Complete Restoration of Cell Surface Activity of Transmembrane-truncated MT1-MMP by a Glycosylphosphatidylinositol Anchor

IMPLICATIONS FOR MT1-MMP-MEDIATED PROMMP2 ACTIVATION AND COLLAGENOLYSIS IN THREE-DIMENSIONS*

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MT1-MMP is a potent collagenase not only required for skeletal development but also implicated in tumor invasion and metastasis. The mechanism through which cells deploy MT1-MMP to mediate collagenolysis remains largely unknown. C-terminally truncated MT1-MMP lacking its transmembrane and cytoplasmic domains, although proteolytic active in purified forms, is known to be deficient in cell-mediated proMMP2 activation and collagenolysis, suggesting that cells regulate its activity through both domains. Indeed, the cytoplasmic domain is recognized by the trafficking machinery that mediates its internalization and recycling. Here we demonstrate that its transmembrane domain can be functionally substituted by the glycosylphosphatidylinositol (GPI)-anchor of MT6-MMP. The GPI-anchored MT1-MMP, or MT1-GPI, activates proMMP2 on the cell surface and promotes cell growth in a three-dimensional type I collagen matrix. On the other hand, a GPI-anchored MMP13 with a functional furin activation signal fails to promote cell growth in a three-dimensional collagen matrix, whereas remaining competent in collagenolysis on a two-dimensional cell growth in a three-dimensional collagen matrix. Our results suggest that both membrane tethering and proteolytic activity encoded by MT1-MMP are required for its ability to promote cell growth and invasion in a three-dimensional collagen matrix.

Matrix metalloproteinases (MMPs) are a superfamily of proteolytic enzymes capable of degrading almost all components of the extracellular matrix (ECM) collectively (1). However, each individual MMP is expected to cleave its respective substrates in a time- and tissue-dependent fashion during development to sculpt a specific tissue or organ (1, 2). Since these MMPs provide the necessary remodeling activities to ensure proper growth and differentiation of many cell types, they must be modulated in precision by cells producing them. Currently, there are 21 members within this superfamily, which can be subdivided into two major categories: seven membrane-bound and 14 soluble MMPs. Although the soluble MMPs may be secreted to the extracellular milieu, where they may attack the ECM components freely, the seven membrane-bound MMPs, including one MMP anchored by a type II transmembrane domain, four anchored by type I transmembrane domains, and two anchored by GPI anchors, may be regulated at the cell surface to focus their proteolytic attack more precisely (3–6). It has been hypothesized that these membrane-bound MMPs are regulated by their host cells through trafficking events to deliver their proteolytic activities in precision (6).

The archetypal MT1-MMP is the most studied and has been shown to play a critical role in the development of skeleton, cartilage, and other bone tissues (7, 8). It has emerged as a model system to study cell surface-mediated proteolysis in both normal and malignant cells. We and others have shown that MT1-MMP undergoes dynamic trafficking between intracellular compartments and the plasma membrane (9–11). Furthermore, the signals responsible for membrane-type MMP (MT-MMP) trafficking appear to be coded in the cytoplasmic tails (5, 6, 9, 10). For example, the LLY motif has been demonstrated as the internalization signal for MT1-MMP, whereas the C-terminal motif DKV mediates recycling of internalized MT1-MMP to the cell surface (5, 6, 9, 12). These regulatory processes should ensure the appropriate amount of MT1-MMP activity to be displayed on the cell surface to mediate precise degradation of ECM beneath the plasma membrane. Indeed, truncation of its transmembrane and cytoplasmic domains from MT1-MMP generated a mutant deficient in mediating proMMP2 activation and promoting cell growth in a three-dimensional collagen matrix at the cellular level, whereas fully functional in purified forms (13). However, removal of the cytoplasmic domain alone yields a gain-of-function mutant with enhanced activities against proMMP2 and three-dimensional collagen matrix due to impaired internalization (9, 13). These data suggest that tethering to the cell surface would be sufficient to...
allow MT1-MMP to express its proteolytic activity against its substrates at the cellular level. To test this idea, we engineered a GPI anchor at the carboxyl end of MT1-MMP ectodomain and demonstrated that the GPI anchor can rescue the activity of transmembrane-truncated MT1-MMP on the cell surface. However, tethering a fully active collagenase MMP13 to the cell surface by a GPI anchor failed to yield a MT1-MMP-like activity toward three-dimensional type I collagen matrix, suggesting that MT1-MMP encodes collagenolytic activity different from that of MMP13.

