C TGAGACA-3′, antisense 5′-CTATTTCTTCTTCCTCGCCTTCT-3′; and (c) syntaxin 7 mutant: sense 5′-TCTAGATCTTACACTCCAGG AGTTGG-3′, anti-sense 5′-CTACTATCTGGATTTGCGCTGAT AATC-3′. Amplified cDNAs were verified by sequencing, subcloned into the pcDNA3.1/NT-GFP-TOPO vector (Invitrogen, Carlsbad, CA), and expressed as GFP-fusion proteins. A pcDNA3.1/NT-GFP vector was used as a control plasmid for mock transfection (Invitrogen).

Transfection. Transfection was performed using LipofectAMINE PLUS Reagent (Life Technologies, Rockville, MD), according to the manufacturer's instructions. AGS cells were seeded at a density of 10^5/cm^2 and transfected with the plasmids described above. Stable transfected cell lines and geneticin-resistant clones were selected and then their potential to express transfected GFP-tagged proteins was confirmed by fluorescence microscopy.

Cell surface biotinylation assay. The amount of MT1-MMP on the plasma membrane was examined with cell surface biotinylation assay as described previously [13]. Briefly, cells are incubated in triethanolamine (TEA buffer, pH 8.5) containing 0.5 mg Sulfo-NHS-LC Biotin (EZ-Link; Piierce, Rockford, IL) at 4 °C for 1 h. After washing with PBS, cells are resuspended in lysis buffer (20 mM Tris–HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, and 0.25% polyoxyethylene ether) at 4 °C for 1 h. Lysates were then centrifuged at 13,000*g* for 15 min and the supernatants were incubated with protein A beads at 4 °C for 1 h to remove non-specific binding proteins. After low-speed centrifugation, supernatants were incubated with Streptavidin beads (Pierce, Rockford, IL) at 4 °C for 16 h. Biotinylated MT1-MMP bound to the beads was analyzed with Western blotting with anti-MT1-MMP antibody as described above.

Cellular viability determination. To examine cellular viability, we used the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay described previously [14]. Cells were seeded (5×10^5/cm^2) into 24-well plates and incubated in PBS with 0.5 mg/ml MTT (Sigma, St. Louis, MO) for 2 h at 37 °C. Following incubation, the cells were dissolved in 0.04 N HCl in isopropanol to solubilize the dye which had been converted to purple. The amount of converted dye was measured (A_{570}) and any potential differences in the number of cells in each preparation were corrected by measuring their respective protein concentrations.

Cell invasion assay. Cell invasion was measured using a BioCoat Matrigel chamber according to manufacturer's instruction (Discovery Labware, Bedford, MA). Cells were seeded (5×10^5/cm^2) on the Matrigel-coated upper surface of the filter membrane and incubated for 22 h. Following incubation, cells on the upper surface of the membrane were removed and the cells that penetrated the Matrigel onto the lower surface of the membrane were fixed with 100% methanol and stained with Giemsa solution. Eight fields of stained cells per filter were counted under a microscope and the results were expressed as the percentage of invaded cells relative to control cells stably transfected with GFP alone.

Statistical analysis. Statistical significance was determined using ANOVA. A value of $P < 0.05$ was considered to be statistically significant.

Results

Expression of syntaxin 3 and 4 proteins in AGS cells

Four syntaxin isoforms, syntaxins 1, 2, 3, and 4, are known to participate in vesicular transport toward the plasma membrane in mammalian cells [5]. Western blots revealed that syntaxins 3 and 4 are expressed in AGS cells (Fig. 1). Although anti-syntaxin 1 and 2 antibodies recognized the respective proteins in rat crude brain lysate, no labeling was observed in AGS cell lysates. Therefore, we focused our subsequent experiments on syntaxins 3 and 4.

