The Involvement of the Fibronectin Type II-like Modules of Human Gelatinase A in Cell Surface Localization and Activation*

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Recombinant collagen-binding domain (rCBD) comprising the three fibronectin type II-like modules of human gelatinase A was found to compete the zymogen form of this matrix metalloproteinase from the cell surface of normal human fibroblasts in culture. Upon concanavalin A treatment of cells, the induced cellular activation of gelatinase A was markedly elevated in the presence of the rCBD. Therefore, the mechanistic aspects of gelatinase A binding to cells by this domain were further studied using cell attachment assays. Fibroblasts attached to rCBD-coated microplate wells in a manner that was inhibited by soluble rCBD, blocking antibodies to the β1-integrin subunit but not the α2-integrin subunit, and bacterial collagenase treatment. Addition of soluble collagen rescued the attachment of collagenase-treated cells to the rCBD. As a probe on ligand blots of octyl-glucoside-solubilized cell membrane extracts, the rCBD bound 140- and 160-kDa protein bands. Their identities were likely pro-collagen chains being both bacterial collagenase-sensitive and also converted upon pepsin digestion to 112-kDa bands that co-migrated with collagen types I, V, and X and elastin and also contains a heparin-binding deficient mutant (Ly8→Ala) with reduced collagen affinity showed less cell attachment, whereas a heparin-binding deficient mutant (Ly357→Ala), heparinase treatment, or heparin addition did not alter attachment. Thus, a cell-binding mechanism for gelatinase A is revealed that does not involve the hemopexin COOH domain. Instead, an attachment complex comprising gelatinase A-native type I collagen-β1-integrin forms as a result of interactions involving the collagen-binding domain of the enzyme. Moreover, this distinct pool of cell collagen-bound proenzyme appears requisite to cellular activation.

The plasma membrane of various human cancer cells contains high levels of collagenolytic and gelatinolytic proteinases (1, 2) with a positive correlation shown between the expression of the matrix metalloproteinase (MMP) gelatinase A and invasive potential (3). Moreover, certain tumor cell lines, which do not express gelatinase A, can bind the enzyme to their cell membranes by a membrane-associated receptor in trans (2, 4). Activation of progelatinase A by cell membranes of concanavalin A (ConA)-stimulated (5, 6) or 12-O-tetradecanoyl-phorbol-13-acetate-stimulated (7, 8) normal cells requires a specific mode of enzyme-cell interaction that utilizes the COOH-terminal domains of gelatinase A and the tissue inhibitor of MMPs, TIMP-2 (8–10). Four membrane type (MT)-MMPs possessing a hydrophobic transmembrane domain have been shown to activate progelatinase A at the cell surface (11, 12) in an activation complex comprising progelatinase A, TIMP-2, and MT-MMP (12, 13). Here, the active site of MT-MMP functions as a receptor for the inhibitory NH2 domain of TIMP-2, leaving the TIMP-2 COOH domain free to interact with progelatinase A. Recent site-directed mutagenesis studies have mapped the TIMP-2-binding site on gelatinase A to the junction of the outer rim of β-blades III and IV of the hemopexin-like COOH-terminal domain (C domain) (2). However, alternative interactions of the gelatinase A C domain with TIMP-4 (14) and cell surface components such as the α2β1 integrin receptor (15), fibronectin (16), and heparin (16–18) have also been identified.

The C domain of MMPs is involved in several important protein-protein interactions. In gelatinase B the C domain binds TIMP-1, whereas interstitial and neutrophil collagenases utilize the C domain for binding and cleavage of native type I collagen (19). However, the gelatinase A C domain does not bind collagen (16, 20). Instead, a different collagen-binding domain (CBD) is found in gelatinases A and B consisting of three fibronectin type II-like modules inserted in the catalytic domain (21, 22). In addition to binding denatured type I collagen (23–25), our characterization of recombinant human gelatinase A (rCBD) showed that this domain accounts for all of the binding properties of the enzyme to native and denatured collagen types I, V, and X and elastin and also contains a heparin-binding site (17, 25). The importance of these functions is shown by CBD deletion, which reduces gelatinase A cleavage of denatured type I collagen by 90% (20) and abolishes elastin binding and cleavage (26).

