Jpn. J. Cancer Res. **90**, 116–121, January 1999

Preventive Effect of Matrix Metalloproteinase Inhibitor, R-94138, in Combination with Mitomycin C or Cisplatin on Peritoneal Dissemination of Human Gastric Cancer Cell Line TMK-1 in Nude Mice

Naoki Igarashi,[1] Tetsuro Kubota,[1,5] Yoshihide Otani,[1] Shinjiro Wilson Matsuzaki,[1] Masahiko Watanabe,[1] Tatsuo Teramoto,[1] Koichiro Kumai,[1] Kazuhiko Tamaki,[3] Kazuhiko Tanzawa,[3] Tomowo Kobayashi[4] and Masaki Kitajima[1]

[1]Department of Surgery, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, [2]Medical Chemistry Research Laboratories, [3]Biological Research Laboratories and [4]R&D, Planning and Management Department, Sankyo Co., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710

R-94138, a matrix metalloproteinase inhibitor, was examined for the ability to prevent peritoneal dissemination of a human gastric cancer xenograft, TMK-1. When the supernatant of a co-culture of TMK-1 cells and human normal fibroblast cells was subjected to gelatin zymography, it was clear that the protein expression of MMP-2 had been inhibited by R-94138. When TMK-1 was injected intraperitoneally (i.p.) into nude mice at 5×10^5 cells/body, the resulting peritoneal dissemination mimicked clinical carcinomatous peritonitis. When the maximum tolerated dose of mitomycin C (MMC) or cisplatin (DDP) was given 12 h after the tumor inoculation, peritoneal dissemination was completely inhibited, while the effect of R-94138 was limited when it was given i.p. at a dose of 20 mg/kg in a schedule of q.d. ×5 starting 12 h after tumor injection. MMC and DDP also suppressed peritoneal dissemination when they were administered 1 week after the tumor inoculation at a single dose of 2 and 3 mg/kg i.p., respectively. R-94138 inhibited peritoneal dissemination when it was administered i.p. at a dose of 30 mg/kg in a schedule of q.d. ×5 starting from 1 week after tumor injection. The combination of MMC and R-94138 increased the preventive effect on peritoneal dissemination. R-94138 seems to be a promising candidate to prevent peritoneal dissemination of gastric cancer.

Key words: MMP inhibitor — R-94138 — Gastric cancer — Nude mouse — Peritoneal dissemination

In spite of the declining tendency of gastric cancer,[1)] this cancer remains one of the most important causes of death among Japanese people.[2)] In particular, peritoneal dissemination at the final stage of gastric cancer remains untreatable. Although some trials were reported on the control of peritoneal dissemination of gastric cancer, including chemotherapy and hyperthermia,[3,4)] no significant prolongation of survival was found. Peritoneal dissemination involves several steps, including tumor cell attachment, invasion and growth in the peritoneum.[5)] We have been investigating the role of matrix metalloproteinase (MMP) in the invasion and metastasis of gastric cancer, and found a close relation between MMP expression and malignant potential of gastric cancer.[6–8)] If the initial invasion can be controlled by an appropriate inhibitor of MMP, peritoneal dissemination may be prevented. In the present paper, we describe the preventive effect of a newly developed MMP inhibitor, R-94138, in a peritoneal dissemination model using the human gastric cancer cell TMK-1 in nude mice. The effectiveness of R-94138 alone and in combination with the conventional antitumor agents mitomycin C (MMC) or cisplatin (DDP) was assessed.

[5] To whom correspondence should be addressed.
E-mail: tkubota@med.keio.ac.jp

MATERIALS AND METHODS

Tumor A human stomach carcinoma cell line (TMK-1) was used for this investigation. TMK-1 was established as a serially transplantable human tumor xenograft in nude mice by Tokuda *et al*.[9)] from cancerous tissue of a 21-year-old male patient with gastric cancer, and was established as a cultured cell line by Tasaka *et al*.[10)] This cell line was kindly supplied by Dr. S. Hirohashi, National Cancer Center Research Institute.

