Jpn. J. Cancer Res. 86, 298–303, March 1995

A Possible Role of 92 kDa Type IV Collagenase in the Extramedullary Tumor Formation in Leukemia

Masanobu Kobayashi,[1,4] Jun-ichi Hamada,[2] Young-Quing Li,[1] Noriaki Shinobu,[1] Masahiro Imamura,[3] Futoshi Okada,[2] Noritoshi Takeichi[2] and Masuo Hosokawa[1]

[1]*Laboratory of Pathology,* [2]*Laboratory of Cell Biology, Cancer Institute,* [3]*The Third Department of Internal Medicine, Hokkaido University School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060*

Production of metalloproteinases such as collagenases has been reported to be involved in the metastasis of cancer cells. Granulocytic sarcoma in extramedullary sites can be formed by similar steps to other cancers. In this study, we have examined the secretion of type IV collagenases and a tissue inhibitor of metalloproteinase-1 (TIMP-1) in several human leukemia cell lines, including a granulocytic sarcoma-derived cell line established from a patient with granulocytic sarcomas in dermal tissues. We have also examined the invasive capacity of these leukemia cell lines into reconstituted basement membrane, Matrigel, which was used for *in vitro* invasion assay. Among the human leukemia cell lines used in this study, only the granulocytic sarcoma cell line was found to secrete type IV collagenase constitutively. Other myeloid leukemia cell lines such as HL-60 and U-937 produced type IV collagenase only after treatment with 12-O-tetradecanoylphorbol-13-acetate. All the cell lines secreted similar amounts of the tissue inhibitor of metalloproteinases. *In vitro* invasion assay revealed that the granulocytic sarcoma cell line showed higher invasive capacity than the other cell lines. These results suggest that the secretion of 92 kDa type IV collagenase plays a role in the leukemia cells' invasion of extramedullary tissues.

Key words: Type IV collagenase — Granulocytic sarcoma — Leukemia — TIMP-1 — Invasion

Granulocytic sarcoma (GS) is defined as an extramedullary tumor composed of immature cells of a granulocytic lineage, and develops in patients with acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML).[1,2] Usually the proliferation and differentiation of hematopoietic cells are restricted within hematopoietic tissues, including bone marrow, spleen, lymph nodes, and liver.[3] However, extramedullary tumor formations are found frequently in patients with leukemia, especially acute myelomonocytic leukemia and chronic myelogenous leukemia.[4,5] As cancer cells generally leave the blood vessels and invade extravascular sites in the process of metastasis, leukemia cells which form extramedullary tumors should have capacities to: (1) attach to endothelial cells; (2) traverse endothelial cells; (3) degrade basement membranes; and (4) lodge and proliferate in extravascular sites. Previously, we have reported that only the granulocytic sarcoma cell line among several leukemia cell lines could adhere to skin fibroblasts,[6] which suggested that the attachment to skin fibroblasts is a necessary step for the granulocytic sarcoma cells to lodge and proliferate in skin tissues.

Recently, involvement of the production of collagenases in the invasion and distant metastasis of cancer cells has been documented.[7–12] However, there has been no report demonstrating the involvement of collagenase production in the extramedullary tumor formation in leukemia, since few granulocytic sarcoma cell lines have been established. Therefore, we have examined the secretion of type IV collagenases and a tissue inhibitor of metalloproteinase-1 (TIMP-1) in several leukemia cell lines, including a granulocytic sarcoma cell line, and we have further examined the invasive capacity of the leukemia cell lines by means of *in vitro* invasion assay using Matrigel-coated Transwells.

MATERIALS AND METHODS

Cell lines HL-60 (promyelocytic), U-937 (monocytic), K-562 (undifferentiated), Daudi (B lymphocytic), MOLT-4 (T lymphocytic), and CEM (T lymphocytic) were obtained from the Japanese Cancer Resources Bank and maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS). HSM-1 was established from a patient with granulocytic sarcoma and myelofibrosis and maintained for more than four years in RPMI-1640 supplemented with 10% FCS. The HSM-1 cells expressed CD4, CD11b, CD13, CD71, and HLA-DR. Cytochemical analysis revealed that the HSM-1 cells were myeloperoxidase-, Sudan black B-, and naphthol AS-D chloroacetate esterase-positive, but α-naphthyl butyrate-negative.[6]

Zymography The cells were harvested, washed twice with phosphate-buffered saline (PBS), seeded in 6-well tissue culture plates in RPMI-1640 medium supplemented with 0.5% bovine serum albumin (BSA) at 5×

[4] To whom reprint requests should be addressed.

