Type I Collagen Abrogates the Clathrin-mediated Internalization of Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) via the MT1-MMP Hemopexin Domain*

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Type I collagen (Col I)-stimulated matrix metalloproteinase-2 (MMP-2) activation via membrane type 1 MMP (MT1-MMP) involves both a transcriptional and a nontranscriptional response mediated by preexisting MT1-MMP. In order to identify which MT1-MMP domains were required for the nontranscriptional response, MCF-7 cells that lack endogenous MT1-MMP were transfected with either wild type or domain mutant MT1-MMP constructs. We observed that mutant constructs lacking the MT1-MMP cytoplasmic tail were able to activate MMP-2 in response to Col I but not a construct lacking the MT1-MMP hemopexin domain. Col I did not alter total MT1-MMP protein levels; nor did it appear to directly induce MT1-MMP oligomerization. Col I did, however, redistribute preexisting MT1-MMP to the cell periphery compared with unstimulated cells that displayed a more diffuse staining pattern. In addition, Col I blocked the internalization of MT1-MMP in a dynamin-dependent manner via clathrin-coated pit-mediated endocytosis. This mechanism of impaired internalization is different from that reported for concanavalin A, since it is not mediated by the cytoplasmic tail of MT1-MMP but rather by the hemopexin domain. In summary, upon Col I binding to its cell surface receptor, MT1-MMP internalization via clathrin-coated pit-mediated endocytosis is impaired through interactions with the hemopexin domain, thereby regulating its function and ability to activate MMP-2.

Matrix metalloproteinases (MMPs) are a family of zinc-binding, calcium-dependent proteolytic enzymes with the ability to remodel the extracellular matrix (ECM) as well as cleave a number of cell surface modulators (1). Due to their ability to influence a plethora of cell functions, aberrant MMP activity has been associated with several pathological situations such as rheumatoid arthritis, diabetic nephropathy, angiogenesis, and tumor invasion and metastasis (2, 3). Although several MMPs have been associated with tumor progression, particular attention has been focused on MMP-2 and MT1-MMP, which are often produced by peritumoral fibroblasts within a tumor (4, 5). MT1-MMP can break down a variety of extracellular proteins, including collagens type I, II, and III, fibronecron, vitronectin, laminin, fibrin, and proteoglycans, as well as modulating cell function by processing a number of non-ECM proteins (6–9). An important role of several of the MT-MMPs (MT1-, MT2-, MT3-, MT5-, and MT6-MMP) is the cell-localized activation of pro-MMP-2 (10). MT1-MMP is also a key enzyme involved in cell migration and invasion and has been localized to the leading edge of migrating cells, thus facilitating matrix remodeling (7, 11). MT1-MMP null mice display abnormal development and aberrant collagen degradation and die prematurely due to these defects (12, 13). Although unchallenged MMP-2 null mice appear healthy, double knock-out MMP-2/MT1-MMP null mice show a more severe phenotype compared with the single MT1-MMP null mice, since the double null mice die within minutes of birth (14). This important observation highlights the cooperativity of MMP-2 and MT1-MMP in vivo, indicating that both enzymes have unique and overlapping functions.

MMP-2 activation proceeds on the cell surface in a two-step process involving a trimolecular complex of MT1-MMP, tissue inhibitor of metalloproteinases-2, and MMP-2. The initial cleavage of the pro-domain of MMP-2 is mediated by the membrane-tethered MT1-MMP, generating an intermediate form of MMP-2 (15–17). The remainder of the propeptide is removed by an autocatalytic mechanism generating the fully active enzyme (18, 19). In addition to gene transcription, zymogen activation, and inhibition by tissue inhibitors of metalloproteinases, MT1-MMP activity can be regulated by complex events, including autocatalytic processing, ectodomain shedding, differential glycosylation (20), homodimerization via the HPX domain (21) or cytoplasmic tail of MT1-MMP (22, 23), microdomain compartmentalization, and internalization involving both clathrin-dependent and -independent mechanisms (24–26). The regulation of internalization of MT1-MMP has been postulated as a rapid short term level of control of net MT1-MMP proteolytic activity on the cell surface.

Primary fibroblasts grown in three-dimensional fibrillar collagen gels have previously been shown to activate MMP-2 (27, 28). This has also been extended to other cell types, such as endothelial cells and tumor cell lines such as HT1080, MDA-MB-231, and BT549 cells (29–31).
Type I collagen (Col I)-stimulated MMP-2 activation functions principally via MT1-MMP in fibroblasts, since no MMP-2 activation occurs in MT1-MMP-deficient mouse fibroblasts in response to Col I (27), and reduced levels of active MMP-2 are seen in MT1-MMP-deficient mice (12). Col I can activate MMP-2 through 2 distinct mechanisms. First, Col I can increase MT1-MMP mRNA and protein levels, thereby activating MMP-2 (transcriptional response). Indeed, endothelial cells cultured in a three-dimensional Col I matrix induced MT1-MMP expression, which was dependent on the Egr-1 site in the MT1-MMP promoter (32). Col I induction of MT1-MMP mRNA also appeared to be dependent on Rac1 function in HT1080 cells (33). Furthermore, treatment of HT1080 cells with Col I induced MT1-MMP-dependent extracellular signal-regulated kinase activation, which in turn increased MT1-MMP protein levels and cell migration (31). However, an alternate pathway has been observed in which Col I increases the activity of preexisting MT1-MMP molecules in order to activate MMP-2 (non-transcriptional response) (34, 35). Due to the importance of MT1-MMP and MMP-2 in several pathological conditions, including tumor invasion and metastasis, we aimed to study the nontranscriptional mechanism of MMP-2 activation via MT1-MMP induced by Col I, the latter being abundant in the tissue stroma. We determined that Col I blocks MT1-MMP internalization through a clathrin-coated pit-mediated mechanism involving the MT1-MMP hemopexin domain, thereby stimulating MMP-2 activation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—MCF-7-MT1-MMP cells (36) were generated by stably transfecting MT1-MMP cDNA under the control of the cytomegalovirus promoter (pCNCT1-MMP) into an MCF-7 clone previously transfected with β-galactosidase and maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (JRH Biosciences, Lenexa, KS). BT549 breast adenocarcinoma cells were obtained from the ATCC (Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum. Human breast fibroblasts were isolated from human breast tissue with appropriate consent from women undergoing reduction mammoplasty in accordance with the standards of the St. Vincent’s Hospital Human Ethics Committee. Tissue was minced and digested with 200 units/ml collagenase (Sigma) and 100 units/ml hyaluronidase (Sigma) in Dulbecco’s modified Eagle’s medium/F-12 with 10% (v/v) fetal bovine serum (JRH Biosciences, Lenexa, KS). BT549 breast adenocarcinoma cells were obtained from the ATCC (Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum. Human breast fibroblasts were isolated from human breast tissue with appropriate consent from women undergoing reduction mammoplasty in accordance with the standards of the St. Vincent’s Hospital Human Ethics Committee. Tissue was minced and digested with 200 units/ml collagenase (Sigma) and 100 units/ml hyaluronidase (Sigma) in Dulbecco’s modified Eagle’s medium/F-12 with 10% (v/v) fetal bovine serum and penicillin/streptomycin/amphotericin B (Invitrogen) overnight at 37 °C and 5% CO2 with gentle rocking. Digested tissue was centrifuged at 600 g for 10 min, and the cell pellet was resuspended in medium and filtered through a 40-μm cell strainer. The cells were allowed to attach overnight, cultured in growth medium as above, and used within 3 passages.

