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GI24 enhances tumor invasiveness by regulating cell surface membrane-type 1 matrix metalloproteinase

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GI24, an immunoglobulin superfamily member, has been cloned from a placenta cDNA library as a gene product that promoted activation of matrix metalloproteinase (MMP)-2 mediated by membrane type (MT) 1–MMP. Co-expression of GI24 with MT1-MMP in HEK293T cells increased the cell-surface level of MT1-MMP concomitant with the cleavage of the GI24 at the juxtamembrane site to shed the extracellular domain. HT1080 fibrosarcoma cells stably transfected with the GI24 gene expressed a higher level of MT1-MMP and showed more invasive ability in collagen gel than the control cells. GI24 was cleaved in HT1080 cells, which was blocked by the administration of MMP inhibitor BB94 or transfection of small interfering RNA (siRNA) targeting MT1-MMP. GI24 expression is relatively high in some squamous carcinoma and hepatocarcinoma cell lines. Transfection of siRNA for GI24 into oral squamous carcinoma-derived HSC-4 cells, which express GI24 and MT1-MMP genes reduced the expression of not only GI24 but also MT1-MMP, and attenuated invasive growth in the collagen matrix. These results suggest that GI24 contributes to tumor-invasive growth in the collagen matrix by augmenting cell surface MT1-MMP. (*Cancer Sci* 2010; 101: 2368–2374)

Cells in tissues are surrounded by an extracellular matrix (ECM) that interacts with cells to regulate their activity.[1,2] Matrix metalloproteases (MMP) are a family of zinc-dependent enzymes that degrade components of the ECM and have been implicated in the pathological turnover of ECM in tumor invasion and metastasis.[3–5] Contemporary research divides the MMP family into two categories: (i) soluble-type MMP, which are enzymes secreted in the extracellular milieu that can diffuse and target cells in trans; and (ii) membrane-type MMP (MT-MMP), another subset of MMP, display the common structural domains of the MMP family, but present an additional C-terminal extension that tethers them to the plasma membranes, making them important effectors of pericellular ECM degradation and proteolytic activities.[6] Membrane type 1 matrix metalloprotease (MT1-MMP) was the first MT-MMP to be discovered as an activator of MMP-2 (gelatinase A),[7] but it also cleaves multiple proteins in the pericellular milieu and thereby regulates various cell functions, and has been shown to promote cancer invasion and metastasis.[8] Many substrates of MT1-MMP were identified, including ECM proteins (type I collagen, fibronectin, vitronectin, laminin-1 and -5, and others), cell adhesion molecules (CD44, syndecan-1, and αv integrin), cytokines (stromal cell-derived factor 1, transforming growth factor-β, growth differentiation factor-1 (GDF-15), and others), and latent forms of MMP-2 and MMP-13.[9–13] Processing of these proteins by MT1-MMP alters their activities and thereby regulates a variety of cellular functions, such as motility, invasion, growth, differentiation and apoptosis. In addition, to cleave different types of substrates, MT1-MMP associates with various cellular molecules such as β1 integrins,[14] CD44[15] and tetraspanin proteins such as CD63,[16] CD151,[17] CD81 and others,[18] which regulate MT1-MMP activity either positively or negatively.[19,20] Subcellular localization and internalization/recycling of MT1-MMP are considered important factors for its activity at the cell surface.[21] Thus, expression cloning strategy as a screening for molecules that regulate MT1-MMP activity on the cell surface may provide a systemic method to identify not only potential regulators but also substrates of MT1-MMP.

Platelet receptor GI24 precursor, also named stress-induced secreted protein 1 (SISP1) is a novel member of the immunoglobulin superfamily; however, the function of GI24-encoding protein remains unsolved thus far.[22] In this study, we demonstrated for the first time that GI24 serves as not only as a positive regulator but also as a substrate of MT1-MMP, and contributes at least in part to tumor invasion.

Materials and Methods

Materials. Dulbecco's modified Eagle's medium (DMEM) was from Sigma (St Louis, MO, USA). Primers were synthesized by Greiner Japan (Tokyo, Japan). A human placenta cDNA library constructed in pEAK8 expression vector was obtained from EdgeBio Systems (Gaithersburg, MD, USA). Recombinant MT1-MMP catalytic domains tagged with FLAG epitope at the COOH-terminus were prepared as described previously.[13] Monoclonal antibody against HA and GST were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. Monoclonal antibodies against FLAG epitope and tubulin were purchased from Sigma. The monoclonal antibody against MT1-MMP (222-3ER) was a gift from Daiichi Fine Chemical Co. Ltd (Takaoka, Japan). Recombinant GI24 extracellular domain protein was expressed in *Escherichia* (*E.*) *coli*, and the polyclonal antibody against GI24 was prepared by injecting the recombinant protein into rabbits.

Cell culture. Human embryonic kidney HEK293T, fibrosarcoma HT1080, breast cancer MCF-7 and human oral squamous cell carcinoma HSC-3 and HSC-4 cells were obtained from the Health Science Research Bank (Osaka, Japan) and were cultured in DMEM supplemented with 5% fetal calf serum. Hepatoma Hep3B, PLC5 and Huh7, and cholangiocarcinoma Huh28 cells were obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer of Tohoku University, and cultured in DMEM supplemented with 10% fetal calf serum.

