Growth Inhibition of Human Prostate Cancer Cells in Human Adult Bone Implanted into Nonobese Diabetic/Severe Combined Immunodeficient Mice by a Ligand-Specific Antibody to Human Insulin-Like Growth Factors

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ABSTRACT

Advanced prostate cancer frequently involves the bone that has the largest content of insulin-like growth factors (IGFs). However, the role of bone-derived IGFs in bone metastasis of prostate cancer has not been studied extensively because of the lack of a reliable animal model. Therefore, we investigated whether a novel antibody directed against human IGF-I and IGF-II (KM1468) could inhibit the development of new bone tumors and the progression of established bone tumors in nonobese diabetic/severe combined immunodeficient mice implanted with human adult bone. We first confirmed that KM1468 bound specifically to human IGF-I, human IGF-II, and mouse IGF-II but not to insulin. It also blocked autophosphorylation of the type I IGF receptor induced by the binding of IGFs in human-type I IGF receptor-overexpressing BALB/c 3T3 cells, and it inhibited the IGF-stimulated growth of MDA PCa 2b cells in vitro. Then mice were injected intraperitoneally with KM1468 once weekly for 4 weeks either immediately or 4 weeks after inoculation of MDA PCa 2b cells. KM1468 markedly and dose-dependently suppressed the development of new bone tumors and the progression of established tumor foci, as determined by histomorphometry, and it also decreased serum prostate-specific antigen levels, compared with the control. This is the first report of an IGF ligand-specific inhibitory antibody that suppresses the growth of human prostate cancer cells in human adult bone. These results indicate that the IGF signaling axis is a potential target for prevention and treatment of bone metastases arising from prostate cancer.

INTRODUCTION

It has been well documented that advanced prostate cancer preferentially metastasizes to bone (1–3) that is generally related to skeletal morbidity and mortality in the prostate cancer patients (4, 5). Although androgen deprivation is the only effective systemic therapy available for metastatic prostate cancer, its efficacy is eventually limited because of the development of resistance to androgen deprivation by prostate cancer. Meanwhile, 15% of patients do not respond to androgen deprivation. Despite the importance of this clinical problem, only a few alternative treatments that are satisfactory for the prevention or treatment of prostate cancer metastasis to bone are currently available. Therefore, more effective treatment is urgently needed.

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Bone contains the largest store of insulin-like growth factor (IGF)-I and IGF-II in the body (6, 7), and IGFs have been implicated in the pathogenesis, cell proliferation, and cell survival in many cancers (8–10). IGFs exert their activity predominantly via the type I IGF receptor (IGF-IR), and IGF-IR is present on prostate cancer cells. A recent study of matched pairs of primary prostate cancers and corresponding bone lesions showed that up-regulated expression of IGF-IR in primary prostate cancers persists in metastatic lesions (11), and prostate cancer cells have been found to secrete certain IGF binding protein proteases, as represented by PSA (12, 13). Therefore, it is conceivable that the presence of IGF binding protein protease increases the local availability of IGFs, which in turn induces additional growth of prostate cancer in bone. These above findings suggest that IGFs play an important role in the metastasis of prostate cancer to bone, which would support the concept of the IGF axis as a potential target for the treatment of metastatic prostate cancer. Some investigators have recently reported successful in vivo suppression of tumor growth and progression in vivo by controlling IGF-IR function by an antisense strategy (14), with neutralizing antibody (15) or by dominant-negative truncation of IGF-IR (16), but the inhibitory effect of IGFs neutralizing antibody itself on tumor formation and progression by prostate cancer remains to be established.

To confirm the importance of bone-derived IGFs, not circulating IGFs in the blood, to the development of new bone tumors and the progression of established bone tumor in prostate cancer, and to evaluate the therapeutic utility of anti-IGF antibody against bone metastasis by prostate cancer, we used a NOD/SCID—human adult bone model in which human adult bone fragment was implanted subcutaneously into the flank of the mice and provided an appropriate human bone environment with anatomic and hematopoietic features (17). MDA PCa 2b cells are the currently available human prostate cancer cell line that was established from a bone metastasis of prostate cancer, and they exhibit typical features, i.e., express prostate-specific antigen (PSA) and androgen receptors, and display androgen-mediated growth (18). MDA PCa 2b cells are also capable of giving rise to osteoblastic bone tumor, the same as developed clinically (19). Therefore, because the clinical pathological changes in bone are reproduced, they are suitable for research on bone metastasis.