Dominant-negative mutant syntaxin 4 reduced MT1-MMP localized on plasma membrane

In order to determine whether or not syntaxin 3 or 4 functions in the transport of MT1-MMP to the plasma membrane, we generated AGS cells that transiently express either GFP-tagged dominant-negative mutant syntaxin 3 or 4. GFP-tagged dominant-negative mutant syntaxin 7, which functions in the late endocytotic pathway, was used as a control. The cytosolic part of syntaxin proteins interacts with its counter-partner to form a SNARE complex. The SNARE complex subsequently facilitates the docking and fusion of the two membrane compartments by functioning as a linker. However, the mutant syntaxin, which lacks the carboxyl-terminal hydrophobic region essential for localization to intracellular membranes, competes with endogenous syntaxin in binding to its counter-partner SNARE proteins. Thus, mutant syntaxin acts as a dominant-negative syntaxin [15]. As anticipated, the GFP-tagged dominant-negative mutant syntaxins 3 (Fig. 3B), 4 (Figs. 2A and D), and 7 (Fig. 3C) are localized to the cytoplasm in AGS cells. In order to study MT1-MMP localization to the plasma membrane, immunocytochemistry using anti-MT1-MMP antibody was performed without membrane permeabilization. In AGS cells, MT1-MMP on the plasma membrane was observed as small dot signals mainly at cytoplasmic region (Figs. 2 and 3). In contrast, little appreciable signals were observed on the plasma membrane at nucleus region, suggesting that MT1-MMP is not expressed uniformly on the plasma membrane (Figs. 2 and 3). Cells transiently transfected

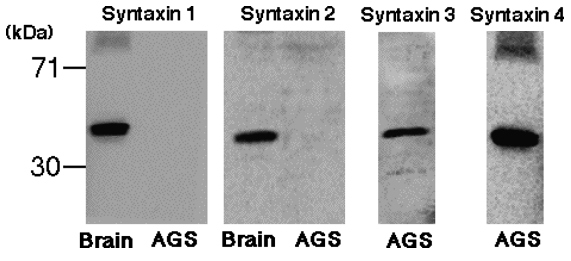

Fig. 1. Western blots of syntaxin proteins in AGS cells. Ten micrograms of AGS cell total homogenate (AGS) and rat brain (Brain) was separated on 10% sodium dodecyl sulfate mini gels and transferred to nitrocellulose membranes. Membranes were probed with anti-syntaxin 1, 2, 3, or 4 antibodies, followed by horseradish peroxidase-conjugated secondary antibodies and visualized by enhanced chemiluminescence. The molecular mass standards are indicated on the left.

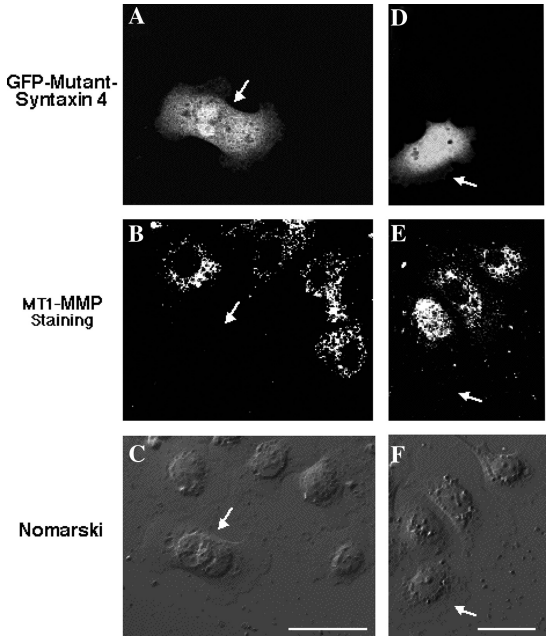

Fig. 2. Effect of dominant-negative mutant syntaxin 4 on the expression of plasma membrane MT1-MMP. (A,D) GFP-fluorescence images of transiently transfected GFP-tagged dominant-negative syntaxin 4 in AGS cells. (B,E) Anti-MT1-MMP antibody staining of the same fields as in (A) and (D), respectively. Since cell permeabilization with Triton X was omitted, only the plasma membranes are stained. (C,F) Nomarski images of the same fields as in (A) and (D), respectively. In cells transiently transfected with GFP-tagged dominant-negative mutant syntaxin 4, no discernible signals of MT1-MMP were observed on the plasma membranes (arrows). Bars: 10 μm.

