Organ Heterogeneity of Host-derived Matrix Metalloprotease Expression and Its Involvement in Multiple-Organ Metastasis by Lung Cancer Cell Lines1


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ABSTRACT

Cancer metastasis is tightly regulated by the interaction of tumor cells and host organ microenvironments. Matrix metalloproteinases (MMPs), produced by both tumor cells and host stromal cells, play a central role in tumor invasion and angiogenesis. We determined whether metastatic potential of lung cancer to multiple organs is dependent solely on the expression of MMPs by tumor cells, using two metastasis models of human lung cancer cell lines expressing various levels of MMPs and a MMP inhibitor (ONO-4817). In the lung metastasis model, tumor cells (PC14, PC14PE6, H226, A549) inoculated i.v. into nude or SCID mice metastasized only in the lung. In the multiple-organ metastasis model, tumor cells (RERF-LC-AI, SBC-3/DOX, H69/VP, which express low levels of MMPs) inoculated i.v. into natural killer cell-depleted SCID mice metastasized into the liver, kidneys, and systemic lymph nodes. Film in situ zymography analysis revealed that the nontumor parenchyma of the lung had no gelatinolytic activity, whereas gelatinolytic activity of the liver and kidney was high and low, respectively. In the lung metastasis model, gelatinolytic activity of lung nodules directly correlated with the in vitro expression of MMP-2 and MMP-9 by tumor cells. Inhibition of MMP activity by ONO-4817 suppressed lung metastasis by the cell lines that expressed MMPs, but not those that did not express MMP, via the inhibition of MMP activity of lung tumors. In the multiple-organ metastasis model, liver parenchyma, but not liver nodules, showed gelatinolytic activity. The MMP inhibition reduced metastasis to the liver, but not to the kidney or lymph nodes, via inhibition of MMP activity of liver parenchyma. These findings suggest that MMP expression varies among the host organ microenvironments and that stromal MMPs may promote metastasis of lung cancer from metastatic lesions. Therefore, antimitostatic effects based on MMP inhibition may be dependent on MMPs derived not only from tumor cells but also from organ-specific microenvironments.

INTRODUCTION

Lung cancer is the major cause of malignancy-related deaths worldwide, and its incidence is rising in many countries. The high mortality of this disease is attributable to difficulties in early diagnosis. In many cases, local invasion and metastasis to distant organs have already occurred by the time of the diagnosis.

MMPs3 are a family of structurally related zinc-ion-dependent endopeptidases that include the collagenses, stromelysins, gelatinases, and MT-MMPs (1). The first three types are secreted as proenzymes, are activated by cleavage of the NH2 terminus, and have highly conserved regions, particularly at the catalytic site, which encloses a zinc ion. MMP activity is tightly controlled in several ways: at the transcriptional level, by regulated activation of latent proenzymes, and by the presence of natural inhibitors (such as α2-macroglobulin) and a specific family of tissue inhibitors of metalloproteinase (2). MMPs play crucial roles in tumor invasion and angiogenesis by mediating degradation of extracellular matrix (3). In many types of neoplasm, including lung cancer, higher levels of activated MMPs have been demonstrated in more invasive and/or metastatic tumors and may give prognostic information independent of stage (4–8). Gelatinases (MMP-2 and MMP-9) are the major proteases in lung cancer, and they closely correlate with invasive and metastatic potentials (9, 10).

As reviewed by Zucker et al. (11), because host stroma cells, as well as tumor cells, produce a variety of MMPs and promote tumor progression, it is possible that host cell-derived MMPs promote tumor progression. In this study, we used two metastasis models (12, 13) of human lung cancer cell lines that express various levels of MMPs and a selective MMP inhibitor, ONO-4817 (14), to determine whether metastasis of lung cancer to multiple organs is dependent solely on the expression of MMPs by tumor cells.

