Targeting Platelet-Derived Growth Factor Receptor on Endothelial Cells of Multidrug-Resistant Prostate Cancer

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Background: Inhibiting phosphorylation of platelet-derived growth factor receptor (PDGFR) by treatment with the PDGFR kinase inhibitor imatinib and the chemotherapeutic agent paclitaxel reduces the incidence and size of human prostate cancer bone lesions in nude mice. Because tumor cells and tumor-associated endothelial cells express activated PDGFR, the primary target for imatinib has been unclear.

Methods: We selected multidrug-resistant human PC-3MM2 prostate cancer cells (termed PC-3MM2-MDR cells) by culturing them in increasing concentrations of paclitaxel. PC-3MM2-MDR cells were implanted into one tibia of 80 nude mice. Two weeks later, the mice were randomly assigned to receive distilled water (control group), paclitaxel, imatinib, or imatinib plus paclitaxel for 10 weeks (20 mice per group). Tumor incidence and weight, bone structure preservation and osteolysis, and the incidence of lymph node metastasis were determined. The phosphorylation status of PDGFR on tumor cells and tumor-associated endothelial cells and levels of apoptosis were examined with immunohistochemical analyses. Microvessel density was assessed as the number of cells expressing CD31/platelet endothelial cell adhesion molecule 1 (PECAM-1). All statistical tests were two-sided.

Results: PC-3MM2-MDR cells were resistant to paclitaxel and imatinib in vitro. Treatment of implanted mice with imatinib plus paclitaxel led to statistically significant decreases in bone tumor incidence (control = 19 mice with tumors of 19 mice total; imatinib plus paclitaxel = four of 18 mice; \( P < .001 \)), median tumor weight (control = 1.3 g, interquartile range [IQR] = 1.0–1.9; imatinib plus paclitaxel = 0.1 g, IQR = 0–0.3; \( P < .001 \)), bone lysis, and the incidence of lymph node metastasis (control = 19 of 19 mice total; imatinib plus paclitaxel = three of 18 mice; \( P < .001 \)). Treatment with imatinib alone had similar effects, and imatinib treatment also inhibited phosphorylation of PDGFR on tumor cells and tumor-associated endothelial cells and increased the level of apoptosis of endothelial cells, but not tumor cells. Treatment with imatinib and more so with imatinib and paclitaxel decreased mean vessel density (three CD31/PECAM-1–positive cells, 95% confidence interval [CI] = 0 to 9; and control group = 38 CD31/PECAM-1–positive cells, 95% CI = 17 to 59) (\( P < .001 \)), which was followed by apoptosis of tumor cells.

Conclusion: Tumor-associated endothelial cells, rather than tumor cells themselves, appear to be the target for imatinib in prostate cancer bone metastasis.


The major cause of death from prostate cancer is from metastases that are resistant to conventional therapies. Genetic instability of tumor cells, leading to biological heterogeneity, is largely responsible for the development of hormone-refractory prostate cancer (I–4), and selection pressures by chemotherapeutic agents result in the emergence of cells that are resistant to chemotherapy (5–10). These resistant cells often express the MDR1 gene and its product, P-glycoprotein (11–13). Methods to overcome multidrug resistance by blocking the action of the transmembrane drug efflux pump (14–16) or by using liposomal encapsulation of cytotoxic drugs (17), immunotherapeutic monoclonal antibody targeting P-glycoprotein (18), or protein toxins that selectively kill cells expressing P-glycoprotein on their surfaces (19) are under clinical investigation.

Another protein family, multidrug resistance–associated protein (MRP), is found in multidrug-resistant prostate cancer cell lines that do not express P-glycoprotein (20). MRP1 (13,21) and MRP2 (22) have been reported to be expressed in prostate cancer cell lines, but presently few compounds that can modulate MRP1–associated multidrug resistance are known. Glutathione and glutathione S-transferase detoxification systems are another drug-resistance mechanism that may protect cancer cells against the lethal effects of chemotherapy (23). Inactivation of glutathione S-transferase \( \pi \) is related to early steps of prostate carcinogenesis (24), whereas hormone-independent disseminated prostate cancers clearly express glutathione S-transferase \( \pi \) (25). Also, reversal of multidrug resistance in prostate cancer by challenging the glutathione pathway has been observed in vitro (26). Many changes in the regulatory processes of apoptosis apparently contribute to the malignant phenotype of prostate cancer cells (27–29), and methods to stimulate apoptosis are currently under development to overcome drug resistance. Despite progress in our understanding of the biology of multidrug resistance, however, therapeutic approaches that directly target tumor cells have failed.

To devise treatments to overcome multidrug resistance in prostate cancer, we are exploring the effects of host factors in the organ microenvironment on the progressive growth of the tumor (30). One example is the development and maintenance of vascularity (9,31–33). Because all cells in the body depend on an adequate supply of oxygen and nutrients and on the ability of the circulatory system to remove toxic molecules, therapeutic regimens directed against tumor-associated endothelial cells can destroy tumor cells, regardless of their biological heterogeneity.
We have reported statistically significant therapeutic results in an experimental model of human prostate cancer bone metastasis in nude mice treated with a combination of paclitaxel and various tyrosine kinase inhibitors directed toward platelet-derived growth factor receptor (PDGFR) or epidermal growth factor receptor (EGFR) (35–38). These experimental results have been successfully translated to clinical trials (39,40). Because both tumor cells and tumor-associated endothelial cells can express these activated growth factor receptors (35–38), however, it was not clear whether the combination treatment of paclitaxel and a tyrosine kinase inhibitor targeted tumor cells or endothelial cells. The purpose of the study was to determine whether tumor-associated endothelial cells can serve as the primary target for the PDGFR tyrosine kinase inhibitor imatinib and whether killing the endothelial cells can lead to the death of all surrounding cells that depend on a viable circulation.

