Osteoprotegerin/Osteoclastogenesis Inhibitory Factor Decreases Human Prostate Cancer Burden in Human Adult Bone Implanted into Nonobese Diabetic/Severe Combined Immunodeficient Mice

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ABSTRACT

Human prostate cancer frequently metastasizes to bone, where it gives rise to osteoblastic bone metastases with an underlying osteolytic element and subsequent bone pain. However, the importance of osteoclastogenesis in the development of prostate cancer bone lesions in humans is unclear. Osteoprotegerin/osteoclastogenesis inhibitory factor (OCIF) is a member of the tumor necrosis factor receptor family and a novel secreted protein, and it is a negative regulator of osteoclast differentiation, activation, and survival both in vitro and in vivo. In the present study we used a model in which human LNCaP prostate cancer cells that give rise to osteoblastic bone tumors were injected directly into the intramedullary space of human adult bone implanted into nonobese diabetic/severe combined immunodeficient mice to investigate whether the new bone-resorption inhibitor osteoprotegerin/OCIF would inhibit the development of new bone tumors and the progression of established osteoblastic bone tumors. The mice were given consecutive daily s.c. injections of recombinant human OCIF (rhOCIF; 100 μg/mouse/day) for 2 weeks starting either immediately or 2 weeks after injection of the LNCaP cells. In both protocols, rhOCIF markedly inhibited both the development of bone tumors and the progression of established bone tumors. These results demonstrate that osteoclasts play an important role in bone tumor by tumor lesions, and prevents the development of new lesions. These results suggest that inhibition of osteoclastic bone resorption may be an effective therapy for the treatment of prostate cancer that has colonized bone.

INTRODUCTION

Bone has long been recognized as the most common target organ for prostate cancer metastasis (1), with >80% of prostate cancer patients developing bone metastases (2). In the past the bone metastases of prostate cancer were thought to form only osteolytic lesions, but a number of histomorphometric studies have indicated that bone resorption is increased even at sites where there is a high degree of bone formation, and that there is increased bone formation and numbers of osteoblasts, as well as increased bone resorption, especially at sites close to the tumors. It is now thought that osteolytic components are included in the bone metastases of prostate cancer and that they are present in the early stage (3–6). The presence of osteolytic lesions in the bone metastatic foci of prostate cancer suggests that osteoclastogenesis may play an important role in the establishment of these lesions.

The RANK,3 RANKL, and OPG, also called OCIF, have been identified recently as the three major molecules that modulate osteoclast differentiation and maturation. RANKL is a cell-bound protein that is induced on osteoblasts and stromal cells by bone-resorbing factors (7). Osteoclast progenitor cells possess RANKL receptors (RANK), and on receiving a signal from RANKL as a result of adhering to osteoblasts and stromal cells, they differentiate toward osteoclasts (8–10). OPG/OCIF is a decoy receptor that by binding directly to RANKL obstructs binding between RANKL and its receptor, and it inhibits osteoclast formation, survival, and bone resorption activity (11–13). Because expression of RANKL, which induces osteoclast formation, has been found recently to be increased significantly in the bone metastases of prostate cancer (14), it may be possible to use OPG/OCIF to inhibit preceding bone resorption by osteoclasts as a means of inhibiting bone metastasis by prostate cancer.

Unfortunately, no suitable model that consistently reproduced the osteoblastic bone metastases of prostate cancer in experimental animals was available until we succeeded in using a human prostate cancer cell line, LNCaP, and human adult bone to establish a model of bone metastasis capable of producing osteoblastic bone metastases in human adult bone the same as clinically (15). In the present study we used this model to investigate whether the new bone-resorption inhibitor OPG/OCIF would inhibit the development of new bone tumors and the progression of established osteoblastic bone tumors.

MATERIALS AND METHODS

Animals

Male NOD/SCID mice, 5 weeks of age, were purchased from CLEA Japan, Inc. (Tokyo, Japan) and maintained under specific pathogen-free, temperature-controlled air conditions throughout this study at the National Cancer Center Research Institute East (Chiba, Japan) in accordance with Institutional Guidelines. The mice used in all of the experiments were 6–8 weeks of age. All of the animal manipulations were performed inside a laminar-flow hood under sterile conditions while maintaining them under inhalation anesthesia with ethyl ether, unless otherwise noted.