The gelatinase A CBD may also serve to localize the enzyme to matrix components in tissues (17, 20, 25). These properties may similarly provide another mode of cell binding to membrane-associated matrix proteins, including collagen and heparin.

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ran sulfate proteoglycans, and thus may play a role in gelatinase A activation (18) and its physiological function on the cell surface. Here we report experiments that establish that the fibronectin-like CBD localizes gelatinase A to fibroblast cell surfaces by the formation of a gelatinase A-type I collagen-β1 integrin complex. Notably, this distinct pool of cell-bound enzyme shows a lowered cellular activation potential compared with soluble gelatinase A. This finding has important implications for the role of cell membrane-bound stromal gelatinase A on tumors cells.

**EXPERIMENTAL PROCEDURES**

**Recombinant Gelatinase A Domains and Antibodies—** rCBD (Val191–Gln446) and the rC domain (Gln447–Cys631) of human gelatinase A were expressed in Escherichia coli and purified by Zn2+-chelate and gelatin-Sepharose chromatography as appropriate (14, 25). Electrospray mass spectrometry of the recombinant proteins was performed on a SCIEX API 300 (Perkin-Elmer) mass spectrometer. The convention used in this paper to distinguish between the recombinant protein comprised of the gelatinase A triple fibronectin type II-like repeat and the domain present in the natural enzyme will be to refer to the recombinant collagen-binding domain as the rCBD and to the domain in the enzyme as the CBD.

Rabbit polyclonal antibody (αCBD) was raised against rCBD injected with sarcosyl-extracted rCBD inclusion bodies and was then affinity purified over rCBD-AffiGel 10 (Bio-Rad) columns. Anti-peptide antibody (αHis4) to the NH2-terminal His6 fusion tag on the recombinant proteins was affinity purified as before (16).

**Cell Culture—** Human gingival fibroblasts, kindly provided by Drs. D. Brunette and H. Largaja (University of British Columbia), were maintained in α-minimal essential medium (α-MEM) (Life Technologies, Inc.) containing 10% newborn calf serum (Life Technologies, Inc.) and antibiotics at 37 °C. To minimize proteolysis of membrane proteins during cell harvesting for cell attachment assays, 0.2 mM EDTA with a low concentration of trypsin (0.05%) in phosphate-buffered saline (PBS) (140 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4) was used for 30–60 s only.

**Competition Experiments—** Fibroblasts in 96-microwell tissue culture plates were treated with soluble rCBD (1.0 × 10−8 to 1.0 × 10−4 μg) or rC domain (5.6 × 10−8 to 1.0 × 10−8 μg) for 24–28 h during and/or after ConA treatment (20 μg/ml) (5) of quiescent cells in serum-free conditions. Conditioned medium and cell extracts were analyzed by zymography on 10% polyacrylamide/4% gelatin SDS-PAGE gels (27). To determine whether gelatinase A could bind unstimulated cells by the CBD, quiescent cells were thoroughly rinsed with PBS to remove unbound secreted enzyme. Gelatinase A was then competed from cell surfaces by incubation of the cell layers with 1.2 or 12 × 10−6 μg rCBD in serum-free α-MEM at 22 °C for 5 min only. This short time selection was used to minimize contributions from newly synthesized enzyme to the total in the incubation. After medium harvesting, the remaining cell-associated enzyme was assessed after lysis of the cell layer with SDS-PAGE sample buffer.

