CDCP1 Regulates the Function of MT1-MMP and Invadopodia-Mediated Invasion of Cancer Cells

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Abstract
Complement C1r/C1s, Uegf, Bmp1 (CUB) domain-containing protein 1 (CDCP1) is a transmembrane protein that regulates anchorage-independent growth and cancer cell migration and invasion. Expression of CDCP1 is detected in a number of cancer cell lines and tissues and is closely correlated with poor prognosis. Invadopodia are actin-based protrusions on the surface of invasive cancer cells that promote the degradation of the extracellular matrix (ECM) via localized proteolysis, which is mainly mediated by membrane type 1 matrix metalloproteinase (MT1-MMP). MT1-MMP is accumulated at invadopodia by targeted delivery via membrane trafficking. The present study shows that CDCP1 is required for ECM degradation by invadopodia in human breast cancer and melanoma cells. CDCP1 localized to caveolin-1-containing vesicular structures and lipid rafts and was detected in close proximity to invadopodia. Further biochemical analysis revealed that substantial amounts of CDCP1 existed in the Triton X-100 insoluble lipid raft fraction. CDCP1 was coimmunoprecipitated with MT1-MMP and colocalized with MT1-MMP at the vesicular structures. The siRNA-mediated knockdown of the CDCP1 expression markedly inhibited MT1-MMP–dependent ECM degradation and Matrigel invasion and reduced the accumulation of MT1-MMP at invadopodia, as shown by immunofluorescence analysis. These results indicate that CDCP1 is an essential regulator of the trafficking and function of MT1-MMP– and invadopodia-mediated invasion of cancer cells.

Introduction
Complement C1r/C1s, Uegf, Bmp1 (CUB) domain-containing protein 1 (CDCP1), also described as SIMA135 and TRASK (1, 2), is a type 1 transmembrane protein with 3 CUB domains as the extracellular domains and several tyrosine residues that can be phosphorylated by Src family kinases (SFK) in the intracellular domain (1–6). The expression of CDCP1 was reported in several human malignancies, such as colon and breast cancers (3, 7). CDCP1 expression is strongly associated with cancer progression and poor prognosis in renal cell carcinoma, lung adenocarcinoma, and pancreatic cancer (8–10). We previously reported that CDCP1 is a critical regulator of anoikis resistance in lung cancer cells (11), peritoneal dissemination of gastric scirrhus carcinoma (12), and invasion of pancreatic cancer cells (8). We showed that knockdown of CDCP1 resulted in the inhibition of extracellular matrix (ECM) degradation by pancreatic cancer cells (8). Tyrosine phosphorylation of CDCP1, especially at tyrosine 734 residue, plays an essential role in these oncogenic processes (8, 11, 12). However, the exact molecular mechanisms by which CDCP1 regulates cancer cell invasion remain to be determined.

Matrix metalloproteinases (MMP) have been implicated in many aspects of cancer progression, such as tumor growth, angiogenesis, invasion, and metastasis (13). Among MMPs, membrane type 1 (MT1)-MMP, the first discovered membrane-type MMP (14), plays a pivotal role in ECM degradation at the leading edge of invasive cancer cells (15). Clinically, MT1-MMP expression is strongly associated with cancer progression and metastasis (16) and poor prognosis of patients (17, 18). A cell–surface complex consisting of MT1-MMP oligomers binds to inactive proMMP-2 and mediates its cleavage and activation (19). In addition to activating secreted MMPs, MT1-MMP plays a key role in cancer invasion through its interaction and processing of cell surface proteins including CD44 (20). MT1-MMP function can be regulated by clathrin-mediated or caveolar endocytosis followed by degradation in the lysosomal compartment (21).

Invadopodia are unique protrusions observed at the cell adhesion sites of invasive cancer cells that are rich in cell-ECM adhesion molecules, actin assembly regulators, membrane-remodeling proteins, tyrosine kinases, tyrosine-phosphorylated proteins, and MMPs (22, 23). Because they offer an environment that supports ECM

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degradation and thus cancer invasion and metastasis, the ability to form invadopodia is closely associated with the invasive and metastatic potentials of cancer cells (24, 25). The matrix degradation activity at invadopodia is dependent on the accumulation and activation of MT1-MMP at the cell surface of invadopodia (26–28). Recent studies have revealed that endocytic and exocytic trafficking are critical for the targeted delivery of MT1-MMP to invadopodia (21, 29–31). However, the molecular mechanisms that control the delivery and function of MT1-MMP at invadopodia are not fully understood. In this study, we show that CDCP1 regulates the function of MT1-MMP and plays an essential role in ECM degradation at invadopodia in human cancer cells.