For transfection experiments, cells were plated in 6-well plates (3 × 105 cells/well) and transfected with FuGENE 6 (Roche Applied Science) in serum-containing medium as per the manufacturer’s instructions using 2 μg of plasmid DNA with 6 μl of FuGENE per treatment.

**Plasmids and Reagents**—Wild-type MT1-MMP and mutant constructs (Fig. 1) were generated as previously described (21, 37, 38): briefly, wtMT1-MMP (wild type MT1-MMP), MT1-ΔCT (MT1-MMP lacking the cytoplasmic tail (Met7–Val159)), MT1-ΔHPR (the ectodomain of MT1-MMP (Met1–Gly335) with the TM/CT of the interleukin 2 receptor α (Val239–Ile790)), MT1-NGRF (the ectodomain of MT1-MMP (Met1–Asp315) with the TM/CT of the NGFR (neurotrophic tyrosine kinase receptor type I, NTRK1) that possesses an intrinsic kinase domain (Glu386–Gly790)), MT1-ΔCAT (MT1-MMP lacking the catalytic domain (ΔTyr112–Pro115)), and MT1-ΔHPX (MT1-MMP lacking the HPX domain (ΔLeu318–Gly335)). All constructs include the FLAG epitope, Asp-Tyr-Lys-Asp-Asp-Asp-Lys, in the catalytic domain as described (21). MT1-ΔHPX, wild type domain, and K44A dominant-negative domain were a kind gift from Dr. Duanqing Pei (University of Minnesota School of Medicine, Minneapolis, MN). Eps15S95/295 in pEGFP vector was kindly provided by Dr. Alexandre Benmerah (Faculté Necker-Enfants Malades, Paris, France) and Dr. Jennifer Stow (Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia).

Recombinant pro-MMP-2 expressed in vaccinia virus (rMMP-2) was a kind gift from Dr. Rafael Fridman (Wayne State University, Detroit, MI). Concanavalin A (ConA), nystatin, methyl-β-cyclodextrin, cycloheximide, gelatin-agarose beads, and protein G-agarose beads were purchased from Sigma. Vitrinogen 100 Col I was obtained from Cohesion (Palo Alto, CA). Sulfo-NHS-LC-biotin was obtained from Pierce. Anti-phosphotyrosine monoclonal antibody (P-Tyr-100) was purchased from Cell Signaling Technologies (Beverly, MA). Nonspecific rabbit IgG, ExtrAvidin-horseradish peroxidase, anti-FLAG M2 monoclonal antibody, and FLAG peptide were purchased from Sigma. Mouse anti-human MT1-MMP antibody (clone 114-1F2) was purchased from Calbiochem, and rabbit anti-human polyclonal MT1-MMP antibody (ABB15) was obtained from Chemicon (Boron, Australia). A rabbit anti-human polyclonal MMP-2 antibody (PAB 753) was kindly provided by Dr. Jack Windsor (University of Alabama at Birmingham). Horseradish peroxidase-conjugated secondary antibodies were obtained from Pierce. Alexa Fluor 568-labeled transferrin and donkey anti-rabbit secondary Alexa Fluor 488-conjugated antibody were obtained from Molecular Probes, Inc. (Eugene, OR). IRDye700DX-conjugated purified anti-rabbit antibody was obtained from Rockland Immunochemicals (Gilbertsville, PA). ODYSSEY infrared imaging system blocking buffer was purchased from LI-COR Biosciences (Lincoln, NE).

**Cell Lysates for Western Blot Analysis**—Cells were washed twice in PBS and lysed with 1.5% (v/v) chilled Triton X-114 in Tris-buffered saline (10 mM Tris, pH 8.0, 150 mM NaCl) with EDTA-free complete protease inhibitor mixture (Roche Applied Science). Samples were centrifuged at 1500 g for 15 min at 4 °C to remove insoluble material. Supernatants were then collected, placed at 37 °C for 5 min, and centrifuged at top speed in a microcentrifuge at 4 °C to remove insoluble material. Supernatants were then collected, placed at 37 °C for 5 min, and centrifuged at top speed in a microcentrifuge at room temperature. The bottom detergent phase containing cell membranes was then collected.

**Cell Lysates for Immunoprecipitation**—Cells were washed twice in PBS, and the cells were harvested in fresh chilled PBS with a rubber cell scraper. The cell/PBS mixture was then transferred to 1.5-ml tubes and centrifuged at 3000 × g for 10 min at 4 °C to pellet the cells. The supernatant was then removed, and the cells were resuspended in lysis buffer (10 mM Tris-HCl, pH 7.6, 10 mM NaCl, 3 mM MgCl2, 1% (v/v) Igepal CA-630, EDTA-free complete protease inhibitor mixture, 1 mM NaF, and 1 mM Na3VO4). Cells were left on ice for 1 h with periodic mixing, followed by centrifugation at 3000 × g for 10 min, and the supernatant was collected.

**MMP-2 Capture**—MMP-2 in the conditioned medium of transfected MCF-7 cells was captured with gelatin-agarose beads overnight at 4 °C. Beads were then washed in 0.1% (v/v) TBS-Tween 20 and eluted with SDS sample buffer.

**MT1-MMP Immunoprecipitation**—Equal amounts of protein lysates (100 μg) determined by a BCA protein quantification assay (Pierce) were incubated with prewashed anti-FLAG M2-agarose beads overnight at 4 °C on a rotating wheel. The next day, beads were pelleted by gentle centrifugation, washed three times with Tris-buffered saline.
(TBS) and eluted with 200 µg/ml FLAG peptide in TBS for 30 min at 4 °C. Equal amounts of eluted fractions were analyzed by Western analysis for the presence ofFLAG-tagged MT1-MMP or phosphorylated MT1-NGFR.