Cell Line and Cell Culture

Human prostate cancer cell line LNCaP (16) was purchased from the American Type Culture Collection (Rockville, MD). LNCaP is a PSA-producing androgen-responsive human prostate cancer cell line. It was cultured in

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3 The abbreviations used are: RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; OCIF, osteoclastogenesis inhibitory factor; rhOCIF, recombinant human OCIF; NOD/SCID, nonobese diabetic/severe combined immunodeficient; PSA, prostate-specific antigen; TRAP, tartrate-resistant acid phosphatase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.
RPMI 1640 supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO₂ incubator, and the medium was changed three times per week. LNCaP prostate cancer cells were harvested after trypsinization (0.05% trypsin-0.02% EDTA), and the cells were washed in medium, counted, and resuspended.

**Protocol for Inoculation of Human Adult Bone Fragments into NOD/SCID Mice**

After obtaining the informed consent of the patients, normal-appearing human adult bone tissue was dissected from surgical material obtained during pulmonary lobectomy for lung cancer (patient age range, 52–75 years; mean, 64.9) in the Division of Thoracic Oncology, National Cancer Center Hospital East. Infected material and suspected material of being infected was rejected. The bone fragments were maintained under sterile conditions in RPMI 1640 at 4°C and implanted into NOD/SCID mice within 1 h of procurement, as described elsewhere (15, 17). Briefly, morcellized bone fragments (−5 × 5 × 5 mm) were implanted s.c. in the back of the NOD/SCID mice through a small skin incision, and the animals were used 4 weeks after implantation.

**Bone Tumor Model**

In all of the experiments, 4 weeks after bone implantation single-cell suspensions (1 × 10⁷/50 μl/mouse) of LNCaP prostate cancer cells in PBS were injected through the skin into the intramedullary space of the implanted human adult bone with a 27-gauge needle. rhOCIF

**rhOCIF** (12, 13) was kindly provided by Sankyo Co., Ltd. (Tokyo, Japan) and Snow Brand Milk Products Co., Ltd (Tochigi, Japan). It was prepared as reported elsewhere (18), diluted in a stock solution [1 mg/ml of OCIF in 10 mM PBS containing 150 mM NaCl, and 0.01% Tween 80 (pH 7.0)], and kept frozen at −70°C until used. The buffer was used as a vehicle.

**Experimental Protocols for Administration of rhOCIF**

The experiments are summarized in Fig. 1. Human adult bone implanted into male NOD/SCID mice was inoculated with LNCaP prostate cancer cells (day 0). In all of the experiments, mice were s.c. injected daily with either vehicle containing rhOCIF (100 μg/mouse/day; 100 μl) or vehicle alone [10 mM PBS in 150 mM NaCl, 0.01% Tween 80; 100 μl] at the times indicated. The same protocol was performed in another vehicle-treated group of mice (LNCaP 2 week + vehicle; n = 10) to histologically assess the natural state of early bone tumor in human adult bone implanted in NOD/SCID mice after 2 weeks.

**Protocol 1: Effect of rhOCIF on the Development of Bone Tumors**

In the prevention model, administration of rhOCIF was initiated within hours of the intrabone injection of LNCaP prostate cancer cells, and the animals were randomly divided into a group injected s.c. with vehicle for 14 days after LNCaP prostate cancer cell injection (LNCaP 4 week + early vehicle; n = 10) and a group injected with rhOCIF (100 μg/mouse/day; LNCaP 4 week + early OCIF; n = 10).

**Protocol 2: Effect of rhOCIF on Established Bone Tumors**

One group of mice (LNCaP 4 week + late vehicle; n = 10) was s.c. injected with vehicle once a day from day 14 to 28, and a second group of mice (LNCaP 4 week + late OCIF; n = 10) was injected with rhOCIF (100 μg/mouse/day).

**Histological Examination**

Mice were killed by cervical dislocation under anesthesia 4 weeks after direct injection of LNCaP prostate cancer cells. The implanted human adult bone and certain mouse organs (femur, lumbar vertebrae, heart, lungs, brain, liver, kidneys, spleen, pancreas, adrenal glands, and testes) were harvested for histological analysis, and all of the other mouse organs were inspected visually. Any obvious or suspicious lesions were submitted for histological analysis.

Portions of each organ were fixed in 10% neutral buffered formalin and embedded in paraffin. All of the bone specimens were decalciﬁed with EDTA solution (Wako, Osaka, Japan) before parafﬁn embedding, and 4-μm-thick sections were cut from each specimen and stained with H&E for routine histological examination.