**Materials and Methods**

**Cells and Cell Culture Methods**

Human prostate cancer cells PC-3MM2 (41) and PC-3MM- MDR (described below) and human MG63 osteogenic sarcoma cells (42) were maintained as monolayer cultures in minimal essential medium (MEM; Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum, sodium pyruvate (110 µg/mL), nonessential amino acids (glycine at 7.5 mg/L, L-alanine at 8.9 mg/L, L-asparagine at 13.2 mg/L, L-aspatic acid at 13.3 mg/L; l-glutamic acid at 14.7 mg/L, l-proline at 11/5 mg/L, and l-serine at 10.5 mg/L), 4 mM l-glutamine, a twofold concentrated vitamin solution (Life Technologies), and penicillin (100 IU/mL)-streptomycin (100 µg/mL) (Flow Laboratories, Rockville, MD) containing doxorubicin at 40 ng/mL in an atmosphere of 5% CO₂ and 95% air at 37 °C. Doxorubicin was present to maintain the phenotype of resistant cells. Cultures were free of mycoplasma and the following murine viruses (as assayed by M. A. Bioproducts, Walkersville, MD): reovirus type 3, pneumonia virus, K virus, Theiler encephalitis virus, ectomelia virus, and a tyrosine kinase inhibitor targeted tumor cells or endothelial cells.

**Selection of Multidrug-resistant PC-3MM2 Cells**

Cultured PC-3MM2 cells (41) were exposed to increasing concentrations of doxorubicin (0.001–100 ng/mL). The concentration of doxorubicin was increased by 0.1 to 0.5 ng/mL every 2–3 weeks. After 9 months of chronic exposure to doxorubicin at 50–60 ng/mL, these cells were established as the multidrug-resistant cell line designated PC-3MM2-MDR.

**Assay for Multidrug Resistance**

To determine whether PC-3MM2-MDR cells were resistant to multiple chemotherapeutic agents (including vinblastine at 0.001–0.5 µg/mL, actinomycin D at 0.1–250 ng/mL, doxorubicin at 0.001–100 µg/mL, and 5-fluorouracil at 0.001–0.1 µg/mL), we incubated the cells with various concentrations of the indicated chemotherapeutic agent for 96 hours and then determined the number of metabolically active cells by a tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; product M2128)] assay (43). MTT was purchased from Sigma Chemical Co. (St. Louis, MO). A MTT stock solution was prepared by dissolving 5 µg of MTT in 1 mL of phosphate-buffered saline (PBS) and filtering the solution to remove particulate matter. The solution was protected from light with aluminum foil and stored at 4 °C. After a 2- to 4-hour incubation in medium containing MTT at a final concentration of 0.42 µg/mL, cells were lysed in 100% dimethyl sulfoxide, and the amount of MTT that was metabolically converted to its formazan derivative by the viable cells was monitored in an Mr-5000 96-well microtiter plate reader at 750 nm (Dynatech, Chantilly, VA). Percent growth inhibition was calculated by the following formula: cytostasis (%) = [1 – (A/B)] × 100, where A is the absorbance of treated cells and B is the absorbance of the control cells. The concentration that inhibits the proliferation by 50% (IC₅₀) was determined by Cricket Graph program (Cricket Software, Malvern, PA). PC-3MM2-MDR cells expressing a high level of P-glycoprotein by Western blot and immunohistochemistry analyses (data not shown) were maintained in medium containing doxorubicin at 20–40 ng/mL. In vitro cytotoxicity mediated by paclitaxel and/or imatinib in the presence or absence of exogenous PDGF A AND B (5 ng/mL), as determined by cell viability, was assessed by adding increasing concentrations of paclitaxel, paclitaxel plus PDGF A AND B (5 ng/mL; human PDGF A AND B, Upstate, Charlestown, VA), paclitaxel plus imatinib (3 µg/mL), or paclitaxel plus PDGF A AND B (5 ng/mL) plus imatinib (3 µg/mL) in serum-free MEM at 37 °C for 96 hours.

**Semiquantitative Reverse Transcription–Polymerase Chain Reaction Analysis for ABC Transporters**

Total RNAs were isolated with TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA), according to the manufacturer’s recommended instructions, and cDNAs for the ABC transporters were synthesized from 1 µg of each total RNA preparation by use of oligo(dT) primers and reverse transcriptase (Reverse Transcription System; Promega, San Luis Obispo, CA). We prepared appropriate dilutions of each single-stranded cDNA for subsequent polymerase chain reaction (PCR) amplification and monitored the reactions by use of β-actin (ACTB) as a quantitative control. The primer sequences were 5′-CATCAGCAGAAGATACCTCATTCACT-3′ and 5′-TCTCCCTTAGAGAGATGGGTTG-3′, for ACTB; 5′-AGGAAAGACTGACCGTATCG-3′ and 5′-CCAACATCGTCACATCAAC-3′ for ABCB1 (44); 5′-ATGTCACGTGGAATACCAGC-3′ and 5′-GAAGACTGAACCTCCCTCT-3′ for ABC1 (45), and 5′-GAGGACTCTGCTCTAC-3′ and 5′-CAGATTGCCCATGAGAG-3′ for ABC2 (46); 5′-GCAGTCTCAGAGATGTG-3′ and 5′-AGGAAGCTGGTCTACG TGAT-3′ for ABCG2 (47). All reactions had an initial denaturation step at 94 °C for 4 minutes, followed by 18 cycles (for ACTB), 40 cycles (for ABCB1 or ABC2), 35 cycles (for ABC2), or 31 cycles (for ABC2) at 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds. All reactions took place in a Mastercycler gradient 5331 PCR machine (Eppendorf AG, Cologne, Germany). The PCR products were separated by electrophoresis on 2% agarose gels.

**Western Blot Analysis**

PC-3MM2-MDR and MG63 cells were cultured for 24 hours in serum-free MEM. PC-3MM2-MDR cells were washed in PBS, and the medium was changed to serum-free medium containing paclitaxel at 0.1 ng/mL, imatinib at 3.0 µg/mL, or both. After
1 hour, we added PDGF BB at 10 ng/mL for 15 minutes. MG63 cells were incubated with PDGF BB as a positive control. Adherent cells were washed with PBS containing 5 mM EDTA and 1 mM sodium orthovanadate and then scraped into lysis buffer (20 mM Tris–HCl [pH 8.0], 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 20 μM leupeptin, and aprotonin at 0.15 U/mL), and the mixture was incubated for 20 minutes on ice. The lysed cells were centrifuged at 16,000g for 15 minutes at 4 °C, and the supernatant was collected. Proteins in the supernatant were quantified by spectrophotometry, and a constant amount of protein was loaded per lane, resolved by sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (pore size = 0.45 μm). The membranes were incubated with 3% bovine serum albumin in Tris-buffered saline (TBS = 20 mM Tris–HCl [pH 7.5] and 150 mM NaCl) to block nonspecific binding and then probed with either a rabbit anti-human phosphorylated PDGFR polyclonal antibody (1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) or a sheep anti-mouse immunoglobulin G (IgG; 1:2000 dilution) in Tween–TBS (TTBS = 0.1% Tween 20 in TBS). Blots were then incubated with horseradish peroxidase–conjugated donkey anti-sheep IgG (1:2000 dilution; Sigma) in TTBS. Antibody-reactive protein bands were visualized with an enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ).