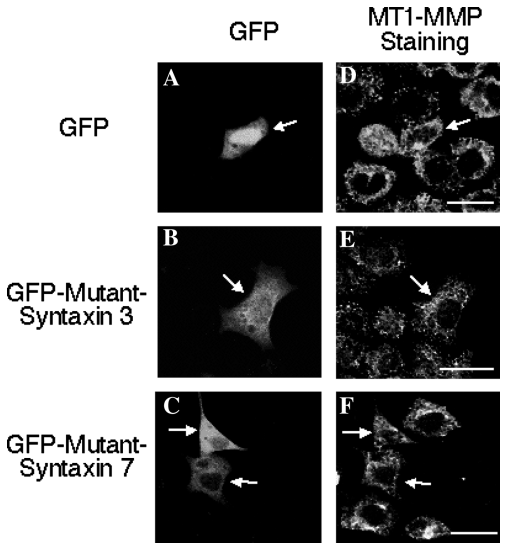

Fig. 3. Effect of dominant-negative mutant syntaxins 3 and 7 on the expression of MT1-MMP on the plasma membranes. (A, C, and E) GFP-fluorescence images of transiently transfected GFP alone (A), GFP-tagged dominant-negative syntaxin 7 (C), and GFP-tagged dominant-negative syntaxin 3 (E) in AGS cells. (B, D, and F) Images of anti-MT1-MMP antibody staining of the same fields as in (A), (C), and (D), respectively. Since cell permeabilization with Triton X was omitted, MT1-MMT signals were observed only on the plasma membranes. In cells transiently transfected with GFP alone or GFP-tagged dominant-negative mutant syntaxins 3 or 7, MT1-MMP was expressed on the plasma membranes (arrows), as well as in non-transfected cells. Bars: 10 μm.

with GFP alone (Figs. 3A and D, arrows) or dominant-negative mutant sytaxin 3 (Figs. 3B and E, arrows) or 7 (Figs. 3C and F, arrows), as well as non-transfected cells, exhibited MT1-MMP on their plasma membranes. However, there was no discernible MT1-MMP signal on the plasma membranes of cells transiently transfected with dominant-negative mutant sytaxin 4 (Fig. 2, arrows). These data suggest that the expression of dominant-negative mutant sytaxin 4 inhibits MT1-MMP localization to the plasma membrane, i.e., syntaxin 4 participates in the vesicular transport of MT1-MMP to the plasma membrane.

Effect of stable transfection with dominant-negative mutant syntaxin 4 on the amount of MT1-MMP on the plasma membrane

For quantitative estimation of the inhibitory effect of dominant-negative syntaxin 4 on MT1-MMP localization to the plasma membrane, we constructed AGS cell lines stably transfected with GFP-tagged mutant syntaxin 4, mutant syntaxin 3, mutant syntaxin 7, or GFP alone. Using these cell lines, we examined the amount of MT1-MMP on the plasma membrane with cell surface biotinylation assay. We established clones that expressed GFP-tagged mutant syntaxin 4, mutant syntaxin 3, or mutant syntaxin 7 (Fig. 4A). As anticipated, GFP-tagged mutant syntaxin proteins were localized to cytoplasm. Their intracellular localizations were consistent with those in transiently transfected cells (Figs. 2 and 3). As shown in Fig. 4B, Western blotting with anti-MT1-MMP antibody of whole lysate of these cell lines revealed that three forms of MT1-MMP, latent form (63 kDa), mature active form (60 kDa), and proteolytically inactive form (43 kDa) are expressed in these cell lines. No significant difference of MT1-MMP expression pattern was observed among these cell lines. In contrast, cell surface biotinylation assay showed that MT1-MMP mature active from (60 kDa) was predominantly present on the plasma membrane (Fig. 4C). Moreover, the amount of mature MT1-MMP on the plasma membrane in AGS cells stably transfected with dominant-negative syntaxin 4 was smaller than those in other cell lines (Fig. 4C). These data indicate that dominant-negative syntaxin 4 attenuated MT1-MMP localization on the plasma membrane, suggesting that syntaxin 4 is involved in MT1-MMP transport toward the plasma membrane.