10^5/ml, and cultured for 24 h. In some experiments, the cells were cultured with 10^{-7} *M* 12-O-tetradecanoylphorbol-13-acetate (TPA) (purchased from Sigma Chemical Co., St. Louis, MO), 10 ng/ml of interleukin (IL)-1β (kindly provided by Shionogi Pharmaceutical Co., Ltd., Osaka), 10 ng/ml of IL-6 (kindly provided by Toray Industry Corp., Tokyo), 100 U/ml of tumor necrosis factor (TNF)-α (purchased from Genzyme), 0.2 ng/ml of transforming growth factor (TGF)-β (purchased from Genzyme), or 100 U/ml interferon (IFN)-α (purchased from Genzyme). Supernatants were collected, sequentially centrifuged at 1,200 rpm for 10 min and at 12,000 rpm for 10 min, filtered through 0.45 μm membrane filters, and then stocked at -30°C until use. Gelatinolytic activity was analyzed by zymography using a gelatin-bedded sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). SDS-gelatin-embedded gels were prepared by the methods of Nakajima *et al.* and Albini *et al.*[13, 14] Gelatin type A from porcine skin (G2625; Sigma Chemical Co.) was dissolved in 2% SDS. The SDS-gelatin solution was filtered through 0.22 mm membrane filters, and copolymerized with 7.5% acrylamide (final concentration of gelatin; 1.0 mg/ml). The serum-free samples were mixed with sample buffer (0.185 *M* Tris-HCl), pH 6.8; 30% glycerol; 0.00375% Bromophenol Blue; 6% SDS) at 2:1. Electrophoresis was performed as described by Laemmli[15] under cooling. After electrophoresis, the gels were soaked with 2.5% Triton X-100 in 50 m*M* Tris-HCl buffer (pH 7.5) containing 0.05% sodium azide on a rocker platform at room temperature for 3 h. Then the gels were incubated at 37°C for 16 h in 0.15 *M* NaCl, 10 m*M* $CaCl_2$ and 50 m*M* Tris-HCl buffer (pH 7.5) containing 0.05% sodium azide. In some experiments, 2 m*M* EDTA or 1 m*M* 1,10-phenanthroline was added in this incubation buffer. After the incubation, the gels were stained with 0.05% Coomassie Blue R-250 in isopropanol:acetic acid:water (1:1:8), destained with isopropanol:acetic acid:water (1:1:8), and dried on GelBond PAG film (FMC Bioproducts, Rockland, ME) and cellophane membrane backing sheets (BIORAD, Richmond, CA) to obtain permanent records. Gelatinolytic activities were detected as transparent bands on the blue background of Coomassie Blue-stained gels.

Immunoblotting Supernatants were separated on 12% SDS-polyacrylamide gel under reducing conditions according to the method of Laemmli,[15] electrophoretically transferred to nitrocellulose membrane, and then blotted using anti-TIMP antibody (Fuji Chemical Industries, Toyama) and ECL Western blotting detection reagents (Amersham International plc, Amersham, UK).