MATERIALS AND METHODS

Human Lung Cancer Cell Lines and Tissue Culture. PC14 (13) and H69/VP (12) cells were provided by Dr. N. Sajiio (National Cancer Center Hospital, Tokyo, Japan). PC14PE6, a highly metastatic variant of PC14, and H226 cells (13) were kind gifts from Dr. I. J. Fidler (M. D. Anderson Cancer Center, Houston, TX). A549 cells were from American Type Culture Collection. RERF-LC-AI cells were provided by Dr. M. Akiyama (Radiation Effects Research Foundation, Hiroshima, Japan; Ref. 12). SBC-3/DOX cells were established in our department as a doxorubicin-resistant variant of SBC-3 cells (15). Cell cultures were maintained in MEM supplemented with 10% heat-inactivated FBS and gentamicin at 37°C in a humidified atmosphere of 5% CO2 in air.

Chemicals. ONO-4817 [2S,4S]-N-hydroxy-5-ethoxymethoxy-2-methyl-4-(4-phenoxybenzoyl)aminopentanamide] was synthesized by Ono Pharmaceutical Co. (Osaka, Japan) as reported previously (14). ONO-4817 is a novel synthetic hydroxamic acid-based nonpeptide compound designed to be administered p.o. It binds reversibly to the zinc-binding region of MMPs such as MMP-2, -8, -9, -12, and -13, but not MMP-1, -3, or -7, and has a selective inhibitory spectrum. ONO-4817 suppressed the release of proteoglycan from the cartilage of the knee joints in an arthritis model of guinea pigs. Pharmacokinetic studies showed that plasma concentrations of ONO-4817 were >10 μmol/l at 1 h and 1 μmol/l at 4 h after p.o. administration at a dose of 100 mg/kg.

Measurement of MMP Activity. The activities of MMP-1, -2, and -9 were measured by commercially available assay kits (Biotrack; Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom), as reported previously (16). To evaluate the effect of ONO-4817 on the inhibition of MMP activity, samples (culture supernatants of PC14PE6 cells in serum-free conditions) were incubated with various concentrations of ONO-4817 for 1 h at 37°C. The resultant solutions were measured for MMP-1, -2, and -9 activity by the respective ELISAs. The detection limits of the ELISAs for MMP-1, -2, and -9 were 0.3, 0.75, and 0.25 ng/ml, respectively.

MT1-, MT2-, and MT3-MMP mRNA Expression. mRNA expression of MT1-, MT2-, and MT3-MMP was determined by RT-PCR. The total cellular RNA was extracted from the culture of seven lung cancer cell lines by use of ISOGEN (Nippon Gene Co., Toyama, Japan) according to the protocol recommended by the manufacturer. One μg of total RNA was reverse transcribed.
The primers for MT1-, MT2-, and MT3-MMP and β-actin were as follows: MT1-MMP sense (5'-CCG TAC GCC ATC CAG GGT CTC AAA-3') and antisense (5'-CCG TCA TCG GGC AGC ACA AAA-3'); MT2-MMP sense (5'-ACA ACC ACC ATC TGA CCT TTA GCA-3') and antisense (5'-AGC TTG AAG TTA TCA ACG TCC TCC-3'); MT3-MMP sense (5'-TTA CTT GTG GGC GGG CTT GCC TCT CCT AT-3') and antisense (5'-AGA CTA TAT GTT GCG CCG TGT TCC TCC-3'); β-actin sense (5'-AGC CAT TTG GTC GTA TTG GG-3') and antisense (5'-TAG ATT GGG GGA TCT CGC-3'); MT2-MMP antisense (5'-CGC TAC GCC ATC CAG GGT CTC AAA-3').

Amplification was performed as reported previously (17) with some modifications. The reaction mixture containing the cDNA was derived from 1 μg of total RNA, 200 pmol of the sense and antisense primers, 1 unit of Taq DNA polymerase, and 200 pmol of each deoxynucleotide triphosphate, in a final volume of 50 μl. The Taq DNA polymerase, reaction buffer, and deoxynucleotide triphosphates were from the commercial kit (TaKaRa Taq; Takara Shuzo, Kyoto, Japan). Amplification was performed in sequential cycles and included 30 s of denaturation, 1 min of primer annealing at 62°C (MMP-16 at 58°C), and 1 min and 50 s of extension at 72°C. Amplification was carried out for 28 cycles for MT1-, MT2-, and MT3-MMP. Amplification of β-actin involved 30 sequential cycles of 1 min of denaturation, 1 min of primer annealing at 58°C, and 2 min of extension at 72°C, with the last cycle including incubation for 4 min at 72°C.