**EXPERIMENTAL PROCEDURES**

*Cell Culture*—MDCK cells, MCF-7 cells, and HEK293 cells are from ATCC and maintained in DMEM and 10% fetal bovine serum as described (5, 9, 14).

*Antibodies and Reagents*—Rabbit anti-MT1-MMP antibody is a gift from Dr. Jorma Keski-oja (Helsinki, Finland). Monoclonal antibody against MMP13 was purchased from R&D systems (Minneapolis, MN). Rat-tail type I collagen is from BD Biosciences (Temecula, CA). Biotin is from Pierce. Secondary antibody conjugates are from Sigma. Proteinase inhibitor mix tablets are from Roche Applied Science. Phosphatidylinositol-specific phospholipase C (PI-PLC) was from Invitrogen.

*Expression Constructs, DNA Transfection, and Stable Line Generation*—pCR3.1uni-MT1-MMP, MT1-ΔC (cytoplasmic tail truncation), and wild-type MMP13 were described previously (5, 9, 14–16). MMP13-F was generated using QuikChange site-directed mutagenesis method (Stratagene, CA) to insert in-frame a 12-amino-acid sequence from MMP11 into the COOH terminus of MMP13-F. Chimeras were generated using overlap-primers designed for recombinating the GPI anchor signal in MT6-MMP with the C terminus of the hemopexin-like domain of MT1-MMP or MMP13 through a PCR strategy. Thus, the MT1-GPI chimera contains Met1–Cys508 of MT1-MMP and Pro510–Arg562 of MT6-MMP; MMP13-F-GPI contains Pro510–Arg562 of MT6-MMP in the C terminus. Liposome-mediated DNA transfections into MDCK cells was performed by using Lipofectamine (Invitrogen), and stable lines were established as described (5, 6, 9, 14–16). HEK293 was transfected using calcium phosphate transfection methods as described (5). Two-dimensional and Three-dimensional Collagen Cell Culture—Collagen gel was prepared by neutralizing rat tail type I collagen (BD Biosciences) in acetic acid solution with 10× PBS and 1 N NaOH at the final concentration of 2.5 mg/ml as described (14). For cell growth assay in three-dimensional collagen gels, 1 × 10⁶ cells were mixed with collagen solution at 4 °C prior to gelling at 37 °C. Cells were maintained in DMEM containing 10% FBS. The diameters of each cyst were measured via microscopically as described (14). For collagenolysis by cells on two-dimensional collagen gel, 5 × 10⁴ cells were seeded on top of collagen gels and maintained in DMEM containing 10% FBS or serum-free DMEM as described.

*Cell Surface Biotinylation*—Cells grown on 6-cm dishes were chilled to 4 °C by washing with ice-cold PBS three times before being allowed to incubate with biotin-S-SH (30 mg/ml, Pierce) for 30 min on ice with constant shaking. Cells were then washed with PBS again and lysed in radioimmune precipitation buffer in the presence of a proteinase inhibitor mixture (14). After centrifugation at 14,000 rpm for 30 min to remove any debris, cell lysates were immunoprecipitated with streptavidin-conjugated beads and analyzed by Western blotting with rabbit anti-MT1-MMP antibody (6).

*Immunostaining*—Cell stainings were carried out as described previously (6). For cell surface staining, cells grown on coverslips were washed with ice-cold PBS (3×) and allowed to chill at 4 °C. Then cells were incubated with rabbit anti-MT1-MMP antibody in DMEM for 2 h on ice. The cells were then washed with ice-cold PBS, fixed, permeabilized, stained, and scanned by confocal microscopy as described (6, 14).

*SDS-PAGE, Western Blotting, and Zymography*—These procedures were carried out as described previously (5, 6).

*PI-PLC Treatment*—cells were treated with or without PI-PLC (10 units/ml) in serum-free DMEM in the presence of 5 μM GM6001 at 37 °C for 1 h as described (17).