***In vitro* invasion assay** *In vitro* invasion assays were done according to the method described previously.[13] Briefly, Matrigel was diluted in cold RPMI-1640 medium with 0.5% BSA at 5 μg/ml. The Matrigel solution was poured into the upper chambers of Transwell plates (pore size 8 μm; Costar, Cambridge, MA) and allowed to gel at room temperature. Then 5×10^5 cells in RPMI-1640 medium supplemented with 0.5% BSA in the presence or absence of TPA were dispensed into the upper chambers, which were subsequently placed in the outer wells of the Transwell plates. Skin fibroblast conditioned medium, which was prepared by incubating confluent skin fibroblasts in serum-free RPMI-1640 medium supplemented with 0.5% BSA and which has already been found to have chemotactic activities for the leukemia cells used in this study (unpublished data), was added to the outer wells of the Transwell plates. After 1 day of incubation, the numbers of cells in the outer wells of the Transwell plates were counted. In some experiments, 100 U/ml of TNF-α, 0.2 ng/ml of TGF-β, 2 m*M* EDTA, or 1 m*M* 1,10-phenanthroline was added to the upper chambers.

RESULTS

Secretion of type IV collagenase Fig. 1 shows the gelatinolytic (collagenase) activities in the supernatants of the leukemia cell lines. No lysis was detected in the lanes loaded with supernatants of U-937, HL-60, HSM-1, K-562, Daudi, CEM, and MOLT-4. Only HSM-1 cells secreted 92 kDa type IV collagenase. Next we tested the effects of inflammatory cytokines which could affect myelomonocytic cells on the secretion of 92 kDa type IV collagenase. Both TNF-α and TGF-β clearly enhanced, while IFN-α faintly enhanced, the secretion of 92 kDa type IV collagenase in HSM-1 cells, whereas IL-1β and IL-6 had no effect (Fig. 2). TPA enhanced the secretion of 92 kDa type IV collagenase in HSM-1 cells (Fig. 3). U-937 and HL-60 cells secreted the 92 kDa type IV col-

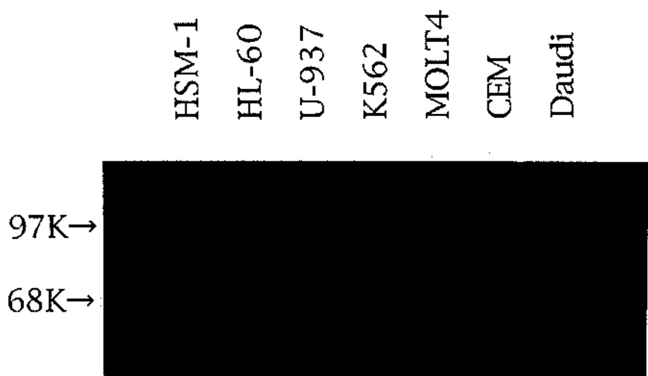

Fig. 1. Type IV collagenase secreted by leukemia cell lines. Supernatants were harvested and analyzed for type IV collagenase according to the methods described in "Materials and Methods." Molecular sizes are indicated by arrows.

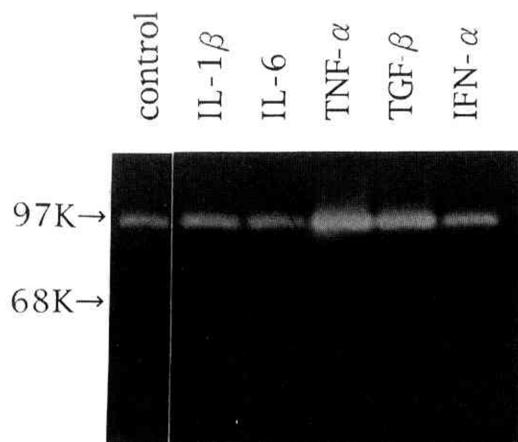

Fig. 2. Secretion of type IV collagenase in HSM-1 cells stimulated by IL-1β, IL-6, TNF-α, TGF-β, and IFN-α. Supernatants of HSM-1 cells stimulated by 10 ng/ml of IL-1β, 10 ng/ml of IL-6, 100 U/ml of TNF-α, 0.2 ng/ml of TGF-β, or 100 U/ml of IFN-α for 24 h were harvested and analyzed for type IV collagenase. Molecular sizes are indicated by arrows.

