Lung cancer is the major cause of malignancy-related death worldwide and its incidence is increasing in many countries. More than 50,000 new cases of lung cancer are detected annually in Japan, and the mortality rate of nearly 90% makes it the leading cause of cancer-related deaths (1). The high mortality of this disease is predominantly due to the difficulty of early diagnosis and the highly metastatic potential of lung cancer. In many cases, metastases to multiple organs have already developed by the time of the diagnosis. Over one third of patients with advanced lung cancer develop osteolytic bone metastasis, which cause pain, pathologic fractures, spinal cord compression, and hypercalcemia (2). It leads to a considerable reduction in quality of life in lung cancer patients. Currently, no curative therapy exists for bone metastasis, and clinical management is generally palliative. Therefore, the prevention and treatment of osteolytic bone metastasis are clinically important.

Bone metastasis is a multistep event regulated not only by cancer cells but also by host microenvironments. Of the host microenvironmental cells, osteoclasts are suggested to play the critical role. Osteoclasts cause bone resorption, which supplies the space for cancer cells to grow and leak out various growth factors from bone matrix (3, 4). Therefore, osteoclasts are the ideal therapeutic target of osteolytic bone metastasis.

Reveromycin A is an antibiotic that was discovered in the culture medium of Actinomycetes because of its inhibitory activity on epidermal growth factor–dependent responses of mouse epidermal cells (5). Then, reveromycin A was shown to have an antitumor effect against a human ovarian carcinoma BG-1, which is a transforming growth factor-α–secreting and estrogen receptor–expressing cell line (6). We recently reported that reveromycin A targets isoleucyl-tRNA synthetase in osteoclasts (7). In addition, reveromycin A has a high potential to inhibit bone resorption by inducing osteoclast apoptosis via the suppression of isoleucyl-tRNA synthetase in osteoclasts (7). Therefore, reveromycin A seems to be a unique agent that has activity toward both tumor cells and the host microenvironmental cells (osteoclasts).

We previously established an osteolytic bone metastasis model with a human small-cell lung cancer (SCLC) cell line, SBC-5, in natural killer (NK) cell–depleted severe combined immunodeficient mice (SCID) mice (8). In this model, SBC-5 cells produce experimental metastatic foci not only in the bone
but also in the visceral organs, such as the lung, liver, and kidneys, representing a patient-like metastasis pattern of SCLC. The purpose of this study was to determine whether reveromycin A can inhibit the production of osteolytic bone metastasis. We evaluated the therapeutic effect of reveromycin A on bone metastasis, comparing with the visceral metastases, produced by SBC-5 cells in NK cell–depleted SCID mice and explored the underlying molecular mechanism.

Materials and Methods

Cell culture. The human SCLC cell line, SBC-5, was provided by Dr. K. Hiraki (Okayama University, Okayama, Japan; ref 8). The SBC-5 cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and 1% gentamicin. The cells were incubated at 37°C in a humidified atmosphere of 5% CO2 in air.

Reagents. Antimouse interleukin 2 receptor β chain monoclonal antibody, TM-β1 (IgG2b), was supplied by Drs. M. Miyasaka and T. Tanaka (Osaka University, Osaka, Japan; ref. 9). Reveromycin A was purified at RIKEN (the molecule weight was 660) as described previously (10).

Animals. Male ddY mice, ages 4 to 8 weeks, and male S EB-17/ICr-SCID mice, ages 6 to 8 weeks, were obtained from CLEA Japan (Osaka, Japan) and maintained under specific pathogen-free conditions in type I collagen-coated dishes in Sigma M8042 MEM (Sigma, St. Louis, MO) with 10% fetal bovine serum in the presence of guidelines of our university.

Effect of reveromycin A on the viability of mouse osteoclasts. Osteoclasts were generated from mouse bone marrow cells. Briefly, bone marrow cells were harvested from the femora of ddY mice and cultured in type I collagen-coated dishes in Sigma M8042 MEM α modification (Sigma, St. Louis, MO) with 10% fetal bovine serum in the presence of 50 mg/mL macrophage colony-stimulating factor (Kyowa Hakko, Tokyo, Japan) and 1 ng/mL transforming growth factor-β1 (R&D Systems, Minneapolis, MN) for 3 days. The resultant cells were harvested and further incubated (5,000 cells/100 μL/well) in 96-well tissue culture plates coated with type I collagen in MEM α modification with 50 mg/mL receptor activator of nuclear factor κB ligand (Peptech, London, United Kingdom) and 50 ng/mL macrophage colony-stimulating factor for 3 days. Then, the cells were treated with reveromycin A for 20 hours at 37°C, fixed, and stained for tartrate-resistant acid phosphatase (TRAP). The number of TRAP-positive multinucleated cells was scored.