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Plsamids. The expression plasmid for MT1-MMP or MT1-MMP tagged with FLAG epitope (MT1-F) was described previously.[23] The expression plasmid for GI24 tagged with FLAG or HA epitope at the COOH-terminus (GI24-F or GI24-HA, respectively) was constructed as follows: a GI24 cDNA fragment containing EcoRI restriction sites in place of the stop codon was generated by PCR using GI24 cDNA in a pEAK8 plasmid as a template and PCR primers of pEAK8 forward primer (TTCATTCTCAAGCCTCAGACAGTGG) and flanking reverse primer with an extra EcoRI site (underlined) starting at nucleotide 1074 (TCAGAATTCGATGACCTCAAAGTTGG) of the GI24 gene (Gene bank accession no. NM_022153). An amplified fragment was digested with EcoRI and inserted into the EcoRI site of the pEAK8-FLAG, and pEAK-HA vectors to make GI24-F and GI24-HA, respectively, as described previously.[23] The expression plasmid for GI24 tagged with FLAG epitope at NH2-terminus was constructed as follows: a GI24 cDNA fragment encoding full-length GI24 lacking the signal sequence was generated using the PCR forward primer with an extra EcoRI site (underlined) starting at nucleotide 237 (TCAGAATTCTTCAAGGTCGCCACGC) and reverse primer with an extra XbaI site (underlined) starting at nucleotide 1080 (TCA- TCTAGAGGGCTAGATGACCTCAAAGTTTGG). The PCR-amplified fragment was digested with EcoRI and XbaI and inserted into the pEAK8 vector-containing signal sequence of the testican-1 gene and the FLAG sequence.

Preparation of recombinant GI24 protein. cDNA fragment encoding amino acids 56–194 (ectodomain) was generated by PCR using a flanking forward primer with an extra *HindIII* site (underlined) starting at nucleotide 307 (Gene bank accession no. NM_022153) (GATCAAGCTTCTTGGGCCCTGTGGACAA) and a flanking reverse primer with an extra *EcoRI* site (underlined) starting at nucleotide 708 (TCAGAATCCTGCAG CCGTGATGTTT). The amplified DNA fragment was digested with *HindIII* and *EcoRI* and inserted into the *HindIII* and *EcoRI* sites of glutathione *S*-transferase (GST) plasmid.[13] GI24–GST fusion proteins were purified using glutathione-Sepharose beads according to the manufacturer's instructions (Amersham Biosciences, Upsala, Sweden).

Expression cloning. Expression cloning to identify the candidate genes whose products interact with MT1-MMP was carried out using the modified method of Miyamaori *et al.*[23] In brief, plasmid DNA from placenta cDNA library aliquots (400 ng) was co-transfected with an MT1-MMP plasmid (100 ng) into HEK293T cells cultured in a 24-well microplate. At 48 h post-transfection, cells were incubated with MMP-2 sample for 1 h, and were then subjected to gelatin zymography. A pool of cDNA, transfection of which promoted MMP2 processing, was used to transform *E. coli*. A plasmid DNA from an individual bacterial colony, expression of which promoted activation of MMP-2 by MT1-MMP, was selected and the nucleotide sequence was determined as described previously.[23]

Western blotting. Cell lysates or proteins precipitated from conditioned medium with 10% Trichloroacetic acid were analyzed using western blotting with the indicated antibodies. Goat anti-mouse or anti-rabbit IgG antibodies conjugated with Alexa Fluor 680 (Molecular Probes Inc., Eugene, OR, USA) were used as a second antibody. The signal was monitored using a LI-COR Odyssey IR imaging system (Lincoln, NE, USA). Precision Plus Protein Standards were used as molecular weight standards (Bio-Rad, Hercules, CA, USA). HT1080 cells stably expressing GI24 were established by selecting cells transfected with GI24-F plasmid (HT1080/GI24-F) in culture medium containing 0.5 μg/mL puromycin.

Cell surface biotinylation. At 48 h post-transfection, cell-surface biotinylation and immunoprecipitation were performed as described previously.[24] The immunoprecipitated materials were separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then blotted and detected with IR-Dye800-conjugated streptavidin.

Immunofluorescence staining. HT1080/GI24-F cells were fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, stained with anti-FLAG M2 antibody, and examined on a Zeiss LSM5 EXCITER confocal microscope.

Quantitative real-time PCR assay. Real-time PCR analysis was performed as described previously.[25] The GI24 mRNA level was examined using forward primer (GTTGCTAACCAG-GATGCTGG) starting at nucleotide 4296 and reverse primer (CAGACATGGCTATCTATCTCG) starting at nucleotide 4543 of the GI24 gene. Hs_GAPDH_1_SG QuantiTect Primer (Qiagen, Gaithersburg, MD, USA) was used to monitor the amplification of GAPDH gene transcript as a control.

RNA interference. Small interfering RNA (siRNA) targeting GI24 mRNA was designed and prepared by Nippon EGT (Toyama, Japan). The siRNA target sequences were as follows: MT1-MMP, 5-AACAGGCAAAGCTGATGCAGA); GI24-1, TAGGAGATCAATCAGGAATTA; GI24-2, 5′-ATGAAGC-GGGATGCTATTAAA. Scrambled control RNA duplex was used to serve as a negative control for the RNA interference response. Transfection was carried out using LipofectaminR-NAiMAX (Invitrogen, Carlsbad, CA, USA) by the reverse transfection method, according to the manufacturer's instructions.