Agents R-94138 (Fig. 1), a synthetic derivative of matlystatins isolated from *Actinomadura atramentaria*,[11,12)] was synthesized as described previously.[13)] The *in vitro* IC_{50} values against the following matrix metalloproteinases are: 28 n*M* for stromelysin (MMP-3), 38 n*M* for gelatinase A (MMP-2) and 1.2 n*M* for gelatinase B (MMP-9).[11)] DDP and MMC were purchased from Nippon Kayaku Co., Ltd., Tokyo and Kyowa Hakko Kogyo Co., Ltd., Tokyo, respectively.

Gelatin zymography TMK-1 cells and human fibroblast cells obtained from normal gastric wall of a patient with

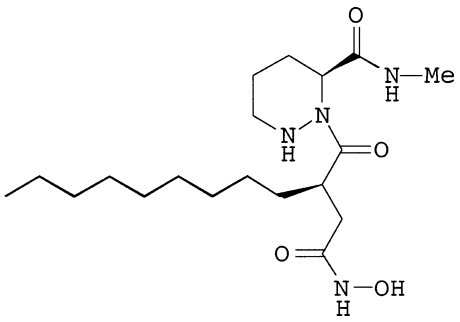

Fig. 1. Molecular structure of R-94138.

gastric cancer were co-cultured (1×10^4 cells each) for 72 h in RPMI-1640 supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and 250 μg/ml fungizone per ml with or without 10 μg of R-94138. The culture was centrifuged twice at 1,500 rpm for 5 min, and the resulting supernatant was subjected to gelatin zymography. As positive control, culture media were obtained from two cultured cell lines of synovial cells obtained from patients with rheumatoid arthritis. These culture media were kindly supplied by Dr. Okada, Kanazawa University. The protein concentration of these samples was determined (Bio-Rad protein assay, Bio-Rad, Hercules, CA), then 10 μg aliquots were diluted in sample buffer and size-fractionated in duplicate on 8.3% polyacrylamide gels containing 1% gelatin (Sigma, St. Louis, MO). The duplicate gels were washed with 2.5% Triton X-100 for 1 h and incubated overnight in 50 m*M* Tris-HCl (pH 7.6) and 10 m*M* $CaCl_2$ at 37°C in the presence or absence of 100 m*M* 1,10-phenanthroline. Gels were subsequently stained with Coomassie brilliant blue at 65°C for 30 min and destained with 10% acetic acid until gelatinolytic bands were clearly visible.

Using the peritoneal dissemination model in nude mouse described below, the disseminated tumor nodule was aseptically removed and homogenized for the detection of MMP-2. One hundred milligrams of the homogenize was diluted with 5 ml of phosphate-buffered saline and gelatin zymography was conducted by the same method mentioned above.

Nude mice BALB/c *nu*/*nu* male nude mice were purchased from CLEA Japan Inc., Tokyo. They were maintained under specific pathogen-free conditions using an Isorack at our experimental animal center and given sterile food and water *ad libitum*. Four- to six-week-old mice weighing 20–22 g were used for the experiment.

Tumor inoculation, counting of tumor nodules and evaluation of drug activity In the preliminary experiment, cultured TMK-1 cells (5×10^5 cells/body) were injected intraperitoneally (i.p.) into 5 nude mice. One mouse was killed every week from 2 to 6 weeks after the tumor injection, and peritoneal dissemination was examined macroscopically.

In the first experiment, using 4 groups of 5 mice, the treatment was initiated 12 h after the i.p. injection of TMK-1 (5×10^5 cells/body). The maximum tolerated dose of MMC (6 mg/kg) or DDP (9 mg/kg) was administered once i.p., or R-94138 was given i.p. in a schedule of q.d. for 5 days at a dose of 20 mg/kg. The control group received no treatment. One mouse from each group was killed every week from 2 to 6 weeks after tumor injection, and peritoneal dissemination was examined macroscopically.

In the second experiment, using 6 groups of 5 mice, the treatment was initiated 1 week after TMK-1 injection (5×10^5/body). DDP was administered i.p. once at a dose of 3 mg/kg, and MMC was given i.p. once at a dose of 2 mg/kg. R-94138 was administered i.p. q.d. for 5 consecutive days at a dose of 30 mg/kg. Combined therapy was also conducted using the combination of DDP and R-94138 or MMC and R-94138 using the same schedules and doses as used in the single agent therapy. All mice were killed at 5 weeks after the tumor injection, and peritoneal dissemination was examined macroscopically.