**Animals**

Male athymic nude mice (NCI-nu) were purchased from the Animal Production Area of the National Cancer Institute–Frederick Cancer Research Facility (Frederick, MD). The mice were housed and maintained in specific pathogen–free conditions. The facilities were approved by the American Association for Accreditation of Laboratory Animal Care and met all current regulations and standards of the U. S. Department of Agriculture, the U. S. Department of Health and Human Services, and the National Institutes of Health. The mice were used in accordance with institutional guidelines when they were 6–8 weeks old.

**Reagents for Immunohistochemistry and the Terminal Deoxynucleotidyltransferase–Mediated Nick-end Labeling Assay**

Antibodies for immunohistochemistry were purchased as follows: rabbit anti-fibroblast growth factor-2 (bFGF), rabbit anti-vascular growth factor/vascular permeability factor (VEGF/VPF), rabbit anti-EFGR, rabbit anti-EFGR, rabbit anti-PDGFA AND B (against PDGF AA and BB), rabbit anti-PDGFRα and -β, and goat polyclonal anti-phosphorylated PDGFRβ (activated PDGFRβ) were from Santa Cruz Biotechnology. Rabbit anti-phosphorylated EGFR (activated EGFR; Tyr-845) polyclonal antibody was from Cell Signaling Technology (Beverly, MA). Rat anti-mouse CD31/platelet endothelial cell adhesion molecule 1 (PECAM-1) was from Pharmingen (San Diego, CA). Mouse anti-fibronectin cell nuclear antigen (PCNA) monoclonal antibody, clone PC-10, was from Dako A/S (Copenhagen, Denmark). Rabbit anti-interleukin 8 (IL-8) polyclonal antibody was from Biosource International (Camarillo, CA). Peroxidase-conjugated goat anti-rabbit IgG, peroxidase-conjugated goat anti-rat IgG, Texas Red–conjugated goat anti-rat IgG, and fluorescein isothiocyanate–conjugated goat anti-rabbit IgG were from Jackson Research Laboratories (West Grove, CA). Peroxidase-conjugated rat anti-mouse IgG2a was from Serotec (Harlan Bioproducts for Science, Indianapolis, IN). Alexa Fluor 594–conjugated goat anti-rabbit IgG and Alexa Fluor 594–conjugated rabbit anti-goat IgG were from Molecular Probes (Eugene, OR). All secondary antibodies were polyclonal.

**Terminal deoxynucleotidyltransferase–mediated nick-end labeling (TUNEL)** was performed with a commercial apoptosis detection kit (Promega, Madison, WI) with modification as described (35–38). In brief, samples were fixed with 4% paraformaldehyde (methanol free) for 10 minutes at room temperature, washed for two 5-minute periods with PBS, and then incubated with 0.2% Triton X-100 for 15 minutes at room temperature. After being washed for two 5-minute periods with PBS, the samples were incubated with equilibration buffer (from the apoptosis detection kit) for 10 minutes at room temperature. The equilibration buffer was drained, and reaction buffer containing equilibration buffer, nucleotide mixture, and terminal deoxynucleotidyltransferase enzyme was added to the tissue sections. The sections were incubated in a humid atmosphere at 37 °C for 1 hour in the dark. The reaction was terminated by immersing the samples in 2× SSC (1× SSC = 8.765 g of NaCl and 4.41 g of sodium citrate in 1 L of H2O) for 15 minutes. Samples were washed for three 5-minute periods to remove unincorporated fluorescein–dUTP.

**Intratibial Injection of Tumor Cells**

To produce bone tumors, PC-3MM2-MDR cells were harvested from subconfluent cultures after a brief exposure to 0.25% trypsin and 0.02% EDTA. Trypsinization was stopped with medium containing 10% fetal bovine serum, and cells were washed once in serum-free medium and resuspended in Ca2+- and Mg2+-free Hanks’ balanced salt solution (HBSS). Cell viability was determined by trypan blue exclusion, and only single-cell suspensions of more than 95% viability (4 × 105 cells per 20 μL of Ca2+- and Mg2+-free HBSS) were used to produce tibial tumors. Nude mice (n = 40 mice per experiment) were anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL), and then each mouse received one 20-μL percutaneous intratibial injection of 4 × 105 cells, as previously described (35, 36). The animals tolerated the surgical procedure well, and no anesthesia-related deaths occurred.

**Therapy of Human Prostate Cancer Cells Growing in the Tibia of Athymic Nude Mice**

Imatinib, a PDGFR tyrosine kinase inhibitor that was synthesized and provided by Novartis Pharma (Basel, Switzerland), was dissolved in distilled water at 6.25 mg/mL for daily oral administration. Paclitaxel, purchased from Bristol Myers Squibb (Princeton, NJ), was diluted as indicated in water for weekly intraperitoneal injections.

Fourteen days after the implantation of PC-3MM2-MDR cells in the tibia, the mice were randomly assigned to one of four treatment groups (each with 10 mice) as follows: 1) daily oral administrations of distilled water and intraperitoneal injection of distilled water once per week (control group); 2) intraperitoneal injection of paclitaxel (8 mg/kg of body weight) once per week and daily oral administrations of distilled water (paclitaxel group); 3) a daily oral imatinib (50 mg/kg) and intraperitoneal...
injection of distilled water once per week (imatinib group); and 4) daily oral imatinib (50 mg/kg) and intraperitoneal injection of paclitaxel once per week (8 mg/kg) (imatinib + paclitaxel group). Mice were treated for 10 weeks and then killed by injection with Nembutal. Tumor incidence, size (measured with calipers, size = longest diameter × shortest diameter), and weight were measured, and the number of lymph node metastases was determined. Osteolysis, defined as decreased radiopacity and/or disruption of bone structure, was determined by digital radiography, as described below. Immunohistochemical analysis for bFGF, VEGF/VPF, IL-8, EGF, EGFR, phosphorylated EGFR, PDGF AA, PDGF BB, PDGFRα and -β, phosphorylated PDGFRα and -β, and CD31/PECAM-1, as well as fragmented DNA (TUNEL assay), was carried out as described below. This experiment was done twice. Because data from the two experiments were similar, they were analyzed together.