Collagen gel invasion assay and culture. Type I collagen was obtained from Nippon Meat Packers (Osaka, Japan). HT1080 cells transfected with the indicated plasmid (2×10^5 cells) were suspended in 100 μL collagen mixture (3.5 mg/mL), and a 50 μL-drop of collagen gel-containing cells was polymerized on the bottom of a 24-well microplate and incubated in 0.5 mL medium for 3 days. For collagen gel culture, 1×10^4 HSC-4 cells transfected with siRNA were embedded in 100 μL collagen gel, and cultured in 0.5 mL medium for 6 days.

Results

Screening of the cDNA library. Aliquots of plasmid DNA from the human placenta cDNA library were co-transfected with MT1-MMP cDNA into HEK293T cells, and the cells were incubated with MMP-2. Then, the cell lysates were analyzed by gelatin zymography (Fig. 1). Transfection of one pool of cDNA

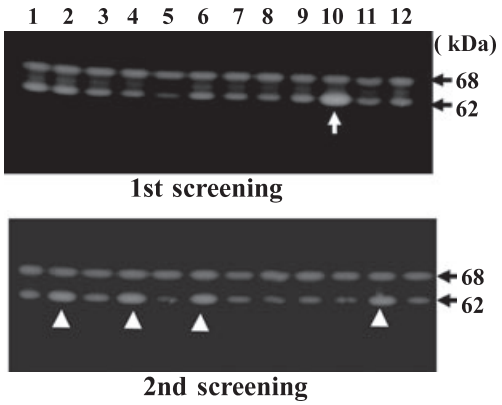

Fig. 1. Expression cloning. In the first screening, plasmid DNA aliquots from the human placenta cDNA library were co-transfected with membrane type 1–matrix metalloproteinase (MT1-MMP) plasmid into HEK293T cells cultured in 24-well microplates. At 48 h post-transfection, cells were incubated with a MMP-2 sample for 1 h, and cell lysates from each well were subjected to gelatin zymography. Note that MMP-2 activation to generate the 62-kDa active form was stimulated in lane 10, as indicated by an arrow (upper panel). In the second screening, single clones of plasmid DNA were examined as described above. Note that MMP-2 activation was enhanced in lanes 2, 4, 6 and 11, as indicated by arrowheads (lower panel).

partially stimulated processing of MMP-2 to the 62-kDa active form. Four cDNA clones of 12 clones from this cDNA pool were isolated by a second screening, transfection of which enhanced MMP-2 processing to the active form. The size of all four cDNA clones was 1.1 kb. Homology search analysis of its nucleotide sequence revealed that this cDNA encodes platelet receptor GI24 precursor (GI24) (GenBank accession no. NM_022153), which has many synonyms, including SISP1, and chromosome 10 open-reading frame 54 (C10orf54). Gene GI24 encoded a 34-kDa of 311 amino acids consisting of an extracellular region, a transmembrane segment, and a cytoplasmic tail. The extracellular region comprised a signal peptide and Ig V-set domain (Ig-like) as predicted by SMART.[22] Three *N*-linked glycosylation sites were identified in the extracellular region, which may contribute to the glycosylation of GI24 protein. Overall, the structure of GI24 closely resembles Ig-like cell adhesion molecules.

GI24 enhances MT1-MMP-mediated MMP-2 activation. GI24 tagged with HA epitope at the COOH-terminus was co-expressed with MT1-MMP in HEK293T cells, and MMP-2 activation and expression of MT1-MMP were examined by gelatin zymography and western analysis, respectively (Fig. 2A). Co-expression of GI24 with MT1-MMP augmented MMP-2 activation as shown above, and induced accumulation of the active form of MT1-MMP (52 kDa) on the cell surface. GI24 protein was detected as two bands of approximately 50 and 60 kDa. Co-expression of MT1-MMP with GI24 reduced the level of the 60 kDa form, and generated major 25-kDa and minor 35-kDa COOH-terminal fragments; however, the level of the 50 kDa form was not altered. Next, GI24 tagged with FLAG epitope at the NH2-terminus was co-expressed with MT1-MMP, and shedding of the GI24 NH2-terminal extracellular domain into the culture medium was examined (Fig. 2B). An NH2-terminal 30 kDa fragment of GI24 was detected in the culture medium from cells co-expressing GI24 and MT1-MMP. Cell-surface labeling detected only a 60 kDa but not a 50 kDa species of GI24, indicating that a 60 kDa species appears on the cell surface after modification of the 50 kDa immature form, and only the cell-surface 60 kDa form of GI24 was cleaved to shed the extracellular domain by MT1-MMP (Fig. 2C).