Materials and Methods

Cell culture and transfection

The human breast cancer cell line MDA-MB-231 and the human melanoma cell line RPMI 7951 were obtained from the American Type Culture Collection. MDA-MB-231 and RPMI 7951 cells were maintained in a 1:1 mixture of high-glucose Dulbecco’s modified Eagle’s medium and RPMI-1640 supplemented with 10% FBS, 10 U/mL penicillin, and 10 μg/mL streptomycin. The cells were transfected with the indicated plasmids, using Lipofectamine 2000 or Lipofectamine LTX (Life Technologies Corporation) according to the manufacturer’s instructions.

siRNA treatment

Two sets of siRNA against CDCP1 were synthesized and purchased from Life Technologies Corporation as described elsewhere (12). The control siRNA for CDCP1 was synthesized as follows: CDCP1 control siRNA-1, 5’-CGGAGCGAAACAACAUCUUA-3’ (sense), and 5’-AGAUGUUGUUUCGCUCCGGUAGC-3’ (antisense). siRNAs (40 pmol) were incorporated into cells using Lipofectamine 2000 or Oligofectamine (Life Technologies Corporation) according to the manufacturer’s instructions. The cells were subjected to further experiments 72 hours after the siRNA treatment.

Plasmids, antibodies, and reagents

To generate the GFP-MT1-MMP construct, human MT1-MMP cDNA was subcloned into the pEGFP-N1 vector (Clontech Laboratories Inc.; ref. 29). Human MT1-MMP having a FLAG-tag at the N-terminus (MT1-F) was subcloned into the pSG5 vector (Sigma; ref. 32). The polyclonal antibodies against CDCP1 and tyrosine-phosphorylated CDCP1 (Tyr734) were prepared as described previously (8). The anti-MT1-MMP antibody (1D8) was prepared as described previously (33). Other antibodies used were α-tubulin (B-5-1-2; Sigma), cortactin (4F11; Millipore), caveolin-1 (610493; BD Biosciences), early endosome antigen 1 [EEA-1 (610457; BD Biosciences)], MT1-MMP (ab38971; Abcam), GFP (ab1218, Abcam; 04363-24, Nacalai Tesque), CDCP1 (ab1377; Abcam), Syntaxin 6 (C34B2; Cell Signaling Technology Inc.), and Tks5 (sc-9945; Santa Cruz Biotechnology).

Western blotting and immunoprecipitation

The Western blotting and immunoprecipitation were conducted as described previously (8). The cells were lysed with a buffer (20 mmol/L Tris-HCl, at pH 7.4, 150 mmol/L NaCl, 1 mmol/L EDTA, 20 mmol/L NaF, 1% Nonidet P-40, 5% glycerol, and 2% octyl-β-D-glucoside plus protease inhibitor) for dissolution of lipid rafts and subjected to Western blotting and immunoprecipitation. Protein concentration was determined using the Bicinchoninic Acid Protein Assay Kit (Thermo Fisher Scientific Inc.), and equal amounts of protein were separated by SDS-PAGE. A polyvinylidene difluoride membrane (Immobilon-P, Millipore) was used as the transfer membrane, and Blocking One (Nacalai Tesque) was used for blocking the membrane. For immunoprecipitation, 1 mg of protein was mixed with 5 μg of CDCP1 antibody and rotated with Protein G Sepharose beads (GE Healthcare) or 10 μL FLAG M2 agarose beads (Sigma).

Invadopodia assay

Fluorescent gelatin-coated coverslips were prepared as described previously (29, 34). MDA-MB-231 and RPMI 7951 cells were cultured for 3 to 7 hours on coverslips coated with fluorescent gelatin. In the case of the cells overexpressing MT1-MMP, they were cultured on the gelatin matrix for 2 hours. To quantitate the degradation activity of invadopodia, 20 randomly selected fields, usually containing 20 to 40 cells, were imaged with a × 60 objective for each determination. The degradation area was determined by using the ImageJ software version 1.41o and normalized for the number of cells. In each analysis, the mean value of the control cells was set at 100%, and the relative values of the cells treated with siRNAs were then calculated. The relative number of invadopodia per cell was determined by counting punctate F-actin structures that were positive for MT1-MMP. The mean (SE) of at least 3 independent determinations was calculated.