Immunohistochemistry for Tumor-Associated Endothelial Cells and Assessment of Apoptosis

To determine whether apoptosis of tumor-associated endothelial cells preceded apoptosis of tumor cells, we carried out another experiment that used 20 mice per treatment group; treatment groups were as described above. In this study, we randomly selected two mice with bone lesions (as determined by digital radiography) every week of treatment (for 10 weeks). The mice were killed by injection of Nembutal (1.0 mg/g of body weight), and their tibias were prepared for immunohistochemical analysis for CD31/PECAM-1 proteins to identify endothelial cells and for a TUNEL assay to identify apoptotic cells. Frozen sections of tibia tumor tissues were incubated with a protein-blocking solution (5% normal horse serum and 1% normal goat serum in PBS) for 20 minutes at room temperature and then incubated with a rat anti-mouse CD31/PECAM-1 monoclonal antibody, which recognizes human and mouse PECAM-1 (1:400 dilution; PharMingen) for 18 hours at 4 °C. After the samples were rinsed for four 3-minute periods with PBS, the slides were incubated in the dark with Texas Red–conjugated goat anti-rat antibody (1:200 dilution) for 1 hour at room temperature. Samples were then washed for two 5-minute periods with PBS containing 0.1% Brij (Fisher Scientific, Pittsburgh, PA) and for one 5-minute period with PBS. The TUNEL assay was carried out as described above. Images of stained cells were captured via a fluorescent microscope and merged digitally.

Digital Radiography, Harvest of Tumors, and Examination of Lymph Nodes

After 2, 4, 6, 8, and 10 weeks of treatment, all mice were anesthetized with Nembutal (0.5 mg/g of body weight) and placed in a prone position. Digital radiography of both hind limbs was carried out with a Faxitron digital radiography instrument (Faxitron X-Ray Corporation, Wheeling, IL). After 10 weeks of treatment, mice were killed by injection of Nembutal (1.0 mg/g). Both hind limbs of each mouse were again examined with digital radiography, and tumor incidence and size were recorded. The tumor-bearing hind leg and the tumor-free, uninjected contralateral leg of each mouse were resected at the head of the femur and weighed. Tumor weight was calculated by subtracting the weight of the tumor-free leg from that of the tumor-bearing leg. Macroscopically enlarged lymph nodes were also collected and examined histologically for the presence of metastases.

Preparation of Tissues

Tumor tissues, including the tibia and surrounding muscles, were fixed in 10% buffered formalin and embedded in paraffin for sectioning at 4 °C or were fixed in 4% paraformaldehyde containing 0.075 M lysine and 0.01 M sodium periodate (PLP) solution and embedded in Optimal Cutting Temperature (OCT) compound (Miles, Elkhart, IN) for frozen sectioning. Fixed bone tissues were decalcified and processed as previously described (48).

Immunohistochemical Determination of bFGF, VEGF/VPF, IL-8, EGF, EGFR, Phosphorylated EGFR, PDGF A and B, PDGFRα and -β, Phosphorylated PDGFRβ, PCNA, and CD31/PECAM-1 Expression in Tissues and the TUNEL Assay

Paraffin-embedded tibia tissues were cut into sections 4–6 μm thick, and the expression of bFGF, VEGF/VPF, IL-8, EGF, EGFR, phosphorylated EGFR, PDGF A and B, PDGFRα and -β, phosphorylated PDGFRβ, and PCNA was determined immunohistochemically. Sections of PLP-fixed frozen tissues were used to detect the expression of CD31/PECAM-1 and of colocalized CD31/PECAM-1 and phosphorylated PDGFRβ on TUNEL-positive apoptotic cells. Immunohistochemical staining procedures were performed as described previously (36). Internal negative controls for staining exposed to secondary antibody alone showed no specific staining. Dilutions of primary antibodies were as follows: bFGF (1:100 dilution), VEGF/VPF (1:100 dilution), IL-8 (1:25 dilution), EGF (1:100 dilution), EGFR (1:50 dilution), phosphorylated EGFR (1:50 dilution), PDGF A, B (1:100 dilution), PDGFRα and -β (1:100 dilution), phosphorylated PDGFRβ (1:100 dilution), PCNA (1:100 dilution), and CD31/PECAM-1 (1:400 dilution). Sections for the detection of bFGF, VEGF/VPF, IL-8, EGF, EGFR, phosphorylated EGFR, PDGF A and B, PDGFRα and -β, and PCNA were incubated with a protein blocking solution containing 5% normal horse serum and 1% normal goat serum for 20 minutes. Sections for the detection of phosphorylated PDGFRβ were incubated with protein-blocking solution containing 4% fish gel (cold water fish skin gelatin, 40%, Aurion; Electron Microscopy Science, Fort Washington, PA) in PBS for 20 minutes.

Immunohistochemical reactions with bFGF, VEGF/VPF, IL-8, EGF, EGFR, PDGF A and B PDGFRα and -β, PCNA, and CD31 or PECAM-1 were visualized by staining with stable diamino benzidine and counterstaining with Gill’s hematoxylin. Counterstaining with Gill’s hematoxylin or staining with Alexa Fluor 594–conjugated secondary antibody was used for immunohistochemistry of phosphorylated EGFR and phosphorylated PDGFRβ. The sections stained with immunofluorescence-tagged secondary antibody were rinsed with distilled water and mounted with Vectashield (mounting medium with 4'-6-diamidino-2-phenylindole; Vector Laboratories, Burlingame, CA), which stained nuclei with a blue fluorescence. Immunofluorescence microscopy was performed with a ×10 or ×20 objective on an epifluorescence microscope equipped with narrow-band-pass excitation filters mounted on a filter wheel (Ludl Electronic Products, Hawthorne, NY). Cells were counted at a magnification of ×100 in 0.14-mm² fields. Cells were counted in six fields per each slide.
Double Immunofluorescence Staining for CD31/PECAM-1 (Endothelial Cells), PDGFRα and -β, and phosphorylated PDGFRβ and the TUNEL Assay