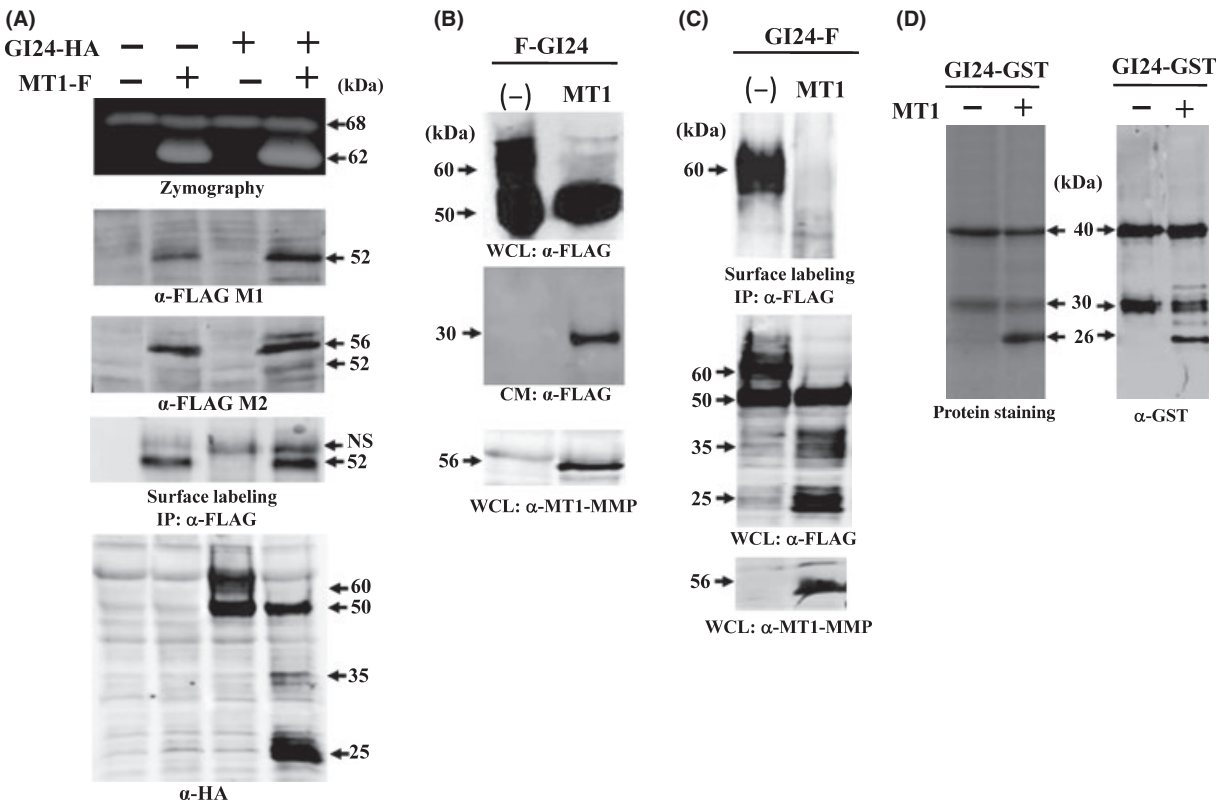

Fig. 2. GI24 stimulates membrane type 1–matrix metalloproteinase (MT1-MMP) activity. (A) Control plasmid or expression plasmid for GI24-HA (400 ng) was co-transfected with control or MT1-F plasmid (100 ng) into HEK293T cells plated in 24-well plates. Twenty four hours after the culture, cells were either incubated with a MMP-2 sample for gelatin zymography (top panel) or subjected to western blotting with anti-FLAG M1 antibody to detect the MT1-MMP active form (α-FLAG M1), anti-FLAG M2 antibody to detect active and latent MT1-MMP (α-FLAG M2), and anti-HA antibody to detect GI24-HA (α-HA). MT1-F plasmid (500 ng) was co-transfected with either control or GI24-HA plasmid (500 ng) into HEK293T cells cultured in a 6-well microplate. The cells were labeled and immunoprecipitated by anti-FLAG-M2 beads, and the precipitated materials were blotted with IRDye800-conjugated streptavidin as indicated. NS, non-specific band. (B) F-GI24 plasmid (200 ng) was co-transfected with either control plasmid or expression plasmid for MT-MMP (400 ng). At 24 h after transfection, the culture medium was replaced with serum-free medium, and cells were incubated for a further 24 h. Culture supernatants (panel CM) and cell lysates (panels WCL) were then analyzed by western blotting using anti-FLAG M2 and MT1-MMP antibody as indicated. (C) GI24-F plasmid (500 ng) was co-transfected with either control or MT1-MMP plasmid (500 ng) into HEK293T cells cultured in a 6-well microplate, and cells were labeled with biotin at 48 h after transfection. Immunoprecipitation was performed using anti-FLAG-M2 beads, and the precipitated materials were blotted with IRDye800-conjugated streptavidin (top panel). Expression of GI24-F and MT1-MMP was confirmed with cell lysates using anti-FLAG M2 and anti-MT1-MMP antibodies as indicated. (D) The recombinant GI24-GST protein (2 μg) was incubated with or without the recombinant MT1-MMP catalytic domain (0.2 μg) for 3 h, separated by 12% SDS-PAGE, and then detected by either Coomassie Brilliant Blue staining (protein staining) or western blotting using anti-GST antibody (α-GST). Note that 40 kDa GI24-GST protein and co-purified 30 kDa degradation product were cleaved by recombinant MT1-MMP to generate the 26 kDa fragment.

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Cleavage of GI24 by MT1-MMP was examined by an *in vitro* experiment using recombinant MT1-MMP and GI24 proteins (Fig. 2D). MT1-MMP cleaved GI24 at a site close to the transmembrane domain.

GI24 enhances invasion of HT1080 cells into collagen matrices. The GI24 gene was identified, the product of which stimulated MT1-MMP-mediated MMP-2 activation. To examine the physiological relevance of interaction between GI24 and MT1-MMP, the GI24-F gene was stably transfected into HT1080 fibrosarcoma cells (HT1080/GI24-F), which express endogenous MT1-MMP. GI24 COOH-terminal fragments of 35 and 25 kDa were detected in HT1080/GI24-F cells (Fig. 3A).

