Inhibits the Cell-mediated Activation of Progelatinase A

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Tissue inhibitor of metalloproteinases-2 (TIMP-2) is supposed to play a regulatory role in the cell-mediated activation of progelatinase A. To investigate the mechanism of the regulation, we prepared and characterized a chemically modified TIMP-2, and examined its effects on the activation of progelatinase A. We found that treatment of TIMP-2 with cyanate ion led to loss of inhibitory activity toward matrilysin or gelatinase A. Structural and functional analyses of the modified TIMP-2 showed that carboxamylation of the α-amino group of the NH₂-terminal Cys of TIMP-2 led to complete loss of the inhibitory activity. When the reactive-site-modified TIMP-2 was added to culture medium of concanavalin A-stimulated HT1080 cells, the conversion of endogenous progelatinase A to the intermediate form was partially inhibited, whereas that of the intermediate form to the mature one was strongly inhibited. The reactive site-modified TIMP-2 also prevented an accumulation of active gelatinase A on the cell surface. We speculate that occupation of the hemopexin-like domain of gelatinase A by the reactive site-modified TIMP-2 makes it unable for gelatinase A to be retained on the cell surface, thus preventing the autocatalytic conversion of the intermediate form of gelatinase A to its mature form.

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade components of extracellular matrix and play an essential role in tissue remodeling under physiological and pathological conditions such as morphogenesis, angiogenesis, tissue repair, and tumor invasion (1–3). Most MMPs are secreted as a zymogen and are activated by serine proteases or some activated MMPs. The activities of activated MMPs are regulated by a family of specific inhibitors known as tissue inhibitor of metalloproteinases (TIMPs). Among the MMP family, gelatinase A (MMP-2) and gelatinase B (MMP-9) are critical in the invasion of tumor cells across basement membranes because of their strong activity against type IV collagen, a major component of basement membranes (4–6). Unlike other zymogen of MMPs, progelatinase A is not activated by serine proteases or soluble MMPs and had been reported to be activated by a MMP-like activity on the surface of cancer and fibroblastic cells (7–10). Sato et al. (11) recently identified a novel membrane-type MMP, named MT-MMP, as an activator of progelatinase A on the cell surface. The cell-mediated activation of progelatinase A includes two steps of processing: MT-MMP-catalyzed cleavage of progelatinase A at a peptide bond between Asn³⁷ and Leu³⁸, first converts the zymogen into an intermediate form, and then autocatalytic cleavage of an Asn⁵⁰-Tyr⁸¹ bond converts the intermediate form into a mature one (12). Several studies suggest that both steps are greatly accelerated by binding of (pro)gelatinase A onto the cell surface, and therefore, the receptor of (pro)gelatinase A on the cell surface is important for the activation. Carboxy-terminal hemopexin-like domain of gelatinase A is reported to be essential for the interaction with the cell surface receptor (12, 13). The NH₂-terminal reactive site of TIMP-2 binds to the active site of MT-MMP to form a protease-inhibitor complex, whereas the COOH-terminal region of TIMP-2 has an affinity to the hemopexin-like domain of gelatinase A. Therefore, it is hypothesized that a complex formed between MT-MMP and TIMP-2 acts as a receptor of progelatinase A. This hypothesis appears to be supported by a finding that overexpression of MT-MMP results in an accumulation of gelatinase A on the cell surface (11). Another candidate for the gelatinase A receptor is integrin αvβ, which forms a sodium dodecyl sulfate stable complex with gelatinase A also by binding to the hemopexin-like domain (14, 15). TIMP-2 is a bifunctional regulator of the cell-mediated activation of progelatinase A. Strongin et al. (13) demonstrated that a small amount of TIMP-2 facilitates the activation of gelatinase A by the MT-MMP-containing cell membrane, whereas excess TIMP-2 strongly inhibits the activation. This could be explained that the binding of TIMP-2 to MT-MMP provides a receptor for progelatinase A and also leads to an inhibition of catalytic activity of MT-MMP. However, the detailed mechanism remains to be clarified. Recently, we examined expression levels of gelatinase A, TIMP-2, and three MT-MMPs in human cancer cell lines and found that activation of progelatinase A has a strong inverse correlation only with the level of TIMP-2 secreted into culture medium (16), suggesting that TIMP-2 is a key regulator of the activation of progelatinase A. In this study, we prepared a chemically modified TIMP-2 of which the reactive site is destroyed, and the modified inhibitor was examined for its effect on the cell-mediated activation of progelatinase A. Mechanisms related to the TIMP-2 regulation of the activation of progelatinase A are discussed.