Statistical analysis The statistical analysis was performed with Student's *t* test, paired *t* test or the χ^2 test, and $P<0.05$ was taken as the criterion of statistical significance.

RESULTS

A gelatin zymogram illustrating the inhibition of MMP-2 by R-94138 is shown in Fig. 2. The positive controls showed strong activity of 62 kDa and 94 kDa proteins, representing the active forms of MMP-2 and MMP-9, respectively. The activity of MMP-2 was also observed in the culture medium obtained from TMK-1 and human fibroblast cell, though no MMP-9 was expressed in that medium [R-94138(–)]. MMP-2 was completely inhibited by the addition of R-94138 at a concentration of 10 μg/ml [R-94138(+)]. On the other hand, this MMP-2 activity was not detected in the culture medium obtained from TMK-1 cells or human fibroblast cells alone. This result is consistent with our previous finding[14] that MMP-1 activity was observed only in the co-culture with human fibroblasts and in the supernatant of MKN-74 culture.

Fig. 3 shows the peritoneal dissemination of TMK-1 5 weeks after tumor inoculation in untreated nude mouse. Multiple peritoneal dissemination of the tumor was obvious in the visceral peritoneum of nude mice, while no metastasis was observed in the parietal peritoneum. No ascites, lymph nodes metastasis or liver metastasis was observed in untreated mice throughout the experiments. The nodule size was usually 1–3 mm, and in cases where the nodules piled up to form a large tumor, we counted each small nodule.

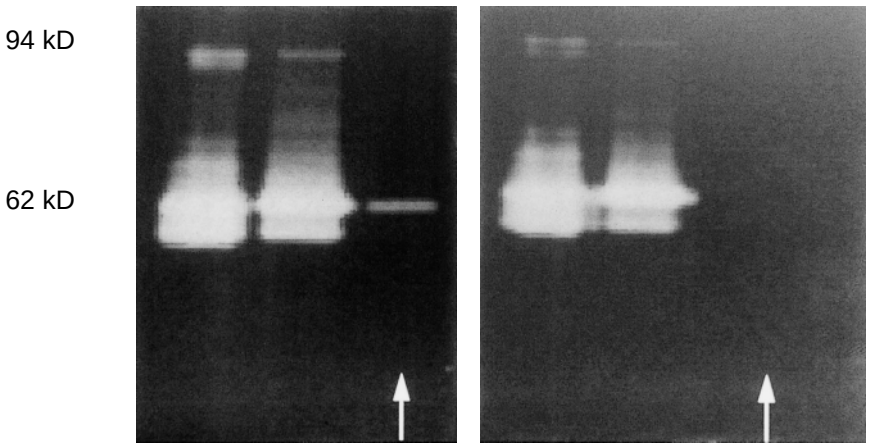

Fig. 2. Gelatin zymogram of MMP-2. Two positive controls showed strong activity of 62 kDa and 94 kDa proteins, the active forms of MMP-2 and MMP-9. MMP-2 was observed in the culture medium obtained from TMK-1 and human fibroblasts in the absence of R-94138, but was completely inhibited by the addition of R-94138 at a concentration of 10 μg/ml.

Fig. 3. Peritoneal dissemination of TMK-1 5 weeks after tumor injection in untreated nude mouse. Multiple peritoneal dissemination of the tumor was obvious in the visceral peritoneum of nude mice, while no metastasis was observed in the parietal peritoneum. The nodule size was usually 1–3 mm, and the nodules sometimes piled up.

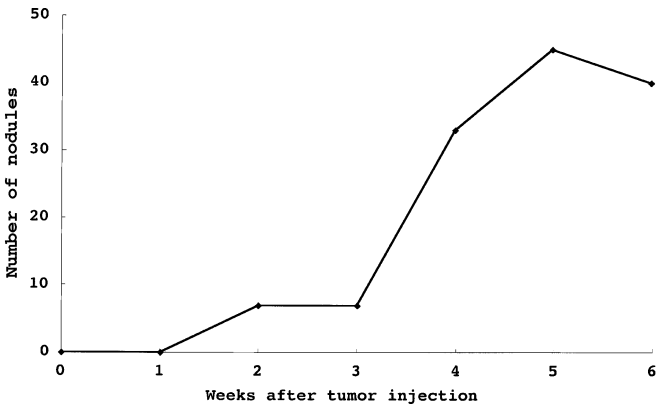

Fig. 4. The time course of increase of tumor nodules after tumor inoculation. In the preliminary experiment, after i.p. injection of TMK-1 (5×10^5 cells/body) into nude mice, the mice were killed every week starting from 2 to 6 weeks after the tumor injection, and peritoneal dissemination was counted macroscopically. At 2 weeks after injection, 7 nodules were already established in the untreated mouse, and the number of nodules increased gradually until 5 weeks after tumor inoculation, then seemed to reach a plateau.