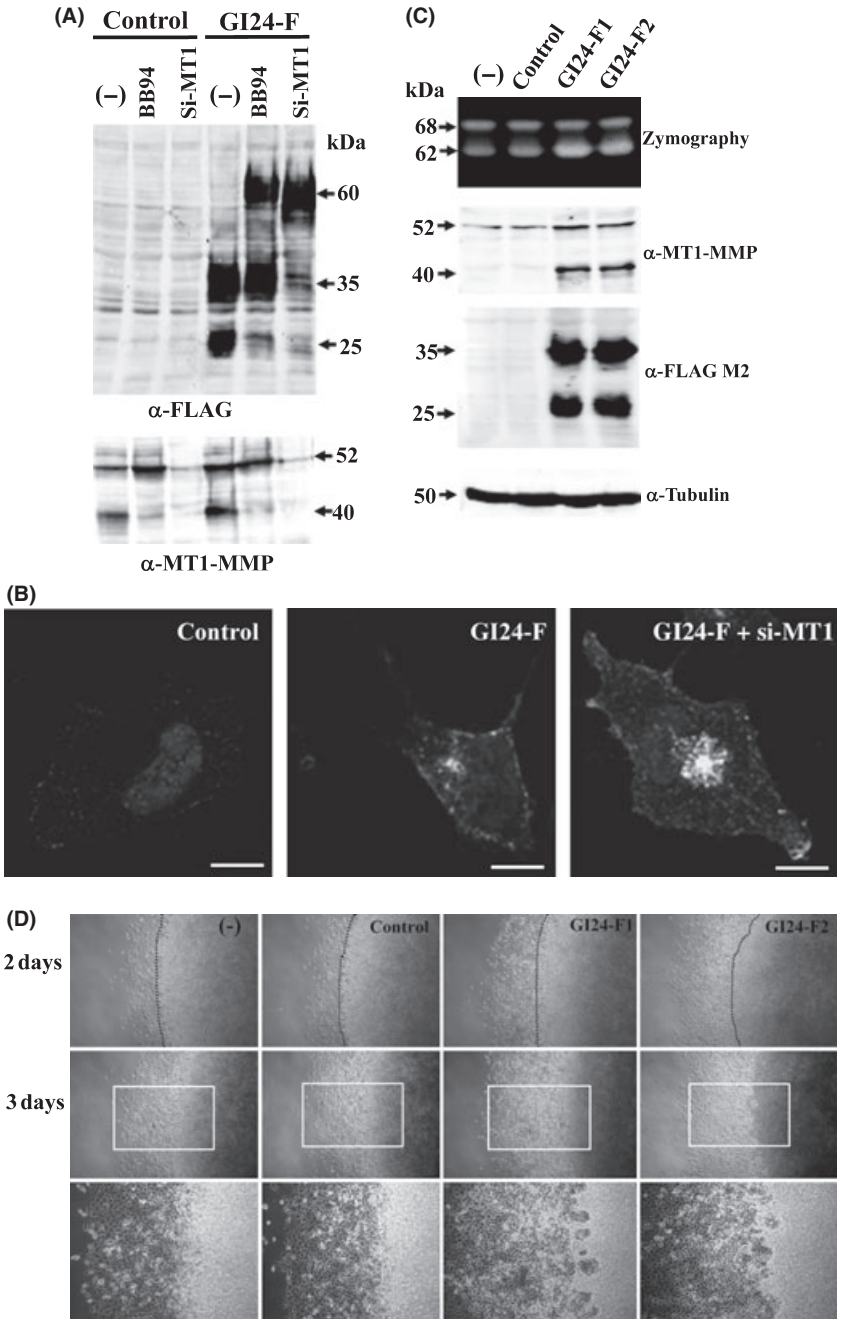

Fig. 3. GI24 enhances invasiveness of HT1080 cells. (A) HT1080 cells transfected with control or GI24-F plasmid were mock-treated (lane -) or treated with 1 μM BB94 (lanes BB94) or siRNA for membrane type 1–matrix metalloproteinase (MT1-MMP) (lanes si-MT1) for 24 h, and subjected to western blotting using anti-FLAG M2 or anti-MT1-MMP antibody. (B) Control HT1080 cells or HT1080/GI24-F cells were mock-treated or transfected with si-RNA for MT1-MMP, and then immunostained using anti-FLAG M2 antibody. Scale bar, 10 μm. (C) Parental HT1080 cells (–), HT1080 cells transfected with control plasmid and two independent clones of HT1080/GI24-F were either incubated with MMP-2 sample for gelatin zymography or subjected to western blotting using anti-MT1-MMP, anti-FLAG M2 or anti-tubulin antibody as indicated. (D) Parental HT1080 cells (–), control HT1080 cells and two independent clones of HT1080/GI24-F (1×10^5 cells) embedded in a 50 μL drop of collagen gel were cultured in 0.5 mL culture medium. Photography was taken 2 and 3 days after the culture. The dotted line represents the border of the collagen gel. Magnification, ×40. The area framed in white is expanded in the bottom panels. Note that HT1080/GI24-F cells invaded out of the collagen gel and degraded the collagen gel more than the control cells.