EXPERIMENTAL PROCEDURES

Materials—The sources of materials used were as follows: 3167v (7-methoxycoumarin-4-yl)-acetyl-Arg-Pro-Lys-Pro-Tyr-Ala-norvalyl-Trp-Met-N (+2,4-dinitrophenyl)-lysine amide) was from Peptide Institute, Inc. (Osaka, Japan); potassium cyanate was from Wako Pure Chemical Industries (Osaka); p-aminophenyl mercuric acetate (APMA)
TBS as described under "Experimental Procedures." In matrilysin had been coupled to 5000.01% NaN₃ (Tris-buffered saline; TBS). The mixture was incubated at 37 °C for 15 min. The mixtures were added with 10 μmol of 1 mM 3167v, and further incubated for 40 min. The reaction was terminated by adding 100 μmol of 0.1 mM EDTA (pH 7.5). The hydrolyzed 3167v was measured as described above.

Reduction and S-Carboxyamidomethylation of KNCO-treated TIMP-2 Forms in Matrilysin-bound and Matrilysin-unbound Fractions—Each of the KNCO-treated TIMP-2 forms in matrilysin-bound and matrilysin-unbound fractions (10 μmol) was incubated with 100 mM dithiothreitol in TBS containing 1 mM guanidine hydrochloride and 20 mM EDTA at 50 °C for 30 min. After incubation, the samples were transferred to a container of ice water and further incubated with 240 mM iodoacetamide. After 2 h, the samples were dialyzed against TBS.

Cell Culture and Preparation of CM and Cell Lysate—HT1080 fibrosarcoma cell line was grown to semi-confluency in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (Life Technologies, Inc., Grand Island, NY), Dulbecco's modified Eagle's/Ham's F-12 medium. After 24 h, the resultant CM was collected, clarified by centrifugation, and dialyzed against distilled water at 4 °C. The sample was then lyophilized and dissolved in a small volume of a sodium dodecyl sulfate-sampling buffer consisting of 50 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate, and 10% glycerol. By these procedures, the initial CM was concentrated 20-fold. To prepare cell lysates, the cells were rinsed three times with phosphate-buffered saline, and then dissolved in a small volume of the sodium dodecyl sulfate-sampling buffer.

Ligand Blotting Analysis—TIMP-2 or modified TIMP-2 was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, under nonreducing conditions. After electrophoresis, the proteins on the gel were transferred onto nitrocellulose membrane, using a Bio-Rad Mini Trans-Blot apparatus. The membrane was blocked with TBS-
**FIG. 2.** Inhibitory activity of KNCO-treated TIMP-2 forms in matrilysin-bound and matrilysin-unbound fractions. After treatment with KNCO, the partially modified TIMP-2 was separated, using a matrilysin-Sepharose 4B column as described under “Experimental Procedures.” Matrilysin (30 nM, panel A) and APMA-activated gelatinase A (80 nM, panel B) were incubated, respectively, with 0.1 mM 3167v at 37 °C for 40 min in the presence of various concentrations of the KNCO-treated TIMP-2 forms in the matrilysin-bound (●) and matrilysin-unbound (○) fractions. All the reaction mixtures contained TBS, 10 mM CaCl₂, and 0.01% Brij 35. The amount of 3167v hydrolyzed by enzyme was taken as 100%, and the relative amount of 3167v hydrolyzed by enzyme in the presence of each concentration of the KNCO-treated TIMP-2 forms is shown on the ordinate.