**Cell Attachment Assay—** Tissue culture surface treatment 96-microwell plates were coated with 2-fold serially diluted rCBD (50–0.25 μg/ml) in 100 μl PBS/well for 18 h at 4 °C. After blocking with 10 mg/ml heat-denatured bovine serum albumin (BSA) for 30 min, 4 × 104 fibroblasts were added per well in serum-free α-MEM (to avoid cell attachment from serum proteins) and incubated for 90 min at 37 °C. Cells were then thoroughly rinsed with PBS and fixed with 4% formaldehyde in PBS. The attached cells were stained with 0.1% crystal violet in 200 mM boric acid, pH 6.0 (28). After extensive rinses, cellular stain was dissolved in 10% acetic acid, pH 6.0 (28). After extensive rinses, cellular stain was dissolved in 10% acetic acid, pH 6.0 (28). Chemiluminescence reagents (Amersham Pharmacia Biotech). The rCBD-binding proteins were characterized by digestion with pepsin (0.1 mg/ml) (29) at pH 2.0 for 4 h at 15 °C. The resultant material was then separated under nonreducing (65 mM DTT) conditions by

**RESULTS**

**Recombinant Protein Expression—** The rCBD mass was measured by electrospray mass spectrometry to be 21,218 Da, confirming NH2-terminal methionine processing of the recombinant protein (predicted mass 21,212 Da); fidelity of expression, and homogeneity of the protein preparation. The typical yield of purified rCBD from 3.6 liters of culture was 120 mg.

**The Collagen-binding Domain Mediates Binding of Gelatinase A to Cells—** When rCBD was incubated with human fibroblasts for 24 h during and after ConA treatment (Fig. 1A) or for 24 h after ConA treatment only (not shown), an increase in gelatinase A activation was apparent in six separate experiments. At high rCBD concentrations, essentially all the soluble gelatinase A was converted to the 59-kDa (−DTT) activated form (5). Although quantitation of enzyme levels from zymograms is only semiquantitative, less than −3% of the total soluble gelatinase A remained as the 66-kDa (−DTT) zymogen form in the presence of 100 μg rCBD (lane 100 +) compared with −28−34% in those cells not treated with rCBD (lanes 0 +). This trend was also apparent at 50 μg rCBD. In contrast, recombinant gelatinase A C domain reduced cellular activation of the enzyme as before (17) (not shown).

Cell lysates containing rCBD that was bound to cells via the C domain of the enzyme or was intracellular in the cell secretary pathway were also prepared after rCBD treatment. Unlike the effect of rCBD on gelatinase A levels in the medium (Fig. 1A), addition of rCBD to cells during and/or after ConA treatment did not alter the ratios of latent (66 kDa) to active (59 kDa) gelatinase A in the lysates (Fig. 1B). As estimated...
Panel C gelatinase A was present in the 59-kDa active form. In contrast, the SDS-PAGE/40 serum-free confluence, the cells were rinsed with PBS and then incubated with mm were seeded in uncoated wells or wells coated with 25 mm rCBD as before. Medium samples (lane M). The medium was then changed, and the cells were grown in -MEM with or without ConA (20 µg/ml) in the presence of serially diluted rCBD as indicated (µM). The medium was then changed, and the cells were incubated for a further 24 h in the presence of the same amounts of rCBD as before. Medium samples (panel A) were analyzed on 10% SDS-PAGE/40 µg/ml gelatin zymograms. Essentially identical results were obtained when rCBD was only added after ConA treatment for 24 h. Cell lysates from this single addition of rCBD were analyzed on zymograms (panel B). In panel A, the first lane (0 →) shows that in the absence of both ConA (→) and rCBD (0), essentially 100% of the gelatinase A present in the medium is in the zymogen form with an apparent molecular mass of 66 kDa (DTT). Upon ConA treatment of cells for 24 h in the absence of added rCBD protein (lanes 0 →), activation of gelatinase A occurred. Laser densitometry showed that ~66–72% of the enzyme was converted to the fully active 59-kDa form (DTT) as indicated. A minor amount of the 62-kDa (DTT) activation intermediate was detected. In cells treated with ConA (+) and at the highest rCBD concentration shown (100 µM) (100 +), essentially all of the gelatinase A was present in the 59-kDa active form. In contrast, the adjacent control lane (far right lane) retains a strong latent gelatinase band. Panel C, cell layers were rinsed three times with PBS (PBS, lanes 1, 2, and 3) to remove all unbound secreted enzyme from the medium (lane M). Cells were then pulsed with 12 or 1.2 µM rCBD in PBS for 5 min at 22 °C. Gelatinase A, competitively released by the rCBD, was analyzed by zymography. No gelatinase A was released without rCBD (→+ CBD, lane 0). Gelatinase A was then extracted from the corresponding cells (Cells) with SDS-PAGE sample buffer. Panel D, fibroblasts were seeded in uncoated wells or wells coated with 25 µg/ml rCBD. At confluence, the cells were rinsed with PBS and then incubated with serum-free α-MEM with or without ConA (ConA + or –) for 18 h before zymographic analysis of the medium.