Treatment of HT1080/GI24-F cells with an MMP inhibitor BB94 attenuated production of 25 and 35 kDa fragment and recovered 60 kDa species. Tranasfection of siRNA for MT1-MMP reduced the MT1-MMP level by more than 90%, and 25 and 35 kDa species of GI24 were lost and 60 kDa species recovered in HT1080/GI24-F cells. The MT1-MMP level was significantly higher in HT1080/GI24-F cells than in the control cells. GI24-F of the 60 kDa form was immunolocalized on the cell membrane, and the cleavage products of 25 kDa and 35 kDa COOH-terminal fragments were also detected predominantly at the cell membrane (Fig. 3B).

To confirm the stimulatory effect of GI24 expression on MT1-MMP activity, two independent clones of HT1080/GI24-F cells were examined for MMP-2 activation (Fig. 3C). Both clones of HT1080/GI24-F cells activated MMP-2 more effectively, and express a significantly higher level of 52 kDa active MT1-MMP and 40 kDa its auto-degradation product than parental HT1080 cells or cells transfected with control plasmid.

To examine the possible contribution of GI24 to invasive growth, HT1080/GI24-F cells were cultured in collagen matrix, and cells which invade out from the gel were monitored

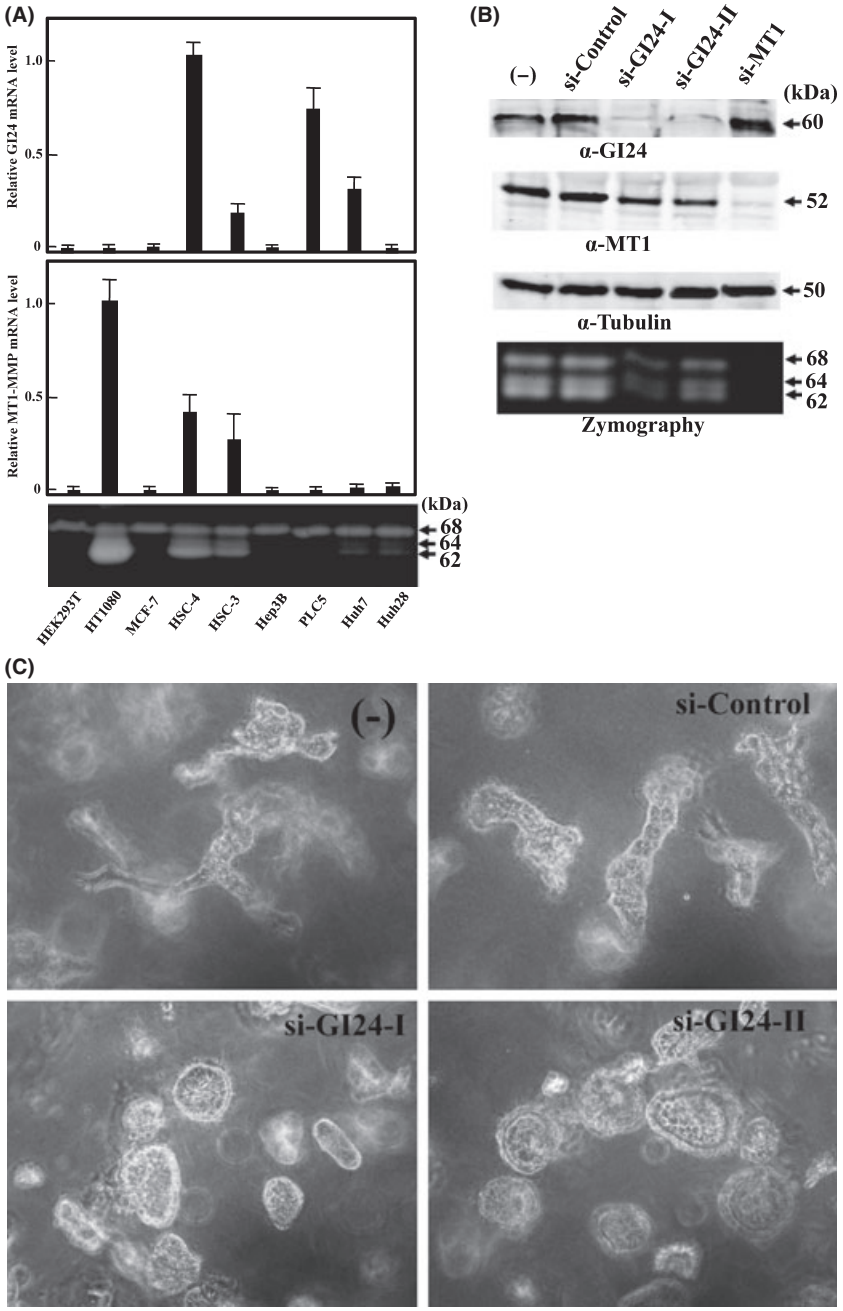

Fig. 4. GI24 stimulates invasive growth of HSC-4 cells. (A) GI24 and membrane type 1–matrix metalloproteinase (MT1-MMP) mRNA levels were compared using quantitative real-time PCR analysis. For gelatin zymography, cells were incubated with a MMP-2 sample for 1 h, and then washed with PBS before being dissolved in the sample buffer. (B) HSC-4 cells were mock-treated or transfected with control siRNA or siRNA for GI24 or MT1-MMP, and analyzed by western blotting using anti-GI24, anti-MT1-MMP or anti-tubulin antibody. Gelatin zymography was performed as described above. (C) HSC-4 cells were mock-treated or transfected with control siRNA or siRNA for GI24, and cultured in collagen gel for 6 days. Original magnification, ×100.