**FIG. 3.** High performance liquid chromatography separation of tryptic peptides of KNCO-treated TIMP-2 forms in matrilysin-bound and matrilysin-unbound fractions. Each of the KNCO-treated TIMP-2 forms in the matrilysin-bound (panel A) and matrilysin-unbound (panel B) fractions was reduced and S-carboxymethylated as described under “Experimental Procedures,” and then digested with trypsin in an enzyme to substrate ratio of 1:100 (w/w) at 37 °C for 24 h. The digest was applied to an Ultrasphere ODS 5 µ column (2.0 × 150 mm) and eluted at a flow rate of 0.5 ml/min with a linear gradient of acetonitrile containing 0.05% trifluoroacetic acid. The column eluate was monitored at 206 nm (solid lines), and the broken line shows the percentage of acetonitrile in the elution medium.

RESULTS

**Effect of KNCO Treatment of TIMP-2 on the Inhibitory Activity**—The recently determined crystal structure of the complex formed between TIMP-1 and stromelysin suggests that the α-amino group of the NH₂-terminal Cys₁ of TIMP-1 binds to the catalytic zinc atom at the active site of stromelysin, thus playing an essential role in the inhibitory action of TIMP-1 (19). As the structure of the NH₂-terminal region of TIMP-2 is homologous to that of TIMP-1, the α-amino group of Cys₁ of TIMP-2, corresponding to that of TIMP-1 may be critical for the inhibitory activity of TIMP-2. To examine this possibility, we attempted to carbamylate the α-amino group of Cys₁ by treating TIMP-2 with KNCO under various conditions, and the chemically modified derivatives of TIMP-2 were examined for their abilities to inhibit the matrilysin-catalyzed hydrolysis of 3167v. As shown in Fig. 1A, the incubation of TIMP-2 with KNCO led to increase in the IC₅₀ value of the inhibition, where IC₅₀ represents a concentration of the modified derivatives of TIMP-2 giving a 50% inhibition of the activity of matrilysin. When the inverse values of the IC₅₀ versus incubation time with KNCO were plotted, the 1/IC₅₀ value diminished with increasing time of incubation with KNCO, and 50% reduction of the 1/IC₅₀ value was observed when the incubation time was 25 min (Fig. 1B). The inhibitory activity of TIMP-2 was abolished after 4 h incubation with KNCO.

Separation of Active and Inactive Fractions after Partial
Modification of TIMP-2—As described under “Experimental Procedures,” TIMP-2 was treated with 0.2 M KNCO at 37 °C for 25 min. This modification led to loss of 50% inhibitory activity of TIMP-2 (Fig. 1). The partially modified TIMP-2 was then separated on an matrilysin-Sepharose 4B column. After separation, matrilysin-bound and matrilysin-unbound fractions contained almost the same amount of protein (data not shown), suggesting that about 50% of the modified TIMP-2 before separation had essentially no affinity for matrilysin. The matrilysin-bound fraction and native TIMP-2 showed comparable abilities to inhibit the matrilysin-catalyzed hydrolysis of 3167v (Fig. 2A). In contrast, the matrilysin-unbound fraction had no inhibitory activity, as expected. The matrilysin-unbound fraction was also inactive against APMA-activated gelatinase A (Fig. 2B). These data are consistent with the view that treatment of TIMP-2 with KNCO leads to modification of the reactive site of TIMP-2, thus preventing formation of the protease-inhibitor complex.