PLP-fixed tibia tissues were used for colocalization of CD31 and PDGFRα and -β, and phosphorylated PDGFRβ and the TUNEL assay as previously described (36). The dilution used for CD31/PECAM was 1 : 400 and that for anti-PDGFRα, anti-PDGFRβ, and anti-phosphorylated PDGFRβ was 1 : 100. CD31/PECAM-positive endothelial cells were identified by red fluorescence from the Texas Red chromophore attached to the secondary antibody, and tumor cells and endothelial cells positive for PDGFRα or -β or phosphorylated PDGFRβ were identified by green fluorescence from the fluorescein isothiocyanate attached to the secondary antibody. Colocalization of endothelial cells stained with antibodies against PDGFRα or -β or phosphorylated PDGFRβ (endothelial cells, red + EGFR), phosphorylated EGFR, PDGFRα or -β, and phosphorylated PDGFRβ (green) yielded yellow signal obtained by two overlapping images, one red and the other green. TUNEL staining was performed as described above to yield green signal. Stained slides were observed with epifluorescence microscopy to individually select for green, red, and blue fluorescence. Blue signals were expressed by nuclei. Endothelial cells were identified by red fluorescence, and DNA fragmentation in apoptotic cells was detected by colocalized green and yellow fluorescence within the nucleus of apoptotic cells. Apoptotic endothelial cells and total endothelial cells were counted and expressed as an average of the ratio of apoptotic endothelial cells to total number of endothelial cells in five to 10 random fields of 0.011 mm². Images were captured with a Sony three-chip camera (Sony, Montvale, NJ) mounted on a Zeiss universal microscope (Carl Zeiss, Thornwood, NY) and Optimas Image Analysis software (Bioscan, Edmond, WA) installed on a Compaq computer with Pentium 5 chip, a frame grabber, an optical disk storage system, and a Sony Movigraph UP-D7000 digital color printer (Sony, Tokyo, Japan). To produce prints, images were further processed with Adobe PhotoShop software (Adobe Systems, Mountain View, CA) to make figures.

Quantification of Mean Vessel Density and PCNA

Mean vessel density was analyzed to determine the effects of treatment on tumor-associated endothelial cells. For the quantification of microvessel density and cellular proliferation in bone tumors, 10 random 0.14-mm² fields at ×100 magnification were captured for each tumor. The mean vessel density (number of CD31/PECAM-1-positive cells per field) was calculated as described previously (35–38). In brief, microvessel-like structures consisting of endothelial cells that were stained with the anti-CD31/PECAM-1 antibody were captured and counted. Cells positively stained with anti-PCNA antibody in the same fields were counted to determine the effects of treatment on the cell proliferation.

Statistical Analysis

The study was based on a 2 x 2 factorial design. Incidences of tumors and of lymph node metastasis were compared between groups with Fisher’s exact test. Differences in tumor weight among groups were assessed with the Mann–Whitney U test. The Bonferroni correction was used to adjust for multiple comparisons and to control for the overall type I error rate of .05. With a total of 25 comparisons, the Bonferroni-adjusted significance level was .002. All statistical tests were two-sided.

Analysis-of-variance models were fitted to evaluate the treatment effects of imatinib alone or paclitaxel alone on the number of PCNA-positive cells, mean vessel density (CD31/PECAM-1–positive cells), the number of TUNEL-positive, CD31/PECAM-1–expressing cells (ratio of TUNEL-positive, CD31/PECAM-1–expressing cells to total CD31/PECAM-1–expressing cells), respectively. The statistical interaction between imatinib and paclitaxel was also evaluated by including a product term of the two main effects into the model. For adjustments of unequal variances among the groups, we used the log-transformation for TUNEL-positive cells and the ratio of CD31/PECAM-1 to TUNEL, the X₁/2 and X₁/1.5 transformation for TUNEL and CD31/PECAM-1, and X₁/1.5 transformation for PCNA-positive cells. All analyses were carried out in S-plus (Insightful Corp., Durham, NC) (49).

RESULTS

Characterization of PC-3MM2-MDR Cells

The resistance of the PC-3MM2-MDR cells to chemotherapeutic agents was determined by the MTT assay, which reflects cell proliferation (42). The cells were resistant to vinblastine, actinomycin D, and doxorubicin but not to 5-fluorouracil, which acts independently of multidrug resistance–associated mechanisms (50) (data not shown). The IC₅₀ for paclitaxel was 0.03 ng/mL for PC-3MM2 cells, and it was 2.0 ng/mL for PC-3MM2-MDR cells; i.e., the IC₅₀ was 67-fold (95% confidence interval [CI] = 51-fold to 83-fold; p < .001) higher in PC-3MM2-MDR cells than in PC-3MM2 cells. The IC₅₀ of paclitaxel was not influenced by the addition of PDGF A and B (5 ng/mL), imatinib (3 μg/mL), or the combination of PDGF A and B (5 ng/mL) plus imatinib (3 μg/mL) to reaction mixtures containing paclitaxel at 0.6, 0.3, 0.15, 0.072, 0.036, or 0.018 ng/mL. Thus, PC-3MM2-MDR cells are resistant to paclitaxel alone or in combination with imatinib, regardless of the activation status of PDGFR (Fig. 1).

Because recent reports have concluded that multidrug-resistant cells express different ABC transporters that pump drugs out of cells than their parental cells (45–48), we examined the type of ABC transporters expressed by PC-3MM2-MDR and PC-3MM2 cells by reverse transcription–PCR. In these cells, we did not find a difference in expression of transporters C₁ and G₂; however, PC-3MM2-MDR cells expressed a higher level of ABC transporter B₁, which plays a critical role in resistance to paclitaxel (45–48). Neither parental nor multidrug-resistant cells expressed ABC transporter C₁ (data not shown).