**RESULTS**

*GPI Anchor Restores the Cell-mediated proMMP2 Activation Activity of Transmembrane Domain Truncated MT1-MMP*—As the first member of the MT-MMP family, MT1-MMP was shown to be the long sought membrane-associated activator for proMMP2 (18). However, truncation of its transmembrane domain led to the release of a fully active ectoenzyme into culture medium that can activate proMMP2 and degrade ECM components upon purification (19). However,
this transmembrane-deletion mutant lost its ability to activate proMMP2 at the cellular level (20). The transmembrane domain of interleukin-2 receptor α chain was able to restore the cell-based proMMP2 activation when fused to MT1-MMP ectoenzyme (20). Based on these findings, we hypothesize that the cell surface provides an ideal environment for MT1-MMP to recognize, bind, and cleave its substrates. If this is correct, any means that tethers the ectoenzyme of MT1-MMP should be able to restore its cell-associated activity. To this end, we selected the GPI anchor of MT6-MMP (21) to tether the MT1-MMP ectoenzyme to the cell surface as shown in Fig. 1A. To assess the function of MT1-GPI, we evaluated its ability to activate proMMP2 on the cell surface. As shown in Fig. 1B, MT1-GPI efficiently activates proMMP2 supplied in the medium (lane 10), whereas soluble MT1-MMP does not (lane 7). Surprisingly, MT1-GPI is more efficient than wild-type MT1-MMP (Fig. 1B, lane 10 versus lane 8) but less efficient than MT1-ΔC (Fig. 1B, lane 10 versus lane 9), perhaps reflecting the amount of active enzymes on the cell surface as demonstrated previously (9). Immunostainings shown in Fig. 2, A and B, demonstrate that indeed, MT1-GPI is presented mostly on the cell surface. The staining data were confirmed using cell surface labeling by biotinylation as shown in Fig. 2C. Also, PI-PLC treatment of the MT1-GPI expressing cells indeed released cell surface MT1-GPI proteins as indicated by diminished immunostaining signal (Fig. 2D). These data suggest that GPI is capable of anchoring MT1-MMP ectodomain on the cell surface and providing an ideal environment for MT1-MMP to cleave its substrates.

GPI Anchor Can Substitute the Transmembrane Domain of MT1-MMP in Cell Growth in Three-dimensional Collagen Matrix—To further demonstrate that GPI-anchored MT1 functions equivalently as the wild-type MT1-MMP as shown in Figs. 1 and 2, we analyzed their ability to promote cell growth and invasion in a three-dimensional collagen matrix. To this end, we generated stable cell lines that express MT1-GPI in MDCK as described previously (22). As shown in Fig. 3A, the stable clones MT1-GPI-8 and -9 were generated and found to express the protein well but less than MT1-WT and MT1-ΔC (lanes 5 and 6 versus lanes 2–4). To assess their ability to grow and invade into three-dimensional collagen, we embedded the cells in three-dimensional collagen and measured their growth as described (22). As shown in Fig. 3B, MT1-GPI-expressing cells are also able to promote cell growth and invasion very well in a three-dimensional collagen matrix, more effectively than cells expressing MT1-WT (B and C). The enhancements are inhibited by GM6001, a MMP inhibitor. Therefore, MT1-GPI, tethered on the cell surface by a GPI anchor (Figs. 2 and 3), can promote cell invasion and metastasis. This set of data demonstrates strongly that MT1-MMP is a potent protease for cell invasion and metastasis if it can be displayed on the cell surface, suggesting that a malignant switch may be the translocation of MT1-MMP from intracellular pools to the cell surface as we demonstrated previously with concanavalin A treatment (9).

Membrane-associated MMP13 Is Not Able to Promote Cell Growth in Three-dimensional Collagen Matrix but Maintains Its Collagenase Activity in the Absence of Serum—Our data presented so far suggest that MT1-MMP can promote cell invasion and growth in type I collagen three-dimensional matrix as long as it can be displayed on the cell surface (Fig. 3), suggesting that collagenolytic activity presented on the cell surface should be able to generate the same effects. To test this idea, we engineered a construct that should be able to deliver collagenolytic activity on the cell surface as shown in Fig. 4A. Previously, Weiss and colleagues (13) have indicated that MMP13 is...
another strong collagenase but is not able to usurp cell growth in three-dimensional collagen, even when they generated active MMP13 by inserting that furin activation motif RXKR in front of its catalytic domain. In light of our data, we interpret that this molecule should be inactive because it is not on the cell surface since soluble MT1-MMP is also a good collagenase but fails to promote cell growth in a three-dimensional collagen matrix. Thus, it is of interest to test whether the cause for the negative phenotype of MMP13 is due to the fact that it is not membrane-associated. To accomplish this goal, we generated membrane-associated active MMP13, MMP13F-GPI, as shown in Fig. 4A. MMP13F-GPI is activated by a furin-mediated process and displayed on the cell surface as expected (Fig. 4B and C). The GPI anchor of MMP13F-GPI is verified by PI-PLC treatment, which shed soluble MMP13 into conditioned medium (Fig. 4D). However, it failed to promote cell growth in a three-dimensional collagen matrix (Fig. 4E, MMP13F-GPI), suggesting that MT1-MMP differs from MMP13 in a fundamental way that mere presentation on the cell surface is not sufficient to generate an invasive and growth phenotype in three-dimensional collagen under identical condition.