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(Fig. 3D). HT1080/GI24-F cells invaded out more frequently than parental cells or cells transfected with control plasmid. Collagen gel degradation was more intense in the culture of HT1080/GI24 cells than that of the parental or control cells.

Roles of GI24 in invasive growth of squamous carcinoma cells. The mRNA levels of GI24 and MT1-MMP in tumor cell lines were compared using real-time PCR analysis (Fig. 4A). Squamous carcinoma cell lines HSC-3 and HSC-4 and hepatocarcinoma cell lines PLC5 and Huh7 express GI24 mRNA at a relatively high level, and HT1080, HSC3 and HSC-4 cells express relatively high levels of MT1-MMP. Consistent with the MT1-MMP mRNA level, MMP-2 activation was observed in these cells. Thus, HSC-4 cells were used for further study to examine the interaction between GI24 and MT1-MMP. GI24 expression was knocked down by transfection of siRNA for GI24, and MT1-MMP expression and MMP-2 activation by these cells were analyzed (Fig. 4B). Transfection of siRNA for GI24 reduced its expression level, which significantly attenuated the MT1-MMP level and MMP-2 activation. Knockdown of MT1-MMP significantly increased the GI24 protein level. HSC-4 cells transfected with GI24 siRNA were cultured in 3-D collagen gel (Fig. 4C). HSC-4 cells or cells transfected with control siRNA formed a branched rod-like structure, but GI24 siRNA-transfected cells formed a more spherical shape in the collagen gel.

Discussion

We have identified KiSS-1/metastin, syndican-1, testican/N-Tes, lumican, amyloid-β precursor protein (APP), apolipoprotein E and GDF-15 using the expression cloning strategy as molecules which serve as regulators or substrates for MT1-MMP.[13,24,26–30] Among these molecules, APP and GDF-15 not only served as substrates for MT1-MMP, but also stimulated MT1-MMP-mediated MMP-2 activation. In contrast, the lumican gene was isolated, the product of which suppressed MT1-MMP-mediated MMP-2 activation but served as a substrate like other positive regulators. In the present study, we found that GI24 also serves not only as a substrate but also a positive regulator of MT1-MMP. In HEK293T cells, co-expression of GI24 augmented the level of active MT1-MMP, which consequently enhanced MMP-2 activation. Stable transfection of the GI24-F gene into HT1080 cells augmented the MT1-MMP level and MMP-2 activation, and thus HT1080/GI24-F cells showed more intensive degradation of collagen gel and invasion into it. GI24 expression was high in some squamous carcinoma and hepatocellular carcinoma cells. Down-regulation of GI24 expression by transfection of siRNA for GI24 attenuated MT1-MMP activity and invasive growth in the collagen matrix. These results suggest that GI24 contributes to the invasive growth of carcinoma cells by up-regulating cell-surface MT1-MMP. Cell-surface MT1-MMP is regulated through a variety of processes such as a dimer formation, internalization, interaction with cell-surface molecules, tetraspanin-regulated cell surface localization and autodegradation.[19,20] Interaction of GI24 with MT1-MMP positively regulates cell surface localization of MT1-MMP, although the detailed mechanism still remains to be solved. Deletion of transmembrane and cytoplasmic domains of GI24 abolished the positive regulatory effect on MMP-2 activation, indicating that cell-surface localization of GI24 is essential for it (data not shown). Another synonym of GI24 is SISP-1. MT1-MMP expression is induced in diverse physiological events such as tumor progression and tissue injury, which may shed the extracellular domain of GI24. The structure of GI24 closely resembles Ig-like cell adhesion molecules (PECAM-1 HepaCAM, ICAM-1 and ICAM-2).[31–33] Actually, GI24 was shown to enhance cellular adhesion to fibronectin (data not shown). GI24 is cleaved at the juxtamembrane site to shed the extracellular domain, which consequently abrogates enhanced adhesion to fibronectin. Thus far, the physiological significance of GI24-mediated cell adhesion still remains to be solved, but it might be possible that shed GI24 negatively regulates cellular adhesion to fibronectin.

In conclusion, we demonstrated for the first time that GI24 not only serves as a substrate for MT1-MMP but also stimulates MT1-MMP activity, and the stimulatory function of GI24 may contribute, at least in part, to tumor invasion and metastasis. Thus, the association between MT1-MMP and GI24 could be a target to regulate MT1-MMP activity and subsequent tumor growth in extracellular matrices.