To determine whether paclitaxel affects phosphorylation of PDGFR on PC-3MM2-MDR cells, we treated PC3-MM2-MDR cells with medium alone, imatinib (3 μg/mL), paclitaxel (0.1 ng/ml), or a combination of imatinib and paclitaxel for 60 minutes. The cells were washed, medium containing PDGF BB (10 ng/mL) was added to the cultures for 15 minutes, and the cells were prepared for Western blot analysis to examine the level of phosphorylated PDGFR. PC-3MM2-MDR cells treated with medium alone or medium containing paclitaxel expressed phosphorylated PDGFRβ. Treatment of PC-3MM2-MDR cells with imatinib alone or imatinib plus paclitaxel inhibited phosphorylation of the PDGFRβ (data not shown). MG63 osteosarcoma cells served as positive control (data not shown). Thus, these data confirm that
and had lymph node metastases. In comparison with the control developed large tumors (median weight = 1.1 g, IQR = 0.9 – 1.7 g) of the injected leg, and all 19 mice had lymph node metastasis (seven mice with lymph node metastases of 18 mice, P<.001). The data of two independent therapy experiments (n = 10 mice) were similar and so were combined (n = 20 mice) for analysis of tumor incidence, size, and incidence of lymph node metastasis (Table 1). All mice in the control groups (19 of 19 mice) had large tumors in the tibia and surrounding muscles (median weight of bone tumors = 1.3 g, interquartile range [IQR] = 1.0–1.9 g) of the injected leg, and all 19 mice had lymph node metastases. All mice in the paclitaxel group (18 of 18 mice) also developed large tumors (median weight = 1.1 g, IQR = 0.9–1.7 g) and had lymph node metastases. In comparison with the control group, a daily oral dose of imatinib (50 mg/kg) statistically significantly decreased tumor incidence (nine mice with tumors of 18 mice, P<.001), bone tumor weight (median weight = 0.3 g, IQR = 0–1.2 g, P<.001), and the incidence of lymph node metastasis (seven mice with lymph node metastases of 18 mice, P<.001). Tumor incidence (four mice with tumors of 18 mice, P<.001), bone tumor weight (median = 0.1 g, IQR = 0–0.3 g, P<.001), and the incidence of lymph node metastasis (three mice with lymph node metastasis of 18 mice, P<.001) were further reduced in mice treated with the combination of imatinib and paclitaxel. Digital radiographs of hind legs of representative mice from each of the four treatment groups are shown in Fig. 2. Lysis of the tibia was pronounced in control mice and in mice treated with paclitaxel, less severe in mice treated with imatinib, and least pronounced in mice treated with a combination of paclitaxel and imatinib (Fig. 2).

**Histology and Immunohistochemical Analyses**

Routine histologic examination after staining with hematoxylin–eosin revealed that PC-3MM2-MDR cells grew in the tibia and caused lysis of that bone, which allowed tumor cells to invade and grow in the surrounding musculature. Similar to previous
findings with PC-3MM2 experimental models (35,36), the expression of bFGF, IL-8, VEGF, EGF, EGFR, PDGF AA and BB, and PDGFRA and -β (Fig. 3) was elevated in tumor cells growing or adjacent to the bone but not in tumor cells growing in the muscles; however, PDGFRA was phosphorylated only in cells growing in the bone but not in tumor cells growing in the muscles, in accordance with our previous reports (33–35) that PDGFRA phosphorylation depends on cross-talk between tumor cells and host microenvironment.

The rate of tumor and endothelial cell proliferation, as measured by the presence of PCNA-positive cells, was similar in control mice (mean number of PCNA-positive cells [10 fields of 0.14 mm² at a magnification of ×10] = 58, 95% CI = 35 to 80) and mice treated with paclitaxel (mean number of PCNA-positive cells [10 fields of 0.14 mm² at a magnification of ×10] = 60, 95% CI = 32 to 88), indicating that the multidrug-resistant cells continued to proliferate in paclitaxel-treated mice. Treatment with imatinib, however, statistically significantly decreased the mean number of both PCNA-positive tumor (31 cells, 95% CI = 7 to 56 cells; and control = 58 cells, 95% CI = 35 to 80; P < .001) and endothelial cells (10 cells, 95% CI = 1 to 19; and control = 38 cells, 95% CI = 17 to 59; P < .001), compared with those of the control group. The combination of imatinib and paclitaxel further decreased the mean number of PCNA-positive cells to 14 (95% CI = 4 to 25; P < .001) (Table 2 and Fig. 4). A statistically significant interaction between paclitaxel and imatinib was also found (P < .001).

Treatment with paclitaxel did not increase the mean number of apoptotic tumor cells in paclitaxel-treated tumors (20 cells, 95% CI = 4 to 37 cells) compared with control untreated tumors (13 cells, 95% CI = 6 to 21 cells) (P = .59). However, treatment with imatinib (34 cells, 95% CI = 7 to 62 cells; P < .001) or with the combination of imatinib and paclitaxel (53 cells, 95% CI = 24 to 83 cells; P < .001) did statistically significantly increase the mean number of apoptotic cells (Fig. 4). No statistically significant interaction (P = .20) was found between paclitaxel treatment and imatinib treatment. These data demonstrate that treatment with imatinib and, especially, with the combination of imatinib and paclitaxel induced a statistically significantly increased level of apoptosis in tumor and endothelial cells.

Table 1. Treatment of multidrug-resistant human prostate carcinoma (PC-3MM2-MDR) growing in the tibia of nude mice

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>Incidence†</th>
<th>Median weight of bone lesion (g) (IQR‡)</th>
<th>Incidence of LN metastasis†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19/19</td>
<td>1.3 (1.0–1.9)</td>
<td>19/19</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>18/18</td>
<td>1.1 (0.9–1.7)</td>
<td>18/18</td>
</tr>
<tr>
<td>Imatinib</td>
<td>9/18</td>
<td>0.3 (0–1.2)</td>
<td>7/18</td>
</tr>
<tr>
<td>Imatinib + paclitaxel</td>
<td>4/18</td>
<td>0.1 (0–0.3)</td>
<td>3/18</td>
</tr>
</tbody>
</table>

*PC-3MM2-MDR cells (4 × 10⁶ cells) were injected into one tibia of 40 nude mice. Mice were randomly assigned to one of four treatment groups (each with 10 mice). Two weeks later, treatment was begun with water (daily oral and weekly intraperitoneally) paclitaxel (8 mg/kg of body weight, intraperitoneally, once per week), imatinib (50 mg/kg, oral, daily), or a combination of the two drugs. Mice were killed after 10 weeks of treatment. Tumor incidence, tumor weight, and regional lymph node metastasis were determined. Results of two independent experiments were similar and were therefore combined for this analysis. (Mice dying of causes unrelated to bone tumors were excluded.)