In the present study we investigated the ability of a novel monoclonal antibody against human IGFs (KM1468) to inhibit the development of new bone tumors by prostate cancer and the progression of established bone tumor of prostate cancer in a MDA PCa 2b cell-bearing NOD/SCID—human adult bone model.

MATERIALS AND METHODS

Cell Culture. BALB/c 3T3 fibroblasts overexpressing human IGF-IR (3T3-IGF-IR) were kindly provided by Drs. Axel Ullrich and Reiner Lammers, and androgen-responsive human prostate cancer cell line MDA PCa 2b was
purchased from American Tissue Culture Collection (Manassas, VA). 3T3-IGF-IR cells were propagated in DMEM (Sigma Chemical Co., St. Louis, MO) with 10% fetal bovine serum (FBS, Sigma), and MDA PCa 2b cells were maintained in F12K(+)+ medium with 20% FBS and 50 μg/ml gentamicin (Sigma). F12K(+) medium was F12K (Sigma) supplemented with 25 ng/ml cholera toxin (List Biological Laboratories, Inc., Campbell, CA), 10 ng/ml recombinant human epidermal growth factor (Invitrogen Life Technologies, Inc., Carlsbad, CA), 100 μg/ml hydrocortisone (Sigma), 1.0E-10 M dithiothreitol (Sigma), and ITS-X (Life Technologies, Inc.).

Monoclonal Antibody. A monoclonal antibody KM 1468 (rat IgG2b) was established by immunizing female SD rats (SLC, Shizuoka, Japan) with mBSA-hIGF-I, the recombinant human IGF-I (R&D Systems, Minneapolis, MN) conjugated with methylated bovine serum albumin (methylated BSA; Sigma Chemical Co.) as described previously (20). KM1468 antibody was purified from ascites fluid by caprylic acid precipitation and ammonium sulfate precipitation. A rat monoclonal antibody, KM1762 (IgG 2a), was used as a control antibody in appropriate experiments.

Competitive Inhibition Enzyme-Linked Immunosorbent Assay for KM1468 Binding to mBSA-hIGF-I. Various dilutions of growth factor proteins in PBS were added to each well of enzyme-linked immunosorbent assay plates coated with 0.1 μg/ml mBSA-hIGF-I and an equal volume of 0.6 μg/ml KM1468 was added. The plates were incubated at room temperature for 1 hour, and bound monoclonal antibodies were detected with horseradish peroxidase-labeled goat antirat IgG (Dako, Glostrup, Denmark). The binding activity was quantified by measuring the absorption at 415 nm on a microplate reader Enmax (Wako Pure Chemical, Tokyo, Japan). The growth factor proteins used were human IGF-I, human epidermal growth factor, human Platelet-derived growth factor-AB, and human basic fibroblast growth factor, all purchased from Peprotech (London, United Kingdom), human insulin purchased from Wako, Tokyo, Japan, and human transforming growth factor (TGF)-β1, mouse IGF-I, and mouse IGF-II purchased from R&D Systems.

Immunoprecipitation and Western Blot Analysis. 3T3-IGF-IR cell lysates were prepared as described previously (21). Briefly, lysates were centrifuged at 14,000 × g for 30 min at 4°C, and the protein concentration was measured using the Bio-Rad protein assay dye reagents. Then lysates containing 500 μg of protein were subjected to immunoprecipitation followed by Western blot analysis. IGF-IR was immunoprecipitated with anti-IGF-IR monoclonal antibody (clone oIR3; Oncogene Research Products, Boston, MA) for 3 hours at 4°C. The precipitated proteins (50 μg) were separated by 7.5% SDS-PAGE, transferred to a polyvinylidene difluoride membrane, probed overnight at 4°C with an antiphospho-tyrosine monoclonal antibody (clone 4G10; Upstate Biotechnology, Lake Placid, NY), and visualized by enhanced chemiluminescence (Amersham). Phosphorylation of IGF-IR was quantified by scanning the gel with an AE-690M densitometer (Atto, Tokyo, Japan). The 50% neutralizing dose (ND50) of KM1468, which blocked 50% of the phosphorylation of the IGF-IR induced by various IGFs relative to that in KM1468-unreated cells, was calculated using Graphpad Prism software for Windows (ver. 3.03, GraphPad Software, San Diego, CA).