from enzyme levels per microliter, the total enzyme recovered in the lysates of ConA-activated cells was ~10-fold less than that in the medium. In other experiments, zymography also demonstrated that cell-bound progelatinase A (the 66-kDa zymogen form) was competitively displaced from unstimulated cells that had not been ConA-treated. This was found even after a short 5 min pulse of rCBD intended to minimize accumulations of newly secreted progelatinase A during the experiment (Fig. 1C). Extraction of the cell layer with SDS-PAGE sample buffer revealed that additional gelatinase A remained associated with the cells that was either not fully released by the short exposure to the rCBD or was bound by the C domain or was intracellular. That the increased gelatinase A activation upon ConA addition combined with rCBD treatment was not because of a direct cellular response to binding rCBD was shown in cultures incubated in the absence of ConA where rCBD addition for 24 h did not induce gelatinase A activation (not shown). Moreover, neither gelatinase A expression nor activation was altered in cells that were attached to rCBD-coated plates (see “The Collagen-binding Domain of Gelatinase A Mediates Cell Attachment”) without ConA treatment (Fig. 1D).

Thus, these data show that in addition to interactions involving the C domain, progelatinase A can bind to cells via another domain of the enzyme, the CBD. Because only latent and not active gelatinase A was displaced in unstimulated cultures by the rCBD, these competition experiments also show that cell binding via the CBD of progelatinase A is not sufficient for enzyme activation. Indeed, because gelatinase A activation upon ConA treatment increases in the presence of excess rCBD, we conclude that cellular progelatinase A bound by the CBD has a lower cellular activation potential than the soluble enzyme in the medium. Hence, displacement of CBD-bound gelatinase A by the rCBD in ConA-treated cells may facilitate entry of the latent enzyme into the cellular activation pathway.

The Collagen-binding Domain of Gelatinase A Mediates Cell Attachment—The mechanistic aspects of gelatinase A cell binding via the CBD were further investigated by adaptation of cell attachment assays. Fibroblasts attached to rCBD-coated microwells in a concentration-dependent manner (Fig. 2A), but this was less efficient than cell attachment to fibronectin (Fig. 2B). Incubation of fibroblasts with soluble rCBD prior to seeding inhibited attachment to rCBD-coated wells in a concentration-dependent manner, confirming binding specificity (Fig. 2C). Attachment was not observed in wells coated with 10 µg/ml BSA, whereas cell attachment to tissue culture-treated plastic alone or to type I collagen-coated wells was similar to that on fibronectin under saturating conditions. As assessed by phase contrast microscopy significantly fewer fibroblasts displayed cytoplasmic spreading on rCBD coated at 10 µg/ml (23%) compared with fibronectin (50%) after 30 min. Greater differences in cell spreading were apparent between rCBD and fibronectin using 25 µg/ml coated protein with 23 and 90%, respectively, of the cells spreading after 30 min. Although the kinetics of cell attachment and spreading differed at these early time points, spreading of cells on both substrates plateaued at 80–90% of the attached cells by 60 min. Scanning electron microscopy confirmed both cell attachment to rCBD protein and these differences. After 1 and 2 h on fibronectin (Fig. 3, A and C,
respectively), cells demonstrated typical cytoplasmic spreading (arrows) with a diameter of ~100 μm. In contrast, cells on rCBD were smaller (diameter of ~50 μm) and more rounded after 1 h (Fig. 3B) with limited spreading and extension of only delicate filopodia (arrowheads) after 2 h (Fig. 3D). Thus, this novel use of cell attachment assays confirmed the potential for gelatinase A binding to cells via the CBD of the enzyme.