\( \alpha_5 \) Blocks Membrane-associated MMP13 Collagenase Activity but Not MT1-MMP-mediated Cell Invasion into Collagen Gel—To unravel the functional difference between MMP13 and MT1-MMP, we reasoned that they might have different sensitivity toward serum. We have observed previously that the ectodomain of MT1-MMP can degrade blocks of collagen under serum-free conditions but is totally inactive in the presence of 10% FBS (data not shown). We wondered whether MMP13 could be subjected to the regulation in serum. We removed the serum when culturing MMP13-expressing cells on collagen and found that MMP13F-GPI shows robust collagenase activity much like soluble active MMP13-F, as shown in Fig. 5A (left column). MMP inhibitor GM6001 is effective in blocking the observed collagenolysis (Fig. 5A, middle column). Interestingly, 10% FBS also blocked the degradation, suggesting that serum components inhibited the MMP13 activity or inactivated MMP13 (Fig. 5A, right column). \( \alpha_5 \)M is the major nonspecific proteinase inhibitor in serum. \( \alpha_5 \)M can inhibit all soluble MMPs in test tubes. Thus, we hypothesize that \( \alpha_5 \)M is the serum component that blocked the collagenase activity of MMP13.

Indeed, \( \alpha_5 \)M blocked the collagen degradation by MMP13F-GPI when added to serum-free medium (Fig. 5B, panel c versus panel b), whereas it had little effect on MT1ΔC (Fig. 5B, panel g versus panel f). As expected, GM6001 blocked both MMP13F-GPI and MT1ΔC completely (Fig. 5B, panels d and h). Taken together, these data suggest that MT1-MMP on the cell surface can escape \( \alpha_5 \)M inhibition, whereas membrane-anchored MMP13 cannot.

**DISCUSSION**

MT1-MMP is critical for cell migration, cell invasion, tumor progression, and skeletal development. A better understanding of MT1-MMP regulation may help target this important enzyme for anticancer drug development. Here we present the evidence that MT1-MMP relies on membrane anchorage and a unique catalytic mechanism to execute efficient collagenolysis and promote cell invasion and growth in a three-dimensional collagen matrix.

MT-MMPs play important roles in multiple physiological and pathological processes. MT-MMPs have some unique functions when compared with other soluble MMPs. For example, MT-MMPs are able to activate soluble MMPs such as MMP-2, -9, and -13 (23–25). Membrane association helps cells present these proteinases on membrane and allows cells to target these proteinases to the invading front of cells through protein sorting (26). Moreover, membrane association plays a critical role in maintaining the proteinase activity for MT1-MMP.

Although GPI-anchored MT-MMPs (MT4, 6-MMP) have very low activity in collagen degradation and proMMP-2 activation (27, 28), our results suggest that the GPI anchors are the functionally equivalent to type I transmembrane domains, and thus, may not be responsible for the low activity observed. As
GPI-anchored MT1-MMP and MMP-13