**PSA Immunohistochemistry**

Immunohistochemical staining was performed by the avidin-biotin-peroxidase complex technique (19). Nonspeciﬁc binding sites were blocked with 2% BSA and 5% skim milk in PBS for 30 min at room temperature. The primary antibody used was a rabbit polyclonal anti-PSA antibody (DAKO, Glostrup, Denmark) diluted to 1:1000. After immunostaining, the sections were counterstained with Meyer’s hematoxylin before dehydration and mounting. As a negative control, the primary antibody was replaced with normal swine serum.

**Determination of Serum PSA Levels**

Blood samples (50–100 μl) were obtained by retro-orbital venipuncture before LNCaP prostate cancer cell injection, and at 2 and 4 weeks after injection. The blood was centrifuged, and the serum was stored at −20°C until assayed. Total serum PSA levels were determined with an immunoradiometric assay kit (Tandem-R; Hybritech, San Diego, CA) according to the manufacturer’s protocol.

**Histomorphometric Examination**

**Tumor Burden.** We histomorphometrically analyzed tumor burden in the implanted human adult bone by examining sections stained with H&E (×200 magnification). Serial sections were cut at three levels far apart enough (≥200 μm) to avoid replicate sampling a single surface event. The total tissue section area and tissue area occupied by the tumor cells were measured with a computed image analyzer, the KS 300 system version 2.00 (Carl Zeiss, Oberkochen, Germany). The results are shown as tumor area (mm²) and tumor area as a percentage of tissue area.

**Osteoclast Number.** Osteoclasts were identiﬁed as TRAP-positive multinucleated (>3 nuclei) cells. The numbers of TRAP-positive multinucleated osteoclasts at the interface between tumor and bone were counted in ﬁve random ﬁelds of each section (×400 magniﬁcation), and the results are expressed as number per millimeter length of tumor-bone interface.

**s.c. Tumors**

LNCaP prostate cancer cells were resuspended in RPMI 1640 plus 10% fetal bovine serum and injected into the flank at 1 × 10⁶ cells/100 μl/site with a 22-gauge needle. As soon as the LNCaP prostate cancer cells had been injected, daily s.c. injection of rhOCIF (100 μg/mouse/day, n = 10; 200 μg/mouse/day, n = 10) or vehicle (n = 10) for 4 weeks was instituted. The tumors were allowed to grow for another 8 weeks, and the mice were sacriﬁced at 12 weeks after the LNCaP cell injection. s.c. tumor growth was monitored by palpation, and two perpendicular axes were measured. Tumor volume was calculated using the formula described previously (15).
Statistical Analysis

The statistical significance of differences in tumor burden and serum PSA level between the rhOCIF groups and control groups was determined by the Mann-Whitney test for nonparametric samples. Osteoclast number in the treatment and control groups was statistically analyzed by ANOVA. All of the data are shown as means ± SD. Differences with a P < 0.05 were considered statistically significant.

RESULTS

Osteoblastic Bone Tumors Produced by LNCaP Prostate Cancer Cells in Implanted Human Adult Bone. In our preliminary study, LNCaP cells were injected directly into implanted human adult bone fragments, which were subjected to freeze-thaw treatment. As a result, no LNCaP tumors were detected (data not shown). This demonstrated that the proliferation of tumor cells in the bone was promoted by bone marrow cells rather than by the bone matrix alone.

When LNCaP cells (1 × 10⁶ cells) were injected directly into the intramedullary space of implanted human adult bone fragments, localized LNCaP (PSA-positive) tumor foci were observed in all of the implanted human adult bone in the untreated group 2 weeks after injection (Fig. 2, A and B). When compared with tumor-free sites, examination of bone that had been partially invaded by tumor or that was located adjacent to tumor revealed the presence of numerous TRAP-positive osteoclasts along the bone, and bone resorption had increased (Fig. 2 C). Most of the intramedullary space had been replaced by PSA-positive tumor cells (Fig. 2 D).}