Flow Cytometry. Cells were incubated for 15 minutes on ice with 2 μg/ml mouse anti-human IGF-IR antibody (Ab-1, Oncogene, Boston, MA) or a control mouse IgG2b antibody (Dako) in PBS containing 3% FBS and 0.1% NaN3. The cells were then washed and incubated for 15 min on ice in the dark with fluorescein-isothiocyanate-labeled anti-mouse secondary antibody. After washing twice in PBS, the cells were stained with propidium iodide before trypan blue exclusion assay.

Proliferation Assay. MDA PCa 2b cells (5 × 106/well) were seeded on six-well plates in F12K(+) supplemented with 20% FBS and were allowed to adhere for 48 hours before the procedure. The cells were then treated for 48 hours with various concentrations of IGF-I (1–100 ng/ml; R&D Systems, Inc.) and/or KM1468 (0.1–10 μg/ml) in serum-deprived F12K alone. At the end of the period, the cells were dislodged by trypsinization and counted by trypan blue exclusion assay.

Animal Care and Human Adult Bone Implantation. Male NOD/SCID mice were obtained from CLEA Japan, Inc. (Tokyo, Japan). Mice were maintained at the National Cancer Center Research Institute East under specific pathogen-free, temperature-controlled-air conditions throughout this study, according to the Institutional Guidelines. The mice used in all of the experiments were in the age range of 42 to 73 years; mean, 60.8) who underwent pulmonary lobectomy in the Division of Thoracic Oncology, National Cancer Center Hospital East. Implantation of bone fragments into NOD/SCID mice was performed as described previously with minor modifications (17). Briefly, after anesthetizing the animals with diethyl ether, ~1 cm3 of morcellized bone fragments were subcutaneously implanted into the left flank through a small skin incision within 1 hour after their procurement.

Induction of Bone Tumors. At 4 weeks after bone implantation, single-cell suspensions (4 × 106 cells/100 μl of serum-free medium) of MDA PCa 2b cells were injected into the marrow spaces of the implanted human adult bone using a 27-gauge needle. Only cells with >90% viability (determined by trypan blue exclusion) were injected to produce tumors.

Induction of Subcutaneous Tumors. Single-cell suspensions were prepared as described previously. Twenty mice were injected s.c. into the left flank with 4 × 106 cells in 100 μl of serum-free medium using a 22-gauge needle. Ten mice were each examined immunohistochemically by staining with anti-PSA antibody at 4 and 8 weeks after injection.

Protocol of KM1468 Antibody Treatment. KM1468 and KM1762 solutions were prepared with sterile PBS and stored at -80°C until used. As shown in Fig. 1, both solutions were injected intraperitoneally according to the following two protocols.

Protocol 1: Effect of KM1468 on the Development of Bone Tumors. KM1468 at a dose of 0.01, 0.1, or 2 mg/kg (n = 10, 6, or 9 per dose group, respectively) was injected intraperitoneally into male NOD/SCID mice at weekly intervals for 4 weeks starting on the day of human adult bone injection with MDA PCa 2b cells. KM1762 (n = 9; 2 mg/kg) was used as the negative control.

Protocol 2: Effect of KM1468 on Established Bone Tumors. KM1468 at a dose of 0.1, 2, or 10 mg/kg (n = 9, 6, or 7 per dose group) was injected intraperitoneally into male NOD/SCID mice at weekly intervals for 4 weeks starting from 4 weeks after human adult bone injection with MDA PCa 2b cells. KM1762 (n = 9; 10 mg/kg) was used as the negative control.

In both protocols, at the end of the experiment, all of the mice were sacrificed for immunohistochemical staining.

Immunohistochemical Staining of IGF-IR and PSA. Harvested human adult bone was fixed in 10% neutral-buffered formalin, decalcified in 10% EDTA solution (Wako, Osaka, Japan), and paraffin-embedded 4-μm-thick sections were prepared and stained with H&E by standard procedures. Immunostaining was performed by the streptavidin-biotin method as described previously (22). Briefly, tissue sections were stained either with a mouse antihuman IGF-IR monoclonal antibody (Chemicon, Temecula, CA) at a 1:100 dilution with a rabbit anti-PSA polyclonal antibody (Dako) at a 1:200 dilution. A negative control for each section was prepared by substituting normal mouse and rabbit serum (Dako), respectively, for the primary antibody.