**Figure 4.** MMP13-F-GPI is not able to promote cell growth in three-dimensional collagen culture. A, schematic presentation of MMP13 wild-type (MMP13), MMP13-F, and MMP13-F-GPI. S, signal peptide; Pro, prodomain; R, furin-cleavage site of MMP11; CAT, catalytic domain; H, hinge region; PEX, hemopexin-like domain. The GPI anchor is represented by wavy lines. B, protein characterization of MMP13 wild type and mutants. MDCK stable lines expressing empty vector (lanes 1 and 8), MMP13 (lanes 2, 3, 9, and 10), MMP13-F (lanes 4, 5, 11, and 12), or MMP13-F-GPI (lanes 6, 7, 13, and 14) were incubated with 5 μM GM6001 in serum-free medium for 16 h and lysed in radioimmune precipitation buffer. After being cleared of debris by centrifugation, cell lysate (lysate) and conditioned medium (CM) were analyzed with Western blotting with monoclonal antibody against MMP13. The active form of MMP13 is indicated with an asterisk. MW, molecular weight markers. C, immunostaining of MMP13-F-GPI MDCK cells. Left, MMP13-F-GPI expression cells were fixed and stained with monoclonal antibody against MMP13 and FITC-conjugated antibody against mouse IgG. Right, MMP13-F-GPI expression cells were incubated with MMP13 antibody on ice before being fixed and stained with the FITC-conjugated secondary antibody. D, MCF-7 cells were transiently transfected with MMP13-F-GPI expression vector. 48 h after transfection, cells were incubated (37 °C, 1 h) with or without PI-PLC (10 units/ml) in the presence of GM6001. Cell lysate and conditioned medium were analyzed via Western blotting with monoclonal antibody against MMP13. E, three-dimensional collagen culture: control, MMP13-WT (MMP13)-, MMP13-F-, MMP13-F-GPI-, and MT1-WT (MT1)-transfected MDCK cells were cultured within type I collagen gel in the absence or presence of 5 μM GM6001 for 6 days (200×). Mock, MDCK control cells.

shown in this report, the GPI anchor promotes the localization of MT1-MMP on the cell surface and enhances MT1-MMP activity as efficiently as a transmembrane domain. GPI-anchored protein is known to be targeted to lipid rafts. Indeed, the internalization of MT1-MMP is partially through the caveolin-dependent pathway (29, 30), and the interaction between MT1-MMP and caveolin-1 is required for MT1-MMP dependent cell locomotion (31). These observations are consistent with our observation that a GPI anchor could functionally substitute the transmembrane domain of MT1-MMP. We further speculate that MT1-GPI mutant may serve as a good model in the investigation of the function and activity of MT1-MMP in lipid rafts.

As a unique collagenase in the MMP family, MT1-MMP could promote cell cyst growth and tumor progression in a three-dimensional collagen culture, whereas other collagenases did not (13). This observation poses two questions. Is membrane association the only requirement for collagenase activity in a three-dimensional collagen culture? Also, does membrane anchorage cause the conformational change in collagenases to be able to escape the inhibition by α,M? Here we demonstrated that MMP13 could degrade collagen in a two-dimensional collagen culture in the absence of serum but not in a three-dimensional matrix. α,M or serum is sufficient to inhibit membrane-anchored MMP13 activity in collagen degradation but not against MT1-MMP. These results are consistent with the notion that both membrane anchorage and MMP and caveolin-1 are required for MT1-MMP dependent cell migration.
and the unique activity of MT1-MMP contribute to the robust collagenolytic activity observed in a three-dimensional matrix.

Proteinases are destructive enzymes that may cause irreparable damage to tissues or organs in the body. Therefore, their activities must be tightly controlled in vivo, most likely by endogenous inhibitors such as tissue inhibitors of metalloproteinase. Serum contains multiple types of proteinase inhibitors that can quench any free proteolytic activity instantaneously so that homeostasis can be maintained in the body. So far, only MT1-MMP and its relatives, MT3 and MT5-MMPs, can circumvent the inhibitory effects of 10% serum in cell cultures, whereas none of their secreted counterparts can. MT1-MMP significantly promotes cell cyst growth in a three-dimensional collagen culture, whereas soluble MT1-MMP does not display growth-enhancing activity (13). One of the reasons could be that MT1-MMP can escape the inhibition of serum but soluble MT1-MMP cannot. Here we demonstrated that MT1-MMP can escape the inhibition of serum but soluble MT1-MMP cannot. We also determined that α2M is one of the inhibitory components in serum that is sufficient to inhibit membrane-anchored MMP13 activity in collagen degradation.

α2M inhibits proteinases by entrapping them and preventing them from their protein substrates. However, certain proteinases can find a way to evade these inhibitors. For example, serine proteinases can escape endogenous inhibitors in serum such as antitrypsin, antichymotrypsin, and α2M (32). It remains to be determined how proteinases maintain their activity in the presence of those inhibitors present in serum. The mechanism for MT1-MMP to escape the inhibition effect of α2M needs further investigation and may serve as a model system to study the interactions between proteinases and their inhibitors. Understanding this mechanism may provide a novel way to design specific inhibitors against MT1-MMP. Given the important function of MT1-MMP in tumor cell proliferation, migration, and metastasis, specific inhibitors against MT1-MMP may inhibit tumor growth and invasion, and thus, may be developed into treatments for various human cancers.