The time course of the number of tumor nodules in the preliminary experiment is shown in Fig. 4. At 2 weeks after tumor inoculation, 7 nodules were established in the untreated mouse, and the number of nodules increased gradually until 5 weeks after tumor injection when it seemed to reach a plateau. The results of the first experiment are summarized in Table I. When the tumor-bearing mice were treated with DDP at a dose of 9 mg/kg, no peritoneal dissemination was observed 2 and 3 weeks after tumor inoculation, while only one or two nodules

were found at 4, 5 and 6 weeks. No tumor dissemination was observed 2, 3 and 5 weeks after tumor inoculation in the mice treated with MMC at a dose of 6 mg/kg, while only one nodule was observed at 4 and 6 weeks in this group. No peritoneal dissemination was observed at 2 weeks after tumor injection in the group given R-94138 at a dose of 20 mg/kg q.d. for 5 days, although the number of nodules increased gradually from 3 to 6 weeks, reaching 21 nodules at 6 weeks (about half the value in the control group). There were statistically significant differences between the control and the three treated groups (paired *t* test).

In the second experiment, 28.2±10.3 nodules were observed in the control group at the end of experiment (Table II). This peritoneal dissemination was significantly suppressed in the mice treated with MMC and DDP (6.4±4.5 and 3.2±2.6 nodules, respectively). R-94138 also inhibited the peritoneal dissemination of TMK-1 to 9.8±5.7 nodules ($P<0.05$). Although the combination of DDP and R-94138 resulted in an average number of nodules of 7.0±5.1, the combination of R-94138 and MMC suppressed the peritoneal nodules to 1.6±2.3, significantly lower than the numbers in mice treated with MMC or R-94138 alone. In addition, peritoneal dissemination was completely suppressed in 3 of 5 mice treated with this combination. This inhibition rate was significant compared to the control ($P<0.05$ by the χ^2 test).

The activity of MMP-2 was observed in a disseminated nodule of TMK-1 i.p. inoculated into a control nude mouse, as shown in Fig. 5, although it was also observed in the nodules of the mice treated with R-94138 alone in the second experiment.

DISCUSSION

Peritoneal dissemination of gastric carcinoma is considered to be a complex process involving invasion and metastasis. We have previously established an important role of MMP in the progression of gastric cancer.[6] In the present paper, we have evaluated the efficacy of a newly developed matrix metalloproteinase inhibitor, R-94138 to prevent peritoneal dissemination of human gastric cancer cells in nude mice, as compared with other conventionally

Table I. Preventive Effect of the Agents on the Peritoneal Dissemination of Human Gastric Cancer Cells in Nude Mice

Treatment[a]	Weeks after injection				
	2[b]	3	4	5	6
Control	7[c]	7	33	45	40
DDP[d],*	0	0	2	2	1
MMC[e],*	0	0	1	0	1
R-94138[f],*	0	2	15	17	21

a) The treatment was initiated 12 h after i.p. injection of TMK-1 (5×10^5 cells/mouse).
b) One mouse was killed in each treatment group every week from 2 to 6 weeks after tumor injection.
c) The number of nodules of peritoneal dissemination was counted macroscopically.
d) Cisplatin was administered once i.p. at a dose of 9 mg/kg.
e) Mitomycin C was administered once i.p. at a dose of 6 mg/kg.
f) R-94138 was administered daily i.p. at a dose of 20 mg/kg for 5 consecutive days.
* $P<0.05$ relative to control by paired *t* test.