To histomorphometrically analyze tumor burden, tissue sections were cut at four levels far apart enough to avoid replicate sampling a single surface event. Total tissue area and total tumor area were determined with a computed image analyzer (KS 300 system version 3.00, Carl Zeiss, Oberkochen, Germany). In brief, all of the tumor tissue areas that were immunostained by anti-PSA antibody on each specimen were traced. Then the traced outlines were used to calculate the total bone tissue area and total tumor tissue area on the display for

Protocol 1: Early treatment protocol

0
Cell
KM1468
Sacrifice

Week 4

Week 8

Protocol 2: Late treatment protocol

0
Cell
KM1468
Sacrifice

Week 4

Week 8

Fig. 1. Summary of the treatment protocol. MDA PCa 2b cells were inoculated into human adult bone implanted in male NOD/SCID mice on day 0. Various weekly doses of KM1468 were administered intraperitoneally. After 4 weeks, all mice were sacrificed for immunohistochemistry and measurement of serum PSA as described in Materials and Methods.
The half-life time was 85 hours, and the serum KM1468 concentration decreased gradually. As shown in Fig. 2, KM1468 inhibited human IGF-I, human IGF-II, and mouse IGF-II. The calculated ND50 of KM1468 for various IGFs was 0.201 ± 0.024 μg/mL, and 0.036 μg/mL, respectively (Fig. 2C). In the same assay using 3T3-IGF-IR cells, even at a KM1468 concentration of up to 100 μg/mL, the antibody did not show a ND50 for insulin.3

Pharmacokinetics of KM1468 Antibody in Mice. To assess the pharmacokinetics of KM1468 in mice, we measured serial serum concentrations after a single intraperitoneal injection (200 μg). As shown in Fig. 3, the time to reach the maximum serum concentration and the maximum serum concentration of KM1468 were 20 to 40 hours and 50 to 55 μg/mL, respectively. After reaching maximum serum concentration, the serum level of KM1468 decreased gradually. The half-life time was 85 hours, and the serum level of KM1468 concentration was still in the range of 15 μg/mL at 168 hours after intraperitoneal injection.

RESULTS

Characterization of KM1468 Antibody for Binding Specificity and Suppression of Autophosphorylation of the IGF-IR. A competition assay was performed against various growth factors to confirm the specificity of the KM1468 antibody (Fig. 2A). The binding of KM1468 to methylated BSA-IGF-I conjugate was dose-dependently inhibited by three growth factors, which were human IGF-I, human IGF-II, and mouse IGF-II. Mouse IGF-I and human insulin did not exhibit any affinity for KM1468. Human epidermal growth factor, basic fibroblast growth factor, platelet-derived growth factor-AB, and TGF-β1, which are the other major growth factors present in bone, did not inhibit the binding of KM1468 to IGF-I. These results indicated that KM1468 only showed reactivity with human IGF-I, human IGF-II, and mouse IGF-II among the growth factors tested. Autophosphorylation of the tyrosine residues of the β-subunits of the type I IGF receptor is essential for IGF-induced signaling (23, 24). Accordingly, we investigated the effect of KM1468 on autophosphorylation of the IGF-IR after exposure of 3T3-IGF-IR cells to 10 ng/mL of IGF-I or 10 ng/mL of IGF-II. As shown in Fig. 2B, KM1468 inhibited human IGF-I, IGF-II, and mouse IGF-II-stimulated receptor autophosphorylation in a dose-dependent manner, whereas it did not inhibit mouse IGF-I-stimulated autophosphorylation. These in vitro findings indicated that KM1468 inhibited autophosphorylation of the IGF-IR in a dose-dependent manner and neutralized the actions of human IGF-I, human IGF-II, and mouse IGF-II. The calculated ND50 of KM1468 for human IGF-I, human IGF-II, and mouse IGF-II was 0.201 μg/mL, 0.024 μg/mL, and 0.036 μg/mL, respectively (Fig. 2C). In the same assay using 3T3-IGF-IR cells, even at a KM1468 concentration of up to 100 μg/mL, the antibody did not show a ND50 for insulin.3