β1-Integrins Are Involved in Cell Attachment to rCBD—A role for β1-integrins in CBD-mediated gelatinase A cell binding was demonstrated using mAb13, an anti-β1-integrin blocking monoclonal antibody. At 2.5 μg/ml antibody, more than 50% of the cell attachment to rCBD-coated wells was inhibited (Fig. 4). This inhibition increased to 90% at antibody concentrations >5 μg/ml. In comparison, α2- and α6-integrin blocking antibody and affinity purified αCBD and αHIs control antibodies showed no significant blocking effects at these concentrations. Ligand blotting was performed to identify cell proteins that may interact with the rCBD. On polyvinylidene difluoride blots of octyl-β-D-thioglucopyranoside solubilized cell membrane proteins, rCBD bound two distinct protein bands having apparent masses of 140 and 160 kDa under reducing conditions (Fig. 5) in the approximate positions of α- and β-integrin subunits or procollagen chains. However, both bands were degraded by bacterial collagenase. The 140- and 160-kDa bands were also partially pepsin-sensitive, being degraded to pepsin-resistant, but collagenase-sensitive, 112- and 126-kDa proteins. These co-migrated with collagen α(I) and α(II) chains that were also bound by the rCBD (Fig. 5). Thus, these data exclude the identity of the 140- and 160-kDa protein bands as integrin chains. Rather, the data provide strong evidence that the rCBD can interact with procollagen chains in cell membrane protein extracts. Nonetheless, other proteins, including those that do not renature on these blots or that require subunit interactions, might also be involved in the CBD interaction.

The Role of Pericellular Collagen in Cell Attachment to rCBD—in addition to any direct interaction with other cell collagen proteins, the ligand blots indicated that binding of gelatinase A CBD to native cellular collagen might represent one mode of gelatinase A cell binding. To test this, rCBD-coated wells were incubated with 10 μg of soluble type 1 collagen in 100 μl of PBS/well to saturate rCBD collagen-binding sites prior to cell seeding. On the rCBD-collagen complexes, cell attachment levels approached that for wells coated with 1.0 μg/well collagen alone (Fig. 6). Cell attachment diminished with decreasing amounts of collagen bound to the rCBD.

In other experiments, cells pretreated with bacterial collagenase showed a concentration-dependent decrease in cell attachment to rCBD-coated wells (Fig. 7A). In control experiments, 0.75 unit of collagenase completely digested 100 μg of purified native type I collagen after a 15-min incubation at 37°C and did not cleave rCBD or BSA (not shown). The digests were not cytotoxic as evident from unaltered attachment of the treated cells to fibronectin or uncoated tissue culture wells (not shown). That the reduced cell attachment was because of pericellular collagen removal and not to integrin degradation was supported by antibody blocking experiments. Incubation of bacterial collagenase-treated cells (0.75 units/100 μl) with β1-integrin blocking antibody further reduced cell attachment to rCBD (not shown) or to the coated rCBD collagen complexes by ~75% (Fig. 7B). In positive control experiments, binding of collagenase-treated cells to wells coated with colla-
Fig. 6. Cell attachment to rCBD-type I collagen complex. 96-well plates were coated with either rCBD (1.5 or 25 μg/ml) or native type I collagen (1, 3, or 10 μg/100 μl PBS coated in each well). After blocking with BSA, rCBD-coated wells were then incubated with soluble native type I collagen (1, 3, or 10 μg/100 μl PBS/well). Tissue culture treated plastic (P) or wells blocked with BSA (B) served as positive and negative controls (Cont), respectively. Human fibroblasts were seeded, and cell attachment was analyzed after 90 min. Data points are the means of duplicate wells (n = 3).