Invasion assay

The invasion assay was conducted using modified Transwell chambers with a polycarbonate nuclopopore membrane (BD Falcon), as described previously (8). The top side of the membrane was coated with 0.02% collagen (Cellmatrix Type I-A, Nitta Gelatin Inc.). The cells treated with each siRNA were seeded onto the top part of each chamber. After incubation for 30 hours, the cells on the membrane were fixed. The number of migrated or invaded cells was determined by counting the cells on the bottom side of the membranes from 2 wells (2 fields per membrane) at a magnification of × 100, and the extent of invasion was expressed as the average ratio (number of cells transfected with siRNA per field/average number of cells transfected with control siRNA per field). The results were expressed as the mean of 3 independent experiments.

Immunofluorescence

Immunofluorescence was conducted as previously described (34). Briefly, the cells were fixed in 4%
paraformaldehyde for 15 minutes and permeabilized with 0.1% Triton X-100 for 5 minutes. The cells were blocked in 1% bovine serum albumin and 1% goat serum for 30 minutes, incubated with primary antibodies for 1 hour, and then incubated with Alexa Fluor-conjugated secondary antibodies and phalloidin (Life Technologies Corporation) for 30 minutes. The samples were observed with an IX81-ZDC-DSU confocal microscope (Olympus) equipped with a cooled CCD camera (ORCA-ER, Hamamatsu Photonics), and the imaging system was driven by the MetaMorph software (Universal Imaging). All the images were acquired using /C2 60 (PLAPON60XO, NA 1.42) or /C2 100 oil objectives (UPLSAPO100XO, NA 1.4). The images were analyzed and processed with various software packages, including MetaMorph, ImageJ version 1.41o (NIH, Bethesda, MD; http://rsbweb.nih.gov/ij/), and Adobe Photoshop CS4. The relative distribution of CDCP1 at the cell periphery versus intracellular vesicles was determined by calculating the ratio between the fluorescence intensities of peripheral and perinuclear vesicular CDCP1 from confocal images. For colocalization analysis, Mander overlap coefficient was calculated using ImageJ with Just Another Colocalization Plugin. All calculations were conducted on 8 to 20 cells.

**Labeling cells with CTxB**

The cells cultured on gelatin-coated coverslips were washed with ice-cold PBS and incubated with 10 μg/mL Alexa Fluor-conjugated cholera toxin B subunit (CTxB; Life Technologies Corporation) in PBS for 20 minutes on ice. For the internalization experiments, the cells were labeled with CTxB in the growth medium for 20 minutes on ice and then incubated for the indicated times at 37°C. The cells were stained with anti-CDCP1 antibody as described previously.

**Cell fractionation**

Cell fractionation was conducted as described previously (29). The cytosolic fraction was extracted with the...
ProteoExtract Subcellular Proteome Extraction Kit (Millipore). Triton X-100 soluble materials were then extracted with a buffer (25 mmol/L Tris-HCl at pH 7.5, 150 mmol/L NaCl, 5 mmol/L EDTA, and protease inhibitors) containing 1% Triton X-100. Insoluble materials were further extracted with TNE buffer containing 1% SDS. Equal amounts of protein from each fraction were analyzed by immunoblotting.

**Results**

**CDCP1 regulates invadopodia-mediated ECM degradation**

The expression of CDCP1 in various cancers, including breast cancer, has been reported previously (7). We examined several human breast cancer cell lines and detected significant CDCP1 expression in MDA-MB-453, BT549, and MDA-MB-231 cells, using Western blotting (Supplementary Fig. S1). The immunoblotting with a phospho-specific antibody against Y734 of CDCP1 revealed tyrosine phosphorylation of CDCP1 in the highly invasive cell line BT549 and MDA-MB-231 (Fig. 1A and Supplementary Fig. S1), suggesting that CDCP1-mediated signaling might play a role in breast cancer cell invasion. Because invadopodia formation in MDA-MB-231 cells has been reported previously (35), we used this cell line to investigate the potential role of CDCP1 in invadopodia formation and function.