Effect of stable transfection with dominant-negative mutant syntaxin 4 on cell migration activity of AGS cells

Using these AGS, we next examined the effect of dominant-negative syntaxin 4 expression on cell inva-

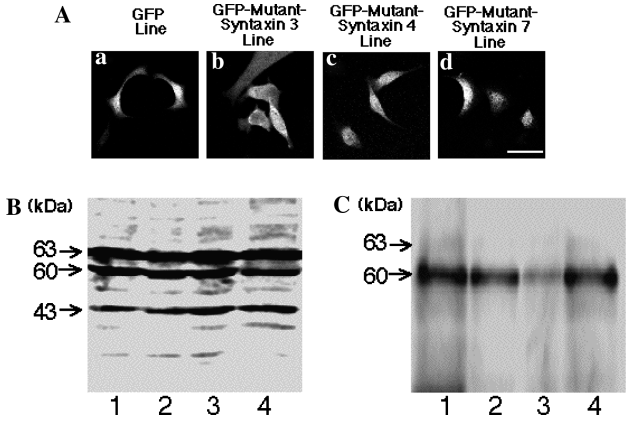

Fig. 4. Effect of stable transfection with dominant-negative mutant syntaxins on the amount of MT1-MMP on the plasma membrane. (A) Stable expression of GFP-tagged mutant syntaxin 3 (panel b), mutant syntaxin 4 (panel c), mutant syntaxin 7 (panel d), and GFP alone (panel a) was confirmed by observing the respective GFP signals by fluorescence microscopy. Bar: 10 μm. (B) Western blotting with anti-MT1-MMP antibody of whole cell lysates revealed the expression of 63-kDa band (latent MT1-MMP), 60-kDa band (mature active MT1-MMP), and 43-kDa band (proteolytically inactive MT1-MMP) in AGS cell lines stably transfected with GFP-tagged mutant syntaxin 3 (lane 2), 4 (lane 3), 7 (lane 4), and GFP alone (lane 1). (C) Cell surface biotinylation assay followed by Western blotting with anti-MT1-MMP antibody showed the smaller amount of MT1-MMP protein on the plasma membrane of AGS cell line stably transfected with dominant-negative mutant syntaxin 4 (lane 4) than those of other cell lines stably transfected with mutant syntaxin 3 (lane 2), 7 (lane 4), and GFP alone (lane 1).

sion of AGS cells. We investigated the invasiveness of these cell lines using Matrigel assay. The numbers of cells reached the lower surface of the Matrigel filter were compared among the AGS cell lines stably transfected with mutant syntaxins. Cells stably transfected with mutant syntaxins 3 or 7 or GFP alone reached the lower surface of the filter through the Matrigel (Fig. 5A; a, b, and d), whereas cells stably transfected with mutant syntaxin 4 barely reached the lower surface (Fig. 5A; c). Quantitative analysis revealed that the invasiveness of the mutant syntaxin 4 transfected cells was significantly repressed in comparison to that of the other AGS cell lines (Fig. 5B). These data suggest that the expression of dominant-negative mutant syntaxin 4 attenuates the invasiveness of AGS cells.