In vitro effect of reveromycin A on proliferation of SBC-5 cells. SBC-5 cells at 80% confluency were harvested, plated into 96-well tissue culture plates (5,000 cells/100 μL/well) and incubated for 24 hours at 37°C in 5% CO2. Then, various concentrations of reveromycin A were added to the cultures. After a 72-hour incubation at 37°C, 50 μL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stock solution (2 mg/mL) was added to each well, and the cells were further incubated for 2 hours at 37°C (11). Then, the culture medium was removed and 100 μL of DMSO was added to dissolve the dark blue crystals. Absorbance was measured with a MTP-32 Microplate Reader (Corona Electric, Ibaraki, Japan) at test and reference wavelengths of 550 and 630 nm, respectively.

Effect of reveromycin A on the production of parathyroid hormone–related peptide and vascular endothelial growth factor of SBC-5 cells. SBC-5 cells at 80% confluency were harvested, plated into six-well tissue culture plates (1 × 105 cells/2 mL/well), and incubated for 24 hours at 37°C and 5% CO2. Then, the cultures were washed and various concentrations of reveromycin A were added. After a 48-hour incubation at 37°C, the culture supernatants were collected, and the concentrations of parathyroid hormone–related peptide (PTHrP) and vascular endothelial growth factor (VEGF) were determined using RIA (Otsuka Assay, Tokushima, Japan) and ELISA (R&D Systems), respectively.

Quantitative real-time reverse transcription–PCR analysis for parathyroid hormone–related peptide expression. Total RNA was reverse transcribed to cDNA using SuperScript II reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA) and random hexamers (Roche, Mannheim, Germany). The PCR primers and probes were purchased from Applied Biosystems (Foster City, CA) for PTHrP (TaqMan Gene Expression Assays: assay ID Hs00174969_m1) and human β2-microglobulin (Pre-Developed Taqman Assay reagent: assay ID. 4326319E). The primers and probes were designed based on sequences from Genbank. The probe sequence for PTHrP was as follows: 5′-FAM-AGCGCCGCCCTCAAAAGACCTGTGTCG-MGB-3′. PCR was conducted using the ABI 7700 sequence detector system (Applied Biosystems) in a 25 μL reaction mixture containing 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems), 1.25 μL of 20× primers and probe mixture, and 11.25 μL of cDNA diluted in RNase-free H2O. Samples were preincubated for 2 minutes at 50°C, and denatured for 10 minutes at 95°C, then subjected to 40 cycles of amplification at 95°C for 15 seconds for denaturing and at 60°C for 1 minute for annealing-extension. The expression of each target cDNA relative to β2-microglobulin was calculated using a comparative Ct method described in the User Bulletin 2 provided by the manufacturer (Applied Biosystems) and was determined for each sample.

Model of multiple-organ metastasis by SBC-5 cells and antimetastatic effect of reveromycin A. To facilitate the metastasis of SBC-5 cells, NK cells were depleted in SCID mice (12). For NK cell depletion, TM-β1 monoclonal antibody (300 μg/300 μL PBS/mouse) was injected i.p. into SCID mice 2 days before tumor cell inoculation. SBC-5 cells in the subconfluent condition were harvested and washed with Ca2+– and Mg2+-free PBS. Cell viability was determined with the trypan blue exclusion test and only single cell suspensions of >90% viability were used. SBC-5 cells (1 × 106 cells/300 μL) were injected into the lateral tail vein of mice on day 0. At the indicated periods, tumor-bearing mice were treated with i.p. administration of reveromycin A daily from day 7 or s.c. administration twice every day. Five weeks after the tumor cell inoculation, the mice were anesthetized with an i.p. injection of pentobarbital (0.5 mg/body), and X-ray photographs of the mice were taken to evaluate bone metastasis (8). Then, the mice were sacrificed by cutting the subclavian artery and all major organs were removed. The lungs were fixed in Bouin’s solution for 24 hours. The number of metastatic lesions larger than 0.5 mm in diameter on the surface of the major organs was counted macroscopically. Osteolytic bone metastasis on X-ray photographs were evaluated by two authors (H. Muguruma and S. Yano) independently.