Fig. 7. Effect of bacterial collagenase treatment on cell attachment to rCBD. Panel A, fibroblasts were treated with highly pure bacterial collagenase (0.075, 0.75, or 7.5 units/ml) in serum-free α-MEM for 15 min at 37 °C. Collagenase-treated cells (4 × 10^4) were then seeded in wells coated either with 25 μg/ml rCBD alone or subsequently bound with native type I collagen (1, 3, or 10 μg/100 μl PBS in each well). Panel B, collagenase (0.75 units/100 μl)-treated fibroblasts were plated for 90 min in the presence of anti-β1-integrin antibody/serum-free α-MEM in wells coated with either 25 μg/ml rCBD subsequently complexed with native type I collagen (10 μg/100 μl PBS in each well) or native type I collagen alone (0.5 μg/well). Means (n = 3) and S.D. bars are shown.

DISCUSSION

By studying fibroblast cell attachment to the recombinant CBD of human gelatinase A we have developed a novel approach to mechanistically explore cell-binding mechanisms of gelatinase A. Our data indicate that the interaction between rCBD and cells in the attachment assays is representative of gelatinase A utilizing this domain to bind cell surfaces. Notably, the potential for gelatinase A interaction with cells via the CBD of the enzyme was shown by the competitive release of progelatinase A from unstimulated fibroblasts by the rCBD.

The importance of the gelatinase A C domain-TIMP-2 C domain interaction for activation by MT-MMPs is also thereby demonstrated because cell surface localization of progelatinase A through the CBD was not sufficient for activation. This confirms previous reports using CBD and C domain deletion mutants of the enzyme (20). Although the rCBD binds heparin (25), we found no evidence of rCBD binding to cell membrane heparan sulfate proteoglycans. Rather, our studies overall in-
Gelatinase A Cell Surface Binding by Fibronectin Modules