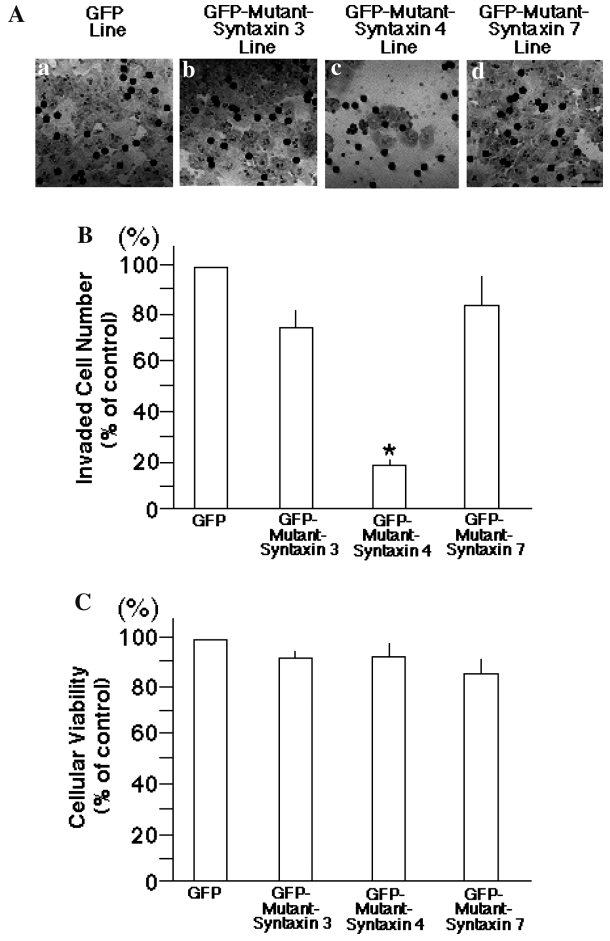

Fig. 5. Effect of stable transfection with dominant-negative mutant syntaxins on the cell invasiveness and viability of AGS cells. (A) Phase-contrast images of Giemsa-stained AGS cells on the lower surface of the Matrigel filter membranes. Cells are stably transfected with GFP alone (panel a), mutant syntaxin 3 (panel b), mutant syntaxin 4 (panel c), or mutant syntaxin 7 (panel d), and invaded the lower surface by penetrating through the Matrigel during 22 h incubation. Small black dots are pores of the filter membranes. Bar: 10 μm. (B) The numbers of each stable mutant syntaxin transfectant invading the lower surface of the filter membranes. Results are expressed as a percentage of invaded cell number of GFP mock transfected cells. Values are means ± SE for three independent experiments. Dominant-negative mutant syntaxin 4 transfection significantly attenuated the invasiveness of AGS cells. $^{*}P < 0.01$. (C) Cell viability was determined for each mutant syntaxin transfectant by MTT assay. Results are expressed as a percentage of the viability of GFP mock transfected cells. Values are means ± SE for three independent experiments. Dominant-negative mutant syntaxin 4 transfection did not affect the viability of AGS cells.

Effect of stable transfection with dominant-negative mutant syntaxin 4 on the viability of AGS cells

Although transfection with the dominant-negative mutant syntaxin 4 significantly inhibited the invasiveness of AGS cells, the possibility remains that this suppression reflects attenuated cell viability. Therefore, we compared cell viability in these AGS cell lines. As shown in Fig. 5C, the viability of cells stably transfected with dominant-negative mutant syntaxin 4 was similar to that of the other AGS cell lines. These results suggest that dominant-negative mutant syntaxin 4 attenuated the invasiveness of AGS cells by reducing MT1-MMP in the plasma membrane without affecting cell viability.

Discussion

We present evidence for the participation of syntaxin 4 in MT1-MMP transport to the plasma membrane. Western blotting documented the expression of syntaxin 4 in AGS cells. Immunocytochemistry revealed that transient transfection with dominant-negative mutant syntaxin 4 inhibited MT1-MMP localization to the plasma membrane. Further, cell surface biotinylation assay quantitatively demonstrated that stable transfection with dominant-negative mutant syntaxin 4 decreased the amount of MT1-MMP on the plasma membrane. Finally, cell invasion assay with Matrigel chamber demonstrated that cell invasiveness of AGS cells was weakened by stable transfection with dominant-negative mutant syntaxin 4. From these lines of evidence, we concluded that syntaxin 4 is involved in MT1-MMP transport to the plasma membrane. These data suggest that cell invasiveness can be attenuated by repressing MT1-MMP localization on the plasma membrane with the dominant-negative mutant syntaxin 4 expression.