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Histologic Characteristics and PSA and IGF-IR Expression in MDA PCa 2b Cells. To determine whether KM1468 was capable of inhibiting ligand-stimulated prostate cancer cell proliferation, we used cell line MDA PCa 2b established from a bone metastasis of prostate cancer. On histopathological examination, all 9 of the mice injected with MDA PCa 2b cells (4 × 10⁶ cells) into implanted human adult bone developed bone tumors. An osteoblastic reaction, characterized by new bone formation and osteoblast proliferation on the bone around the tumors, was apparent compared with the control mice at 4 weeks after injection (Fig. 4A). No palpable tumors were found during 8 weeks of observation in the subcutaneous tumor model. At 4 weeks after s.c. injection, no tumors were detected by immunohistochemical examination. At 8 weeks after injection, 3 of 10 mice had small PSA-positive tumors (8.24 ± 1.78 mm³). Moreover, the serum PSA values of the mice without tumors on immunohistochemical examination were below the detection limit. This cell line is known to exhibit typical characteristics of human prostate cancer, such as expression of PSA (Fig. 4B) and androgen receptors, and androgen...
responsive proliferation (19). MDA PCa 2b cells expressed IGF-IR on
their membrane surfaces as confirmed by flow cytometry and
immunohistochemistry. Intense peripheral staining with anti-IGF-IR an-
tibody was observed after MDA PCa 2b cells were injected into
human adult bone-engrafted NOD/SCID mice, reflecting the location
of IGF-IR on the cell membrane (Fig. 4C).

Neutralizing Activity of KM1468 on IGF-Stimulated Prolifer-
ation of MDA PCa 2b Cells In vitro. As shown in Fig. 5, human
IGF-I stimulated proliferation of MDA PCa 2b cells in a dose-
dependent manner. These findings suggested that MDA PCa 2b cells
possess a functional IGF signal pathway. KM1468 inhibited IGF-I-
stimulated proliferation in MDA PCa 2b cells in a dose-dependent
manner. The inhibition of MDA PCa 2b cell proliferation by KM1468
alone indicates the presence of an IGF autocrine loop in MDA PCa 2b
cells. In our preliminary study, the IGF-I and IGF-II mRNA levels of
MDA PCa 2b cells determined by quantitative real-time reverse
transcription-PCR were 1.36 ± 0.11 *10^6 copies/100 ng total RNA and
1.23 ± 0.40 *10^4 copies/100 ng total RNA, respectively. The negative
control, KM1762, had no effect on IGF-I–dependent cell prolifera-
tions (Fig. 5, lane 9). Because KM1468 inhibits IGF-binding to
IGF-IR and the resultant proliferation, we extended the analysis in
vivo to investigate KM1468 for an inhibitory effect on tumor growth.

Effect of KM1468 on the Development of Bone Tumors in
NOD/SCID Mice Injected with MDA PCa 2b Cells (Protocol 1). To
determine the effect of KM1468 on the development of new bone
tumors in NOD/SCID mice, age-matched mice were randomly as-
signed to four subgroups based on animal weight at the start of the
experiment and then intraperitoneally injected with KM1468 at a dose
of 0.01, 0.1, or 2 mg/kg in 200-μL volume immediately after injection
of MDA PCa 2b cells. Antibody injections were performed once
weekly for 4 weeks. The control group was administered rat IgG2a
(KM1762) at a dose of 2 mg/kg. Histomorphometric studies of the
control group 4 weeks after the injection revealed the mean tumor area
of 2.33 ± 0.25 mm^2 and the percentage of tumor area of the implanted
human adult bone of 5.8 ± 0.4%. A dose-dependent markedly smaller
mean tumor area and smaller proportion of implanted human adult
bone occupied by tumor was observed in the animals treated with
KM1468 compared with the control (Fig. 6, A and B). At the highest
dose of KM1468 (2.0 mg/kg), mean tumor area and mean percentage
of tumor area were significantly lower (P < 0.001), and no tumor at
all was detected in 3 of the 10 animals. In protocol 1, an osteoblastic
reaction was hardly detected in the specimens after KM1468 treat-
ment. As shown in Fig. 6C, the serum PSA values in the MDA PCa 2b
tumor-bearing animals treated with KM1468 were markedly and

Fig. 3. Profile of serum KM1468 antibody levels after a single intraperitoneal injection
in mice. Eight time points were tested using 3 mice per time, and data are shown as the
mean; bars, ±SD. (Cmax, maximum serum concentration; Tmax, time to reach the maxi-
mum serum concentration; T1/2, half-life time).