Immunohistochemical and immunofluorescent analyses. The hind limbs of the mice were taken and fixed in 10% formalin. The bone specimens were decalcified in 10% EDTA solution for 1 week and then embedded in paraffin. From paraffin-embedded tissue samples, 3 μm sections were cut and picked up on slides. For the detection of osteoclasts, TRAP staining was done using a Sigma Diagnostics Acid Phosphatase kit (Sigma Diagnostics, St. Louis, MO; ref. 13). The number of TRAP-positive osteoclasts at the tumor-bone interface was counted under a microscope in five random fields at ×200 magnification. The liver with metastatic foci was also taken and fixed in 10% formalin and then embedded in paraffin. Tissue sections (3-μm thick) were processed and stained with H&E for routine histologic examination. Proliferating tumor cells were determined using immunohistochemistry for Ki-67 (14). Slides were deparaffinized and hydrated with an ethanol series (100%, 95%, and 70%, PBS). The endogenous peroxidase activity was blocked for 10 minutes in 3% hydrogen peroxide. Nonspecific binding was blocked by treatment with normal goat serum for 50 minutes at room temperature. Antigen retrieval for the Ki-67 antigen was done using citrate buffer (pH 6.0) at 95°C for 10 minutes. The slides were then incubated with a 1:50 dilution of monoclonal mouse anti-Ki-67 antibody (MIB1, PharMinigen, San Diego, CA) for 1 hour at room temperature. The sections were then rinsed four times with PBS and incubated for 60 minutes at room temperature with the appropriate dilution of peroxidase-conjugated antimouse IgG. The slides were rinsed with PBS and incubated for...
5 minutes with diaminobenzidine. The sections were then washed thrice with distilled water and counterstained with Mayer’s hematoxylin. For quantification, the number of stained cells in the nucleus was counted in five random fields at ×200 magnification. Negative control slides processed without the primary antibody were included for each staining. The Ki-67 proliferation index was defined as the ratio of labeled cells to total cells.

To determine the apoptotic cells, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining was done using the Apoptosis Detection System (Promega, Madison, WI; ref. 15). Briefly, after the deparaffinization and rehydration, the slides were washed with PBS and permeabilized with 20 μg/mL proteinase K. The samples were then equilibrated and DNA strand breaks were labeled with fluorescein-12-dUTP by adding a nucleotide mix and terminal deoxynucleotidyl transferase enzyme. The reaction was stopped with saline sodium citrate and the localized green fluorescence of apoptotic cells was detected by fluorescence microscopy. For quantification, the number of stained cells in the nucleus was counted in five random fields at ×200 magnification.

**Statistical analysis.** The Mann-Whitney U test was used to determine the significance of differences in the number of metastases into multiple organs (the bone, liver, lung, and kidney) between the reveromycin A–treated groups and the untreated groups. The significance of differences in the number of TRAP-positive cells was analyzed using Student’s t test (two tailed). P < 0.05 was considered significant in all experiments.

**Results**

**Effects of reveromycin A on the viability of osteoclasts and the proliferation of SBC-5 cells in vitro.** In the first set of experiments, we examined the direct effect of reveromycin A against murine osteoclasts and the human SCLC cell line (SBC-5) in vitro. Osteoclasts were highly susceptible to reveromycin A (IC50 of 0.05 μg/mL; Fig. 1A). On the other hand, SBC-5 cells were less sensitive, compared with osteoclasts, to reveromycin A (IC50 was 2.1 μg/mL). Reveromycin A at concentrations of ≤1.25 μg/mL did not significantly inhibit the proliferation of SBC-5 cells, whereas it suppressed the proliferation of SBC-5 cells at concentrations of 2.5 μg/mL or more (Fig. 1B).