Table II. Preventive Effect of the Agents on the Peritoneal Dissemination of Human Gastric Cancer Cells in Nude Mice

Treatment[a]	Number of nodules[b]					Average[c]
Control	27	12	33	39	40	28.2±10.3
DDP[d]	10	11	4	7	0	6.4±4.5**
MMC[e]	4	7	0	3	2	3.2±2.6***
R-94138[f]	12	14	13	0	10	9.8±5.7*
DDP+R-94138[g]	0	4	8	0	13	7.0±5.1**
MMC+R-94138[h]	0	0	0	3	5	1.6±2.3****,#

a) The treatment was initiated 1 week after i.p. injection of TMK-1 (5×10^5 cells/mouse).
b) Each number indicates the number of nodules in one mouse counted macroscopically 5 weeks after tumor injection.
c) Mean±SD of number of nodules.
d) Cisplatin was administered once i.p. at a dose of 3 mg/kg.
e) Mitomycin C was administered once i.p. at a dose of 2 mg/kg.
f) R-94138 was administered daily i.p. at a dose of 30 mg/kg for 5 consecutive days.
g) *d*)+*f*).
h) *e*)+*f*).
* $P<0.05$, ** $P<0.005$, *** $P<0.001$, **** $P<0.0005$, relative to control by Student's *t* test.
\# $P<0.05$ relative to single therapy with mitomycin C or R-94138.

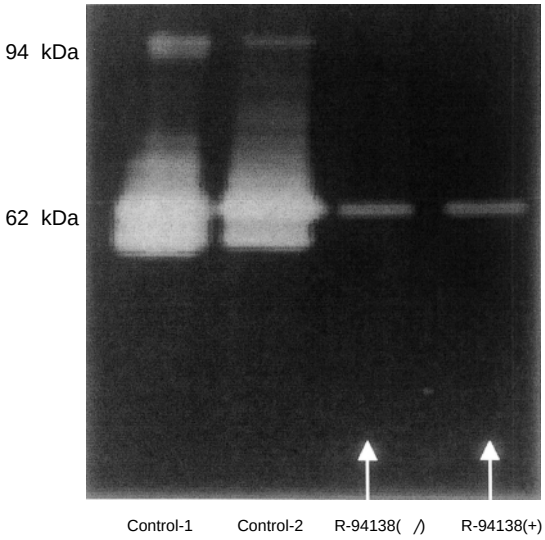

Fig. 5. The activity of MMP-2 observed in a disseminated nodule of TMK-1. The activity of MMP-2 was observed in a disseminated nodule of TMK-1 after i.p. inoculation into a control nude mouse (left), and was also seen in a nodule from a mouse treated with R-94138 alone in the second experiment (right).

available antitumor agents, DDP and MMC. The previous *in vitro* study indicated that R-94138 strongly inhibits MMP-9 with an IC_{50} of 1.2 n*M*, but has much less effect on MMP-3 (IC_{50}=28 n*M*) and MMP-2 (IC_{50}=38 n*M*). Actually, in the present study, the activity of MMP-2 in the culture medium obtained from TMK-1 and human fibroblast cells was completely inhibited by the addition of R-94138 at a concentration of 10 μg/ml. R-94138 inhibited the peritoneal dissemination when 20 mg of this agent per kg was administered daily for 5 days. Since side effects such as body weight loss and death were not observed in this first experiment, the dose was elevated to 30 mg/kg in the second experiment, in which peritoneal dissemination was significantly reduced. In addition, the combination of R-94138 and MMC showed a superior effect to either single agent or to the combination of R-94138 and DDP. These data suggested that the two agents may suppress the dissemination of gastric carcinoma via different mechanisms. Although MMP-2 activity was not detected in the culture medium obtained from TMK-1 cells or human fibroblast cells alone, the activity of MMP-2 was found in the disseminated nodules of TMK-1 cells, as observed in the co-culture medium of TMK-1 and human fibroblasts in the present study. These results suggested that the dissemination of TMK-1 was inhibited through the inhibition of MMP by R-94138. However, no significant histological change was observed in the nodules of treated mice, including those in the MMC alone and R-94138 alone groups (data not shown), suggesting that the established nodules have escaped the inhibition of MMP by R-94138, as shown in Fig. 5.