Fig. 4. The histologic characteristics of MDA PCa 2b tumors growing in human adult
bone implanted into male NOD/SCID mice at 4 weeks after inoculation of tumor cells, and
effect of IGF-I and KM1468 on in vitro proliferation of MDA PCa 2b cells. A, H&E-
stained section of implanted human adult bone (×200). WB, woven bone; LB, lamellar

Fig. 5. Serum-free cultures of MDA PCa 2b cells were treated for 48 hours with
different doses of IGF-I (lanes 1–4), KM1468 antibody in the presence or absence of 100
ng/mL of IGF-I (lanes 5–8) or 10 μg/mL of KM1762 antibody plus 100 ng/mL of IGF-I
(lane 9). At the end of treatment, cells were counted by trypan blue dye exclusion. Data
are expressed as the mean of triplicate determinations in duplicate experiments; bars,
±SE. *, P < 0.05, **, P < 0.001 compared with control. *, P < 0.05, **, P < 0.001
compared with 100 ng/mL of IGF-I.
dose-dependently lower than in the control, and they paralleled the reduction of tumor area.

**Effect of KM1468 on Progression of Bone Tumor Growth in NOD/SCID Mice Injected with MDA PCa 2b Cells (Protocol 2).** To determine the effect of KM1468 on the progression of established bone tumors in NOD/SCID mice, animals were randomized into four equal groups based on animal weight at the start of the experiment, and KM1468 and control solution were administrated by intraperitoneal injection starting 4 weeks after injection of MDA PCa 2b cells. KM1468 was tested at doses of 0.1, 2, and 10 mg/kg. Control injections contained KM1762 at a dose of 10 mg/kg. Treatment was continued once weekly for 4 weeks as described above. The control group had the mean tumor area of 13.7 ± 0.83 mm² and the percentage of tumor area of implanted human adult bone of 37.2 ± 1.53% at 8 weeks after cell injection. Compared with the control (8 weeks), the mean tumor area and percentage of tumor area were significantly smaller at all doses of KM1468 tested (P < 0.001; Fig. 6, D and E). The mean tumor area and percentage of tumor area increased by 6-fold and 6.4-fold during the 4-week period from 4 to 8 weeks after MDA PCa 2b cell injection in...
the control group. However, the respective increases were only 1.5-fold and 1.7-fold when 10 mg/kg KM1468 was administered for 4 weeks from 4 weeks after cell injection, so tumor progression was markedly inhibited by KM1468. In protocol 2, an osteoblastic reaction weaker than that in control mice was recognized around the residual tumors after KM1468 treatment. The serum PSA value in the control group was 45.4 ± 8.9 ng/mL at 8 weeks after cell injection, and it was markedly and dose-dependently decreased by KM1468. The highest dose of KM1468 (10 mg/kg) resulted in 60% reduction of the serum PSA values compared with the control (Fig. 6F). Numerous cancer cells with pyknotic nuclei were apparent in hematoxylin and eosin-stained sections from tumors treated with KM1468 compared with the control, indicating the induction of apoptosis by KM1468 (Fig. 6G).

**Body Weight Measurement.** In none of the studies was any change in body weight noted between the KM1468-treated group and the control group, nor were there any differences in the weight (P = 0.89, P = 0.82, P = 0.51, and P = 0.183, respectively) or histologic findings of the internal organs, including lung, liver, spleen, and kidney.

**DISCUSSION**

In the present MDA PCa 2b cells injected into implanted human adult bone yielded a higher incidence of tumor formation than when injected subcutaneously (9 of 9 mice for injection into human adult bone versus 0 of 10 mice for subcutaneous injection at 4 weeks; 9 of 9 mice versus 3 of 10 mice at 8 weeks). The tumor area was larger for bone tumors than subcutaneous tumors (2.33 ± 0.25 mm² versus 0 mm² at 4 weeks; 14.25 ± 0.83 mm² versus 8.24 ± 1.78 mm² at 8 weeks). MDA PCa 2b tumors appeared to grow more rapidly in bone than in subcutaneous tissue. These results combined with our previous observation that tail-vein-injected human prostate cancer cells specifically metastasized to subcutaneously implanted human adult bone but not to other implanted adult human lung and mouse organs (17) indicated that human bone provides a favorable environment for the growth of prostate cancer cells as suggested previously by Paget (25) in the “seed and soil” theory of metastasis.