Cell Surface Biotinylation—Transfected MCF-7 cells were cell surface-biotinylated with 0.2 mg/ml sulfo-NHS-LC-biotin in PBS for 30 min at 4 °C. Cells were then washed in chilled PBS and neutralized with 0.1 M glycine in PBS for 10 min at 4 °C. Cells were washed again in chilled PBS and lysed as above for immunoprecipitation. Equal amounts of lysates were precleared with protein G-agarose beads and 1 µg of non-specific rabbit IgG for 5 h at 4 °C. MT1-MMP molecules were then immunoprecipitated with 1 µg of a rabbit anti-human polyclonal MT1-MMP antibody (AB815) and protein G-agarose beads overnight at 4 °C. Beads were then washed with lysis buffer and eluted with SDS sample buffer.

RNA Isolation—RNA was harvested using RNasey minicolumns (Qiagen, Clifton Hill, Australia). Quantification of RNA was established based on A_{260} and A_{280} using an ND-1000 spectrophotometer (Nanodrop Technologies Inc., Rockland, DE).

Zymography—Conditioned medium was mixed with SDS sample buffer (50 mM Tris-HCl, pH 6.8, 1% (w/v) SDS, 0.025% (w/v) bromphenol blue, and 10% (v/v) glycerol) and separated on a 10% polyacrylamide resolving gel co-polymerized with 1 mg/ml gelatin (BDH Laboratory Supplies, Poole, UK). Zymograms were then washed in 50 mM Tris-HCl, 5 mM CaCl2 (pH 8.0), and 2.5% (v/v) Triton X-100 overnight and then incubated in 50 mM Tris-HCl, pH 7.5, 5 mM CaCl2 for 24 h at 37 °C. Gels were stained with 2.5 mg/ml Coomassie Brilliant Blue (R250) dye in 10% (v/v) acetic acid and 10% (v/v) isopropyl alcohol and then destained in 10% (v/v) acetic acid and 10% (v/v) isopropyl alcohol. Densitometry was performed with the ODYSSEY program, version 1.2 (LI-COR Biosciences).

Western Blot Analysis—Equal amounts of total protein from each sample, determined by a BCA protein quantification assay, were prepared in SDS sample buffer as above with 100 mM dithiothreitol and boiled for 5 min. Protein samples were separated with a 10% polyacrylamide resolving gel. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane for 1 h at 100 V. Membranes were then blocked in 5% (w/v) skim milk powder in 0.1% (v/v) TBS-Tween 20 for 1 h at room temperature and probed either with a mouse anti-human MT1-MMP antibody (clone 114-1F2; 1 µg/ml), a rabbit anti-human polyclonal MT1-MMP antibody (AB815; 1 µg/ml), an anti-FLAG M2 monoclonal antibody (0.5 µg/ml), or an anti-phosphotyrosine monoclonal antibody (P-Tyr-100; 0.5 µg/ml) overnight at 4 °C. Membranes were then washed in 0.1% (v/v) TBS-Tween 20 and probed with a horseradish peroxidase-conjugated goat anti-mouse secondary antibody for all monoclonal primary antibodies or a horseradish peroxidase-conjugated donkey anti-rabbit secondary antibody for the polyclonal primary antibodies for 1 h at room temperature. Membranes were then blocked in 5% (w/v) BSA in 0.1% (v/v) TBS-Tween 20 and probed with ExtrAvidin-horseradish peroxidase for 1 h at room temperature. In the case of the MMP-2 Western blot analysis, membranes were blocked for 1 h at room temperature using Odyssey blocking buffer and probed with an MMP-2 antibody (PAB 753; 10 µg/ml) overnight at 4 °C. Membranes were then washed in 0.1% (v/v) TBS-Tween 20 and probed with IRDye700DX-conjugated.
purified anti-rabbit antibody for 1 h at room temperature. Membranes were washed in 0.1% (v/v) TBS-Tween 20, and detection was performed using the fluorescence 700 channel on the ODYSSEY Infrared Imaging System (LI-COR Biosciences). Densitometry was performed with the ODYSSEY program, version 1.2.

cDNA Synthesis and Quantitative PCR—cDNA was prepared from 100 ng of total RNA. cDNA was generated with the ThermoScript RT-PCR System (Invitrogen) using gene-specific priming with primer 5'-CAGCAGGCAATGACCTGC-3' for human L32 and primer 5'-CAGCAGGCAATGACCTGC-3' for dynamin and an annealing/extension temperature of 55 °C. Human ribosomal protein L32 mRNA was used as a housekeeping gene to normalize all samples. Quantitative RT-PCR was performed on an ABI Prism 5700 Sequence Detection System (PerkinElmer Applied Biosystems, Scoresby, Australia) with cDNA generated from an equivalent of 2 ng of RNA in 10 mM Tris-HCl, pH 8.0, 2.5 mM Mg(C$_2$H$_3$O$_2$)$_2$, 50 mM KCl, 200 μM dNTPs, a 1:40,000 dilution of SYBR Green I (Molecular Probes), 1 μg/ml 6-carboxy-X-rhodamine (Molecular Probes), 8% (v/v) Me$_2$SO, 200 nM primers, and 0.625 units of AmpliTaq Gold polymerase (Applied Biosystems) per 25-μl reaction. PCR primers are as follows: human L32 forward primer, 5'-CAGGGAACGCTGAGACTTC-3'; human L32 reverse primer, 5'-CAGGGAACGCTGAGACTTC-3'; forward rat dynamin primer, 5'-CAGGGAACGCTGAGACTTC-3'; and reverse rat dynamin primer, 5'-CAGGGAACGCTGAGACTTC-3'. Reaction conditions were 95 °C for 10 min followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min. Melt curve analysis from 60 to 95 °C was performed at the end of each run as a quality control step.

FIGURE 1. Transcriptional and nontranscriptional mechanisms of MMP-2 activation. Conditioned media were collected from unstimulated or Col I-stimulated (100 μg/ml) cells at the indicated time points and assessed by gelatin zymography for BT549 cells (A), primary human breast fibroblasts (B), and stably transfected MCF-7-MT1-MMP cells (C). D, MCF-7 cells were transiently transfected with MT1-MMP and treated for several hours with cycloheximide to block new protein synthesis and then treated or not with Col I (100 μg/ml) for a further 24 h. Conditioned media were collected and analyzed by gelatin zymography. E, MCF-7 cells were transiently transfected with MT1-MMP or vector control and treated or not with Col I (100 μg/ml) for 24 h. Conditioned media were collected, MMP-2 was captured using gelatin agarose beads, and elutions were analyzed by Western blot for MMP-2. In the case of A, C, D, and E, 100 ng/ml recombinant pro-MMP-2 was added to the cultures. Pro-MMP-2 (pro), intermediate active MMP-2 (inter), and fully active MMP-2 (active) are indicated by arrows. The percentage of active MMP-2 (active/total) calculated by densitometry is indicated below each lane.
relative expression levels relative to wild type dynamin-transfected MCF-7 cells.