The previous study indicated that BB-94 (Batimastat, British Biotech Inc., Oxford, UK), a synthetic MMP inhibitor, inhibited the growth of colorectal cancer in an orthotopic nude mouse model and increased the survival of tumor-bearing mice.[15–17] Furthermore, it was also reported that a combination of gelatinase inhibitor CT1746 and cytotoxic agents (DDP and cyclophosphamide) significantly delayed local tumor growth and reduced pulmonary metastasis in an animal model.[18] BB-94 also underwent clinical phase I and II trials, which indicated low toxicity and some antitumor effect on ovarian carcinomas. Recently, marimastat[19] was also examined in clinical phase I/II studies, and afforded survival benefits for patients with gastric,[20] ovarian,[21] prostate,[22] colon[23] and pancreatic cancers.[19]

Since MMP inhibitors reduce the growth and invasion of malignant tumors through inhibition of MMPs secreted from cancer or interstitial cells, we can hardly expect a reduction of tumor mass by these agents, in contrast with the conventional cytotoxic drugs. As a result, the possible positive effects of MMP inhibitors would be a long no change (NC), stabilization of tumor growth or prolongation of survival. It has already been reported that a long NC resulted in prolongation of survival in patients with breast cancer who were treated with CGS16949A.[24] If we could stabilize the tumor growth by arresting the invasion of cancerous cells into the surrounding normal tissue, cancer might become a chronic, incurable but non-fatal disorder. R-94138 is thought to be a promising new agent that exhibits antitumor activity through a new mode of action, i.e., MMP inhibition, and the combined effect with MMC warrants further investigation of combination therapies of MMP inhibitor and cytotoxic agents.

(Received August 10, 1998/Revised October 12, 1998/Accepted October 22, 1998)

REFERENCES

1) Bunt, A. M., Hermans, J., Smit, V. T., van de Velde, C. J., Fleuren, G. J. and Bruijin, J. A. Surgical/pathological stage migration confounds comparison of gastric cancer survival rates between Japan and Western countries. *J. Clin. Oncol.*, **13**, 19–25 (1995).
2) Korenaga, D., Tsujitani, S. and Haraguchi, H. Long-term survival in Japanese patients with far advanced carcinoma of stomach. *World J. Surg.*, **12**, 236–240 (1988).
3) Okuyama, T., Endo, K., Maehara, Y., Baba, H., Oshiro, T., Adachi, Y. and Sugimachi, K. Continuous hyperthermic