Animals. Animal studies were performed in accordance with guidelines established by the Tokushima University Committee on Animal Care and Use. Male athymic mice and SCID mice 6–8 weeks of age were obtained from Clea Japan (Osaka, Japan) and were maintained under specific pathogen-free conditions throughout this study.

Methods. Production of Metastasis and Expression of MMPs by Human Lung Cancer Cell Lines. We first determined the activity of MMP-1, -2, and -9 constitutively produced by human lung cancer cell lines. As shown in Fig. 1A, PC14 and PC14PE6 cells expressed MMP-1, -2, and -9 activity, whereas H226 cells expressed only MMP-2 activity. A549, SBC-3/DOX, H69/VP, and RERF-LC-AI cells expressed very low or undetectable MMP-1, -2, and -9 activity under in vitro condition. MT-MMPs have been shown to play crucial roles in the activation of MMP-2 and, thus, regulate tumor cell invasion (19). We therefore explored mRNA expression of MT-MMPs in human lung cancer cell lines by RT-PCR. Five of seven cell lines strongly expressed MT1-MMP mRNA, although A549 expressed only a low level (Fig. 1B). H69/VP cells expressed no detectable MT1-MMP mRNA. All seven cell lines expressed MT2-MMP mRNA. Interestingly, none of adenocarcinoma cell lines tested expressed detectable levels of MT3-MMP mRNA, whereas others did.

RESULTS

Expression of MMPs by Human Lung Cancer Cell Lines in Vitro. We first determined the activity of MMP-1, -2, and -9 constitutively produced by human lung cancer cell lines. As shown in Fig. 1A, PC14 and PC14PE6 cells expressed MMP-1, -2, and -9 activity, whereas H226 cells expressed only MMP-2 activity. A549, SBC-3/DOX, H69/VP, and RERF-LC-AI cells expressed very low or undetectable MMP-1, -2, and -9 activity under in vitro condition. MT-MMPs have been shown to play crucial roles in the activation of MMP-2 and, thus, regulate tumor cell invasion (19). We therefore explored mRNA expression of MT-MMPs in human lung cancer cell lines by RT-PCR. Five of seven cell lines strongly expressed MT1-MMP mRNA, although A549 expressed only a low level (Fig. 1B). H69/VP cells expressed no detectable MT1-MMP mRNA. All seven cell lines expressed MT2-MMP mRNA. Interestingly, none of adenocarcinoma cell lines tested expressed detectable levels of MT3-MMP mRNA, whereas others did.

Production of Metastasis and Expression of MMPs by Human Lung Cancer Cell Lines In Vivo. In the lung metastasis model, adenocarcinoma (A549, PC14, PC14PE6) or squamous cell carcinoma (H226) cells injected i.v. into SCID or nude mice produced metastasis only in the lungs, although some of the mice inoculated
with PC14 or PC14PE6 cells developed malignant pleural effusions (Table 1). In the multiple-organ metastasis model, lung squamous cell carcinoma (RERF-LC-AI) or small cell carcinoma (SBC-3/DOX and H69/VP) cells inoculated i.v. into SCID mice depleted of NK cells produced metastases in the liver, kidneys, and systemic lymph nodes (but not to the lungs; Table 2). FIZ analysis revealed that although lung parenchyma had no detectable gelatinolytic activity, liver and kidney parenchyma showed high and low MMP activity, respectively, irrespective of the tumor cell injection (Fig. 2). In the lung metastasis model, gelatinolytic activity was observed in lung lesions produced by A549, PC14PE6, and H226 cells on FIZ-GN films (Fig. 3). The gelatinolytic activity of lung colonies of A549 cells, but not PC14PE6 or H226 cells, was detected on FIZ-GI films, suggesting that the gelatinolytic activity of tumors produced by PC14PE6 and H226 cells was attributable to MMPs but that the activity of A549 tumors was mainly attributable to other proteases, such as trypsin. In the multiple-organ metastasis model, nontumor parenchyma of the liver and kidney had high and low gelatinolytic activity, respectively, on FIZ-GN film, but tumors produced by SBC-3/DOX cells did not, consistent with the lower potential of this cell line to express MMPs in vitro. The gelatinolytic activity was not detected on FIZ-GI film, suggesting that the liver and kidney have high and low MMP activity, respectively, and that SBC-3/DOX cells do not express MMP activity even under in vivo conditions. These findings indicate the heterogeneity of both tumor cells and organ microenvironments in terms of MMP expression.