Internalization Detection by Immunofluorescence—Transfected MCF-7 cells were plated and stimulated as indicated on Teflon-printed glass slides (Electron Microscopy Sciences, Ft. Washington, PA) at 37 °C and 5% CO₂. The following day, cells were equilibrated at 4 °C for 30 min and incubated with a rabbit anti-human polyclonal MT1-MMP antibody (AB815; 1 μg/ml) in serum-free medium for 2 h at 4 °C or 25 μg/ml Alexa Fluor 568-conjugated transferrin in serum-free medium for 30 min at 37 °C. Cells were then washed with chilled PBS to remove unbound material and then untreated or treated with acetic acid (500 mM acetic acid, 150 mM NaCl) for 10 min at 4 °C to remove cell surface-bound MT1-MMP or transferrin. This method has previously been shown to be very effective at removing cell surface-bound antibody without affecting the internalized antibody-antigen complex (25). In addition, after incubation with the MT1-MMP antibody at 4 °C, some cells were washed in chilled PBS, fixed with 3% (w/v) paraformaldehyde/PBS for 20 min at room temperature, and blocked with 5% (w/v) BSA/PBS for 30 min. Primary antibody was detected with a donkey anti-rabbit secondary Alexa Fluor 488-conjugated antibody (4 μg/ml) in 1% (w/v) BSA/PBS for 1 h at room temperature. After several washes in PBS, nuclei were stained with propidium iodide (0.25 mg/ml in 0.25% (v/v) Triton X-100, PBS, RNase) for 10 min at 37 °C. Cells were then washed in PBS and mounted using fluorescent mounting medium and viewed by confocal microscopy.

RESULTS
Nontranscriptional Component of Col I-induced MMP-2 Activation—As previously mentioned, Col I can activate MMP-2 in several cell types (27, 30, 35). In most of these cases, the MMP-2 activation in response to Col I is largely mediated by transcriptional control of MT1-MMP, since an increase at both the mRNA and protein levels is observed for MT1-MMP in response to Col I. However, in addition, a
nontranscriptional but post-translational component of Col I regulation of MT1-MMP activity has also been identified. The transcriptional response is exemplified in Fig. 2, A and B, in BT549 cells and primary human breast fibroblasts, respectively, which express endogenous MT1-MMP that can be transcriptionally increased in response to Col I, as previously reported (27, 35), resulting in increased MMP-2 activation, as seen by gelatin zymography. However, in MCF-7-MT1-MMP cells (MCF-7 cells that lack endogenous MT1-MMP stably transfected with MT1-MMP), Col I caused activation of MMP-2 despite MT1-MMP being under the control of a heterologous promoter (Fig. 2C).

Furthermore, when MCF-7 cells transiently transfected with MT1-MMP were treated with cycloheximide to block new protein synthesis, some reduction in MMP-2 activation was seen, but the Col I response was still observed (Fig. 2D). Thus, MCF-7-MT1-MMP cells provide a model for nontranscriptional regulation of Col I-induced MMP-2 activation.

**Col I-induced MMP-2 Activation Does Not Require the MT1-MMP CT or HPX Domains**—MCF-7 cells do not produce MT1-MMP protein (Fig. 3, B and C) and do not activate MMP-2 in response to Col I, as seen in Fig. 3A. Upon transfection of wtMT1-MMP, we observed MT1-MMP protein by Western blot (Fig. 3B), which was localized to the cell surface, as observed by immunofluorescence (Fig. 3D). MT1-MMP cell surface localization was performed by antibody labeling of live, nonpermeabilized cells at 4 °C, thus only detecting extracellular and not intracellular or internalized MT1-MMP. The MT1-MMP produced renders these cells capable of activating exogenous MMP-2 (Fig. 3A). After Col I stimulation, we saw a further increase in MMP-2 activation, which was not due to increased MT1-MMP protein levels (Fig. 3B) but rather to Col I effects on MT1-MMP activity/localization. In order to determine the contribution of the MT1-MMP transmembrane domain/cytoplasmic tail (TM/CT) on Col I-induced MMP-2 activation, we used an MT1-MMP chimeric molecule composed of the CAT, hinge, and HPX domains of MT1-MMP fused to the TM/CT of the interleukin-2α receptor (MT1-IL2R). The chimeric protein was expressed, albeit at

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**FIGURE 4. Effect of deletion mutants lacking MT1-MMP CAT or HPX domains.** MCF-7 cells were transiently transfected with vector control, wtMT1-MMP, MT1-ΔHPX, or MT1-ΔCAT. The following day, cells were incubated for 24 h in serum-free media with 100 ng/ml rMMP-2 containing 100 μg/ml Col I where indicated. A, conditioned media were analyzed by gelatin zymography for MMP-2 analysis; B, cell lysates were analyzed by Western blot for MT1-MMP using the anti-FLAG M2 monoclonal antibody. Pro-MMP-2 (pro), intermediate active MMP-2 (int), and fully active MMP-2 (active) are indicated by arrows. The percentage of active MMP-2 (active/total) calculated by densitometry of gelatinolytic bands is indicated below each zymogram lane. MCF-7 cells transfected with vector control (C), wtMT1-MMP (D), MT1-ΔHPX (E), or MT1-ΔCAT (F) were analyzed 48 h after transfection by immunofluorescence for cell surface localization of MT1-MMP (green) using the AB815 polyclonal MT1-MMP antibody. Nuclei were stained with propidium iodide (red).
lower levels than the wild type protein (Fig. 3B), and was localized to the cell surface (Fig. 3E). Nevertheless, some basal MMP-2 activation was observed with MT1-IL2R, and there was further activation in response to Col I (Fig. 3A). We thus conclude that the TM/CT elements of MT1-MMP are not required for MMP-2 activation in response to Col I but rather that Col I exerts its regulatory effects on the ectodomain of MT1-MMP.

To study the extracellular domains of MT1-MMP, we used MT1-MMP mutant constructs lacking either the CAT or HPX domains. MT1-ΔCAT lacking the CAT domain, although expressed (Fig. 4B) and localized to the cell surface (Fig. 4F), was unable to activate MMP-2 under basal conditions or with Col I stimulation (Fig. 4A), as expected due to the lack of a functional CAT domain. The MT1-ΔHPX construct was also expressed (Fig. 4B) and localized to the cell surface (Fig. 4F) and was able to activate MMP-2 under basal conditions (Fig. 4A), demonstrating that this mutant was functional at least in terms of basal processing of MMP-2, as previously described (39). However, unlike wtMT1-MMP or MT1-MMP lacking the TM/CT, the MT1-ΔHPX mutant could not further activate MMP-2 in response to Col I (Fig. 4A).