peritoneal perfusion with cisplatin in mice with peritoneal dissemination of B16 melanoma. *Semin. Surg. Oncol.*, **10**, 145–149 (1994).
4) Yonemura, Y., Fujimura, T., Nishimura, G., Falla, R., Sawa, T., Katayama, K., Tsugawa, K., Fushida, S., Miyazaki, I., Tanaka, M., Endou, Y. and Sasaki, T. Effects of intraoperative chemohyperthermia in patients with gastric cancer with peritoneal dissemination. *Surgery*, **119**, 437–444 (1996).
5) Liotta, L. A., Steeg, P. S. and Stetler-Stevenson, W. Cancer metastasis and angiogenesis; an imbalance of positive and negative regulation. *Cell*, **64**, 327–336 (1991).
6) Otani, Y., Okazaki, I., Arai, M., Kameyama, K., Wada, N., Maruyama, K., Yoshino, K., Kitajima, M., Hosoda, Y. and Tsuchiya, M. Gene expression of interstitial collagenase (MMP-1) in gastrointestinal tract cancers. *J. Gastroenterol.*, **29**, 391–397 (1994).
7) Otani, Y. The collagenases activities, interstitial collagenase and Type IV collagenase in human stomach cancer; with special reference to local spreading and lymph node metastasis. *Keio J. Med.*, **39**, 159–167 (1990).
8) Sakurai, Y., Otani, Y., Kameyama, K., Hosoda, H., Okazaki, I., Kubota, T., Kumai, K. and Kitajima, M. Expression of interstitial collagenase (matrix metalloproteinase-1) in gastric cancer. *Jpn. J. Cancer Res.*, **88**, 401–406 (1997).
9) Tokuda, Y., Nagura, H., Maruo, K., Uemura, Y., Yoshimura, S., Tamaoki, K., Kondo, Y., Ogoshi, Y. and Mitomi, T. An immunohistochemical study of human gastric carcinoma in nude mice and athymic rats with special reference to secretory component production. *Jpn. J. Cancer Clin.*, **27**, 1605–1612 (1981) (in Japanese with English abstract).
10) Ochiai, A., Yasui, W. and Tahara, E. Growth-promoting effect of gastrin on human gastric carcinoma cell line TMK-1. *Jpn. J. Cancer Res. (Gann)*, **76**, 1064–1071 (1985).
11) Tanzawa, K., Ishii, M., Ogita, T. and Shimada, K. Matlystatins, new inhibitors of type IV collagenases from *Actinomadura atramentaria*. *J. Antibiot.*, **45**, 1733–1737 (1992).
12) Tamaki, K., Kurihara, S., Oikawa, T., Tanzawa, K. and Sugimura, Y. Matlystatins, new inhibitors of type IV collagenases from *Actinomadura atramentaria*. *J. Antibiot.*, **47**, 1481–1492 (1994).
13) Tamaki, K., Tanzawa, K., Kurihara, S., Oikawa, T., Monma, S., Shimada, K. and Sugimura, Y. Synthesis and structure-activity relationships of gelatinase inhibitor derived from matlystatins. *J. Pharm. Bull.*, **43**, 1883–1893 (1995).
14) Sakurai, Y., Otani, Y., Kameyama, K., Igarashi, N., Kubota, T., Kumai, K. and Kitajima, M. The role of stromal cells in the expression of interstitial collagenase (matrix metalloproteinase-1) in the invasion of gastric cancer. *J. Surg. Oncol.*, **66**, 168–172 (1997).
15) Ogita, T., Sato, A., Enokita, R., Suzuki, K., Ishii, M., Negishi, T., Okazaki, T. and Tanzawa, K. Matlystatins, new inhibitor of type IV collagenases from *Actinomadura atramentaria*. *J. Antibiot.*, **45**, 1723–1732 (1982).
16) Watson, S. A., Moris, T. M., Robinson, G., Crimmin, M. J., Brown, P. D. and Hardcastle, J. D. Inhibition of organ invasion by the matrix metalloproteinase inhibitor batimastat (BB-94) in two human colon carcinoma metastasis models. *Cancer Res.*, **55**, 3629–3633 (1995).
17) Brown, P. Matrix metalloproteinase inhibitors: a novel class of anticancer agents. *Adv. Enzyme Regul.*, **35**, 293–301 (1995).
18) Anderson, I., Shipp, M. and Docherty, A. Combination therapy including a gelatinase inhibition and cytotoxic agent reduces local invasion and metastasis of murine Lewis lung carcinoma. *Cancer Res.*, **56**, 715–718 (1996).
19) Bramhall, S. R. The matrix metalloproteinases and their inhibitors in pancreatic cancer. *Int. J. Pancreatol.*, **21**, 1–12 (1997).
20) Drummond, A. H., Becker, P. and Bone, E. A. BB2516: an orally bioavailable matrix metalloproteinase inhibitor with efficacy in animal cancer models. *Proc. Am. Assoc. Cancer Res.*, **36**, 100 (1995).
21) Malfetano, J., Teng, N. and Moore, D. Marimastat, a novel matrix metalloproteinase inhibitor in patients with advanced cancer of ovary: a dose finding study. *Proc. Am. Soc. Clin. Oncol.*, **15**, 283a (1996).
22) Boasberg, P. and Harvaugh, B. Marimastat, a novel matrix metalloproteinase inhibitor in patients with hormone-refractory prostate cancer. *Proc. Am. Soc. Clin. Oncol.*, **15**, 258a (1996).
23) Zaknoen, S., Wolff, R., Cox, J., Marshall, J., Bordelon, T., Drunheller, A. and Rasmussen, H. Marimastat in advanced progressive colorectal cancer—a dose-finding study. *Proc. Am. Soc. Clin. Oncol.*, **15**, 273a (1996).
24) Tominaga, T., Abe, O., Asaishi, K., Abe, R., Enomoto, K., Kajiwara, T., Yoshida, M., Wada, T. and Nomura, Y. Phase II study of CGS16949A, a new aromatase inhibitor—a dose finding study. *Jpn. J. Cancer Chemother.*, **21**, 465–475 (1994) (in Japanese with English abstract).