Table 1 Antimetastatic effect of treatment with ONO-4817 in the lung metastasis model of human lung cancer cells in SCID or nude mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Incidence</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
<th>Pleural effusion incidence</th>
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<tbody>
<tr>
<td>A549</td>
<td>Control</td>
<td>5/5</td>
<td>86</td>
<td>32-112</td>
<td>780</td>
<td>260-1100</td>
</tr>
<tr>
<td></td>
<td>ONO-4817</td>
<td>5/5</td>
<td>58</td>
<td>24-108</td>
<td>450</td>
<td>260-840</td>
</tr>
<tr>
<td>PC14</td>
<td>Control</td>
<td>10/10</td>
<td>9</td>
<td>3-23</td>
<td>Not determined</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>ONO-4817</td>
<td>10/10</td>
<td>2a</td>
<td>2-4</td>
<td>Not determined</td>
<td>0/10</td>
</tr>
<tr>
<td>PC14PE6</td>
<td>Control</td>
<td>8/8</td>
<td>13</td>
<td>3-9</td>
<td>261</td>
<td>195-399</td>
</tr>
<tr>
<td></td>
<td>ONO-4817</td>
<td>9/9</td>
<td>4b</td>
<td>2-17</td>
<td>189a</td>
<td>169-218</td>
</tr>
<tr>
<td>H226</td>
<td>Control</td>
<td>15/15</td>
<td>All &gt;150</td>
<td>268</td>
<td>191-490</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ONO-4817</td>
<td>15/15</td>
<td>&gt;150</td>
<td>0 to &gt;150</td>
<td>208a</td>
<td>180-234</td>
</tr>
</tbody>
</table>

* P < 0.01; + P < 0.05.

Table 2 Antimetastatic effect of treatment with ONO-4817 in the multiple-organ metastasis model by human lung cancer cell lines in NK-cell-depleted SCID mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Incidence</th>
<th>Liver metastasis</th>
<th>Liver weight (mg)</th>
<th>Kidney metastasis</th>
<th>Lymph node metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>RERF-LC-AI</td>
<td>Control</td>
<td>9/9</td>
<td>73-150</td>
<td>4.75</td>
<td>9/9</td>
<td>18-49</td>
</tr>
<tr>
<td></td>
<td>ONO-4817</td>
<td>10/10</td>
<td>70b 21-150</td>
<td>2.25b 1.93-3.50</td>
<td>10/10</td>
<td>21 9-44</td>
</tr>
<tr>
<td>SBC-3/DOX</td>
<td>Control</td>
<td>21/21</td>
<td>10-150</td>
<td>4.50</td>
<td>11/21</td>
<td>1 0-4</td>
</tr>
<tr>
<td></td>
<td>ONO-4817</td>
<td>20/20</td>
<td>6-102</td>
<td>2.38b 1.09-2.99</td>
<td>8/20</td>
<td>0 0-3</td>
</tr>
<tr>
<td>H69/VP</td>
<td>Control</td>
<td>9/9</td>
<td>2-39</td>
<td>1.10</td>
<td>9/9</td>
<td>11 6-36</td>
</tr>
<tr>
<td></td>
<td>ONO-4817</td>
<td>8/9</td>
<td>1a 0-11</td>
<td>1.23</td>
<td>9/9</td>
<td>9 6-34</td>
</tr>
</tbody>
</table>

* P < 0.05.

