Human gelatinase B is involved in tissue remodeling and angiogenesis. It is thought to be synthesized and rapidly secreted as an inactive precursor. In this report, we have shown that human endothelial cells accumulate active forms of gelatinase B in the cytosol. Microvascular but not macrovascular endothelial cells dramatically increased the expression of cytosolic gelatinase B in response to phorbol myristate acetate. Western blotting showed that tissue inhibitor of metalloproteinase-1 (TIMP1) was also present in the cytosol. Whereas gelatinase B was complexed with TIMP1 in the conditioned medium, it existed as a free enzyme in the cytosol, suggesting that the formation of gelatinase B and TIMP1 complex occurs after their secretion. Immunogold electron microscopy revealed that gelatinase B was localized in secretory vesicles which were especially prominent in invading pseudopodia. In contrast, TIMP1 was found throughout the cytoplasm but was not present in the gelatinase vesicles. The accumulation of intracellular activated gelatinase B, ready for rapid release, may facilitate the migration of microvascular endothelial cells during angiogenesis.

The formation of capillaries from pre-existing microvessels (angiogenesis) occurs in a variety of normal and pathological conditions, including wound healing, tumor growth and metastases, and arthritis (1). During angiogenesis, microvascular endothelial cells secrete proteases, migrate through the underlying extracellular matrix, and proliferate to organize into new blood vessels. The initial step of angiogenesis requires focalized degradation of the basement membrane (2). The process is carried out by at least two matrix metalloproteinases (MMPs), gelatinase A and gelatinase B, both of which degrade basement membrane collagens (3) and are produced by many cell types, including endothelial cells (4, 5). They are unique among the MMPs in that their latent forms are thought to be secreted in physical association with their natural inhibitors, the tissue inhibitors of MMPs (TIMPs) (6, 7). Pro-gelatinase B binds to TIMP1, whereas pro-gelatinase A complexes with TIMP2, both via the COOH-terminal domain of the enzymes (6, 8).

With the exception of neutrophil gelatinase B, which is stored in cytoplasmic granules (9), MMPs are thought to be synthesized and rapidly secreted as latent precursors that must be processed extracellularly to their active forms to express enzymatic activity (10). Gelatinase A binds to and can be activated by the membrane-type MMP (MT1-MMP) at the cell surface of several tumors, transformed and normal cell types (11, 12). The activation of gelatinase B is thought to occur in the extracellular milieu, although the mechanism is not clearly understood. Recent reports have suggested that stromelysin-1 may be responsible (6, 14, 15). Ginestra et al. (13) have recently shown that membrane vesicles shed from human HT1080 fibrosarcoma cells contained active gelatinase B. In this report, we have demonstrated that microvascular endothelial cells are capable of accumulating active gelatinase B in the cytoplasm. Furthermore, this active enzyme is separately compartmentalized from TIMP1 by being located in secretory vesicles.

EXPERIMENTAL PROCEDURES

Cells—Human microvascular endothelial cells (FSE) derived from neonatal foreskin obtained after circumcision were isolated using *Ulex europaeus* 1-coated Dynabeads as described previously (16). Microvascular endothelial cells were obtained from human umbilical veins (HUVE) isolated as described by Jaffe (17). FSE were grown and maintained in Biorich medium (ICN Biomedicals, Aurora, OH) containing 30% normal pooled human serum (derived from healthy volunteers) plus 100 μg/ml endothelial cell growth supplement prepared as described by Macing et al. (18) and 50 μg/ml heparin (Sigma). HUVE were grown in Biorich containing 20% fetal calf serum plus 50 μg/ml endothelial cell growth supplement and 50 μg/ml heparin. Cells were used at passage 4.