These results indicate that the Col I regulatory region lies in the HPX of MT1-MMP. Col I Does Not Appear to Directly Increase MT1-MMP Clustering—It has recently been reported that MT1-MMP forms homophilic interactions on the cell surface via its HPX domains and that these dimerizations are required for MMP-2 activation (21, 22). We thus asked whether Col-I increased MT1-MMP dimerization. This was performed using an MT1-NFGR construct that contains the CAT, hinge, and HPX domains of MT1-MMP with the TM/CT of the NTRK1 nerve growth factor receptor (NGFR) that contains an intrinsic kinase domain. It is well established that growth factor receptors dimerize upon ligand binding, causing autophosphorylation of the receptor (40). The MT1-NFGR mutant has been shown to be autophosphorylated upon MT1-MMP dimerization (21). When transfected into MCF-7 cells, MT1-NFGR was expressed and localized to the cell surface similarly to
wtMT1-MMP (Fig. 5, C and B, respectively). MT1-NGFR was also capable of activating MMP-2, which was further increased in response to Col I (Fig. 5A), similarly to MT1-IL2R. Whole cell lysates from MCF-7 cells transfected with wtMT1-MMP or MT1-NGFR (both tagged with the FLAG epitope) were collected and immunoprecipitated with an anti-FLAG antibody, and the eluted fractions were analyzed by Western blot probed with an anti-phosphotyrosine antibody to look for MT1-NGFR transphosphorylation. No phosphotyrosine signal was detected in wtMT1-MMP-transfected cells, as expected (Fig. 5E), despite the MT1-MMP protein detected (Fig. 5D). However, tyrosine phosphorylation was evident in MT1-NGFR-transfected cells, and the amount of phosphorylated MT1-NGFR was not further increased in response to Col I (Fig. 5E), suggesting that Col I does not directly increase MT1-MMP dimerization in our system. However, we cannot rule out the possibility that the NGFR CT of the chimeric molecule interacts with other accessory molecules that could be interfering with its ability to dimerize in response to Col I.

Col I Blocks Dynamin-mediated MT1-MMP Internalization, Which Differs from the Mechanism of ConA Inhibition of Internalization—Because Col I regulated MT1-MMP activity rather than protein levels and did not appear to directly affect MT1-MMP dimerization, we decided to study Col I effects on MT1-MMP internalization, since this has been recently identified as a mechanism that regulates MT1-MMP activity post-translationally (25, 26, 41). First, MCF-7 cells transfected with wtMT1-MMP, MT1-ΔCT, MT1-ΔCAT, MT1-IL2R, or MT1-ΔHPX were treated or not with Col I overnight. The amount of MT1-MMP present on the cell surface was then determined by cell surface biotinylation. An increase in cell surface MT1-MMP protein was observed upon Col I stimulation for wtMT1-MMP, MT1-ΔCT, MT1-IL2R, and MT1-ΔCAT. However, no increase in cell surface MT1-MMP was seen with the MT1-ΔHPX mutant with Col I stimulation (Fig. 6A (Western blot) and B (densitometry of cell surface/total MT1-MMP)), consistent with its inability to further activate MMP-2 in response to Col I.

Those MT1-MMP domains (CT and CAT) necessary for the ability of Col I to increase cell surface MT1-MMP were further studied using a fluorescence-based internalization assay. MCF-7 cells transfected with wtMT1-MMP, MT1-ΔCT, or MT1-ΔCAT and treated or not with Col I were placed at 4 °C for 30 min to slow cellular events, including the internalization process. The live cells were then incubated at 4 °C with an anti-MT1-MMP antibody in order to label only cell surface MT1-MMP molecules. The cells were then immediately fixed (time point 0 min) or warmed to 37 °C for 20 or 180 min to resume internalization. Also, at each time point, the cells were either not treated or treated with acetic acid in order to strip all cell surface antigen-antibody complexes without interfering with internalized (intracellular) complexes. Fig. 7A shows the cell surface staining for untreated and Col I-treated cells prior to warming to 37 °C. Note that in Col I-treated cells, the localization of MT1-MMP is concentrated around the edge of the cells, whereas in untreated cells, MT1-MMP is more diffusely distributed on the cell surface. This was consistently observed with wtMT1-MMP, MT1-ΔCT, and MT1-ΔCAT. When treated with acetic acid, cell surface staining was removed, and no internalized MT1-MMP was observed in
all treatments (Fig. 7B). However, when cells were brought to 37 °C for 20 min to resume internalization, intracellular MT1-MMP was evident in the acid-treated unstimulated cells; however, very little internalized MT1-MMP was detected in Col I-treated cells transfected with wtMT1-MMP, MT1-ΔCT, or MT1-ΔCAT. A similar pattern was also seen after 180 min at 37 °C, although more internalized MT1-MMP was evident (Fig. 7B). Compared with wtMT1-MMP or MT1-ΔCAT (which showed similar internalization kinetics), MT1-ΔCT showed very little internalized MT1-MMP (Fig. 7B). This is in accordance with previous studies showing reduced internalized MT1-ΔCT, which lacks internalization signals in the CT (25). However, the small amount of internalized MT1-ΔCT in the unstimulated cells was not seen in Col I-treated cells (Fig. 7B). Taken together, these data clearly demonstrate that Col I interferes with MT1-MMP internalization in MCF-7 cells, even when lacking the CT or CAT domains but not the HPX domain.

MT1-MMP internalization has been identified to function by two different mechanisms: clathrin-coated pit-mediated internalization and clathrin-independent mechanisms involving caveolae (24–26). For clathrin-dependent MT1-MMP endocytosis, internalization signals have been identified in the MT1-MMP CT (25). We thus further studied the effect of Col I-induced MMP-2 activation using MT1-ΔCT that lacks the CT. We demonstrated using MT1-IL2R and MT1-NGFR that the TM/CT of MT1-MMP is not required for Col I-induced MMP-2 activation; however, these mutants contain a TM/CT of other proteins that could interfere with the internalization process. Thus, to eliminate any possible interference, we further studied Col I effects on MMP-2 activation using MT1-ΔCT.