Fig. 2. Histological analysis of LNCaP tumors in human adult bone implanted into NOD/SCID mice. Non-treated (control) group at 2 weeks (A–C), control group at 4 weeks (D–F), OCIF early treatment group (G–I), OCIF late treatment group (J–L). A, localized LNCaP tumors were observed in implanted human adult bone in the control group 2 weeks after injection of LNCaP cells (H&E, ×20). B, localized PSA-positive tumor foci were observed (PSA staining, ×20). C, numerous TRAP-positive osteoclasts are present along the bone, and bone resorption has increased (TRAP staining; ×100). D, the majority of the intramedullary space in the implanted human adult bone has been replaced by LNCaP tumors in the control group at 4 weeks after injection of LNCaP cells, and bone thickness has increased in places (H&E, × 20). E, the intramedullary space has been almost entirely replaced by PSA-positive tumor cells (PSA staining; ×20). F, TRAP-positive osteoclasts are present in the control group at 4 weeks, but the osteoclasts have decreased in number in comparison with the control group at 2 weeks (TRAP staining; ×100). G, LNCaP tumors were markedly fewer in the OCIF early treatment group (H&E, ×20). H, PSA-positive cells were observed in only an extremely small area (PSA staining; ×20). I, hardly any TRAP-positive osteoclasts were observed, and staining was attenuated (TRAP staining; ×100). J and K, localized LNCaP tumor foci (PSA-positive cells) were observed in all of the implanted human adult bone in the OCIF late treatment group (J, H&E, ×20; K, PSA staining, ×20). L, hardly any TRAP-positive osteoclasts were observed (TRAP staining; ×100).
Tumor burden and the number of osteoclasts in the implanted human adult bone (sham + vehicle) was 0.28 ± 0.05/mm, whereas the number of TRAP-positive osteoclasts on the bone tumor surface after LNCaP cell direct injection in the control group was 0.89 ± 0.19/mm at 2 weeks and 0.66 ± 0.14/mm at 4 weeks, and had increased 3.2- and 2.4-fold, respectively, over the counts in normal implanted human adult bone (sham + vehicle; Fig. 3C). The level of osteoclastic activity in the direct injection model was similar to that seen in the i.v. injection model.

Tumor growth and tumor uptake rates were considerably higher in the direct injection model than in the i.v. injection model, but the level of osteoclastic activity and the histological features of the bone did not differ between both models.

The serum PSA value in the control group 2 weeks after LNCaP cell injection was 19.7 ± 8.3 ng/ml as opposed to 84.0 ± 11.3 ng/ml at 4 weeks, and it had sharply increased in 2 weeks, reflecting the kinetics of tumor burden (Fig. 4).

Effect of Early Short-Term Treatment with rhOCIF on the Development of New Bone Tumors (Protocol 1). The OCIF early short-term treatment group was given consecutive daily s.c. injections of rhOCIF for 2 weeks starting immediately after injection of the LNCaP cells. Vehicle was administered to the nontreatment group (controls) instead of rhOCIF. We used this protocol to investigate whether rhOCIF would suppress the establishment of bone tumor foci that cannot be detected clinically. Comparison with the control group (4 weeks) revealed that the LNCaP tumors in the OCIF early treatment group were localized inside the implanted human adult bone and were much smaller (Fig. 2G and H). Moreover, no LNCaP tumors were detected in 2 of the 10 animals in the OCIF early treatment group, and no PSA-positive cells were observed in the immunohistochemical studies. Furthermore, hardly any TRAP-positive osteoclasts were observed in the bone adjacent to the tumors or the bone that was tumor-free, and staining was weaker (Fig. 2F).

Tumor burden and the number of osteoclasts in the implanted human adult bone were measured with a computed image analyzer to assess the effectiveness of rhOCIF. Mean tumor area in the OCIF early treatment group was 3.32 ± 0.6 mm² and was only 4.1% of the mean tumor area in the control group (4 weeks; P < 0.0001; Fig. 3A). The proportion of the implanted human adult bone occupied by tumor was 2.12 ± 0.89% and was also much lower, only 3.1% of the proportion in the control group (4 weeks; P < 0.0001; Fig. 3B). The histomorphometric analysis showed markedly less tumor burden in the OCIF early treatment group. The number of osteoclasts in the implanted human adult bone occupied by tumor of 17.93 ± 4.99%, with corresponding values at 4 weeks of 81.32 ± 18.29 mm² and 69.11 ± 12.81% (Fig. 3, A and B). In the 2-week period from 2 to 4 weeks after the injection of LNCaP cells the average amount of tumor area and proportion of implanted human adult bone occupied by tumor had increased 4-fold, and a sharp increase in LNCaP tumor burden was observed. Four weeks after direct or s.c. injection of LNCaP cells, the average size of the LNCaP bone tumors (157.2 ± 26.5 mm²) was significantly larger than that of s.c. LNCaP tumors (55.5 ± 11.9 mm²; Fig. 5).