Experimental Protocol—HUVE and FSE (3 cell lines each) were cultured on 60-mm dishes (Becton Dickinson) at a density of 1.5 × 10^6 cells/dish in growth medium. Endothelial cells were washed twice with Hank’s balanced salt solution and preincubated in basal medium (Biorich plus 1% normal pooled serum) for 6 h. The cells were then replaced with fresh basal medium and incubated in the absence or presence of 100 ng/ml phorbol myristate acetate (PMA) (Sigma) for 24 h.

Human serum was necessary to maintain an intact confluent monolayer of microvascular cells throughout the course of the experiment (5). Gelatinases present in human serum were removed by running the serum through a gelatin-Sepharose affinity column (3) (Pharmacia Biotech Inc.).

Conditioned Media and Triton X-114 Cell Extracts—After incubation, the conditioned medium was collected and the cytosolic fraction was separated from the membrane fraction by Triton X-114 extraction as described by Lewalle et al. (19). The extract was partitioned into the detergent (membrane) fraction and aqueous phases (cytosolic fraction) at 37 °C for 5 min and centrifuged at 5000 × g for 2 min. The aqueous phase was then collected. To eliminate any carry-over effect from the aqueous fraction, the detergent phase was repartitioned three times in 1.5% Triton X-114. It was then concentrated by mixing with 30 μl of packed gelatin-Sepharose (Pharmacia) with end-over-end rotation for 30 min at 4 °C. The bead suspension was centrifuged at 8000 × g for 2 min and the pellet was then analyzed by gelatin zymography.

Flow Cytometric Analysis—Flow cytometry was performed as de-
stromelysin-1, gelatinase B, and TIMP1 were used at 1 
analysis after SDS-polyacrylamide gel electrophoresis. Antibodies to 
ducing conditions as described previously (21). (Coulter Elite, Hialeah, FL).

applied for 30 min and the cells were then analyzed by flow cytometry 
Fluorescein isothiocyanate-conjugated secondary antibody was then 
with 10% Triton X-100, washed, centrifuged, and the primarymono-
temperature. The cells were then washed and permeabilized for 10 min 
for 10 min, the pellet was resuspended and fixed in freshly prepared 2%
paraformaldehyde in phosphate-buffered saline for 30 min at room 
time. A blank control was used for non-specific staining. 

Zymography—Samples were analyzed by zymography under nonre-
ducing conditions as described previously (21).

Immunoblotting—MMPs and TIMP1 were detected by immunoblot 
analysis after SDS-polyacrylamide gel electrophoresis. Antibodies to 
stromelysin-1, gelatinase B, and TIMP1 were used at 1 μg/ml (Oncogene 
Science).

Immunogold Electron Microscopy—FSE (7 × 10^4) were seeded onto 
type 1 collagen gel and prepared as described previously (22) in an 
culture well insert for 24 h before PMA (100 ng/ml) was added for a 
60 min and the cells were then analyzed by flow cytometry 
(Coulter Elite, Hialeah, FL).

To determine whether gelatinase B was localized to the cell membrane or intracellularly, the 
membrane and cytosolic fractions from endothelial cells were sepa-
ated using Triton extraction as described under “Experimental 
Procedures.” Gelatinase B levels were measured in these 
fractiions as well as in the conditioned medium using zymogra-
phic methods. Results are shown in Fig. 1. Under basal conditions, 
gelatinase B was not detected in the conditioned medium or 
membrane fraction of endothelial cells. However, endothelial 
cells expressed gelatinase B in the cytosolic fraction as three 
versus, HUVE expressed markedly lower levels of gelatinase B.

Gelatinase B Present in Endothelial Cell Conditioned Me-
dium and Cell Fractions—To determine whether gelatinase B 
was located on the cell membrane or intracellularly, the mem-
brane and cytosolic fractions from endothelial cells were sepa-
rated using Triton extraction as described under “Experimental 
Procedures.” Gelatinase B levels were measured in these 
fractiions as well as in the conditioned medium using zymogra-
phic methods. Results are shown in Fig. 1. Under basal conditions, 
gelatinase B was not detected in the conditioned medium or 
membrane fraction of endothelial cells. However, endothelial 
cells expressed gelatinase B in the cytosolic fraction as three 
versus, HUVE expressed markedly lower levels of gelatinase B.