**Effect of reveromycin A on the production of parathyroid hormone–related peptide and vascular endothelial growth factor by SBC-5 cells.** We reported previously (8) that SBC-5 cells produced PTHrP and VEGF, which are thought to be crucial regulatory molecules for bone resorption and tumor angiogenesis, respectively. We next examined the effect of reveromycin A on the production of these two molecules by SBC-5 cells. Reveromycin A at concentrations of 1 μg/mL or less and (noncytotoxic concentrations) did not affect the production of VEGF, although reveromycin A at cytotoxic concentrations (2 μg/mL) reduced the production of VEGF (Fig. 2A). On the other hand, reveromycin A significantly inhibited the production of PTHrP protein even at a noncytotoxic concentration (1 μg/mL; Fig. 2B). Real-time reverse transcription-PCR analysis further showed that noncytotoxic reveromycin A suppressed PTHrP at a mRNA level (Fig. 2C).

**Effects of reveromycin A treatment on multiple organ metastases in severe combined immunodeficient mice.** SBC-5 cells i.v. inoculated into NK cell–depleted SCID mice developed osteolytic bone metastasis in the limbs, vertebral bone, pelvis, scapulae, and the hind limbs. These lesions were detected on day 28 with X-ray photography (8). Several mice experienced paralysis (probably associated with spinal cord compression and bone metastases in the hind limbs) 4 weeks after SBC-5 cell inoculation, and the incidence of mice with paralysis became 30% to 50% at 5 weeks after inoculation. In addition, SBC-5 cells had produced macroscopically detectable metastases in the visceral organs, mainly in the liver, lung, and kidney, by day 28 (Fig. 3A).

Daily i.p. administration of reveromycin A (2.5 or 10 mg/kg/d) was commenced on day 7 to the end of experiments to determine the therapeutic effect of reveromycin A against established bone micrometastasis. It significantly inhibited bone metastasis, whereas it did not affect the production of metastases in the visceral organs, such as the liver, lung, and kidney (Fig. 3A; Table 1), indicating the bone-specific antimetastatic activity of reveromycin A. There was no difference on the body weight between the control group and the reveromycin A–treated groups throughout the experiment (data not shown).

We further evaluated the therapeutic effect of s.c. administration of reveromycin A twice a day at lower doses. S.c. administration of reveromycin A twice a day (1 or 2 mg/kg/dose) was also commenced on day 7 to the end of experiments. As shown in Table 2, the s.c. administration of reveromycin A...
significantly inhibited bone metastasis, whereas it did not affect the production of metastases in the visceral organs.

Effect of reveromycin A on the number of osteoclasts in bone lesions. Histologic analysis of the untreated mice revealed that osteolytic bone lesions consisted of cancer cells. Numerous osteoclasts stained using TRAP staining were observed along the trabecular bone surface surrounded by SBC-5 cells (Fig. 3B). The number of osteoclasts was significantly lower in the bone lesions of mice treated with reveromycin A compared with the control mice (Figs. 3B and 4A). There was no significant difference on the number of proliferating tumor cells between the control group and the reveromycin A–treated group in the bone lesions (Figs. 3B and 4D) or between the liver metastasis and bone metastasis (data not shown), when determined with the staining for Ki-67. In the parallel experiments, there were few apoptotic cells in both the bone metastasis and liver metastasis when determined with TUNEL staining. Treatment with reveromycin A increased the number of apoptotic cells in the bone metastasis but not the liver metastasis (Figs. 3B and 4B and C).

Discussion

In the present study, we showed that reveromycin A, an acidic compound with three carboxylic groups, inhibited the expression of PTHrP by a human SCLC cell line, SBC-5, at...
Cancer Therapy: Preclinical

Table 1. Effect of i.p. administration with reveromycin A on multiple-organ metastasis produced by SBC-5 cells in NK cell–depleted SCID mice

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NOTE: SBC-5 cells (1 x 10⁶ per mouse) were injected into the lateral tail vein of the NK cell–depleted SCID mice on day 0. The mice were i.p. administered with PBS or reveromycin A (2.5 or 10 mg/kg daily) from days 7 to 34. The mice were killed on day 35 and the production of metastasis was evaluated.

* Median (range).