MDA-MB-231 cells were treated with 2 independent siRNAs against CDCP1 and subjected to Western blotting, which showed that CDCP1 expression was successfully suppressed by both siRNAs (Fig. 1A). The siRNA-treated cells were cultured on coverslips coated with fluorescent gelatin and tested for invadopodia formation and gelatin degradation. Gelatin degradation, which was primarily localized to the area around the invadopodia in MDA-MB-231 cells, was significantly decreased by treatment with CDCP1 siRNA, as shown by immunofluorescence and quantitative analysis of the degradation area (Fig. 1B and C). In addition, the proportion of cells with apparent degradation of the gelatin matrix was clearly decreased by CDCP1 knockdown (Fig. 1D). We also observed that CDCP1 knockdown significantly suppressed degradation of gelatin matrix by RPMI 7951 human melanoma cells (Supplementary Fig. S2A and S2B). It is known that assembly of actin-core structures and subsequent focal concentration of MMPs are both required for ECM degradation by invadopodia. The number of invadopodia that were punctate F-actin structures positive for an invadopodia marker Tks5 per cell was significantly decreased in cells transfected with CDCP1 siRNA (Fig. 1E). These observations indicate that CDCP1...
is required for both assembly of invadopodia structures and ECM degradation activity. In addition, reduced expression of CDCP1 significantly suppressed invasion of MDA-MB-231 cells through Matrigel (Fig. 1F), which is consistent with our results obtained with gastric and pancreatic cancer cell lines (8, 12). Taken together, these results indicate that CDCP1 is an essential regulator of invadopodia-mediated ECM degradation during cancer cell invasion.

**CDCP1 localizes to lipid raft-containing vesicular structures associated with invadopodia**

To determine the cellular localization of CDCP1 in MDA-MB-231 cells, we conducted immunocytostaining with a specific antibody against CDCP1. Distinct signals for CDCP1 were observed as punctate and vesicular structures within the cells, and the signals were also detected at the cell periphery (Fig. 2). Quantitative analysis of fluorescence intensities showed that the ratio between perinuclear vesicular and peripheral CDCP1 was 0.42 ± 0.12 (SD, N = 23). These CDCP1 signals did not colocalize with invadopodia, which were observed as punctate structures at the cell-matrix adherent site showing positive staining for the invadopodia markers F-actin and cortactin. Nevertheless, strong vesicular CDCP1 signals were consistently observed in close proximity to invadopodia.

To identify the vesicular structures containing CDCP1, we conducted immunofluorescence analysis with several organelle markers. Syntaxin 6 is a trans-Golgi network protein and regulates membrane trafficking (36). Partial colocalization of CDCP1-containing vesicles with syntaxin

![Image of CDCP1 localization](Image)

**Figure 3.** CDCP1 localizes to intracellular vesicles containing caveolin-1. The MDA-MB-231 cells were cultured on gelatin-coated coverslips and costained for CDCP1 with syntaxin 6 (A), EEA-1 (B), and caveolin-1 (C). Inserts are magnified images of the boxed regions. D, Mander coefficient was calculated on the basis of the degree of colocalization between CDCP1 and each vesicular marker from confocal immunofluorescence micrographs. Columns, mean; bars, SE. *, P < 0.0001.
6 (Fig. 3A) indicated that CDCP1 proteins are delivered through and/or function in the trans-Golgi network. CDCP1 did not colocalize with EEA-1, an early endosome marker (Fig. 3B), whereas it markedly colocalized with caveolin-1 in vesicular structures (Fig. 3C). Quantitative analysis of confocal images confirmed these observations and showed significant colocalization of CDCP1 with caveolin-1 (Fig. 3D).

Caveolin-1, an essential component of a subtype of lipid rafts called caveolae, regulates the organization and dynamics of lipid rafts and plays a role in membrane trafficking by regulating caveola/raft-dependent endocytosis (37). To determine whether CDCP1 is present in lipid rafts, MDA-MB-231 cells cultured on gelatin-coated dishes were separated into cytosolic, Triton X-100–soluble, and Triton X-100–insoluble fractions, and analyzed by Western blotting (Fig. 4A). Lipid rafts are known to be enriched in the Triton X-100–insoluble fraction (38, 39). As expected, CDCP1 was hardly detected in the cytosolic fraction but was concentrated in the Triton X-100–soluble membrane fraction. It is important to note that a significant amount of CDCP1 was also detected in the Triton X-100–insoluble fraction (Fig. 4A), indicating that CDCP1 exists in lipid rafts. Cells were then labeled with fluorescent CTxB, a lipid raft marker (40), and the course of CTxB internalization and localization of CDCP1 was simultaneously followed by immunofluorescence (Fig. 4B). Before the internalization, CTxB signals partly colocalized with CDCP1 at the ventral surface of the cell. The internalization of CTxB caused a shift of CTxB fluorescence to intracellular endosomes, where it colocalized with CDCP1 signals, starting 10 minutes after internalization and lasting until at least 60 minutes (Fig. 4B). Quantitative analysis of colocalization of CDCP1 and CTxB confirmed these observations (Fig. 4C). These results indicate that CDCP1 mainly exists in vesicular compartments where internalized lipid raft membranes accumulate.