RESULTS

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.

Cellular Localization of Gelatinase B by Flow Cytometry—

Previous workers have shown that cultured human endothelial 
cells secrete gelatinase B into the conditioned medium in re-
sponse to PMA (4, 5). To determine whether gelatinase B was 
also localized to the endothelial cell, we performed flow cytometry 
using an antibody directed against the latent and active 
forms of gelatinase B. Results are shown in Fig. 1. Under basal 
conditions, FSE showed a 2.1-fold increase in gelatinase B 
expression compared with the nonimmune control. Stimulation 
of FSE with PMA resulted in a 9.6-fold increase in the expres-
sion of gelatinase B compared with unstimulated cells. In con-
trast, HUVE expressed markedly lower levels of gelatinase B.
gelatinase B was first detected after 2 h, whereas the active forms of gelatinase B were detected after 8 h of PMA treatment, the levels of which were substantially higher at 16 h. Gelatinase A first appeared in the cytosol after 2 h but, in contrast to gelatinase B, the levels remained constant over the 16-h incubation period. This suggested that gelatinase A was secreted from the cell, whereas gelatinase B was stored in the cell.

Western blotting disclosed the presence of TIMP1 in the cytosol, which was markedly elevated in FSE but not HUVE after treatment with PMA (Fig. 5). Stromelysin-1 was also detected in the FSE cytosol under basal conditions, the levels of which were increased after treatment with PMA (data not shown).

**Cytosolic Gelatinase B Is TIMP-free, whereas Secreted Gelatinase B Is Bound to TIMP1—** Previous workers have reported that the aminophenylmercuric acetate (APMA) activation of the purified gelatinase B-TIMP complex results in the loss of an 8–10-kDa NH\textsubscript{2}-terminal domain of the enzyme, generating a final product of 82 kDa (24, 25). In contrast, activation of TIMP-free gelatinase B with APMA results in the formation of not only the intermediate 82-kDa species, but also the active species of 67 kDa and 40–50 kDa (24–26). To determine whether gelatinase B in the cytosolic fraction was complexed with TIMP1, the conditioned medium and the cytosolic fraction were assessed for gelatinase activity by zymography.

**Gelatinase B Is Localized to Intracellular Vesicles—** Because both gelatinase B and TIMP1 were present in the cytosol yet were not complexed, we used immunogold electron microscopy to investigate whether TIMP1 and gelatinase B were separately compartmentalized within the cell. FSE were grown on type I collagen gel to provide support for maintaining cell integrity during processing. Immunogold electron microscopy confirmed the presence of gelatinase B and TIMP1 in the cytosol of FSE which had been treated with PMA for 24 h. Gelatinase B gold colloid particles were mainly localized in membrane-bound secretory vesicles (Fig. 7a). These vesicles were slightly more electron dense than the surrounding cytoplasm and were encapsulated by a clearly defined plasma membrane. In quiescent cells, the vesicles were usually found in close proximity to the cell membrane facing the collagen gel. Interestingly, the vesicles were more abundant in pseudopod extensions from the cells and, in some instances, the immunogold labeling showed gelatinase B being secreted from the tips of pseudopodia which were invading the collagen gel (Fig. 7c). Using double immunogold labeling, we found that TIMP1 was spread more diffusely throughout the cytoplasm (Fig. 7d). Although TIMP was occasionally present in small vesicles (Fig. 7d), it was never found in the gelatinase B-containing vesicles.