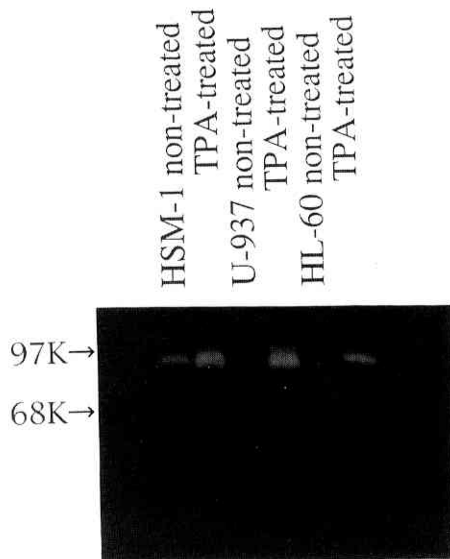

Fig. 3. Enhanced secretion of type IV collagenase by TPA. Supernatants of HSM-1, HL-60, and U-937 cells incubated with or without 10^{-7} M TPA for 24 h were harvested and analyzed for type IV collagenase. Molecular sizes are indicated by arrows.

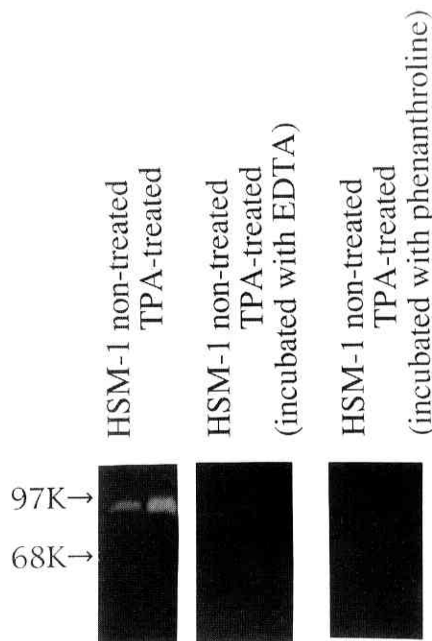

Fig. 4. Effects of EDTA and 1,10-phenanthroline on type IV collagenase activity. Supernatants were harvested and electrophoresed on SDS-PAGE. After electrophoresis, gels were incubated in incubation buffer with or without 10 mM EDTA or 1 mM 1,10-phenanthroline and then stained with Coomassie Blue. Molecular sizes are indicated by arrows.

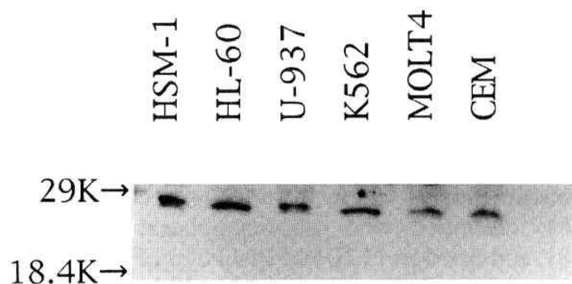

Fig. 5. Immunoblotting of TIMP-1. Supernatants were harvested and electrophoresed on SDS-PAGE. After blotting, the membrane was stained with anti-TIMP antibody and an ECL staining kit according to the methods described in "Materials and Methods."

lagenase only after treatment with TPA (Fig. 3). As shown in Fig. 4, addition of 2 mM EDTA and 1 mM 1,10-phenanthroline to the incubation buffer completely abolished the collagenase activities in the supernatants of non-treated HSM-1 and TPA-treated HSM-1.

Secretion of TIMP-1 We next examined the secretion of TIMP-1 in the supernatant of the cells. As shown in Fig. 5, all cells tested in this assay secreted similar levels of TIMP-1, a 28.5-kDa protein. The treatment of HSM-1 with TPA, IL-1β, IL-6, TNF-α, or TGF-β had no effect on the secretion of TIMP-1 from HSM-1 cells (data not shown).

