Androgen Regulation of Parathyroid Hormone-Related Peptide Production in Human Prostate Cancer Cells

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Parathyroid hormone-related protein (PTHrP) is the major pathogenetic factor for hypercalcemia in several malignancies including prostate cancer. In the current study, we have assessed the ability of androgens to regulate PTHrP production in androgen-insensitive human prostate cancer cells PC-3 and cells transfected with androgen receptor (PC-3T). Androgen responsiveness caused a marked decrease in PC-3T cell growth, and treatment of these cells with dihydrotestosterone led to inhibition of PTHrP production. These inhibitory effects were readily reversed by androgen receptor antagonist flutamide. To determine the effect of androgens on tumor growth and PTHrP production in vivo, PC-3 and PC-3T cells were injected into the right flank of male BALB/c nu/nu mice. Animals inoculated with PC-3 and PC-3T cells developed palpable tumors at wk 2 and 4, respectively. Inoculation of PC-3T cells into castrated animals resulted in rapid tumor growth in PC-3T tumors, effects that were reversed in PC-3T tumors grown in castrated hosts. Using PTHrP promoter luciferase reporter, a 30% decrease in luciferase activity was seen following treatment with dihydrotestosterone. These results indicate that PC-3 cell growth correlates inversely with androgen sensitivity and directly with PTHrP production in vitro and in vivo, androgens can regulate PTHrP production, and the androgen effect on PTHrP is mediated at least in part by transcriptional regulation via the androgen receptor. (Endocrinology 144: 858–867, 2003)

Prostate carcinoma is the leading hormone-dependent malignancy affecting males in North America and results in a high incidence of cancer-related deaths (1, 2). This hormone-dependent malignancy is characterized by a high incidence of osteoblastic as well as osteolytic skeletal lesions (3–5). Malignant transformation of prostatic epithelial cells may be the result of aberrant expression of growth factors or their receptors (6–8). Androgens play an important role in the proper development and physiological function of the prostate (9, 10). The actions of androgens on target cells are initiated by the binding of the steroid to a stereospecific high-affinity intracellular receptor. Because of a conformational change, the androgen-bound receptor complex is then able to interact with specific androgen response elements in the promoter region of target genes that modulate cellular growth and differentiation. Testosterone, synthesized in the testes and to a lesser extent in the adrenals, is converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase, which is localized mainly in the prostate (11, 12). Although testosterone and DHT both stimulate the growth of normal and malignant prostate tissue, DHT is believed to be the more potent androgen (13). Total androgen ablation therapy in combination with antiandrogens has been shown to produce the most beneficial responses in patients with hormone-responsive prostatic tumors (14). However, most patients experience disease progression because of the development of an androgen-independent tumor associated with a lack of or mutation of the androgen receptor (15).

Originally discovered as a product of tumors that produce hypercalcemia, PTHrP has been demonstrated to be a product of many malignant tissues, including prostate carcinoma. PTHrP is also widely distributed in many normal adult and fetal tissues in which it exerts an autocrine or paracrine effect in modulating cellular growth and differentiation (16). Other suggested physiological roles of PTHrP include regulation of ion transport (17, 18) and smooth muscle relaxation (19). The biological effects of PTHrP are mediated, at least in part, through a cell surface seven-transmembrane-spanning G protein-coupled receptor that it shares with PTH, the type I PTH/PTHrP receptor (16). Several growth factors and steroid hormones have been shown to regulate PTHrP production in tumor cells (20–22). Compounds that induce PTHrP production include fetal bovine serum (FBS) and a variety of growth factors, whereas dexamethasone and vitamin D suppress PTHrP in a number of cell types (23, 24). Normal prostate epithelial cells produce PTHrP, however, PTHrP is found in higher levels in prostate carcinoma than in benign prostatic hyperplasia or normal cells (1).

In the current study, we examined the regulation of PTHrP production by androgens in the androgen-insensitive human prostate cancer cell line PC-3, which lacks a functional androgen receptor, and androgen-sensitive PC-3 cells transfected with the full-length human androgen receptor cDNA (PC-3T; Ref. 25). The effects of androgen on tumor cell growth and PTHrP production were evaluated both in vitro and in vivo.

Materials and Methods

Cell culture

Human prostate cancer cell line PC-3 was obtained from the American Type Culture Collection (Manassas, VA). PC-3 cells transfected with a full-length, functional human androgen receptor cDNA (PC-3T) were kindly provided by Dr. T. J. Brown (Toronto Hospital Research Institute,

Abbreviations: DHT, Dihydrotestosterone; FBS, fetal bovine serum; FLU, flutamide; β-gal, β-galactosidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IRMA, immunoradiometric assay; NC, negative control; SFBS, charcoal-stripped FBS.
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Cell proliferation assays

PC-3 and PC-3T cells were plated in triplicate at a density of 10,000 cells/well in 2 ml media in 6-well plates. Cells were grown in FBS or charcoal-stripped FBS (SFBS) (Invitrogen), depleted of androgens. Where indicated, cells in stripped serum were treated with either 100 nM DHT (Sigma-Aldrich Canada) alone or in combination with 1 μM flutamide (FLU) (Sigma-Aldrich Canada). Cells were trypsinized and counted, at the times indicated, using a Coulter Counter (model ZF, Coulter Electronics, Harpenden, Hertfordshire, UK). Cell culture medium was replenished every third day.