**DISCUSSION**

We have demonstrated that gelatinase B accumulates in human microvascular endothelial cells and that the enzyme is present in both its latent and active forms. Previous to this report, only stromelysin-3 and MT1-MMP were known to be
Gelatinase B Accumulates within Endothelial Vesicles

The presence of gelatinase A on the cell membrane of human umbilical vein endothelial cells has been previously reported (19). Our experiments confirmed these findings and also found that both gelatinase A and gelatinase B were expressed in the membrane fraction of FSE in response to PMA. The association of gelatinase B with the plasma membrane has been previously shown in bone metastatic tissue (31) and in basal cell carcinomas (32), although the binding mechanism is not clear. In contrast, the binding and activation of gelatinase A to the cell membrane via TIMP2 and MT1-MMP are well documented (12, 33). Brooks et al. (34) have also found that the C terminus of active gelatinase A is associated with the α5β3 integrin receptor on the cell surface of angiogenic blood vessels and melanoma tumors. Similar interactions may exist for the binding of gelatinase A to the membrane.

In this report, we have demonstrated that FSE have the ability to accumulate and activate gelatinase B intracellularly, especially in the presence of the tumor-promoting chemical PMA. We and others (4, 5) have previously shown that PMA induces the synthesis and secretion of gelatinase B by FSE. To date, there have been few reports on the effects of cytokines or angiogenic/growth factors on gelatinase B induction by human microvascular endothelial cells. Hanemaaijer et al. (4) have shown that tumor necrosis factor-α can enhance the effect of PMA, but it does not stimulate gelatinase B synthesis by FSE when used alone (4, 5). Future studies need to investigate the interplay of physiological agents that can induce gelatinase B accumulation in microvascular endothelial cells.

We have demonstrated that gelatinase B can exist as a free active enzyme in the cytoplasm and to a lesser extent on the cell membrane of FSE. These findings are likely to be relevant in angiogenesis, a phenomenon which only occurs in microvascular endothelial cells. It is feasible that as endothelial cells migrate during angiogenesis, the active forms of gelatinase B are secreted from the cell in short bursts to locally degrade the basement membrane. They are then rapidly inhibited by TIMP and/or degraded in the extracellular milieu (3), rendering them inactive. This inhibitory mechanism is important, as it prevents uncontrolled proteolysis. Pepper et al. (35) have shown that if proteolysis goes uninterrupted the dissolution of the...
matrix prevents endothelial cells from migrating and forming tube-like structures due to the absence of a scaffold. The ability of microvascular endothelial cells to accumulate active gelatinase B in secretory vesicles, ready for release, in addition to the presence of active species of gelatinase A and gelatinase B on the cell membrane, would enable microvascular endothelial cells to localize proteolytic activity to the pericellular environment and thus be very effective in the process of cell migration.

Acknowledgments—We thank Dr. Ross Davey, Assoc. Prof. Leslie Schrieber, Prof. Philip Sambrook, and Kate Gibbons for helpful discussions and review of the manuscript; Peter Jameson (Gore Hill Research Laboratories) and Anne Simpson-Gomes (University of Sydney Electron Microscope Unit) for expert electron microscope assistance; Dr. Malcolm King for performing flow cytometry; and Eddie Jozefiak for photography.