Determination of the Site of Modification Responsible for the Loss of Inhibitory Activity of TIMP-2—To determine the site of modification responsible for the loss of inhibitory activity, the samples in matrilysin-bound and matrilysin-unbound fractions were reduced and S-carboxyamidomethylated and then subjected to tryptic digestion, after which the digests were separated by reversed-phase high performance liquid chromatography. The differences observed between the two elution profiles were only peaks B-20 and U-21 from the matrilysin-bound and matrilysin-unbound fractions, respectively (Fig. 3, A and B). The mass spectrometric analyses of the peptides (Fig. 4, A and B) showed that molecular masses of B-20 and U-21 were 2345.22 and 2388.26, respectively. Based on the determined molecular mass, B-20 is assigned as the peptide corresponding to residues 1–20 of human TIMP-2. On the other hand, difference of molecular masses between B-20 and U-21 corresponds to the mass of a carbamyl adduct, suggesting that U-21 is a peptide corresponding to residues 1–20 of TIMP-2 bearing a single carbamylated amino group. Furthermore, the ZSZSPVHPQQAFZNADVVI sequence corresponding to residues 1–19 of TIMP-2 was determined in the NH2-terminal sequence analysis on B-20, where Z was detected as a phenylthiohydantoin-derivative of S-carboxyamidomethylcysteine. As expected, no phenylthiohydantoin-derivative of amino acid was detected in the NH2-terminal sequence analyses of U-21. These results indicate that B-20 and U-21 are peptides derived from the NH2-terminal region of TIMP-2 corresponding to residues 1–20, and that the ε-amino group of Cys1 of U-21 is carbamylated. The results also suggest that the carbamylation of the ε-amino group of NH2-terminal Cys1 of TIMP-2 leads to the inactivation of TIMP-2.

Effect of KNCO Treatment of TIMP-2 on the Progelatinase A
The chemical modification of TIMP-2 has been shown to affect the progelatinase A binding ability of TIMP-2. Indications from KNCO-treated TIMP-2 forms in the matrilysin-bound and matrilysin-unbound fractions suggested that the carbamylation of TIMP-2 affects the progelatinase A binding ability, the matrilysin-bound and matrilysin-unbound fractions of KNCO-treated TIMP-2 and native TIMP-2 were tested for their progelatinase A binding abilities, using the ligand blotting analysis as described under “Experimental Procedures.” As shown in Fig. 5, native TIMP-2 and the KNCO-treated TIMP-2 in the matrilysin-unbound fraction and that in matrilysin-bound one had comparable abilities to bind with progelatinase A, suggesting that the carbamylation of TIMP-2 has essentially no effect on the interaction with progelatinase A.

Effect of Reactive Site-modified TIMP-2 and Native TIMP-2 on the Cell-mediated Activation of Progelatinase A—It has been hypothesized that a complex formed between MT-MMP and TIMP-2 acts as a receptor of progelatinase A and the formation of the ternary complex is essential for the cell-mediated activation of progelatinase A (12, 13, 20). Since the matrilysin-bound fraction of carbamylated TIMP-2 loses the reactive site to interact with the active site of MMPs while retaining the progelatinase A-binding site, the reactive site-modified TIMP-2 may be able to prevent the formation of the ternary complex by competing for the limited number of the TIMP-2-binding sites of progelatinase A. To examine this possibility, various concentrations of the reactive site-modified and -unmodified TIMP-2 forms and native TIMP-2 were added to the culture medium of concanavalin A-stimulated HT1080 cells. The cell lysates and CMs were prepared from the incubated cells and subjected to gelatin zymography as described under “Experimental Procedures.” Arrowheads indicate the gelatinolytic bands at 66 kDa (upper), the intermediate form at 59 kDa (center), and the mature form at 57 kDa (lower). An arrow at 90 kDa indicates a gelatinolytic band of progelatinase B. Ordinate, molecular size in kDa.

Binding Ability—In addition to the MMPs inhibitory activity, TIMP-2 also has an ability to interact with the hemopexin-like domain of progelatinase A. To examine whether the carbamylation of TIMP-2 affects the progelatinase A binding ability, the matrilysin-bound and matrilysin-unbound fractions of KNCO-treated TIMP-2- and native TIMP-2 were incubated for 24 h in serum-free medium with the indicated concentrations of the KNCO-treated TIMP-2 forms in the matrilysin-unbound fraction (NH2-terminal modified TIMP-2) and matrilysin-bound fraction (unmodified TIMP-2) and a fixed concentration (100 μg/ml) of concanavalin A. Cell lysates (panel A) and CMs (panel B) were prepared from the incubated cells and subjected to gelatin zymography as described under “Experimental Procedures.” Arrowheads indicate the gelatinolytic bands at 66 kDa (upper), the intermediate form at 59 kDa (center), and the mature form at 57 kDa (lower). An arrow at 90 kDa indicates a gelatinolytic band of progelatinase B. Ordinate, molecular size in kDa.
TIMP-2 did not lead to increasing the amount of cell-associated progelatinase A, the cell-associated zymogen may be released at high concentrations of TIMP-2. The effects of native TIMP-2 on the cell-associated gelatinase A and on the cell-mediated activation of progelatinase A were almost the same as those of the active TIMP-2 in the matrilysin-bound fraction (data not shown).