MCF-7 cells were transfected with wtMT1-MMP and MT1-ΔCT, both of which were localized to the cell surface (Fig. 8, D and E, respectively). We observed in MT1-ΔCT-transfected cells an increase in MMP-2 activation compared with wtMT1-MMP (Fig. 8A) as predicted based on its reduced internalization (25). An increase in the amount of active MT1-MMP was also evident in cells transfected with MT1-ΔCT compared with wtMT1-MMP (Fig. 8B), consistent with an impairment
of MT1-MMP internalization and degradation/turnover for MT1-ΔCT, as described (41). Col I further activated MMP-2 in both wtMT1-MMP- and MT1-ΔCT-transfected cells, and this was not due to changes in MT1-MMP protein levels (Fig. 8B). Con A can increase MMP-2 activation via blockade of MT1-MMP internalization (41). Although Con A activated MMP-2 in wtMT1-MMP-transfected cells, it was defective in MT1-ΔCT transfected cells, in contrast to Col I (Fig. 8A). These results confirm that Col I functions through a different mechanism from Con A, independent of the CT-mediated internalization.

Of the two mechanisms identified for MT1-MMP internalization, both are dependent on the activity of the GTPase dynamin, which mediates the scission from the plasma membrane of both endocytic pathways. The activity of dynamin can be inhibited by a dominant negative mutant, K44A that is deficient in GTP hydrolysis (42). This dynamin mutant has been used as a tool to demonstrate that MT1-MMP is internalized via a dynamin-mediated pathway (26, 41). When the dominant-negative K44A mutant was co-transfected with MT1-MMP in MCF-7 cells, basal MMP-2 activation was increased compared with the vector control/MT1-MMP or wild type dynamin/MT1-MMP co-transfectants (Fig. 9A), as previously observed (26, 41). However, no further MMP-2 activation in response to Col I was seen in the K44A dynamin/MT1-MMP co-transfectants, but further MMP-2 activation was seen in the vector control/wtMT1-MMP or wild type dynamin/MT1-MMP co-transfectants (Fig. 9A). The total protein levels of MT1-MMP did not change significantly in all treatments (Fig. 9B). Fig. 9C illustrates that wild type dynamin and K44A dynamin were expressed to similar levels (and not expressed in vector control cells), and these levels were slightly increased in response to Col I. Impairment of MT1-MMP internalization using the K44A dominant-negative mutant was also confirmed by immunofluorescence. Less basal internalized MT1-MMP was observed in the K44A dynamin/MT1-MMP co-transfectants compared with the wild type dynamin/MT1-MMP co-transfectants (Fig. 9D), confirming that the K44A mutant is functional in blocking dynamin-mediated internalization. This suggests that Col I functions by blocking MT1-MMP internalization in a dynamin-mediated mechanism that was mimicked by expression of the K44A dominant-negative mutant. Col I could therefore not further activate MMP-2 in the presence of K44A dynamin.

Col I Blocks MT1-MMP Internalization via Clathrin-Coated Pits—In order to distinguish which endocytic pathway Col I utilizes to impair MT1-MMP internalization, we first used two cholesterol-binding compounds, nystatin and methyl-β-cyclodextrin, which interfere with caveolae-dependent endocytosis without affecting the clathrin-dependent endocytic pathway (43, 44). Fig. 10A illustrates that both compounds increased the basal level of MMP-2 activation in MCF-7 cells stably transfected with MT1-MMP, similarly to the K44A dominant-negative dynamin and consistent with caveolae-disrupting agents, to impair basal MT1-MMP internalization (24, 26). However, Col I further increased MMP-2 activation in the presence of nystatin or methyl-β-cyclodextrin treatment (Fig. 10A), indicating that Col I does not block the caveolin-mediated pathway. Interestingly, with the nystatin treatment, we observed an accumulation of the 45-kDa inactive MT1-MMP form (Fig. 10B), suggesting that the 45-kDa form is normally internal-
ized via caveolae. The 45-kDa form is not normally seen in these cells stably transfected with MT1-MMP. Less internalized MT1-MMP was observed in nystatin-treated cells compared with untreated cells (Fig. 10C), confirming that nystatin was bioactive, blocking basal MT1-MMP internalization.

In order to study the involvement of the clathrin-coated pit-mediated internalization pathway, we used an Eps15/H900495/295 construct that functions as a dominant-negative inhibitor to specifically block Eps15, a crucial component of clathrin-mediated endocytosis, without interfering with clathrin-independent endocytosis (45, 46). By transiently co-transfecting wtMT1-MMP and Eps15/H900495/295 into MCF-7 cells, we saw no further increase in MMP-2 activation in response to Col I; however, increased MMP-2 activation was seen in response to Col I in vector control/wtMT1-MMP cotransfectants (Fig. 10D). No differences in total MT1-MMP protein levels were observed (Fig. 10E). Fig. 10F confirms the co-expression of the green fluorescent protein-tagged Eps15ΔH95/295 mutant protein (green) with MT1-MMP (red). The specificity of the Eps15ΔH95/295 construct for the clathrin-mediated pathway was confirmed, since Eps15ΔH95/295 blocked the internalization of the transferrin receptor (Fig. 10, G–J), a cell surface molecule known to be internalized via clathrin-coated pits (46). Note that no red labeled internalized transferrin is seen in Eps15ΔH95/295-expressing cells (green) but that internalized transferrin is seen in neighboring cells lacking Eps15ΔH95/295 expression (Fig. 10J) or in vector control-expressing cells (Fig. 10I). These data thus indicate that Col I functions to block MT1-MMP internalization via the clathrin-coated pit-mediated pathway.

**DISCUSSION**

Col I is often encountered by cells migrating into a stromal environment and, as such, is an important physiological regulator of MT1-MMP activity and, consequently, MMP-2 activation. MT1-MMP also is essential for fine tuning of deposited collagen by connective tissue cells, as evidenced by the widespread fibrosis evident in MT1-MMP-deficient mice (12). β1-containing integrins are the major cell surface receptors that are required for MMP-2 activation in response to Col I (47). Col I can increase MT1-MMP activity by a transcriptional and/or a nontranscriptional response. The latter may be a more suitable level of regulation if rapid proteolytic activity is required. The nontranscriptional component in response to Col I in MCF-7 cells has also been observed in endothelial cells expressing endogenous MT1-MMP (34). This response is very rapid, observed within hours of stimulation with Col I, and may have evolved alongside the transcriptional response, cooperat-
ing for both rapid and sustained proteolytic activity. A similar nontranscriptional component of MMP-2 activation via MT1-MMP has been observed in U87 glioma cells in response to cytochalasin D (48) and in MDA-MB-231 breast cancer cells in response to ConA (49). In the nontranscriptional response of MMP-2 activation in MCF-7 cells, Col I blocked the internalization of MT1-MMP in a dynamin- and clathrin-dependent fashion, leading to increased MMP-2 activation, and caused a redistribution of MT1-MMP to the cell periphery. In contrast to ConA-induced MMP-2 activation, Col I effects on MT1-MMP internalization were independent of the TM/CT domains.