RT-PCR

Total RNA was extracted from PC-3 and PC-3T cells by a single-step method using Trizol reagent (Invitrogen). The reaction template, denatured for 10 min at 70°C, consisted of 2 μg total RNA in 10 μl diethylpyrocarbonate H2O. To the reaction mixture, 1× PCR buffer (MBI Fermentas, Burlington, Ontario, Canada), dNTP mix 0.2 mM each (MBI Fermentas), 10 μM primers were used as a control. The amplified PCR product was fractionated on a 1.3% agarose gel and visualized by ethidium bromide staining. Band intensities for PTHrP and GAPDH were quantified using denisometric software, Quantity One (Bio-Rad Laboratories, Inc., Mississauga, Ontario, Canada).

Northern blot analysis

Total cellular RNA was isolated by Trizol extraction from control and experimental PC-3 and PC-3T cells and tumors after treatment in the presence or absence of androgens. Briefly, 20 μg total cellular RNA was electrophoresed on a 1.1% agarose-formaldehyde gel, transferred to a nylon membrane (Nytren, Schleicher & Schuell, Keene, NH) by capillary blotting and then fixed by drying and UV cross-linking for 10 min. The integrity of the RNA was assessed by ethidium bromide staining. Hybridization was carried out with [32P]dCTP-labeled human PTHrP and 18S rDNA. After 24-h incubation at 65°C, filters were washed twice under low-stringency conditions [1× standard sodium citrate and 1% SDS at 60°C for 2× 20 min] and under high-stringency conditions [0.1× standard sodium citrate and 0.1% SDS at 60°C for 2× 20 min]. Autoradiography of filters was carried out at −70°C using Biomax film (Eastman Kodak Co., Rochester, NY) with two intensifying screens. The levels of PTHrP and 18S mRNA expression were quantified using the densitometric software, Quantity One (Bio-Rad Laboratories, Inc.).

Immunocytochemistry and immunohistochemistry

PC-3 and PC-3T cells, cultured in 35-mm plates, and paraffin tumor sections were stained immunocytochemically for PTHrP as previously described (26) using the avidin-biotin-peroxidase complex technique. Rabbit antiserum against PTHrP (1–34) peptide was applied to cells and tumor sections overnight at room temperature. As a negative control (NC), the primary antibody was omitted from the reaction mixture. After washing with high Tris-buffered saline salt buffer (50 mM Tris-HCl, 2.5% NaCl, 0.05% Tween 20, pH 7.6) for 10 min at room temperature followed by two 10-min washes with Tris-buffered saline, the samples were incubated with secondary antibody (biotinylated rabbit anti-IgG, Sigma-Aldrich Canada), washed as before, and processed using the Vectastain avidin-biotin-peroxidase complex-AP kit (Vector Laboratories Inc., Burlingame, CA). Red pigment to demarcate regions of immunostaining was produced by a 10- or 15-min treatment with Fast TMB (Naphthol AS-MX-phosphate (Sigma-Aldrich Canada) containing 1 mM levamisole as endogenous alkaline phosphatase inhibitor. The sections were then washed with distilled water, counterstained with methyl green, and mounted with Kaiser’s glycerol jelly.

Western immunoblot analysis

PC-3 and PC-3T cells were grown to 80% confluency, serum starved for 48 h, and the media collected and centrifuged to remove any debris. This conditioned media was concentrated using centricon-10 (Millipore Corp., Napeon, Ontario, Canada), which retains all protein greater than 10,000 kDa, and the protein levels were determined by Bradford analysis (Bio-Rad Laboratories, Inc.). Fifty micrograms total protein from each cell line were electrophoresed on a 15% SDS-polyacrylamide gel and electotransferred to nitrocellulose membranes according to the manufacturer’s specifications (Bio-Rad Laboratories, Inc.). After blocking the membranes overnight (PBS, 5% powdered milk) at 4°C, they were incubated with rabbit antiserum against PTHrP (1–34) peptide diluted in Western antibody buffer (PBS, 5% powdered milk). The membranes were washed three times (PBS, 0.1% Nonidet P-40) and incubated with horseradish peroxidase-conjugated goat-antirabbit IgG antibody (1:20,000). Unbound secondary antibody was removed by washing, and bound antibodies were detected by enhanced chemiluminescence membranes according to the manufacturer’s specifications (Amersham Pharmacia Biotech) and exposed on XAR film (Eastman Kodak Co.).