**DISCUSSION**

To explore the reactive site of TIMP-2 involved in the interaction with the active site of MMPs, we treated TIMP-2 with cyanate ions under controlled conditions, and identified an amino group essential for the inhibitory activity of TIMP-2. The effects of native TIMP-2 on the cell-associated gelatinase A and on the cell-mediated activation of progelatinase A were almost the same as those of the active TIMP-2 in the matrilysin-bound fraction (data not shown).

Fig. 7. Hypothetical model for inhibitory effects of reactive site-modified TIMP-2 and native TIMP-2 on formation of the ternary complex consisting of MT-MMP, TIMP-2, and (pro)gelatinase A. In panel A, the reactive site-modified TIMP-2 inhibits the formation of the ternary complex consisting of MT-MMP, TIMP-2, and (pro)gelatinase A by competing for the hemopexin-like domain of (pro)gelatinase A. The reactive site-modified TIMP-2 cannot interact with the active site of MT-MMP. In panel B, an excess amount of native TIMP-2 inhibits the formation of the ternary complex by occupying both the active site of MT-MMP and the hemopexin-like domain of (pro)gelatinase A. H$_2$N, the $\alpha$-amino group of NH$_2$-terminal Cys$^1$ of TIMP-2; H$_2$NCONH, the carbamylated $\alpha$-amino group of NH$_2$-terminal Cys$^1$ of TIMP-2; Zn$^{2+}$, catalytic zinc atom of metalloproteinases.