†Number of mice that developed bone tumors or lymph node (LN) metastases/number of mice receiving PC-3MM2-MDR cell injections.

‡IQR = interquartile range.

Microvessel Density

Next, we determined the mean vessel density in the bone lesions (Table 2). Treatment with paclitaxel did not affect the mean number of CD31/PECAM-1-positive endothelial cells per field in treated tumors (35 cells per field, 95% CI = 15 to 56 cells per field) compared with that in untreated control tumors (38 cells per field, 95% CI = 17 to 59 cells per field) (P = .07). Treatment with imatinib alone (10 cells per field, 95% CI = 1 to 19 cells per field; P < .001) or with the combination of imatinib and paclitaxel (three cells per field, 95% CI = zero to nine cells per field; P < .001) statistically significantly decreased the mean vessel density compared with that of the control group. A statistically significant interaction between paclitaxel and imatinib was also found (P < .001).

Phosphorylation of PDGFR and Apoptosis of Endothelial Cells

Next, we determined whether treatment with imatinib inhibited phosphorylation of PDGFR on endothelial cells and was associated with apoptosis. We used dual immunofluorescence staining for CD31/PECAM-1 to identify endothelial cells and phosphorylated PDGFR or dual immunofluorescence staining for CD31/PECAM-1 and for TUNEL-positive cells to identify apoptotic endothelial cells. Endothelial cells in tumor-associated vessels of bone lesions expressed surface phosphorylated PDGFβR (Fig. 3), probably in response to the PDGF produced by tumor cells. Treatment of these tumors with imatinib alone or imatinib combined with paclitaxel inhibited the phosphorylation of PDGFR on endothelial cells and on tumor cells (Fig. 3). The colocalization of CD31/PECAM-1 expression and TUNEL positivity indicated that endothelial cells in the bone tumor were apoptotic. At the end of the 10-week treatment, the median percentage of apoptotic endothelial cells in control tumors was 2% (IQR = 1%–4%) and in tumors from mice treated with paclitaxel was 2% (IQR = 1%–4%) (Table 2); the median percentage of apoptotic endothelial cells in tumors from mice treated with imatinib alone was 5% (IQR = 2%–8%) (P < .001) and in tumors from mice treated with a combination of imatinib and paclitaxel was 5% (IQR = 4%–8%) (P < .001), compared with the control group. No statistically significant interaction between paclitaxel and imatinib was found (P = .69).

Another experiment that used a separate set of 80 mice (20 mice per group) was designed to determine whether apoptosis of endothelial cells preceded the apoptosis of tumor cells. After 2 weeks of treatment, we found apoptotic endothelial cells in tumor-associated vessels in bone lesions, but we found no apoptotic tumor cells (Fig. 4). After another 8 weeks of treatment (i.e., a total of 10 weeks of treatment), apoptotic tumor cells were evident, and few tumor-associated blood vessels remained (Fig. 4).

DISCUSSION

We report that multidrug-resistant PC-3MM2-MDR human prostate cancer cells growing in the bone of nude mice were resistant to systemic administration of paclitaxel. Targeting the phosphorylation of PDGFR on tumor-associated endothelial cells by use of imatinib, with or without paclitaxel, led to regression of the multidrug-resistant prostate cancer and to inhibition of lymph node metastasis.
Regardless of sensitivity or resistance to hormones or chemotherapeutic drugs, all tumor cells depend on a viable vasculature for growth and survival (30–34, 51, 52). Tumor cells interact with host factors in the microenvironment to induce growth and the expansion of vasculature (9, 30, 31). We have previously reported (36–38) that PDGF produced by prostate cancer cells growing adjacent to bone induces the expression of PDGFR on tumor-associated endothelial cells and activates PDGFR by a paracrine mechanism. The systemic administration of imatinib inhibits the phosphorylation of PDGFR (but not EGFR) (37), and we report that the combination of imatinib and paclitaxel induces apoptosis of tumor-expressing tumor cells. The expression of phosphorylated PDGFR activates antiapoptotic pathways involving Akt, PI3K, mitogen-activated protein kinase, and Bcl-2 (53, 54), and treatment with imatinib can inhibit this effect (53, 54). Thus, a target for imatinib and paclitaxel therapy appears to be the tumor-associated endothelial cell. We tested this possibility by using multidrug-resistant human prostate cancer cells.

The acquisition of multidrug resistance by leukemia cells (55), hepatocellular carcinoma (56), and human lung carcinoma (57) anti-phosphorylated PDGFRβ (green). Colocalization of CD31/PECAM-1 and phosphorylated PDGFRβ yielded a yellow signal. Tumor cells growing adjacent to bone tissue expressed a high level of PDGFRβ, whereas tumor cells growing in the muscle did not, demonstrating that expression of PDGFRβ on tumor cells depends on the microenvironment. Treatment with imatinib (with or without paclitaxel) inhibits phosphorylation of PDGFRβ on tumor cells and endothelial cells, indicating that this effect of imatinib was active in vivo.

### Table 2. Response to treatment of multidrug-resistant human prostate carcinoma (PC-3MM2-MDR) growing in the tibia of nude mice

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>PCNA+ cells (95% CI)†</th>
<th>TUNEL+ cells (95% CI)†</th>
<th>CD31+ cells (95% CI)‡</th>
<th>CD31/TUNEL+ cells (IQR)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58 (35 to 80)</td>
<td>13 (6 to 21)</td>
<td>38 (17 to 59)</td>
<td>2 (1–4)</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>60 (32 to 88)</td>
<td>20 (4 to 37)</td>
<td>35 (15 to 56)</td>
<td>2 (1–4)</td>
</tr>
<tr>
<td>Imatinib</td>
<td>31 (7 to 56)</td>
<td>34 (7 to 62)</td>
<td>10 (1 to 19)</td>
<td>5 (2–8)</td>
</tr>
<tr>
<td>Imatinib + paclitaxel</td>
<td>14 (4 to 25)</td>
<td>53 (24 to 83)</td>
<td>3 (0 to 9)</td>
<td>5 (4–8)</td>
</tr>
</tbody>
</table>

* PC-3MM2-MDR cells (4 × 10^5 cells) were injected into the tibia of 40 nude mice. Mice were randomly assigned to one of four treatment groups (each with 10 mice). Two weeks later, treatment was begun with water (daily oral and weekly intraperitoneally) paclitaxel (8 mg/kg of body weight, intraperitoneally, once per week), imatinib (50 mg/kg, oral, daily), or a combination of the two drugs. Mice were killed after 10 weeks of treatment. Results of two independent experiments were similar and were therefore combined for this analysis. (Mice dying of causes unrelated to bone tumors were excluded.)