Fig. 2. Gelatinolytic activity in normal organ parenchyma. The lung, liver, and kidney were removed from untreated SCID mice. Gelatinolytic activity of these organs was determined by FIZ, as described in “Materials and Methods.” Similar results were obtained when the organs of nude mice were used (data not shown). Note: liver parenchyma showed high and low MMP activity, whereas liver parenchyma and kidney parenchyma showed high and low MMP activity, respectively. Arrows indicate gelatinolysis.
Inhibition of MMP Activity by ONO-4817 in Vitro. To explore the role of MMPs in our metastasis models, we used a selective MMP inhibitor, ONO-4817. Before in vivo experiments, we evaluated the potential and selectivity of ONO-4817 on MMP inhibition in vitro. Because PC14PE6 cells produced MMP-1, -2, and -9 activity, culture supernatant of PC14PE6 cells was used for this assay. Pretreatment of culture supernatant of PC14PE6 cells with ONO-4817 inhibited MMP-2 and -9 activity in a dose-dependent manner (Fig. 4). In contrast, treatment with ONO-4817 showed no effect on MMP-1 activity produced by PC14PE6 cells, consistent with previous findings (14). ONO-4817 at 10 μM did not affect proliferation of the seven cell lines used in this in vitro study (data not shown).

Effect of MMP Inhibition by ONO-4817 in the Lung Metastasis Model. The mice inoculated with A549, PC14, PC14PE6, or H226 cells were given food mixed with or without 1% ONO-4817 from the day of or 1 day before tumor cell inoculation until the end of the experiments. Seven to eight weeks later, the mice were killed, and metastasis formation in the lung was determined. Treatment of A549 cell-bearing mice with ONO-4817 tended to inhibit lung metastasis, but the difference was not significant (Table 1; Fig. 5A). Treatment of mice bearing PC14 or PC14PE6 cells with ONO-4817 significantly reduced the number of lung metastases. The formation of pleural effusion in the mice was also inhibited by treatment with ONO-4817. H226 cells developed numerous small lesions (<2 mm) on the surface of the lung. Although >150 lesions were observed in the lungs even in the mice treated with ONO-4817, the size of each lesion was clearly smaller in treated mice than in control mice (Fig. 5B). Consistent with the size of the lung lesions, the lung weight of ONO-4817-treated mice was significantly lower than that of control mice (Table 1). FIZ analysis showed that treatment of tumor-bearing mice with ONO-4817 abrogated the gelatinolytic activity of tumors produced by H226 cells, but not A549 cells on FIZ-GN films (Fig. 6). These results suggest that MMPs may be essential for the formation of lung metastases by tumor cells with high MMP expression (such as PC14, PC14PE6, and H226), but not tumor cells without MMP expression (such as A549).

Effect of MMP Inhibition by ONO-4817 in the Multiple-Organ Metastasis Model. The mice inoculated with RERF-LC-AI, SBC-3/DOX, or H69/VP cells were given food mixed with or without 1% ONO-4817 from 1 day before tumor cell inoculation until the end of the experiments. Seven to eight weeks later, the mice were killed, and metastasis formation to the major organs was determined. Although there was a marginal difference in the degree of antimetastatic efficiency of ONO-4817 among these three cell lines, treatment with ONO-4817 inhibited metastasis formation in the liver but not the kidneys or lymph nodes by these three cell lines (Table 2; Fig. 5C). Mice inoculated with RERF-LC-AI, SBC-3/DOX, or H69/VP cells did not develop lung metastases, irrespective of treatment with ONO-4817. FIZ analysis revealed that ONO-4817 treatment inhibited the MMP activity of nontumor parenchyma of the liver and kidney but

Fig. 3. Gelatinolytic activity in metastatic lesions by human lung cancer cell lines. Gelatinolysis in metastatic lesions was determined by FIZ, as described in “Materials and Methods.” Lung lesions of A549 cells in SCID mice 8 weeks after inoculation, lung lesions of PC14PE6 cells in nude mice 7 weeks after inoculation, lung lesions of H226 cells in nude mice 8 weeks after inoculation, and liver lesions of SBC-3/DOX cells in NK-cell-depleted SCID mice 4 weeks after inoculation are shown. T indicates tumor; arrows indicate gelatinolysis.