**Fig. 9.** Model of the influence of cell surface collagen on progelatinase activation at the cell membrane after ConA stimulation. Panel A, in unstimulated cells, secreted progelatinase A accumulates extracellularly (1) or binds (2) to cellular collagen (3), which is bound to the cell membrane (4) via $\beta_1$-integrins (5). The TIMP-2 (6) C domain (shaded square) interacts with the progelatinase A C domain to form progelatinase A-TIMP-2 complexes (7). Synthesis and secretion of progelatinase A is indicated by the arrows originating from within the cell. Collagen binding by the progelatinase A is mediated through the CBD of the enzyme in an equilibrium with the much larger pool of soluble progelatinase A. Panel B, ConA treatment of cells induces the expression of active MT-MMP (8), which is anchored to the cell membrane by a transmembrane domain and cytoplasmic extension (9). The TIMP-2-progelatinase A complex (7) binds to the active site of the MT-MMP via the NH$_2$-terminal "inhibitory" domain of TIMP-2 (shaded half-circle). A second active MT-MMP molecule (10) then cleaves (asterisk) the prodomain of the gelatinase A to generate the 62-kDa ($\sim$DTT) activation intermediate. Full gelatinase A activation then proceeds autocatalytically by another active gelatinase A molecule (not shown) to generate active gelatinase A that is bound to the MT-MMP on the cell surface (7). The 59-kDa ($\sim$DTT) active gelatinase A (11) is then released from the MT-MMP, possibly by MT-MMP degradation as discussed in the text. Active proteinases are indicated by a line in the open circle representing the active cleft in the catalytic domain after removal of the prodomain (shaded circle). Ongoing synthesis and secretion of progelatinase A replenishes the pools of progelatinase A extracellularly and that bound to integrin-linked collagen on the cell surface. Panel C, in unstimulated cells, the addition of recombinant CBD protein (13) competes for cell surface collagen binding with the natural CBD of the collagen-bound progelatinase A (2). As experimentally shown in Fig. 1C, a short incubation time with rCBD displaces progelatinase A (14) only from cell surfaces. Panel D, addition of recombinant CBD (13) with ConA competes off collagen-bound progelatinase A (2) in proximity to the MT-MMP-TIMP-2 activation complexes on the cell membrane (8, 10). This increases the amount of progelatinase A activation and release of active enzyme (11). The presence of recombinant CBD also competes for any binding of newly activated gelatinase A to the cell-bound collagen. Together with the ongoing activation of soluble progelatinase A (1), this results in the accumulation of active gelatinase A extracellularly relative to the zymogen form of the enzyme over time, as shown experimentally in the medium in Fig. 1A.
penetration of basement membranes (35), emphasizing the important role of integrins in gelatinase A function. Thus, gelatinase A may localize to cell surfaces by a number of distinct mechanisms including the CBD, the C domain via the TIMP-2-MT-MMP complex, a distinct TIMP-2 receptor, and the C domain via the αβ3 integrin receptor.

As shown in Fig. 1, when rCBD was added to ConA-treated cells this produced an elevated activation of the progelatinase A in the medium, but not in the cell layer, over that seen by ConA alone as first described by Overall and Sodek (5). Because the total amount of enzyme recovered in the cell lysates, which also includes proenzyme in the secretory pathway, was approximately 10-fold less than that found in the medium, the total cellular response to the rCBD was one characterized by a marked elevation in ConA-induced gelatinase A activation. The explanation we favor for this new finding is presented in Fig. 9. The displacement of progelatinase A by the rCBD in cells treated with ConA would promote enzyme activation before release to the medium because of the proximity of the released enzyme with the cell membrane and MT-MMPs. This is likely to be the mechanism because the active enzyme accumulated in the medium rather than in the cell layer, which retained relatively unaltered levels of latent and active gelatinase A. The progelatinase A on the cell-bound collagen could be replenished from newly synthesized enzyme bound at the time of secretion. Thus, the relative proportions of latent to active gelatinase A in the collagen-bound pool would not necessarily alter significantly upon rCBD addition. Together with the ongoing activation of soluble progelatinase A, the presence of rCBD would also compete for binding of newly activated soluble active gelatinase A to cell-bound collagen. This would result in the accumulation of active gelatinase A in the medium relative to the zymogen form of the enzyme over time. Thus, these data strongly indicate that the pool of gelatinase A that is cell-bound by the rCBD-bound progelatinase A from the MT-MMP degraded cell-collagen. Of note, MT1-MMP can cleave native type I collagen (26). We propose that CBD-mediated cell binding of progelatinase A may provide a means of maintaining a pool of latent enzyme at the cell membrane. Cell binding by the CBD also has the potential to target progelatinase A from one cell to another in trans, a mechanism thought to be important for increasing the proteolytic potential of tumor cells. However, our data indicate that in these cells MT-MMPs would not necessarily readily activate enzyme so targeted unless subsequently released from the collagen. Of note, MT1-MMP can cleave native type I collagen (36, 37). Therefore, on MT-MMP induction, release of CBD-bound progelatinase A from the MT-MMP degraded cell-collagen pool may provide the means for entry of this pool of progelatinase A into the C domain-TIMP-2-MT-MMP activation pathway. Activated gelatinase A would thereby be localized at sites of ongoing cell matrix degradation and gelatinolysis.

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