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inhibitory activity of TIMP-2. There is an alternative explanation that the carbamylated α-amino group of Cys\(^2\) may not be able to interact with the catalytic zinc atom due to steric hindrance. The crystal structures of the two MMP-TIMP complexes also indicate that TIMPs have wide range contacts with the corresponding MMPs. However, the present study showed that the modified TIMP-2 bearing a single carbamylated α-amino group had essentially no affinity with matrilysin. This discrepancy might be explained by sequential interactions: the primary interaction between the Cys\(^3\) of TIMPs and the catalytic zinc atom of MMPs may trigger a rearrangement of residues to make secondary interactions. Further study will be required to clarify this mechanism. Previously, it has been reported that chemical modification of TIMP-1 with diethyl pyrocarbonate abolishes the inhibitory activity. The modified residues are His\(^{95}\), His\(^{144}\), and His\(^{164}\) of TIMP-1, and the modification of His\(^{95}\) is proposed to be responsible for the loss of activity (22). However, mutational study has revealed that replacement of His\(^{95}\) to glutamine does not affect the inhibitory activity of TIMP-1 (22). Furthermore, the H95Q mutant is still sensitive to diethyl pyrocarbonate treatment. So far, there is no explanation for the effect of diethyl pyrocarbonate on the TIMP-1 activity. It is possible, however, to speculate that the α-amino group of Cys\(^2\) of TIMP-1 had been modified during treatment with diethyl pyrocarbonate, because the α-amino group, as well as the imidazole group, are reactive with diethyl pyrocarbonate. As the carbamylated TIMP-2 in the matrilysin-unbound fraction had an ability to bind to gelatinase A, it is likely that a site of TIMP-2 essential for the interaction with the hemopexin-like domain of (pro)gelatinase A is not affected by the carbamylation. We found that the reactive site-modified TIMP-2 could prevent an accumulation of the active form of gelatinase A on the surface of concanavalin A-stimulated HT1080 cells. It is hypothesized that a complex formed between MT-MMP and TIMP-2 acts as a cell surface receptor of (pro)gelatinase A (12, 13). Accordingly, the disappearance of the cell-associated gelatinase A could be explained by speculation that the competitive binding of the reactive site-modified TIMP-2 to the hemopexin-like domain of gelatinase A makes it unable for gelatinase A to be retained on the cell surface, because TIMP-2 cannot interact with MT-MMP. We also found that the reactive site-modified TIMP-2 partially inhibited the conversion of progelatinase A to the intermediate form and strongly inhibited the conversion of the intermediate form to the mature one. As the conversion of progelatinase A to the intermediate form is thought to be facilitated by cell association of progelatinase A (20), the partial inhibition of the processing of progelatinase A is likely to be caused by the prevention of cell association of the zymogen by the reactive site-modified TIMP-2 (Fig. 7A). We also speculate that the conversion of the intermediate form of gelatinase A to the mature one depends upon the cell-associated activity of gelatinase A, and therefore, deprivation of the cell-associated active form of gelatinase A by the reactive site-modified TIMP-2 causes an inhibition of production of the mature form. In the presence of high concentrations of reactive site-modified TIMP-2, the disappearance of the mature form of gelatinase A in the CM was indeed in parallel with the diminution of the cell-associated active gelatinase A (Fig. 6). Recent studies (15, 23–26) suggest that transmembrane domainless variants of MT-MMP convert progelatinase A to the intermediate form but hardly to the mature one. It is also reported that cell-mediated processing of mutant progelatinase A of which the active site residue is replaced does not produce the mature form of the mutant (27, 28). These studies suggest the importance of cell associated activity of gelatinase A for the conversion of the intermediate form of gelatinase A to its mature form. Considering the importance of formation of the ternary complex consisting of MT-MMP, TIMP-2, and (pro)gelatinase A, the inhibition of the cell-mediated activation of progelatinase A by TIMP-2 could be explained in two alternative ways. One explanation is that excess TIMP-2 occupies both the active site of MT-MMP and the TIMP-2-binding site in hemopexin-like domain of (pro)gelatinase A, thus preventing the formation of the ternary complex (Fig. 7B). The other explanation is that TIMP-2 inhibits the catalytic activity of MT-MMP, thus inhibiting the proteolytic processing of gelatinase A. We found that native TIMP-2, as well as reactive site-modified TIMP-2, could prevent accumulation of active gelatinase A on the cell surface, without increasing the cell-associated progelatinase A. These data suggest that prevention of the formation of ternary complex contributes to the TIMP-2 inhibition of the cell-mediated activation of gelatinase A. Native TIMP-2, but not the reactive site-modified TIMP-2, inhibited production of the intermediate form of gelatinase A. Therefore, it is also likely that inhibition of the catalytic activity of MT-MMP by TIMP-2 contributes to inhibition of the processing of gelatinase A. As disappearance of the mature and the intermediate forms of gelatinase A in the CM and diminution of the cell-associated active gelatinase A were observed at similar concentrations of unmodified TIMP-2 (Fig. 6), prevention of formation of the ternary complex and inhibition of MT-MMP activity may occur simultaneously, at a critical concentration of TIMP-2 (Fig. 7B). It is likely that both the mechanisms make TIMP-2 a potent regulator of the cell-mediated activation of gelatinase A. As described here, reactive site-modified TIMP-2 could inhibit the activation of gelatinase A without inhibiting the catalytic activity of MT-MMP. The reactive site-modified TIMP-2 might be a useful tool to distinguish the functions of MT-MMP and cell-associated gelatinase A. We are now using this modified TIMP-2 to explore the role of MT-MMP and/or cell-associated gelatinase A in the processing of cell-surface proteins.