Fig. 4. Inhibition of MMP activity by ONO-4817 in vitro. PC14PE6 cells (2 × 10^5/ml) were cultured in MEM without FBS for 24 h, and the culture supernatants were harvested. The supernatants were incubated with various concentrations of ONO-4817 at 37°C for 1 h. The MMP-1, -2, and -9 activity in the supernatants was determined by ELISA, as described in “Materials and Methods.” Horizontal dashed lines indicate the detection limits of the respective ELISAs.
had no effect on MMP activity of tumors produced by SBC-3/DOX cells (Fig. 6). These findings suggest that MMP activity expressed by liver parenchyma, but not tumors, may be necessary for the production of liver metastases by SBC-3/DOX cells.

**Effect of MMP Inhibition by ONO-4817 Started after Tumor Cell Inoculation.** MMPs have been suggested to promote tumor invasion and angiogenesis (11). To address what events in metastasis formation were interrupted by ONO-4817, the mice inoculated with H226 or SBC-3/DOX cells were treated with ONO-4817 starting on the indicated days until the end of the experiments. As shown in Tables 3 and 4, ONO-4817 treatment started by days 28 and 7 inhibited the formation of lung metastases by H226 cells and liver metastases (but not kidney or lymph node metastases) by SBC-3/DOX cells, respectively, suggesting that ONO-4817 may inhibit early steps of the metastatic process under particular organ microenvironments after tumor cell injection in these animal models. In addition, vascularization in metastatic lesions was evaluated by immunostaining for CD31. There was, however, no significant difference in the microvessel density in the metastatic lesions (lung and liver) between the control group and the ONO-4817-treated group (data not shown).

**DISCUSSION**

In the present study, MMP activity in the tumors and nontumor parenchyma was determined by FIZ. As reported previously (18), FIZ analysis is a useful modality for detecting gelatinolytic activity under *in vivo* conditions, although it is not able to distinguish precisely which types of cells express the activity. We found that normal lungs had no detectable levels of gelatinolytic activity, whereas the liver and kidney had high and low levels, respectively, suggesting heterogeneity of host organ microenvironments on MMP expression. Because MMP activity was detected only in tumors and MMP inhibition by ONO-4817 reduced lung metastasis of MMP-expressing tumor cells, tumor-derived MMPs may be important for metastasis in the lung. On the other hand, because MMP activity was detected only in nontumor parenchyma in the liver and MMP inhibition reduced liver metastasis of tumor cells without discernible MMP expression, host microenvironment-derived MMPs may be important for metastasis in the liver. These findings further suggest that metastasis could be promoted if MMPs were expressed at the sites of tumor growth irrespective of their source (tumors or nontumor parenchyma). However, because A549 cells, which express predominantly other proteases, also formed lung metastases, other gelatinolytic proteases (such as trypsin) could compensate for lower MMP expression and promote metastasis in our models.

Eccles *et al.* (20) reported an effect of MMP inhibition by Bati-
MMPs might not be the major factor involved in the H226 cells in our experimental conditions. Therefore, MMPs is probably not the case because we could not detect gelatinolytic been adequate to inhibit angiogenesis of large nodules. However, this and hence the level of MMP inhibition by ONO-4817 might not have at present. One explanation is that large tumors express more MMPs, and therefore the level of MMP inhibition in these organs may not be adequate for inhibiting metastasis. The second possibility is that the level of MMP activity in the parenchyma of these organs, unlike the liver, may be less than the threshold level for promoting metastasis formation.

MMPs have been suggested to promote tumor invasion and angiogenesis by basement membrane degradation (3). In our experimental metastasis models, H226 and SBC-3/DOX cells distributed to the lung and liver by 1 h after inoculation (data not shown) and developed micrometastases by 10 days (13, 21). MMP inhibition by ONO-4817 started by days 7 and 28 reduced metastasis formation by SBC-3/DOX and H226 cells, respectively, suggesting that MMPs are necessary for early events of the metastatic processes, such as extravasation, of these cell lines. The reason that MMP inhibition by ONO-4817 did not reduce liver metastasis when started on day 14 is unclear at present. One explanation is that large tumors express more MMPs, and hence the level of MMP inhibition by ONO-4817 might not have been adequate to inhibit angiogenesis of large nodules. However, this is probably not the case because we could not detect gelatinolytic activity even in large tumors (5 mm in diameter) produced by SBC-3/DOX cells in our experimental conditions. Therefore, MMPs (MMP-2 and -9) might not be the major factor involved in the invasion or angiogenesis for large nodules, as described previously (11).