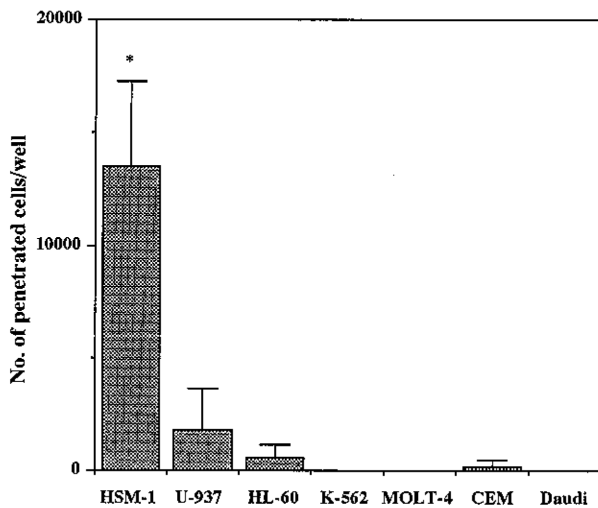

Fig. 6. Invasive activities of leukemia cell lines. Matrigel (100 μl at 5 μg/ml) was placed in the upper chambers of Transwell plates and dried. Cells were placed in the upper chambers at 2×10^5/chamber and skin fibroblast conditioned medium was placed in the lower chambers as a chemoattractant. After one day of incubation, the numbers of penetrated cells in the lower chambers were counted. * $P<0.01$ as compared with the other cells (Student's t test).

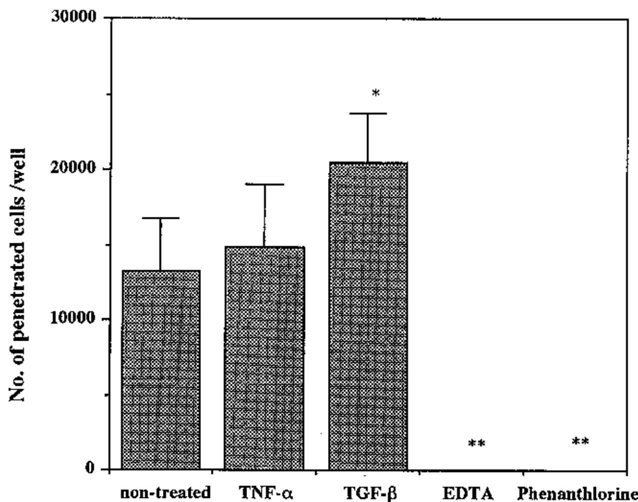

Fig. 7. Effects on TNF-α, TGF-β and inhibitors of type IV collagenase. Matrigel (100 μl, 20-fold-diluted) was placed in the upper chambers of Transwell plates and dried. Cells were placed in the upper chambers at 2×10^5/chamber in the presence or absence of 100 U/ml of TNF-α, 0.2 ng/ml of TGF-β, 1 mM 1,10-phenanthroline, or 2 mM EDTA and skin fibroblast conditioned medium was placed in the lower chambers as a chemoattractant. After one day of incubation, numbers of penetrated cells in the lower chambers were counted. * $P<0.05$, ** $P<0.01$ as compared with non-treated chambers (Student's t test).

***In vitro* invasion assay** Fig. 6 shows the results of *in vitro* invasion assays using Matrigel-coated Transwell plates. The numbers of HSM-1 cells that invaded through Matrigel-coated filters were significantly higher than those of U-937 cells, HL-60, K-562, MOLT-4, CEM, and Daudi cells ($P<0.01$ as compared with the other cells by Student's t test). Invasive activities of HSM-1 cells were significantly enhanced by the treatment with TGF-β. TNF-α did not enhance the invasive activities of HSM-1 cells. In contrast, the treatment of HSM-1 cells with EDTA or 1,10-phenanthroline completely suppressed the invasive activities (Fig. 7).