1 P < 0.01.

Reveromycin A has been reported to have a strong antitumor effect against a human ovarian carcinoma BG-1 (6). Then, it was shown to target isoleucyl-tRNA synthetase in yeast genetics and inhibit their growth and protein synthesis (5). In addition, reveromycin A efficiently induced the apoptosis of osteoclasts (Fig. 1A) in vitro. Therefore, reveromycin A has been expected to have dual actions against tumor cells and host microenvironmental osteoclasts. In the present study, we showed the bone-specific antitumor activity of reveromycin A in a multiple-organ metastasis model with SBC-5 cells. This effect may be predominantly due to the inhibition of osteoclast activity because (a) treatment with reveromycin A up to 1.25 μg/mL for 72 hours did not suppress the proliferation of SBC-5 cells in vitro; (b) reveromycin A was rapidly eliminated from the serum in mice (after i.p. administration with 20 mg/kg reveromycin A, Cₘₐₓ and t₁/₂ were 6.6 μg/mL and 0.66 hours, respectively); (c) treatment with reveromycin A did not inhibit the production of metastasis to the visceral organs, such as the liver, lung, and kidney; and (d) treatment with reveromycin A did not inhibit the proliferation of SBC-5 cells in the bone lesions. In addition, treatment with reveromycin A reduced the number of osteoclasts in the bone lesions (Figs. 3 and 4). Furthermore, in a rat osteoporosis model with a low calcium intake, reveromycin A reduced the number of osteoclasts in the bone (Supplementary Fig. S1) and inhibited bone resorption by inducing osteoclast apoptosis (7). Therefore, reveromycin A may inhibit bone metastasis by reducing the space in which tumor cells are able to grow via the suppression of bone resorption.

Osteoclasts originate from bone marrow stem cells and play a crucial role in physiologic and pathologic bone resorption (16). Several factors, including interleukin 1, interleukin 6, receptor activator of nuclear factor κB ligand, macrophage inflammatory protein-1α, and PTHrP, have been implicated in the differentiation/maturation of osteoclasts and bone destruction in malignant diseases (17). Of these factors, PTHrP has a 70% homology to the first 13 amino acids of the NH₂-terminal protein of PTH. PTHrP binds to the PTH receptor in the bone and kidney, stimulates osteoclast-mediated bone resorption and renal tubular calcium reabsorption and, hence, induces hypercalcemia. Approximately 80% of hypercalcemic patients with solid tumors have detectable or increased plasma PTHrP concentrations (18). In addition, PTHrP has been suggested to play a critical role in the production of bone metastasis. We previously reported that SBC-5 cells overexpressed PTHrP and that treatment with anti-PTHrP-neutralizing antibody inhibited the production of bone metastases of SBC-5 cells in the NK cell–depleted SCID mouse model (19), indicating the critical role of PTHrP in bone metastasis in this model. In the present study, we found that reveromycin A at noncytotoxic concentrations suppressed the production of PTHrP by SBC-5 cells at both protein and mRNA levels. We could not determine whether apoptotic cells in the bone lesions were osteoclasts and/or tumor cells, because immunostaining for the detection of tumor cells (with antihuman keratin antibody) did not work well on decalcified bone lesions.

Together with the findings that reveromycin A strongly inhibited the viability of mouse osteoclasts (Fig. 1A), reveromycin A may suppress osteoclast activity by directly inducing apoptosis and indirectly inhibiting tumor cell–derived PTHrP production. Further experiments are warranted to clarify the mechanism by which reveromycin A inhibits PTHrP production.

For clinical application of new drugs, the profile of toxicity is important. In the present study, we treated NK cell–depleted SCID mice with reveromycin A daily for 4 weeks. This long-term treatment with reveromycin A was feasible and did not cause body weight loss, hypocalcemia, or histologic disorder in the liver or kidney (data not shown). However, the toxicity in humans is still unknown and needs to be evaluated in clinical trials in future.

Bisphosphonates are hydrolysis-resistant PP1 derivatives that have a high affinity for bone and inhibit osteoclastic bone resorption (20). Bisphosphonates are clinically used for treatment of osteoporosis and hypercalcemia of malignancy. In addition, several agents have been approved for treatment of bone metastasis in multiple myeloma and solid tumors, including breast cancer and lung cancer (21). We reported that a third generation bisphosphonate, minodronate

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* Unpublished data.
(YM529), had the potential to inhibit bone metastasis of SBC-5 cells (13). Similar to the results of this study, whereas YM529 did not have a direct inhibitory effect on SBC-5 cell proliferation, it reduced the number of osteoclasts in the bone lesions and inhibited the production of bone metastasis (13). One of the differences between reveromycin A and YM529 was the inhibition of PTHrP expression by reveromycin A, as described above. The combined use of reveromycin A with bisphosphonates may be useful for enhancing the therapeutic efficiency against bone metastasis. On the other hand, because neither reveromycin A nor YM529 suppressed the visceral metastases produced by SBC-5 cells (13), additional therapies directly targeting tumor cells to inhibit the visceral metastases (such as chemotherapy and molecular targeted agents) may be necessary to prolong the survival of the mice bearing multiple-organ metastases.