Several preclinical studies have suggested that MMPs produced by tumor cells have critical importance in tumor progression; therefore, inhibition of MMP activity is suggested to be one promising strategy for molecular targeted modalities against cancer metastasis (11). In fact, many MMP inhibitors have been developed (11, 19). The first generation of MMP inhibitors, such as Batimastat, have broad inhibitory activity against almost all MMPs and is not appropriate for p.o. administration. The second generation, such as Marimastat, MMI270 (CGS27023A), and Prinomastat, also show broad activity spectra, but can be administered p.o. The third generation, such as ONO-4817 and BAY12-9566, were designed to have selective spectra of MMP inhibition, and many compounds of this generation do not inhibit MMP-1 (19). The antitumor potential of many of these compounds has been evaluated in animal models with tumor cells expressing high MMP activity. Recent studies, however, revealed several deficiencies in suppression of cancer progression by MMP inhibitors. Batimastat was reported to inhibit the growth of primary tumors (in the skin, peritoneal cavity, and orthotopic liver) and lung metastasis (20, 22–25), but to promote liver metastasis (26). MMI-166 was shown to inhibit liver metastasis and orthotopic pancreas tumors (27), but not orthotopic colon tumors (28). These results suggest organ heterogeneity in the efficacy of MMP inhibitors. Consistent with these previous findings, we here demonstrated that one of the third-generation MMP inhibitors (ONO-4817) inhibited metastasis of human lung cancer cell lines to the liver and lung (except A549 cells) but not to the kidney or lymph nodes. ELISA and FIZ analyses for evaluating MMP activity in vitro and in vivo, respectively, further suggested that the antitumor effect of this drug by MMP inhibition depends on expression of MMPs derived not only from tumor cells but also from host microenvironments. It was previously documented that the antitumor effect

<table>
<thead>
<tr>
<th>Duration of ONO-4817 treatment</th>
<th>Lung metastasis</th>
<th>n</th>
<th>Lung weight (mg)</th>
<th>n</th>
<th>Kidney metastasis</th>
<th>n</th>
<th>Lymph node metastasis</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>No</td>
<td>Incidence</td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
<td>Incidence</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Days 1 to 28</td>
<td>10/10</td>
<td>99</td>
<td>72–150</td>
<td>5.44</td>
<td>4.5–0.6</td>
<td>3/10</td>
<td>0</td>
<td>0–3</td>
</tr>
<tr>
<td>Days 3–28</td>
<td>10/10</td>
<td>81</td>
<td>25–102</td>
<td>2.77</td>
<td>2.71–2.99</td>
<td>4/10</td>
<td>0</td>
<td>0–1</td>
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<tr>
<td>Days 7–28</td>
<td>10/10</td>
<td>84</td>
<td>35–102</td>
<td>2.63</td>
<td>2.13–3.59</td>
<td>2/10</td>
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<td>Days 14–28</td>
<td>10/10</td>
<td>89</td>
<td>0–101</td>
<td>2.45</td>
<td>1.56–3.25</td>
<td>6/10</td>
<td>1</td>
<td>0–2</td>
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* * * P < 0.01, Mann-Whitney U test.
of MMP inhibitors was directly correlated with the level of MMP expression by tumors (23) and that the effect of MMP inhibitors was sometimes heterogeneous. As described above. In the present study. we demonstrated that the therapeutic efficacy of MMP inhibitors can be differentially affected by heterogeneity of host-derived MMP expression.

In conclusion, we demonstrated that MMP expression by the host microenvironment varied among organs. In addition, metastasis formation could be facilitated if MMPs were expressed at the sites of tumor growth irrespective of their source (tumors or nontumor parenchyma). Therefore, the antitumor effect of MMP inhibitors may be dependent on MMPs derived not only from tumor cells but also from organ-specific microenvironments.

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Organ Heterogeneity of Host-derived Matrix Metalloproteinase Expression and Its Involvement in Multiple-Organ Metastasis by Lung Cancer Cell Lines

Minoru Shiraga, Seiji Yano, Akihiko Yamamoto, et al.


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