References

1 LuKashev ME, Werb Z. ECM signaling: orchestrating cell behavior and misbehaviour. *Trends Cell Biol* 1998; **8**: 437–41.
2 Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. *Cell* 1997; **91**: 439–42.
3 Bosman FT. The border line: basement membranes and the transition from premalignant to malignant neoplasia. *Microsc Res Tech* 1994; **28**: 216–25.
4 Flug M, Kopf-Maier P. The basement membrane and its involvement in carcinoma cell invasion. *Acta Anat (Basel)* 1995; **152**: 69–84.
5 Coussens LM, Werb Z. Matrix metalloproteinases and the development of cancer. *Chem Biol* 1996; **3**: 895–904.
6 Hernandez-Barrantes S, Bernardo M, Toth M *et al.* Regulation of membrane type-matrix metalloproteinases. *Semin Cancer Biol* 2002; **12**: 131–8.
7 Sato H, Takino T, Okada Y *et al.* A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* 1994; **37**: 61–5.
8 Ueno H, Nakamura H, Inoue M *et al.* Expression and tissue localization of membrane-type 1, 2 and 3 matrix metalloproteinases in human invasive breast carcinoma. *Cancer Res* 1997; **57**: 2055–60.
9 Seiki M, Yana I. Roles of pericellular proteolysis by membrane type-1 matrix metalloproteinase in cancer invasion and angiogenesis. *Cancer Sci* 2003; **94**: 569–74.
10 Sato H, Takino T, Miyamori H. Roles of membrane-type matrix metalloproteinase-1 in tumor invasion and metastasis. *Cancer Sci* 2005; **96**: 212–7.
11 Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002; **2**: 161–74.
12 Overall CM, McQuibban GA, Clark-Lewis I *et al.* Discovery of chemokine substrates for matrix metalloproteinases by exosite scanning: a new tool for degradomics. *Biol Chem* 2002; **383**: 1059–66.
13 Abd El-Aziz SH, Endo Y, Miyamaori H *et al.* Cleavage of growth differentiation factor (GDF15) by membrane type 1-matrix metalloproteinase abrogates GDF15- mediated suppression of tumor cell growth. *Cancer Sci* 2007; **98**: 1330–5.
14 Galvez BG, Matias-Roman S, Yanez-Mo M *et al.* ECM regulates MT1-MMP localization with β1 or αv β3 integrins at distinct cell compartments modulating its internalization and activity on human endothelial cells. *J Cell Biol* 2002; **159**: 509–21.
15 Mori H, Tomari T, Koshikawa N *et al.* CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain. *EMBO J* 2002; **21**: 3949–59.
16 Takino T, Miyamori H, Kawaguchi N *et al.* Tetraspanin CD63 promotes targeting and lysosomal proteolysis of membrane-type 1 matrix metalloproteinase. *Biochem Biophys Res* 2003; **304**: 160–6.
17 Yanez-Mo M, Barreiro O, Gonzalo P *et al.* MT1-MMP collagenolytic activity is regulated through association with tetraspanin CD151 in primary endothelial cells. *Blood* 2008; **112**: 3217–26.
18 Kolesnikova T, Kazarov A, Lemieux ME *et al.* Glioblastoma inhibition by cell surface immunoglobulin protein EWI-2, in vitro and in vivo. *Neoplasia* 2009; **11**: 77–86.
19 Itoh Y. MT1-MMP: a key regulator of cell migration in tissue. *IUBMB Life* 2006; **58**: 589–96.
20 Sato H, Takino T. Coordinate action of membrane-type matrix metalloproteinase-1 (MT1-MMP) and MMP-2 enhances pericellular proteolysis and invasion. *Cancer Sci* 2010; **101**: 843–7.
21 Itoh Y, Seiki M. MT1-MMP: a potent modifier of pericellular microenvironment. *J Cell Physiol* 2006; **206**: 1–8.
22 Deloukas P, Erathrwol ME, Grafham DV *et al.* The DNA sequence and comparative analysis of human chromosome 10. *Nature* 2004; **429**: 375–81.

23 Miyamori H, Takino T, Kobayashi Y *et al.* Claudin promotes activation of pro-matrix metalloproteinase-2 mediated by membrane-type matrix metalloproteinases. *J Biol Chem* 2001; **276**: 28204–11.
24 Ahmad M, Takino T, Miyamori H *et al.* Cleavage of amyloid-β precursor protein (APP) by membrane-type matrix metalloproteinases. *J Biochem* 2006; **139**: 517–26.
25 Nishida Y, Miyamori H, Thompson WE *et al.* Activation of matrix metalloproteinase-2(MMP-2) by membrane type 1 matrix metalloproteinase through an artificial receptor for proMMP-2 generates active MMP-2. *Cancer Res* 2008; **68**: 9096–104.
26 Takino T, Koshikawa N, Miyamori H *et al.* Cleavage of metastasis suppressor gene product KiSS-1 protein/metastin by matrix metalloproteinases. *Oncogene* 2003; **22**: 4617–26.
27 Endo K, Takino T, Miyamori H *et al.* Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. *J Biol Chem* 2003; **278**: 40764–70.
28 Nakada M, Yamada A, Takino T *et al.* 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. *Cancer Res* 2001; **61**: 8896–902.
29 Li Y, Aoki T, Mori Y *et al.* Cleavage of lumican by membrane-type matrix metalloproteinase-1 abrogates this proteoglycan-mediated suppression of tumor cell colony formation in soft agar. *Cancer Res* 2004; **64**: 7058–64.
30 Aoki T, Sato D, Li Y *et al.* Cleavage of apolipoprotein E by membrane-type matrix metalloproteinase-1 abrogates suppression of cell proliferation. *J Biochem* 2005; **137**: 95–9.
31 Nakada MT, Amin K, Christofidou-Solomidou M *et al.* Antibodies against the first Ig-like domain of human platelet endothelial cell adhesion molecule-1 (PECAM-1) that inhibit PECAM-1-dependent homophilic adhesion block in vivo neutrophil recruitment. *J Immunol* 2000; **164**: 452–62.
32 Moh MC, Zhang C, Luo C *et al.* Structural and functional analyses of a novel Ig-like cell adhesion molecule, hepaCAM, in the human breast carcinoma MCF7 cells. *J Biol Chem* 2005; **280**: 27366–74.
33 Lina T, James W, Chris O *et al.* Expression of immunoglobulin superfamily cell adhesion molecules on murine embryonic stem cells. *Biol Reprod* 1997; **57**: 561–8.

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