DISCUSSION

Recently, myelomonocytic cells have been reported to secrete 92 kDa type IV collagenase and to traverse the basement membrane as tumor cells do.[16–18] However, the secretion of 92 kDa type IV collagenase and the traverse of the basement membrane were observed only with mature monocytes and macrophages.[19] Indeed, TPA-treated U-937 and HL-60 cells, but not untreated cells, have been shown to secrete type IV collagenase and to degrade type IV collagen.[20, 21] In this study, we have revealed that among the tested leukemia cell lines only a granulocytic sarcoma cell line, HSM-1, secreted type IV collagenase without any stimulation, and that the HSM-1 cells had higher invasive activities through the Matrigel-coated filters than the other leukemia cell lines. Ries *et al.* recently reported that unstimulated as well as TPA-treated HL-60 cells secreted 92 kDa type IV collagenase.[22] However, they prepared the conditioned medium using 10-times-higher numbers of HL-60 cells than ours. As HL-60 cells showed a low invasive activity for Matrigel in our experiments, small amounts of 92 kDa type IV collagenase undetectable in our assays are presumably secreted by HL-60 cells. The secretion of 92 kDa type IV collagenase was enhanced by inflammatory cytokines such as TGF-β and TNF-α. Treatment of HSM-1 cells with TGF-β enhanced the secretion of 92 kDa type VI collagenase with increase of the invasive activities. Furthermore, we have demonstrated that the inhibition of collagenolytic activities in the supernatant of HSM-1 by treatment with EDTA or 1,10-phenanthroline paralleled the decrease of invasive activities in HSM-1 cells treated with EDTA or 1,10-phenanthroline. Although treatment with TNF-α enhanced the secretion of type IV collagenase in HSM-1 cells, the invasive activities of HSM-1 cells were not significantly influenced. This inconsistency may be explained by presuming that other factors necessary for the invasion of Matrigel, such as motility, were influenced by TNF-α treatment. Our results suggest that the secretion of type IV collagenase, found only in a granulocytic sarcoma cell line, HSM-1,

plays an important role in extramedullary tumor formation in leukemia.

Natural proteinase inhibitors, such as TIMPs, have been reported to function as metastasis-suppressing proteins that inhibit latent and active metalloproteinases.[23, 24] TIMP-1, the first member of the TIMP family, is a glycoprotein with an apparent molecular mass of 28.5 kDa that forms a complex of 1:1 stoichiometry with the 92 kDa type IV collagenase.[12, 25] We have demonstrated that all the tested cells secreted similar levels of TIMP-1, although the cells other than the HSM-1 cells did not secrete significant levels of 92 kDa type IV collagenase. Since the net collagenase activities are thought to depend upon the balance between the levels of 92 kDa type IV collagenase and TIMP-1,[24, 26, 27] it is likely that the HSM-1 cells stimulated by TGF-β secrete much higher levels of 92 kDa type IV collagenase than of TIMP-1, and thus acquire the ability to degrade type IV collagen. It has already been reported that type IV collagenase production is enhanced by TNF-α and TGF-β in several tumor cell lines.[28, 29] Although it is unclear why TIMP-1 is secreted in the supernatants of all the tested cell lines, we speculate that the secreted TIMP-1 may act as an autocrine growth factor for all the tested cell lines. Hayakawa *et al.* have reported that TIMP-1 stimulated the growth of leukemia cell lines as well as normal erythroid progenitor cells, such as K-562 and HL-60.[30, 31]

Several metalloproteinases such as 92 kDa type IV collagenase, 72 kDa type IV collagenase, membrane-type matrix metalloproteinase and stromelysin have been reported to be involved in the invasion of tumor cells.[11, 12] Both 92 kDa type IV collagenase and 72 kDa type IV collagenase specifically degrade type IV collagen, whereas stromelysin degrades a variety of matrix components including proteoglycans and glycoproteins, and type IV collagen.[12] Which enzyme plays a major role in extravasation of tumor cells is dependent on the type of the cells.[12] In myelomonocytic lineage cells, 92 kDa type IV collagenase has been reported to play a major role.[19–21] These reports are confirmed by our results that only 92 kDa type IV collagenase was found in the supernatants of both unstimulated and stimulated HSM-1 cells, and in those of stimulated U-937 and HL-60 cells. These findings and our previous results[16] indicate that the HSM-1 cells established from a granulocytic sarcoma have capacities to degrade basement membranes and to lodge in extramedullary sites, which are essential for extramedullary tumor formation. Further studies on the capacities of the HSM-1 cells to attach to and to traverse endothelial cells are needed to clarify the entire mechanisms of extramedullary tumor formation of leukemia cells. A study of the production of type IV collagenases in leukemia cells of patients with granulocytic sarcoma is planned.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture and a Research Grant from the Akiyama Foundation. We thank Ms. M. Yanome for her help in preparing the manuscript.