Angiogenesis is essential for the enlargement of the primary tumor and metastasis of a variety of cancers. VEGF is an important regulator of tumor angiogenesis and its expression correlated directly with tumor vascular density and correlated inversely with the survival of patients with various solid tumors (22–28). VEGF binds with a high affinity to two tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), on endothelial cells (29). Of these two specific

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*Median (range).

Note: SBC-5 cells (1 × 10^6 per mouse) were injected into the lateral tail vein of the NK cell–depleted SCID mice on day 0. The mice were s.c. administered with PBS or reveromycin A (1 or 2 mg/kg twice a day) from days 7 to 34. The mice were killed on day 35 and the production of metastasis was evaluated.

NOTE: *P < 0.01, compared with the control group (Mann-Whitney U test).

Fig. 4. Quantification of osteoclasts, proliferating tumor cells, and apoptotic cells in the bone lesions. Osteoclasts in the bone lesions were determined by TRAP staining (A). Apoptotic cells in the bone lesions (B) and liver lesions (C) were determined by TUNEL staining. The proliferating tumor cells in the bone lesions were determined by staining for Ki-67 (D). Columns, mean of five areas; bars, SD. *, P < 0.05; **, P < 0.01 compared with the control group (Mann-Whitney U test).
receptors. VEGFR-2 has the predominant role in transmitting VEGF-induced signaling responses and has been suggested as an antiangiogenic target (30, 31). As shown in Fig. 2A, SBC-5 cells secreted a large amount of VEGF protein (Fig. 2). In addition, the blocking of VEGF-VEGFR-2 signaling (by the treatment with a VEGF-2 tyrosine kinase inhibitor, ZD6474) inhibited the bone metastasis and the visceral metastases of SBC-5 cells in NK cell–depleted SCID mice (7). Thus, VEGF is suggested to be one of therapeutic targets for bone metastases. Reveromycin A did not affect the expression of VEGF or VEGFR-2 in SBC-5 cells or human dermal microvessel endothelial cells (data not shown), indicating that reveromycin A did not have anti-VEGF activity. Because reveromycin A and VEGFR-2 inhibitors have different antimetastatic mechanisms to the bone lesions, the combined use of reveromycin A with the VEGFR-2 inhibitors may further augment therapeutic efficiency toward bone metastasis.

Much attention has been paid to therapies targeting host-microenviromental factors (including angiogenesis and osteoclasts), in addition to those directly targeting cancer cells (including radiotherapy, chemotherapy, and epidermal growth factor receptor inhibitors). Because tumors consist of cancer cells and host stromal cells, dual targeting by these two modalities seems to be ideal. In fact, an anti-VEGF neutralizing antibody, bevacizumab, has been shown to augment the response rates of chemotherapy and prolong the survival of colorectal cancer patients (32). Reveromycin A has been reported to have activities toward both tumor cells and osteoclasts. Although it inhibited the PTHrP expression of SBC-5 cells, it did not have direct cytotoxicity against SBC-5 cells. Therefore, reveromycin A analogues that have higher cytotoxicity against cancer cells, while maintaining their antiosteoclast activity, may be more beneficial for the treatment of bone metastases.

In conclusion, we showed that reveromycin A inhibited the production of experimental bone metastasis of SCLC cells (SBC-5), presumably through suppression of osteolytic bone metastasis of lung cancer. However, because the antimetastatic effect of reveromycin A was bone specific, additional therapies to control the visceral metastases may be necessary for controlling multiple-organ metastasis of lung cancers.

References

# Clinical Cancer Research

**Reveromycin A Inhibits Osteolytic Bone Metastasis of Small-Cell Lung Cancer Cells, SBC-5, through an Antiosteoclastic Activity**

Hiroaki Muguruma, Seiji Yano, Soji Kakiuchi, et al.


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