Next, changes in number of osteoclasts were investigated by histomorphometric analysis. In our preliminary study, we found that the number of TRAP-positive osteoclasts on the bone tumor surface was 1.02 ± 0.18/mm and 0.56 ± 0.15/mm, respectively, at 4 weeks and 6 weeks after i.v. injection of LNCaP cells, showing an increase of 3.4- and 1.9-fold over the respective values in normal control bone fragments at the same times. The number of osteoclasts in normal implanted human adult bone (sham + vehicle) was 0.28 ± 0.05/mm, whereas the number of TRAP-positive osteoclasts on the bone tumor surface after LNCaP cell direct injection in the control group was 0.89 ± 0.19/mm at 2 weeks and 0.66 ± 0.14/mm at 4 weeks, and had increased 3.2- and 2.4-fold, respectively, over the counts in normal implanted human adult bone (sham + vehicle; Fig. 3C). The level of osteoclastic activity in the direct injection model was similar to that seen in the i.v. injection model.

Fig. 3. Effect of rhOCIF on histomorphometric indices of LNCaP tumor burden. Three nonserial sections of each implanted human adult bone were analyzed for (A) total tumor area, (B) skeletal tumor burden (tumor area as a percentage of tissue area), and (C) number of osteoclasts per millimeter length of tumor-bone interface. rhOCIF significantly reduced all parameters. *P < 0.001; ***P < 0.0001 versus controls (LNCaP 4w + vehicle). Data are means (n = 10 mice/group); bars, ±SD.

Fig. 4. Effect of rhOCIF on serum PSA levels of LNCaP tumor-bearing NOD/SCID mice. PSA levels were measured in serum from early OCIF (tri), late OCIF ( ), and vehicle injected ( ) mice. *P < 0.01; ***P < 0.0001 versus controls (LNCaP 4w + vehicle). Data are means (n = 10 mice/group); bars, ±SD.
OCIF early treatment group, 0.05 ± 0.02/mm², was also significantly smaller in the rhOCIF group than in the control group (4 weeks; \( P < 0.0001 \); Fig. 3C).

The serum PSA values in the OCIF early treatment group were 0.4 ± 0.2 ng/ml at 2 weeks after LNCaP cell injection and 2.2 ± 0.5 ng/ml at 4 weeks, and they were markedly suppressed compared with the values in the control group (\( P < 0.0001 \); Fig. 4).

**Effect of Late Short-Term Treatment with rhOCIF on Established Bone Tumors (Protocol 2).** In the OCIF late short-term treatment group the animals were given consecutive daily s.c. injections of rhOCIF for 2 weeks beginning 2 weeks after injection of the LNCaP cells. This protocol was used to investigate whether rhOCIF would suppress tumor progression by established bone tumors. Localized LNCaP tumors (PSA-positive cells) were observed in all of the implanted human adult bone in the OCIF late treatment group (Fig. 2, J and K), but hardly any TRAP-positive osteoclasts were detected, the same as in the OCIF early treatment group (Fig. 2L).

Histomorphometric analysis showed that whereas mean tumor area in the OCIF late treatment group was 32.81 ± 5.3 mm² and the shrinkage rate was lower than in the early treatment group, tumor size was significantly smaller, only 40.3% of the area in the control group (4 weeks; \( P < 0.001 \); Fig. 3A). The proportion of implanted human adult bone occupied by tumor in the OCIF late short-term treatment group, 27.14 ± 4.25%, was also significantly smaller, only 39.3% of the proportion in the control group (4 weeks; \( P < 0.001 \); Fig. 3B).

Because mean tumor area and proportion of implanted human adult bone occupied by tumor increased up to 4-fold during the 2-week period from 2 to 4 weeks after the LNCaP cell injection in the control group, but only 1.5 -old when rhOCIF was administered for 2 weeks beginning 2 weeks after the LNCaP cell injection, tumor progression was inhibited by rhOCIF administration. There were no significant differences in mean tumor area or proportion of implanted human adult bone occupied by tumor between the control group (2 weeks) and the OCIF late treatment group (\( P > 0.05 \)). Even when rhOCIF was administered after bone tumors had become established, the number of osteoclasts, 0.03 ± 0.01/mm, was much smaller than in the control group (4 weeks; \( P < 0.0001 \); Fig. 3C), and, thus, rhOCIF sufficiently suppressed the number of osteoclasts even under conditions in which tumors were present.