(Received August 26, 1994/Accepted December 5, 1994)

REFERENCES

1) Muss, H. B. and Maloney, W. B. Chloroma and other myeloblastic tumors. *Blood,* **42,** 721–728 (1973).
2) Neiman, R. S., Barcoa, M., Berald, C., Bonner, H., Mann, R., Rydell, R. E. and Benett, J. M. Granulocytic sarcoma: a clinicopathologic study of 61 biopsied cases. *Cancer,* **48,** 1426–1437 (1981).
3) Wintrobe, M. M. "Clinical Hematology," 7th Ed., p. 59 (1974). Lea and Febiger, Philadelphia.
4) Wiernik, P. H. and Serpick, A. A. Granulocytic sarcoma (chloroma). *Blood,* **35,** 361–369 (1970).
5) Liu, P. I., Ishimaru, T., McGregor, D. H., Okada, H. and Steer, A. Autopsy study of granulocytic sarcoma (chloroma) in patients with myelogenous leukemia: Hiroshima-Nagasaki. 1945–1965. *Cancer,* **31,** 948–955 (1973).
6) Kobayashi, M., Imamura, M., Soga, R., Tsuda, Y., Maeda, S., Iwasaki, H., Sugiura, M., Ohizumi, H., Musashi, M., Morioka, M., Okabe, M., Sakurada, K. and Miyazaki, T. Establishment of a novel granulocytic sarcoma cell line which can adhere to dermal fibroblasts from a patient with granulocytic sarcoma in dermal tissues and myelofibrosis. *Br. J. Haematol.,* **82,** 26–31 (1992).
7) Poste, G. and Fidler, I. J. The pathogenesis of cancer metastasis. *Nature,* **283,** 139–146 (1980).
8) Liotta, L. A. Tumor invasion and metastasis — role of the extracellular matrix: Rhoads Memorial Award Lecture. *Cancer Res.,* **46,** 1–7 (1986).
9) Liotta, L. A. and Stetler-Stevenson, W. G. Metalloproteinases and cancer invasion. *Semin. Cancer Biol.,* **1,** 99–106 (1990).
10) Nicolson, G. L. Cancer metastasis: tumor cell and host organ properties important in metastasis to specific secondary sites. *Biochim. Biophys. Acta,* **948,** 175–208 (1988).
11) Nakajima, M. and Chop, A. M. Tumor invasion and extracellular matrix degradative enzymes: regulation of activity of organ factors. *Semin. Cancer Biol.,* **2,** 115–127 (1991).
12) Liotta, L. A., Steeg, P. S. and Stetler-Stevenson, W. G. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell,* **64,** 327–336 (1991).