REFERENCES

1. Folkman, J. (1995) Nature Med. 1, 27–31
2. Mignatti, P., and Rifkin, D. B. (1996) Enzyme Protein 49, 117–137
3. Murphy, G., and Crabbe, T. (1995) Methods Enzymol. 248, 470–484
4. Hanemaaijer, R., Koelwijk, P., Leclercq, L., Devree, W. J. A., and Vanhinsbergh, V. W. M. (1993) Biochem. J. 296, 803–809
5. Jackson, C. J., and Nguyen, M. (1997) Int. J. Biochem. Cell Biol. 29, 1167–1177
6. Goldberg, G. I., Strongin, A., Collier, I. E., Genrich, L. T., and Marmer, B. L. (1992) J. Biol. Chem. 267, 4583–4591
7. 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
8. Stetler-Stevenson, W. G., Krutzsch, H. C., and Liotta, L. A. (1989) J. Biol. Chem. 264, 17374–17378
9. Kjeldsen, L., Bainton, D. F., Sengelov, H., and Borregaard, N. (1993) Blood 82, 3183–3191
10. Matrisian, L. M. (1992) BioEssays 14, 455–463
11. Sato, H., and Seiki, M. (1996) J. Biochem. 119, 209–215
12. Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A., and Goldberg, G. I. (1995) J. Biol. Chem. 270, 5331–5338
13. Gines, A., Monea, S., Seghezzi, G., Dolo, V., Nagase, H., Mignatti, P., and Vittorelli, M. L. (1997) J. Biol. Chem. 272, 17126–17122
14. Itah, Y., and Nagase, H. (1995) J. Biol. Chem. 270, 16518–16521
15. Ogata, Y., Enghild, J. J., and Nagase, H. (1992) J. Biol. Chem. 267, 3581–3584
16. Jackson, C. J., Garbett, P. K., Nissen, B., and Schrieber, L. (1990) J. Cell Sci. 96, 257–262
17. Jaffe, E. A., Nachman, R. L., Becker, C. G., and Minick, C. R. (1973) J. Clin. Invest. 52, 2745–2756
18. Maciag, T., Cerundolo, J., Isdale, S., Kelley, P. R., and Forand, R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5674–5678
19. Lewalle, J. M., Munauf, C., Pichot, B., Cataldo, D., Baramova, E., and Foidart, J. M. (1995) J. Cell. Phys. 165, 475–483
20. Bouharian, G., Osman, J., Black, C., and Olsen, I. (1994) Clin. Chim. Acta 231, 69–78
21. Herron, G. S., Web, Z., Dwyer, K., and Banda, M. J. (1986) J. Biol. Chem. 261, 2810–2813
22. Montesano, R., and Orci, L. (1986) Cell 42, 469–477
23. Bendayan, M. (1982) J. Histochem. Cytochem. 30, 81–85
24. Shapiro, S. D., Fliszar, C. J., Broekelmann, T. J., Mecham, R. P., Senior, R. M., and Welsburg, H. G. (1995) J. Biol. Chem. 270, 6531–6536
25. Okada, Y., Gonoji, Y., Naka, K., Tomita, K., Nakasako, I., Ikawa, K., Yamashita, K., and Hayakawa, T. (1992) J. Biol. Chem. 267, 21712–21719
26. Senior, R. M., Griffin, G. L., Fliszar, C. J., Shapiro, S. D., Goldberg, G. I., and Welsburg, H. G. (1991) J. Biol. Chem. 266, 7870–7875
27. Pei, D. Q., and Weiss, S. J. (1995) Nature 375, 244–247
28. Pei, D., and Weiss, S. J. (1996) J. Biol. Chem. 271, 9135–9140
29. Baramova, E. N., Bajou, K., Remacle, A., Lhoir, C., Krell, H. W., Weidle, U. H., Noel, A., and Foidart, J. M. (1997) FEBS Lett. 405, 157–162
30. Fridman, R., Toth, M., Pena, D., and Mobashery, S. (1995) Cancer Res. 55, 2548–2555
31. Visscher, D. W., Hoyhtya, M., Ottoson, S. K., Liang, C. M., Sarkar, F. H., Crissman, J. D., and Fridman, R. (1994) Int. J. Cancer 59, 339–344
32. Arkena, C., and Wiederanders, B. (1996) Biol. Chem. 377, 695–702
33. Emmertbuck, M. R., Emmard, H. P., Corcoran, M. L., Krutzsch, H. C., Foidart, J. M., and Stetlersteven, W. G. (1995) FEBS Lett. 364, 28–32
34. Brooks, P. C., Stromblad, S., Sanders, I. C., Vonschalscha, T. L., Aines, R. T., Stetlerstevenson, W. G., Quigley, J. P., and Cheres, D. A. (1996) Cell 85, 683–693
35. Pepper, M. S., Montesano, R., Mandriota, S. J., Orci, L., and Vassalli, J. D. (1996) Enzyme Protein 49, 138–162