**FIGURE 10.** Col I-mediated MMP-2 activation mechanism is dependent on clathrin-coated pits. MCF-7 cells stably transfected with wtMT1-MMP (MCF-7-MT1-MMP) were incubated in serum-free medium with 100 ng/ml rMMP-2, with or without 30 μg/ml nystatin or 5 μM methyl-β-cyclodextrin for several hours. Cells were then either treated or not with 100 μg/ml Col I where indicated for 24 h. A, conditioned medium was analyzed by gelatin zymography for MMP-2 analysis; B, cell lysates were analyzed by Western blot for MT1-MMP using the AB815 polyclonal MT1-MMP antibody. Pro-MMP-2 (pro), intermediate active MMP-2 (inter), and fully active MMP-2 (active) are indicated by arrows. The percentage of active MMP-2 (active/total) calculated by densitometry of gelatinolytic bands is indicated below each zymogram lane. C, for internalization studies, MCF-7-MT1-MMP cells were treated with 30 μg/ml nystatin where indicated for 24 h. Cells were then equilibrated at 4 °C, and cell surface MT1-MMP was labeled with the AB815 polyclonal MT1-MMP antibody at 4 °C and then warmed to 37 °C for 0 or 20 min. Cells were then left untreated or treated with acetic acid at 4 °C, washed with PBS, fixed, and permeabilized, and primary antibody was detected with a secondary Alexa Fluor 488 antibody. The percentage of internalized MT1-MMP was determined by measuring the pixel intensity of internalized (acid-treated) samples versus total MT1-MMP. Three representative fields per sample were used for statistical analysis. An unpaired Student’s t test was used to determine statistically significant differences between untreated and nystatin-treated samples (*, p < 0.05). MCF-7 cells were co-transfected with wtMT1-MMP and either vector control or Eps15Δ95/295. The following day, cells were incubated for 24 h in serum-free medium with 100 ng/ml rMMP-2 containing 100 μg/ml Col I where indicated. D, conditioned medium was analyzed by gelatin zymography for MMP-2 analysis; E, cell lysates were analyzed by Western blot for MT1-MMP, using the AB815 polyclonal MT1-MMP antibody. Cells expressing Eps15Δ95/295 (pEGFP) are shown as green, and MT1-MMP is shown in red (red). Internalized transferrin was detected by incubating MCF-7 cells transiently transfected with Eps15Δ95/295 (H and J) or pEGFP vector control (G and I) with no transferrin (G and H) or 25 μg/ml Alexa Fluor 568-conjugated transferrin (I and J) for 30 min at 37 °C. Cells were then treated with acetic acid for 10 min at 4 °C. Internalized transferrin appears as red, whereas Eps15Δ95/295 or pEGFP vector expression is green.
Important Functional Domains of MT1-MMP—MT1-MMP contains several domains that are required for proper function and localization of the enzyme. Membrane anchorage of MT1-MMP through the TM domain is essential for proper localization and function of the enzyme, including MMP-2 activation (37, 50). The CAT domain is also essential for proteolytic activity, since it contains the catalytic active site. Oligomerization via the HPX domain has been shown to be important to keep MT1-MMP molecules in close proximity, an important requirement for MMP-2 activation (21, 22). The HPX domain of MT1-MMP can directly bind native Col I, and it has been suggested that Col I may assemble MT1-MMP on the cell surface through binding to the HPX domain, resulting in an increase in the local concentration of MT1-MMP for collagenolysis and MMP-2 activation (28). Tam et al. (28) have further shown by mutation studies that dileucine residues Leu571-Leu572 and Leu578-Leu579 as well as Tyr573 in the CT of MT1-MMP were critical for its internalization under basal conditions. Furthermore, MT1-MMP was found to co-localize with clathrin-coated pits, and the Eps15 subunit of adaptor protein 2 (AP-2), a component of clathrin-coated pits, was shown to bind to the LLY573 sequence of the CT of MT1-MMP (25).

ConA can induce MMP-2 activation post-translationally by blocking MT1-MMP internalization via clathrin-coated pits (41). Under basal conditions, MT1-ΔCT was more effective at activating MMP-2 than wtMT1-MMP and showed increased cell surface expression compared with wtMT1-MMP, due to the removal of the internalization signals in the CT (38, 50). MMP-2 activation via MT1-ΔCT was found to be resistant to ConA treatment (41), as also observed in our studies, suggesting that ConA regulates MT1-MMP activity through CT-dependent trafficking. Nevertheless, Col I activated MMP-2 via the MT1-ΔCT mutant, illustrating that Col I and ConA block MT1-MMP internalization through two distinct mechanisms, one involving the CT (ConA) and one that
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does not (Col I). Furthermore, although a direct impairment of basal MT1-MMP internalization was observed using MT1-ΔCT, further inhibition of internalization was observed after treatment with Col I, again indicating that Col I blocks MT1-MMP internalization independent of signals in the CT. Taken together, since Col I impaired the internalization of wtMT1-MMP, MT1-ΔCAT, and MT1-ΔCT, and increased cell surface expression was observed in response to Col I for MT1-II2R but not MT1-ΔHPX, these data indicate that the Col I regulatory region lies in the MT1-MMP HPX domain.

Dynamin is a GTPase implicated in endocytosis by mediating scission of newly formed vesicles from the plasma membrane of both clathrin-coated pits and caveolae (S1). Our results using dominant-negative K44A dynamin indicate that this mutant blocks MT1-MMP endocytosis, resulting in increased basal MMP-2 activation, as also previously seen (S2, S3, S4). However Col I could not further activate MMP-2, presumably because internalization had already been blocked by the K44A dynamin. Our results demonstrate that Col I blocks the dynamin-dependent internalization of MT1-MMP.

Caveolae are flask-shaped cholesterol-rich microdomains on or near the cell surface involved in internalization of cell surface receptors, recycling of molecules via the Golgi, and signal transduction pathways (S5). Caveolae were found as the major MT1-MMP endocytic pathway in endothelial cells, and caveolae-disrupting agents or small interfering RNA toward caveolin-1 impaired MT1-MMP internalization, cell surface localization at clusters, activity, and function in migrating endothelial cells. Col I, however, blocked this caveolae-mediated pathway through the action of Rho A (S4). Despite this report, the caveolae pathway was not involved in Col I-mediated inhibition of MT1-MMP internalization in the nontranscriptional component in MCF-7 cells, illustrating that different mechanisms exist in different cell types.