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REFERENCES

1. Docherty, A. J. P., O’Connell, J., Crabbe, T., Angal, S., and Murphy, G. (1992) Trends Biotechnol. 10, 200–207
2. Matrisian, L. M. (1992) Bioessays 14, 455–463
3. Stetler-Stevenson, W. G., Aznavoorian, S., and Liotta, L. A. (1993) Annu. Rev. Cell Biol. 9, 543–573
4. Liotta, L. A. (1986) Cancer Res. 46, 1–7
5. Collier, I. E., Wilhelm, S. M., Eisen, A. Z., Marmer, B. L., Grant G. A., Seltzer, J. L., Kronberger, A., He, C., Buerer, E. A., and Goldberg, G. I. (1988) J. Biol. Chem. 263, 6579–6587
6. Wilhelm, S. M., Collier, I. E., Marmer, B. L., Eisen, A. Z., Grant, G. A., and Goldberg, G. I. (1989) J. Biol. Chem. 264, 17213–17221
7. Overall, C. M., and Sodek, J. (1990) J. Biol. Chem. 265, 21141–21151
8. Brown, P. D., Levy, A. T., Margulies, I. M., Liotta, L. A., and Stetler-Stevenson, W. G. (1990) Cancer Res. 50, 6184–6191
9. Ward, R. V., Atkinson, S. J., Sloembe, F. M., Docherty, A. J., Reynolds, J. L., and Murphy, G. (1991) Biochem. Biophys. Acta 1079, 242–246
10. Azzam, H. S., and Thompson, E. W. (1992) Cancer Res. 52, 4540–4544
11. Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E., and Seiki, M. (1996) Nature 379, 61–65
12. Strongin, A. Y., Marmer, B. L., Grant, G. A., and Goldberg, G. I. (1993) J. Biol. Chem. 268, 14033–14039
13. Strongin, A. Y., Collier, I. E., Lannikov, G., Marmer B. L., Grant, G. A., and Goldberg, G. I. (1995) J. Biol. Chem. 270, 5331–5338
14. Brooks, P. C., Stromblad, S., Sanders, L. C., von Schalscha, T. L., Aimes, R. T., Stetler-Stevenson, W. G., Quigley, J. P., and Cheresh, D. A. (1996) Cell 83, 683–693
15. Brooks, P. C., Sillietti, S., von Schalscha, T. L., Friedlander, M., and Cheresh, D. A. (1998) Cell 92, 391–400
16. Ishiduka, K., Moriyama, K., Nishihashi, A., Higashi, S., Murakami, H., Yasumitsu, H., Miki, K., Sato, H., Seiki, M., and Miyazaki, K. (1998) J. Biochem. (Tokyo) 124, 462–470
17. Miyazaki, K., Fumahashi, K., Numata, Y., Koshikawa, N., Akagi, K.,
Kikkawa, Y., Yasumitsu, H., and Umeda, M. (1993) J. Biol. Chem. **268**, 14387–14393
18. Miyazaki, K., Hattori, Y., Umenishi, F., Yasumitsu, H., and Umeda, M. (1996) Cancer Res. **56**, 7758–7764
19. Gomis-Ruth, F. X., Maskos, K., Betz, M., Bergner, A., Huber, R., Suzuki, K., Yoshida, N., Nagase, H., Brew, K., Bourenkov, G. P., Bartunik, H., and Rode, W. (1997) Nature **389**, 77–81
20. Kinoshita, T., Sato, H., Okada, A., Ohuchi, E., Imai, K., Okada, Y., and Seiki, M. (1998) J. Biol. Chem. **273**, 16098–16103
21. Fernandez-Catalan, C., Bode, W., Huber, R., Turk, D., Calvete, J. J., Lichte, A., Tschesche, H., and Maskos, K. (1998) EMBO J. **17**, 5238–5248
22. Williamson, R. A., Smith, B. J., Angell, S., and Freedman, R. B. (1993) Biochim. Biophys. Acta **1203**, 147–154
23. Kinoshita, T., Sato, H., Takino, T., Itoh, M., Akizawa, T., and Seiki, M. (1996) Cancer Res. **56**, 2535–2538
24. Pei, D. Q., and Weiss, S. J. (1996) J. Biol. Chem. **271**, 9135–9140
25. Will, H., Atkinson, S. J., Butler, G. S., Smith, B., and Murphy, G. (1996) J. Biol. Chem. **271**, 17119–17213
26. Lichte, A., Kolkenbrock, H., and Tschesche, H. (1996) FEBS Lett. **397**, 277–282
27. 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
28. Sato, H., Takino, T., Kinoshita, T., Imai, K., Okada, Y., Stetler-Stevenson, W. G., and Seiki, M. (1996) FEBS Lett. **385**, 238–240