REFERENCES

1. Birkedal-Hansen, H., Moore, W. G., Bodden, M. K., Windsor, L. J., Birkedal-Hansen, B., DeCarlo, A., and Engler, J. A. (1993) Crit. Rev. Oral Biol. Med. 4, 197–250
2. Sternlicht, M. D., and Werb, Z. (2001) Annu. Rev. Cell Dev. Biol. 17, 463–516
3. Seiki, M. (2003) Cancer Lett. 194, 1–11
4. Kojima, S., Itoh, Y., Matsumoto, S., Masuho, Y., and Seiki, M. (2000) FEBS Lett. 480, 142–146
5. Wang, P., Wang, X., and Pei, D. (2004) J. Biol. Chem. 279, 20461–20470
6. Wang, X., Ma, D., Keski-Oja, J., and Pei, D. (2004) J. Biol. Chem. 279, 9331–9336
7. 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
8. Zhou, Z., Apte, S. S., Soininen, R., Cao, R., Baaklini, G. Y., Rauzer, R. W., Wang, J., Cao, Y., and Tryggvason, K. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4052–4057
9. Jiang, A., Lehti, K., Wang, X., Weiss, S. J., Keski-Oja, J., and Pei, D. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 13693–13698
10. Lehti, K., Valtanen, H., Wickstrom, S. A., Lohi, J., and Keski-Oja, J. (2000) J. Biol. Chem. 275, 15006–15013
11. Rozanov, D. V., Deryugina, E. I., Monosov, E. Z., Marchenko, N. D., and Strongin, A. Y. (2004) Exp. Cell Res. 293, 81–95
12. Uekita, T., Itoh, Y., Yama, I., Ohno, H., and Seiki, M. (2001) J. Cell Biol. 155, 1345–1356
13. Hotary, K. B., Allen, E. D., Brooks, P. C., Datta, N. S., Long, M. W., and Weiss, S. I. (2003) Cell 114, 33–45
14. Kang, T., Yi, I., Yang, W., Wang, X., Jiang, A., and Pei, D. (2000) FASEB J. 14, 2559–2568
15. Pei, D., and Weiss, S. J. (1995) Nature 375, 244–247
16. Pei, D., and Yi, J. (1998) Protein Expression Purif. 13, 277–281
17. Itoh, Y., Kajita, M., Kinoh, H., Mori, H., Okada, A., and Seiki, M. (1999) J. Biol. Chem. 274, 34260–34266
18. Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E., and Seiki, M. (1994) Nature 370, 61–65
19. Pei, D., and Weiss, S. J. (1996) J. Biol. Chem. 271, 9135–9140
20. Cao, J., Sato, H., Takino, T., and Seiki, M. (1995) J. Biol. Chem. 270, 801–805
21. Pei, D. (1999) Cell Res. 9, 291–303
22. Wang, P., Nie, J., and Pei, D. (2004) J. Biol. Chem. 279, 51148–51155
23. Toth, M., Cherykova, I., Bernardo, M. M., Hernandez-Barrantes, S., and Fridman, R. (2003) Biochem. Biophys. Res. Commun. 308, 386–395
24. Knauper, V., Will, H., Lopez-Otin, C., Smith, B., Atkinson, S. J., Stanton, H., Hemby, R. M., and Murphy, G. (1996) J. Biol. Chem. 271, 17124–17131
25. Knauper, V., Bailey, L., Worley, J. R., Soloway, P., Patterson, M. L., and Murphy, G. (2002) FEBS Lett. 532, 127–130
26. Itoh, Y., and Seiki, M. (2006) Cell Res. 16, 1–8
27. Nie, J., and Pei, D. (2003) Cancer Res. 63, 6758–6762
28. Wang, Y., Johnson, A. R., Ye, Q. Z., and Dyer, R. D. (1999) J. Biol. Chem. 274, 33043–33049
29. Galvez, B. G., Matias-Roman, S., Yanez-Mo, M., Vicente-Manzanares, M., Sanchez-Madrid, F., and Arroyo, A. G. (2004) Mol. Biol. Cell 15, 678–687
30. Remacle, A., Murphy, G., and Roghi, C. (2003) J. Cell Sci. 116, 3905–3916
31. Labrecque, L., Nyland, C., Langlois, S., Durocher, Y., Roghi, C., Murphy, G., Gingras, D., and Beliveau, R. (2004) J. Biol. Chem. 279, 52132–52140
32. Travis, J. (1988) Am. J. Med. 84, 37–42