† Mean number of cells counted in six 0.14-mm² fields per slide under a ×100 objective. CI = confidence interval.

‡ Median of the ratio of the number of apoptotic endothelial cells to the total number of endothelial cells in five to 10 random 0.011-mm² microscopic fields. IQR = interquartile range.
has been shown to decrease tumorigenicity and prolong doubling time. In accordance with these reports, we found that the in vivo growth of the PC-3MM2-MDR cells was slower than that of the parental PC-3MM2 cells. However, the seed-and-soil theory (30,34,58) (i.e., the interaction of specific metastatic cells with a specific organ microenvironment) held true regardless of the multidrug-resistant phenotype. Similar to the parental PC-3MM2 cells (35,36), multidrug-resistant PC-3MM2-MDR cells growing in the bone (but not in the muscle) expressed IL-8, bFGF, EGF, EGFR, PDGF, and PDGFR. Endothelial cells of tumor-associated vessels in bone lesions also expressed PDGFR, and treatment with imatinib and paclitaxel inhibited the phosphorylation of PDGFR on both tumor cells and endothelial cells.

In vitro, PC-3MM2-MDR cells were highly resistant to paclitaxel, even in the presence of imatinib and PDGF A and B, and their proliferation was not affected by treatment with paclitaxel. Thus, imatinib did not sensitize the multidrug-resistant cells to paclitaxel. In vivo, however, the PC-3MM2-MDR bone lesions responded to systemic administration of imatinib and paclitaxel (but not to paclitaxel administered alone), raising the possibility that imatinib could have sensitized the tumor cells to paclitaxel. The results shown in Fig. 4, however, clearly demonstrate that, after 14 days of treatment with imatinib (daily) and paclitaxel (once per week), apoptosis (as reflected by TUNEL-positive cells) was limited mostly to tumor-associated endothelial cells; i.e., after 14 days of treatment, the multidrug-resistant tumor cells were still resistant to treatment. In contrast, in mice injected with PC-3MM2 parental cells, treatment with imatinib and paclitaxel induced apoptosis in both tumor cells and tumor-associated endothelial cells, leading to extensive apoptosis (data not shown).

The PDGFR has been shown to regulate the interstitial fluid pressure and transcapillary transport of molecules (59), and the administration of imatinib to mice bearing subcutaneous tumors can decrease interstitial fluid pressure, thereby increasing the concentration of a systemically administered chemotherapeutic drug within a tumor by twofold to threefold (60,61). Because the PC-3MM2-MDR cells are 67-fold more resistant to paclitaxel,

Fig. 4. Immunohistochemical analyses of cell proliferation (via localization of proliferating cell nuclear antigen [PCNA]) and immunofluorescent double-labeling analyses of apoptosis of endothelial cells (via colocalization of CD31–platelet endothelial cell adhesion molecule 1 [PECAM-1] and terminal deoxynucleotidyltransferase–mediated nick end labeling [TUNEL]-positive cells). Tumors from each treatment group of mice were harvested weekly, and tissue sections were analyzed with anti-CD31/PECAM antibody (red) and TUNEL (green). Colocalization of CD31–PECAM and TUNEL-positive cells emitted a yellow signal. Two weeks after treatment began (CD31/TUNEL 2W), a combination of imatinib and paclitaxel statistically significantly induced apoptosis of tumor-associated endothelial cells. At the end of the 10-week treatment (CD31/ TUNEL 10W), apoptosis in many tumor cells was observed in tumors from mice treated with imatinib and in mice treated with the combination of imatinib and paclitaxel. Apoptosis also corresponded with decreased proliferation of tumor cells (PCNA 10W).
than are the parental PC-3MM2 cells, even in the presence of imatinib or imatinib plus PDGF BB (Fig. 1), however, the in vivo therapeutic response of the PC-3MM2-MDR bone tumors to imatinib and to imatinib plus paclitaxel was unlikely to have resulted from changes in interstitial fluid pressure.

Antivascular therapy can destroy tumor cells that require oxygen and nutrients for survival. It is important, however, to target tumor-associated endothelial cells rather than all endothelial cells. Endothelial cells in normal tissues rarely divide, whereas 2%–3% of endothelial cells in prostate cancer divide daily (62,63). These dividing endothelial cells should be sensitive to anticycling drugs such as paclitaxel. However, stimulation of endothelial cells with PDGF leads to resistance to paclitaxel (53,54) and, therefore, paclitaxel at a dose administered (54) did not induce apoptosis of endothelial cells (54). Because imatinib inhibited activation of PDGFR, apoptosis in bone tumors from mice treated with imatinib and paclitaxel for only 2 weeks occurred first in tumor-associated endothelial cells and then in tumor cells and was ultimately followed by necrosis. By the fourth week of treatment with imatinib and paclitaxel or imatinib alone, concurrent apoptosis of tumor cells and tumor-associated endothelial cells was observed. Without paclitaxel, imatinib may produce therapeutic effects by the blockade of PDGFR phosphorylation, preventing activation of survival pathways leading to decreased survival of tumor cells (54).

Thus, the imatinib-induced blockade of PDGFR phosphorylation combined with paclitaxel treatment appears to target the tumor-associated endothelial cells (i.e., to be an antivascular therapy). Whether this approach can be useful for other tumor types is unknown. The heterogeneity of angiogenesis in human tumors (54) and the findings that endothelial cells of different organs are phenotypically distinct (53) indicate that further investigation is needed to understand the interaction between various tumor cells and endothelial cells in different organs so that optimal treatment regimens with targeted antivascular agents can be developed. Nevertheless, targeting the phosphorylation of PDGFR on tumor-associated endothelial cells by imatinib and paclitaxel can clearly produce therapeutic results in an experimental model of multidrug-resistant human prostate cancer bone metastasis.

REFERENCES


**NOTES**

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