Organizing ECM degradation, MMPs play pivotal roles in physiological and pathological processes, such as normal cell migration, wound healing, inflammation, cancer invasion, and metastasis [3]. More than 20 different MMPs, secreted or membrane types, have been identified in vertebrates [1]. Membrane-type MMPs (MT-MMPs) are thought to play more important roles in these biological processes than secreted-type MMPs. For instance, during the invasion of cancer cells into adjacent tissues, MT-MMPs at the cancer cell surface degrade the surrounding ECM. MT1-MMP is thought to play a major role in cancer invasion and metastasis [3]. Therefore, we focused our study on the intracellu-

lar transport mechanism of MT1-MMP. MT1-MMP is an integral membrane protein and its transmembrane/cytoplasmic domain is essential for its anchoring and spatial organization relative to the plasma membrane [16]. Recently, it was reported that MT1-MMP moves dynamically between intracellular compartments and the plasma membranes and that MT1-MMP functions on the cell surface are regulated in the balance between trafficking from intracellular pool(s) and internalization from the cell surface [13]. As to the mechanism of MT1-MMP internalization from the cell surface, Jiang et al. [17] reported that MT1-MMP on cell surface is internalized in clathrin-coated vesicles and that dynamin, a large molecular weight GTP-binding protein functioning during endocytosis as a mechanoenzyme, mediates the internalization. In addition to clathrin-mediated pathway, Remache et al. [18] reported that MT1-MMP internalization occurs through clathrin-independent but probably caveolae-dependent pathway, as well. Although molecular mechanism of MT1-MMP internalization from the cell surface has been elucidated, the mechanism of MT1-MMP transport to the cell surface is still unclear. In this respect, Zucker et al. [19] observed rapid trafficking of MT1-MMP to the cell surface possibly from *trans*-Golgi network. Moreover, Remacle et al. demonstrated that internalized MT1-MMP is recycled to the cell surface. These reports indicate that MT1-MMP is dynamically transported to the cell surface through multiple pathways. However, its molecular mechanism remains to be elucidated. In the current study, we demonstrated that inhibition of endogenous syntaxin 4 function by dominant-negative mutant syntaxin 4 expression decreased the amount of MT1-MMP on the cell surface. Since syntaxin 4 is a SNARE protein functioning in vesicle transport toward the plasma membrane, it is reasonable to conclude that syntaxin 4 is involved in the molecular mechanism of MT1-MMP transport to the cell surface.

Tissue inhibitors of metalloproteinases (TIMPs) are endogenous cellular inhibitors of MMPs. Four mammalian TIMPs (TIMP-1, -2, -3, and -4) have been identified and they appear to regulate extracellular matrix homeostasis by inhibiting MMP activity during tissue remodeling [20]. Since MMPs play critical roles in tumor invasion and metastasis, TIMPs have been considered as potential cancer therapeutic agents. Therefore, several synthetic MMP inhibitors have been developed and evaluated in clinical trials [20]. Unfortunately, the results have been disappointing and have culminated in the conclusion that MMP inhibitors have no therapeutic benefits in human cancer [21]. The failure of these clinical trials has been attributed, in part, to the complicated interactions between MMP inhibitors and MMPs in cancers [22]. For examples, in addition to its inhibitory effect on MMPs, TIMP-1 mediates MMP-2 activation by forming a complex with MT1-MMP [1]. Moreover, TIMPs have been shown to function in favor of tumor growth, i.e., TIMPs are mitogenic and anti-apoptotic for cancer cells [20]. These data contribute to the unsatisfactory results of clinical trials on synthetic MMP inhibitors and provide impetus for other therapeutic strategies. In the present study, we have demonstrated that dominant-negative syntaxin 4 expression attenuated MT1-MMP localization on the plasma membrane and inhibited invasiveness of AGS cells, which are derived from human gastric cancer. We therefore propose the possibility that inhibition of MT1-MMP localization on the plasma membrane by dominant-negative syntaxin 4 mutant expression might be a model for novel cancer-therapeutic strategy targeting MMPs.

In conclusion, we have shown that a dominant-negative mutant syntaxin 4 attenuated MT1-MMP localization on the plasma membrane and repressed cell invasiveness of AGS cells. These data provide novel insights into understanding the regulatory mechanism of MT1–MMP functions.

Acknowledgments

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