Although the serum PSA value in the OCIF late treatment group was 23.3 ± 2.1 ng/ml at 2 weeks after the LNCaP cell injection and unchanged compared with the control group, it was only 33.8 ± 17.4 ng/ml at 4 weeks after the LNCaP cell injection, and had been significantly suppressed by rhOCIF administration compared with the control group (\( P < 0.01 \); Fig. 4).

Moreover, no abnormal findings or PSA-positive cells were detected in any of the mouse tissues examined histologically or immunohistochemically in any of the groups, i.e., in the control groups (2 weeks and 4 weeks), or the OCIF early or late treatment groups.

**Effect of rhOCIF on s.c. Growing LNCaP Prostate Cancer.** To determine whether rhOCIF exerts a direct growth-inhibitory effect on LNCaP cells, we investigated its effect after s.c. injection of LNCaP cells into the back of NOD/SCID mice. The s.c. LNCaP tumors grew in the OCIF group (100 or 200 \( \mu \)g/mouse/day) the same as in the control group, and there was no difference in tumor burden between them (\( P > 0.05 \); Fig. 5). LNCaP bone tumor burden decreased significantly in response to rhOCIF administration, but rhOCIF was ineffective against s.c. LNCaP tumors. Moreover, LNCaP growth was unaffected by rhOCIF in vitro even at rhOCIF concentrations of 100-1000 ng/ml, concentrations at which rhOCIF has been found to markedly inhibit osteoclastic bone resorption in several in vitro assays. The results indicate that the inhibitory effect of rhOCIF on prostate cancer is restricted to within bone.

DISCUSSION

Whereas osteoclasts play an important role in osteolytic tumors, their role and importance in bone metastases of prostate cancer was unknown. Histological examination has revealed a heterogeneous mixture of osteolytic and osteoblastic lesions in the bone metastatic foci of prostate cancer (4, 21, 22), and bone resorption appeared to be required for subsequent bone formation (23).

To demonstrate that osteoclasts are important in bone tumors of prostate cancer, in this study we conducted experiments using the osteoclastogenesis inhibitor rhOCIF to determine whether bone tumor of prostate cancer can be suppressed by inhibiting osteoclasts in human adult bone. The results obtained by Zhang et al. (24) showed that osteoclasts are important to the establishment of bone tumors of human prostate cancer cells in mouse bone and that controlling osteoclasts is important in treatment. However, it was unknown whether inhibiting osteoclasts would be efficacious in preventing the bone tumor of prostate cancer to human adult bone or in treating prostate cancer in which bone tumors had already become established and formed tumor masses, which is important from a clinical standpoint. Accordingly, we used a model in which human prostate cancer cells colonize to human adult bone as a means of conducting experiments to assess the effect of rhOCIF on the formation and progression of bone lesions under conditions that mimicked human conditions in vivo as closely as possible.

In the present study, we investigated the in vivo efficacy of rhOCIF on bone tumor of prostate cancer to human adult bone according to two protocols. The early OCIF treatment protocol was devised postulating bone tumor foci that cannot be detected clinically to investigate whether it would prevent the establishment of new bone tumors, and the late OCIF treatment protocol was devised postulating a situation in which the drug is administered after obvious bone tumor has been observed clinically. Histological and histomorphometric examinations in both protocols showed that administration of rhOCIF suppressed both the formation and progression of bone tumors of prostate cancer.

LNCaP tumors appear to grow more rapidly in the bone than in the s.c. tissue. Furthermore, acceleration of bone metabolism has been reported to be associated with increased formation and progression of bone metastases (25). This is thought to be attributable to the fact that a variety of growth factors are stored in bone matrix and that their active forms are released by the matrix into the intramedullary space as a result of bone resorption by osteoclasts (26, 27). Moreover, as proposed by Paget (28), bone can be described as a suitable environ-
ment for the storage of abundant amounts of growth factors and for the growth of tumors that can induce osteoclasts (29). On the other hand, the release of active forms of growth factors as a result of bone resorption can be said to be necessary for prostate cancer to be viable and proliferate in the intramedullary space, and for bone metastases to become established and progress. Accordingly, it was thought that because bone resorption is suppressed and the active form of growth factors is depleted when rhOCIF is administered early, the tumor burden would be much smaller and the formation of bone tumors would be suppressed significantly. Because the results showed that tumor burden was in fact much smaller and the formation of bone tumors was prevented significantly in response to OCIF early treatment, it appears that rhOCIF will be useful as a prophylactic treatment for prostate cancer patients, who are at high risk of bone metastasis. Moreover, whereas even administration of rhOCIF failed to reduce the burden of bone tumors already established in the intramedullary space, it was found to be capable of preventing subsequent progression of the tumors.