13) Nakajima, M., Morikawa, K., Fabra, A., Bucana, C. D. and Fidler, I. J. Influence of organ environment on extracellular matrix degradative activity and metastasis of human colon carcinoma cells. *J. Natl. Cacner Inst.*, **82**, 1890–1898 (1990).
14) Albini, A., Iwamoto, Y., Kleinman, H. K., Martin, G. R., Aaronson, S. A., Kozlowski, J. M. and McEwan, R. N. A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. *Cancer Res.*, **47**, 3239–3245 (1987).
15) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).
16) Murphy, G., Ward, R., Hembry, R. M., Reynolds, J. J., Kuhn, K. and Tryggvason, K. Characterization of gelatinase from pig polymorphonuclear leukocytes. *Biochem. J.*, **258**, 463–472 (1989).
17) Campbell, E. J., Cury, J. D., Shapiro, S. D., Goldberg, G. I. and Welgus, H. G. Neutral proteinases of human mononuclear phagocytes: cellular differentiation markedly alters cell phenotype for serine proteinases, metalloproteinases, and tissue inhibitor of metalloproteinases. *J. Immunol.*, **146**, 1286–1293 (1991).
18) Wilhelm, S. M., Collier, I. E., Marmer, B. L., Eisen, A. Z., Grant, G. A. and Goldberg, G. I. SV40-transformed human lung fibroblasts secreted a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophages. *J. Biol. Chem.*, **264**, 17213–17221 (1989).
19) Pluznik, D. H., Fridman, R. and Reich, R. Correlation in the expression of type IV collagenase and the invasive and chemotactic abilities of monocytic cells during differentiation into macrophages. *Exp. Hematol.*, **20**, 57–63 (1992).
20) Moll, U. M., Youngleib, G. L., Rosinski, K. B. and Quigley, J. P. Tumor promoter-stimulated Mr 92,000 gelatinase secreted by normal and malignant human cells: isolation and characterization of the enzyme from HT1080 tumor cells. *Cancer Res.*, **50**, 6162–6170 (1990).
21) Watanabe, H., Nakanishi, I., Yamashita, K., Hayakawa, T. and Okada, Y. Matrix metalloproteinase-9 (92 kDa gelatinase/type IV collagenase) from U-937 monoblastoid cells: correlation with cellular invasion. *J. Cell Sci.*, **104**, 991–999 (1993).
22) Ries, C., Kolb, H. and Petrides, P. E. Regulation of 92-kD gelatinase release in HL-60 leukemia cells: tumor necrosis factor-α as an autocrine stimulus for basal- and phorbol ester-induced secretion. *Blood*, **83**, 3638–3646 (1994).
23) Alvarez, O. A., Carmichael, D. F. and DeClerck, Y. A. Inhibition of collagenolytic activity and metastasis of tumor cells by a recombinant human tissue inhibitor of metalloproteinases. *J. Natl. Cancer Inst.*, **82**, 589–595 (1990).
24) Testa, J. E. and Quigley, J. P. Reversal of misfortune: TIMP-2 inhibits tumor cell invasion. *J. Natl. Cancer Inst.*, **83**, 740–742 (1991).
25) Carmichael, D. F., Sommer, A., Thompson, R., Anderson, D. C., Smith, C. G., Welgus, H. G. and Stricklin, G. P. Primary structure and cDNA cloning of human fibroblast collagenase inhibitor. *Proc. Natl. Acad. Sci. USA*, **83**, 2407–2411 (1986).
26) Khokha, R., Waterhouse, P., Yagel, S., Lala, P. K., Overall, C. M., Norton, G. and Denhardt, D. T. Antisense RNA-induced reduction in murine TIMP levels confers oncogenicity on Swiss 3T3 cells. *Science*, **243**, 947–950 (1989).
27) Schultz, R. M., Silberman, S., Persky, B., Bajkowski, S. and Carmichael, D. F. Inhibition by human recombinant tissue inhibitor of metalloproteinases of human amnion invasion and lung colonization by murine B16-F10 melanoma cells. *Cancer Res.*, **48**, 5539–5545 (1988).
28) Okada, Y., Tsuchiya, H., Shimizu, H., Tomita, K., Nakanishi, I., Sato, H., Seiki, M., Yamashita, M. and Hayakawa, T. Induction and stimulation of 92-kDa gelatinase/type IV collagenase production in osteosarcoma and fibrosarcoma cell lines by tumor necrosis factor α. *Biochem. Biophys. Res. Commun.*, **171**, 610–617 (1990).
29) Welch, D. R., Fabra, A. and Nakajima, M. Transforming growth factor β stimulates mammary adenocarcinoma cell invasion and metastatic potential. *Proc. Natl. Acad. Sci. USA*, **87**, 7678–7682 (1990).
30) Hayakawa, T., Yamashita, K., Kishi, J. and Harigaya, K. Tissue inhibitor of metalloproteinases from human bone marrow stromal cell line KM 102 has erythroid-potentiating activity, suggesting its possibly bifunctional role in the hematopoietic microenvironment. *FEBS Lett.*, **268**, 125–128 (1990).
31) Hayakawa, T., Yamashita, K., Tanzawa, K., Uchijima, E. and Iwata, K. Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. *FEBS Lett.*, **298**, 29–32 (1992).