**CDCP1 colocalizes and associates with MT1-MMP**

We previously reported that caveolin-1 colocalizes with MT1-MMP at lipid raft-enriched vesicles and regulates ECM degradation by invadopodia (29). The possible interaction between CDCP1 and MT1-MMP was therefore examined by immunocytostaining, which showed that MT1-MMP colocalized with CDCP1 at the vesicular structures (Fig. 5A). Mander coefficient for the degree of CDCP1 signals colocalizing with MT1-MMP was 0.63 ± 0.039 (SE, N = 17), which was very similar to that for CDCP1 colocalizing with caveolin-1 (Fig. 3D). In addition, the coimmunoprecipitation analysis conducted using MDA-MB-231 cells treated with octyl glucoside, which solubilizes...
lipid rafts (41), showed that endogenous CDCP1 is co-immunoprecipitated with FLAG-tagged MT1-MMP and vice versa (Fig. 5B). These results suggested that CDCP1 interacts with MT1-MMP in lipid rafts and may regulate its function.

**CDCP1 regulates MT1-MMP–dependent invasion**

To investigate the role of CDCP1 in the regulation of MT1-MMP–dependent invasion, we used a MDA-MB-231 cell line overexpressing MT1-MMP (MT1-MDA). In a previously reported invadopodia assay, MT1-MDA cells robustly degraded the gelatin matrix within 2 hours, whereas parental MDA-MB-231 cells required 3 to 7 hours to do the same (29). Our results showed that MT1-MDA cells degraded the gelatin at the peripheral regions, along with cortical actin filaments, before degrading the gelatin at invadopodia (Fig. 6B). The downregulation of CDCP1 in the MT1-MDA cells significantly decreased MT1-MMP–dependent gelatin degradation, whereas the total amount of MT1-MMP protein was not affected (Fig. 6A–C). The MT1-MDA cells also showed enhanced invasiveness through the Matrigel in the Transwell invasion assay as compared with the parental or control GFP-expressing cells, and this enhanced invasive activity was clearly suppressed by the knockdown of CDCP1 (Fig. 6D).

Recent studies have shown that targeted trafficking of MT1-MMP to invadopodia, which is mediated by vesicle-associated membrane protein 7, the exocyst complex, caveolin-1, and lipid rafts, is crucial for its accumulation and matrix degradation activity at invadopodia (21, 22, 29–31). Therefore, we next examined the effect of CDCP1 knockdown on the localization of MT1-MMP at invadopodia. While endogenous MT1-MMP accumulated at F-actin-rich invadopodia along with the invadopodia marker Tks5 in control cells, the knockdown of CDCP1 expression significantly hampered MT1-MMP accumulation at the remaining invadopodia but did not affect Tks5 localization at invadopodia (Fig. 6E). Taken together, these results indicate that CDCP1 regulates the function and localization of MT1-MMP and is required for MT1-MMP–dependent invasion.

**Discussion**

We previously reported that CDCP1, a substrate of SFKs, is an essential regulator of anoikis resistance and controls cancer cell migration and invasion (6, 8, 11, 12). CDCP1 expression is positively correlated with poor prognosis in patients with lung, renal, or pancreatic cancer (8–10). These findings strongly suggest that CDCP1 promotes cancer metastasis and invasion. Invadopodia are actin-rich adhesive protrusions that mediate the focal concentration of MMPs and degradation of the ECM. In the present study, we describe a new function of CDCP1 in the regulation of invadopodia-mediated invasion of breast cancer and melanoma cells. Our results indicate that CDCP1 promotes ECM degradation induced by invadopodia in these cancer cells.

Lipid rafts are enriched with a variety of proteins, including caveolins and flotillins (42), and are thought to function as platforms for spatially controlled signal transduction and membrane trafficking (58). Many lipid raft-associated molecules are actively internalized by caveolin-1–dependent endocytosis and trafficked in cells for their proper localization and function (37). Recent studies have confirmed the importance of lipid rafts and associated proteins in the pathogenesis of several diseases, including cancer progression (43). CDCP1 localized at vesicular structures were closely.
associated with invadopodia (Fig. 2). Vesicles enriched with lipid rafts and caveolin-1 were previously shown to be actively trafficked and internalized around invadopodia, which indicates the possible involvement of lipid rafts in the transport of invadopodia components (29). Our current observations showing the colocalization of CDCP1 with caveolin-1 and the lipid raft marker CTxB at the vesicular structures indicate that CDCP1 exists in these compartments (Figs. 3 and 4). This was confirmed by biochemical analysis, which showed a considerable amount of CDCP1 in the Triton X-100–insoluble lipid raft fraction (Fig. 4A). Although a part of CDCP1 colocalized with CTxB on the ventral plasma membrane (Fig. 4B), we could not detect significant colocalization of CDCP1 and caveolin-1 at this region (data not shown). Moreover, CDCP1 partially localized at the trans-Golgi network but not at early endosomes (Fig. 3). Therefore, CDCP1 seems to be involved primarily in intracellular membrane trafficking, most probably in lipid raft-dependent secretory pathway from the Golgi compartment or recycling endosomes, rather than caveolae-mediated endocytosis.