Clathrin-coated vesicles are a major pathway for receptor-mediated endocytosis. Many protein constituents make up clathrin-coated vesicles, including AP-2 adapter protein, clathrin, dynamin, and Eps15 (epidermal growth factor receptor pathway substrate clone 15) (S5). An Eps15 dominant-negative mutant that lacks two of the three Eps15 homology domains (Eps15D95/295) has been useful in the study of endocytic pathways involving clathrin-coated pits (S6) and has determined that Col I specifically blocks MT1-MMP endocytosis via the clathrin-mediated pathway in MCF-7 cells. However, Col I does not function as a general inhibitor of endocytosis, since internalization of the transferrin receptor, a well defined marker of clathrin-coated pit endocytosis, was not affected by Col I treatment (data not shown).

Similarly to our results, endothelial cells cultured on β1 integrin ECM substrates such as Col I, fibronectin, or fibrinogen displayed impaired internalization of MT1-MMP compared with non-β1 integrin ECM substrates (S3). Furthermore, co-immunoprecipitation assays demonstrated a biochemical association between MT1-MMP and β1 and αv integrins when endothelial cells were cultured on Col I (S3). It was hypothesized that clustering of β1 integrins induced phosphorylation or recruitment of cytoskeletal/signaling molecules that could interfere with MT1-MMP endocytosis. Additionally, physical retention of MT1-MMP through interactions with β1 or αvβ3 at the cell membrane could block MT1-MMP internalization (S3). As hypothesized by Galvez et al. (S3), it is possible that Col I interferes with MT1-MMP internalization in MCF-7 cells by inducing a physical association between the MT1-MMP HPX domain and β1 integrin, thereby overriding the non-CT-mediated internalization signals, keeping MT1-MMP on the cell surface. Another candidate molecule that may regulate the internalization of MT1-MMP via Col I is the tetraspanin CD63. It was recently identified that a third MT1-MMP endocytic pathway existed involving the MT1-MMP ectodomain. CD63 formed a complex on the cell surface with the MT1-MMP HPX domain, thereby promoting endocytosis of this complex via internalization signals in the CT of CD63 into lysosomes targeted for degradation (S7). Taken together, our results may support the hypothesis that Col I induces MMP-2 activation through specific inhibition of the CD63-mediated mechanism of MT1-MMP endocytosis involving clathrin-coated pits (Fig. 11). Studies are ongoing to test this hypothesis. Finally, Wu et al. (S8) have shown that focal adhesion kinase/Src-mediated phosphorylation of endophilin A2 prevented endophilin A2 from associating with dynamin, thereby blocking MT1-MMP endocytosis. Considering that focal adhesion kinase can be activated upon integrin ligation, it is tempting to speculate that Col I signaling via Col I-binding integrins feeds into the above mentioned pathway to block MT1-MMP endocytosis via focal adhesion kinase/Src-mediated phosphorylation of endophilin A2.

In conclusion, we have further elucidated the mechanism of the nontranscriptional component of MMP-2 activation in response to Col I, an important physiological activator of MMP-2 often found surrounding tumors. We identified that Col I impairs MT1-MMP internalization, thereby providing a rapid mechanism to increase MMP-2 activation through a dynamin- and clathrin-mediated mechanism involving the HPX domain of MT1-MMP. In addition, Col I induced a redistribution of preexisting cell surface MT1-MMP molecules to the periphery of the cells. Specifically targeting these various components of the MMP-2 activation mechanism may offer novel strategies to limit proteolytic activity required for tumor growth, invasion, and metastasis.

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REFERENCES

1. Egbeblaid, M., and Werb, Z. (2002) Nature Rev. Cancer 2, 161–174
2. Lafleur, M. A., Handsley, M. M., and Edwards, D. R. (2003) Expert Rev. Mol. Med. 5, 1–39
3. Overall, C. M., and Lopez-Otin, C. (2002) Nature Rev. Cancer 2, 657–672
4. Noel, A. C., Polette, M., Lewalle, J. M., Mounaut, C., Emmonard, H., Pirembaut, P., and Foidart, J. M. (1994) Int. J. Cancer 56, 331–336
5. Dalberg, K., Eriksson, E., Enberg, U., Kjellman, M., and Backdahl, M. (2000) World J. Surg. 24, 334–340
6. Belkin, A. M., Akimov, S. V., Zaitsevskaya, I., Ratnikov, B. I., Deryugina, E. I., and Strongin, A. Y. (2001) J. Biol. Chem. 276, 18415–18422
7. Kajita, M., Itoh, Y., Chiba, T., Morii, H., Okada, A., Kinosh, H., and Seiki, M. (2001) J. Cell Biol. 153, 893–904
8. Deryugina, E. I., Ratnikov, B. I., Postnoova, T. I., Rozanov, D. V., and Strongin, A. Y. (2002) J. Biol. Chem. 277, 9749–9756
9. Tam, E. M., Morrison, C. I., Wu, Y. I., Stack, M. S., and Overall, C. M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 6917–6922
10. Seiki, M., Koshikawa, N., and Yama, I. (2003) Cancer Metastasis Rev. 22, 129–143
11. Rotardy, K., Allen, E., Puntari, A., Yama, I., and Weiss, S. J. (2000) J. Cell Biol. 149, 1309–1322
12. Holmbeck, K., Bianco, P., Caterina, J., Yamada, S., Kromer, M., Kuznetsov, S. A., Mankani, M., Robey, P. G., Poole, A. R., Pidoux, I., Ward, J. M., and Birkedal-Hansen, H. (1999) Cell 99, 81–92
13. Zhou, Z., Apte, S. S., Soininen, R., Cao, R., Baiklini, G. Y., Rauser, R. W., Wang, J., Cao, T., and Tryggvason, K. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4052–4057
14. Oh, T., Takahashi, R., Adachi, E., Kondo, S., Kuratomi, S., Noma, A., Alexander, D. B., Motoda, H., Okada, A., Seiki, M., Itoh, T., Itohara, S., Takahashi, C., and Noda, M. (2004) Oncogene 23, 5041–5048
15. Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A., and Goldberg, G. I. (1995) J. Biol. Chem. 270, 5331–5338
16. Butler, G. S., Butler, M. J., Atkinson, S. J., Will, H., Tamura, T., van Westrum, S. S., Crabbe, T., Clements, J., d’Ortho, M. P., and Murphy, G. (1998) J. Biol. Chem. 273, 871–880
17. Kinoshita, S., Hato, O., Okada, A., Ohuchi, E., Imai, K., Okada, Y., and Seiki, M. (1998) J. Biol. Chem. 273, 16098–16103
18. Atkinson, S. J., Crabbe, T., Cowell, S., Ward, R. V., Butler, M. J., Sato, H., Seiki, M., Reynolds, J. J., and Murphy, G. (1995) J. Biol. Chem. 270, 30479–30485
Type I Collagen Abrogates the Clathrin-mediated Internalization of Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) via the MT1-MMP Hemopexin Domain
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