In recent years it has become evident that growth factors are involved in the tumorigenicity, growth, and spread of various cancers from their primary organs (26–29). Bone is a reservoir of growth factors, and IGF-I and IGF-II are two of the most abundant growth factors for a wide variety of cell types. Therefore, the IGF signaling axis is a potential target for the therapy of prostate cancer bone metastases. We were interested in determining whether the rat monoclonal anti-IGF antibody KM1468 would have an inhibitory effect on prostate cancer cell growth in human bone. We investigated the in vivo efficacy of KM1468 on bone tumors produced by prostate cancer in human adult bone according to two protocols. Postulating bone tumor foci that could not be detected clinically, the early treatment protocol was devised to investigate whether KM1468 would prevent the establishment of new bone tumors, whereas the late KM1468 treatment protocol was devised after postulating a situation in which KM1468 is administered after obvious bone tumor has been observed clinically. KM1468 significantly suppressed both bone tumor formation and progression by prostate cancers, and the serum PSA value was also significantly decreased by KM1468. These findings provide strong evidence for potent in vivo antitumor effect of IGF neutralizing antibody on bone tumor formation by prostate cancer.

Recent histomorphometric and biochemical studies have shown a heterogeneous mixture of osteolytic and osteoblastic lesions at the sites of bone involvement by prostate cancer even in metastases characterized by an osteoclastic appearance on imaging, and these findings suggested an increase in osteoclastic activity as well as osteoblastic activity in prostate cancer patients with bone metastases (30–34). Because osteoclastic activity is associated with bone resorption, increasing evidence has indicated that osteoclast-induced osteolysis is necessary for tumor progression and invasion in bone (33, 34). Osteoclastic bone resorption releases bone-stored growth factors, represented by IGF-I and IGF-II, which in turn facilitate the growth of tumor cells in bone (35–37). Because osteoblasts also secrete both IGF-I and IGF-II (38), disruption of IGF binding from their receptors by KM1468 in bone may result in inhibition of both the development of new bone tumors and the progression of established bone tumors by prostate cancer cells.

In our previous study, we demonstrated that PSA secreted by prostate cancer cells proteolytically activates latent TGF-β1 by its serine protease activity (39). Several in vitro studies have shown that PSA also cleaves the IGF binding proteins (12, 13) that modulate IGF function, generally by inhibiting it. The presence of PSA may enhance and liberate free IGFs into the microenvironment, which would enable prostate cancer that had metastasized to bone to produce a high concentration of free IGFs around the prostate cancer cells. These results raise the possibility that growth inhibition of bone-metastatic prostate cancer by anti-IGF antibody may become an important method for treatment.

If KM1468 were capable of inhibiting the growth of prostate cancer in bone with less toxicity to normal cells, KM1468 therapy might considerably improve the quality of life of patients with bone metastases of prostate cancer. Administration of KM1468 did not affect the growth of NOD/SCID mice over 4 weeks of treatments, but long-term observation is needed to confirm its safety, because IGFs are required growth factors for a wide variety of cell types.

In conclusion, the present study is the first to report potent activity of IGF neutralizing antibody in decreasing tumor growth in vivo. KM1468 effectively inhibited both the development of new bone formation and the progression of established bone tumors by MDA PCa 2b cells in a NOD/SCID-human adult bone model and may have a therapeutic effect on the bone tumors formed by prostate cancer. When additional studies are performed to understand the mechanism of tumor growth inhibition by KM1468 in vivo, sections from MDA PCa 2b tumors may need to be stained for the proliferation marker Ki67 and apoptosis markers such as active caspase 3 or terminal deoxynucleotidyl transferase-mediated nick end labeling, and confirmation of the safety of long-term treatment of KM1468 is also needed.

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Growth Inhibition of Human Prostate Cancer Cells in Human Adult Bone Implanted into Nonobese Diabetic/Severe Combined Immunodeficient Mice by a Ligand-Specific Antibody to Human Insulin-Like Growth Factors

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