Because the formation of bone tumor foci by prostate cancer is also suppressed by inhibition of osteoclastogenesis by OPG/OCIF in vivo, osteoclasts are thought to be necessary for the establishment and progression of bone tumors of prostate cancer. Nevertheless, the possibility also exists that tumor formation is suppressed by a direct action of OPG/OCIF on prostate cancer in addition to its inhibitory effect on osteoclastogenesis. However, OPG/OCIF suppresses both osteoclastogenesis and bone resorption in vitro (9, 11–13, 30, 31), and although it promotes apoptosis of osteoclasts (32–34), no effect on LNCaP cell proliferation in culture is observed even at rhOCIF concentrations sufficient to inhibit osteoclastic bone resorption in organ culture assays. Moreover, whereas OPG rapidly induces osteoclast apoptosis in vivo as well (35), it has no significant effect on s.c. LNCaP tumor burden. Thus, the growth-inhibiting effect of rhOCIF on LNCaP cells in bone appears to be a secondary effect that results from suppression of bone resorption by osteoclasts and alteration of the microenvironment of bone, and not a direct action on prostate cancer cells.

RANKL shares some homology with TRAIL, a type II transmembrane protein that induces apoptotic cell death in variety of tumorigenic cells but not in normal cells (36). This similarity suggests that OPG/OCIF might bind to TRAIL, and soluble TRAIL obtained from cell lysates does indeed bind to OPG in vitro (37). The results obtained by Holen et al. (38) showed that OPG is a potential survival factor for human prostate cancer cell lines, protecting them from TRAIL-induced apoptosis. Interestingly, OPG does not appear to bind to membrane-bound (native) TRAIL (9). At physiological temperatures, the binding affinity of OPG to TRAIL is reduced significantly relative to other TRAIL-binding proteins (e.g., DR4, DR5, DcR1, and DcR2; Ref. 39). At 37°C, TRAIL had the highest affinity for DR5 and the lowest affinity for OPG, and OPG is unable to compete with TRAIL binding to immobilized DR5 at 37°C. This is perhaps not surprising, as OPG/OCIF is the least related of the TRAIL receptor genes based on sequence homology, structure, and ligand recognition (i.e., high affinity of OPG/OCIF for RANKL, Ref. 40). The physiological significance of an action of OPG/OCIF through blocking of TRAIL is unknown.

If OPG/OCIF were capable of inhibiting the growth of prostate cancer in bone without any adverse reactions, OPG/OCIF therapy might considerably improve the quality of life of patients with bone metastases of prostate cancer. No abnormal findings have been observed in histological studies in experiments in which OPG/OCIF has been administered to animals (11, 13), and mice that were made to express excessive OPG developed normally and had a normal life expectancy (11). Furthermore, because there have been no reports of administration of rOPG/OCIF having any significant effect on the immune system, OPG/OCIF toxicity appears to be very low. Moreover, because efficacy was also observed when rOPG was used to inhibit bone resorption in postmenopausal women and in patients with breast cancer-related osteolytic or mixed bone metastases clinically without any adverse reactions (41, 42), it seems possible to administer it safely. In addition, it may be possible to use novel therapeutic agents that block RANKL alone without acting on TRAIL (i.e., soluble form of RANK, OPG/OCIF mutant with extremely low affinity to TRAIL) more safely.

In conclusion, osteoclasts play an important role in bone tumor by prostate cancer. Not only does early OCIF treatment suppress the establishment of new bone tumors, but late OCIF treatment suppresses the progression of existing tumors. It seems possible to achieve a therapeutic effect on bone tumors of prostate cancer by administering OPG/OCIF at the proper time. Additional research to establish the efficacy of OPG/OCIF in the treatment of bone metastases of prostate cancer appears to be needed in the future.

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Osteoprotegerin/Osteoclastogenesis Inhibitory Factor Decreases Human Prostate Cancer Burden in Human Adult Bone Implanted into Nonobese Diabetic/Severe Combined Immunodeficient Mice

Hiroyuki Yonou, Naoki Kanomata, Masato Goya, et al.


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