MT1-MMP levels and localization on the cell surface are tightly controlled by membrane-trafficking systems. After its synthesis, MT1-MMP is transported from the trans-Golgi network to the plasma membrane via the secretory pathway. Surface MT1-MMP is internalized by clathrin- and caveolin-dependent endocytosis and is then degraded in lysosomes or recycled back to the plasma membrane (27). Recent studies have revealed that the targeted delivery of MT1-MMP to invadopodia through membrane trafficking is necessary for its accumulation and proteolytic activity in these structures (21, 22, 30, 31). In addition, we previously

Figure 6. CDCP1 is necessary for the localization and function of MT1-MMP. A, the MDA-MB-231 cells were treated with control or CDCP1 siRNAs and subjected to immunoblotting with the indicated antibodies. B, the MDA-MB-231 cells expressing MT1-MMP-GFP were treated with control or CDCP1 siRNAs and tested for invadopodia formation and gelatin degradation for 2 hours, along with the parental MDA-MB-231 cells. The cells were stained for F-actin to visualize invadopodia and observed by confocal microscopy. C, quantification of gelatin degradation areas. Columns, mean; bars, SD. *, P < 0.05. D, the MDA-MB-231 cells expressing MT1-MMP-GFP were treated with control or CDCP1 siRNAs and tested for Matrigel invasion with the Transwell invasion assay. E, the MDA-MB-231 cells cultured on gelatin-coated coverslips were stained for MT1-MMP, Tks5, and F-actin. The inserts are magnified images of the boxed regions. White arrowheads denote invadopodia where Tks5 and F-actin are accumulated.
reported that caveolin-1 colocalizes and traffics with MT1-MMP and controls the MT1-MMP–dependent degradation of the ECM at invadopodia (29). In the present study, CDCP1 was shown to interact and colocalize with MT1-MMP at intracellular vesicles (Fig. 5), indicating that CDCP1 plays a role in the regulation of MT1-MMP. We found that reduction of CDCP1 expression suppressed MT1-MMP–dependent matrix degradation and invasion (Fig. 6) without affecting MT1-MMP expression or proteolysis of MT1-MMP activation by processing (Supplementary Fig. S3). Furthermore, immunofluorescence analysis revealed that CDCP1 reduction hampered the accumulation of MT1-MMP at invadopodia (Fig. 6E). Considering the possible involvement of CDCP1 in lipid raft-dependent secretory pathway as described above, CDCP1 may regulate targeted transport of mature MT1-MMP to invadopodia.

MT1-MMP is reported to accumulate at nascent invadopodia after assembly of F-actin structures, which leads to the stabilization and maturation of proteolytically active invadopodia (26). Importantly, it has been proposed that there is a positive feedback system in which MMP activity in the extracellular space generates new signals for invadopodia formation, based on the observations that compromising expression or delivery of MT1-MMP led to a decrease in invadopodia formation (26, 44). Therefore, inhibition of invadopodia formation in CDCP1-knockdown cells may be caused by disruption of the positive feedback system due to reduced delivery of MT1-MMP to invadopodia. This idea may help explain why CDCP1 affects invadopodia formation without localizing at the core F-actin structures of invadopodia. In conclusion, this study provides evidence that CDCP1 is required for ECM degradation by invadopodia in human cancer cells. Moreover, we showed for the first time that CDCP1 associates with MT1-MMP and regulates its function in lipid rafts. These findings provide a new insight into the role of CDCP1 in ECM degradation and cancer cell invasion that might contribute to the development of new therapeutic strategies for the treatment of cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: Y. Miyazawa, T. Uekita, M. Seiki, H. Yamaguchi, R. Sakai
Development of methodology: Y. Miyazawa
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Miyazawa, R. Sakai

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