Immunoradiometric assay

PTHrP secreted in the conditioned media was measured using a noncompetitive, two-site immunoradiometric assay or immunoradioimmunoassay (IRMA) (Diagnostic Systems Laboratories, Webster, TX). PTHrP is recognized by an NH2-terminal reactive antibody [raised against PTHrP (1–34)] immobilized on a solid phase (capture antibody), and quantitated with a second, 125I-radiolabeled COOH-terminal reactive antibody [(raised against PTHrP (47–86)] for detection (signal antibody). PC-3 and PC-3T cells were plated at a density of 106 cells in 10 ml in 60-mm plates. At confluency, cells were washed twice with SFBS for 24 h and then treated in the presence or absence of androgens for 12 h. The conditioned media were collected and the final cell number was determined using a Coulter counter (Coulter Electronics). The assay was carried out per the manufacturer’s specifications. The reaction mixture was incubated at room temperature for 20 h, washed with deionized water, and the radioactivity from each sample was determined in a gamma counter for 1 min. The detection limit of the assay was 0.5 pmol/liter (3.0 pg/ml).
Luciferase and β-galactosidase (β-gal) assays

Transient transfection for promoter assays was performed by the lipofectamine method (Invitrogen). In brief, 0.8 × 10⁶ H-500 cells were plated in 35-mm dishes a day before transfection. The cells were co-transfected with 1 µg pGL3 vector, into which had been cloned the region representing the luciferase reporter gene, and 1 µg pCMV-β-gal reporter plasmid to determine transfection efficiency (28). The transfected cells were washed the next day and allowed to grow for 24 h in medium containing 10% FBS. After cells were serum deprived for 24 h, they were treated with media containing FBS, SFBS, or SFBS plus 100 nM DHT alone or in combination with 1 µM FLU. The cells were harvested with 1× reporter lysis buffer (Promega Corp., Nepean, Ontario, Canada) and the supernatants collected following centrifugation. Luciferase activity in the supernatants was then analyzed by mixing 45 µl cell lysate, 5 µl luciferase assay reagent (30 mM ATP, 150 mM K2HPO4, 300 mM MgCl2) and 100 µl substrate luciferin (250 µM) for 20 sec in a luminometer (Monolight 2010, BD Biosciences, San Jose, CA). For β-gal activity, 50-µl lysates were mixed with 200 µl β-gal assay buffer (24 mM Na2HPO4, 16 mM NaH2PO4, 4 mM KCl, 400 mM MgCl2, 20 µM β-mercaptoethanol, and 0.3 mg ONPG per sample) in a 96-well plate and incubated for 4 h at 37 C. The reaction was stopped by adding 0.5 ml of 1 M Na2CO3, and the absorbance was measured at 420 nm in a Vmax Plate reader (Molecular Devices, Sunnyvale, CA). Activity was determined by comparison to a standard curve. Luciferase reporter activity in relative luminescence units was normalized to β-gal activity as described (29). All luciferase assay readings were performed in triplicate and corrected for β-gal expression levels in each cell population.

Animal protocols

Six-week-old male BALB/c nu/nu normal and castrated mice were obtained from Charles River Laboratories, Inc. (St. Constant, Québec, Canada). Before inoculation, PC-3 and PC-3T tumor cells, grown in serum-containing medium, were washed with Hanks’ buffer and trypsinized for 5 min. Cells were then collected in Hanks’ buffer and centrifuged at 1500 rpm for 5 min. Cell pellets (3 × 10⁶ cells) were resuspended in 200 µl matrigel and saline mixture (20% matrigel) and injected sc into the flank region of the hind leg of the mice. All animals were numbered, kept separately, and examined weekly for the development of tumors. The tumor mass was measured in two dimensions by calipers, and the tumor volume was calculated according to the equation (l × w²)/2 (l = length, w = width). Animals were killed and the tumors were isolated.

Statistical analysis

Results are expressed as the mean ± SEM of at least triplicate determinations. Statistical comparisons were made using the Student’s t test or ANOVA, with a probability of less than 0.05 being considered significant.

Results

Effect of androgens on prostate cancer cell growth in vitro

A cell proliferation assay was performed to study the effects of restoration of androgen responsiveness on the growth rate of PC-3T cells in comparison with the wild-type PC-3 cells. Wild-type PC-3 cells cultured in media containing SFBS showed no change in cell growth, compared with those grown in FBS (Fig. 1A). In contrast, PC-3T cells, when grown in media with FBS, showed a greater than 45% reduction in proliferation rate and a 30% increase in proliferation rate by 6 d when grown in media containing SFBS and therefore depleted of androgens (Fig. 1B). Media containing SFBS supplemented with 100 nM DHT resulted in a decrease in PC-3T cell growth to a rate comparable with PC-3T cells incubated in media containing FBS. The specificity of the androgen receptor in mediating this androgen growth-inhibitory effect was confirmed by coinoculation of PC-3T cells with 100 nM DHT and 1.0 µM androgen receptor antagonist, FLU, in which the DHT growth inhibition was abolished (Fig. 1). DHT (100 nM) alone or in combination with 1.0 µM FLU did not have any effect on the proliferation rate of PC-3 cells (Fig. 1A).

Effect of androgens on PTHrP gene expression

To examine the effects of the restoration of androgen responsiveness on PTHrP mRNA expression, RT-PCR and Northern blot analysis were performed on PC-3 and PC-3T cultured in vitro in the presence or absence of androgens. Cells in serum-free conditions for 24 h exhibited a maximal induction in PTHrP mRNA levels after a 12-h treatment (data not shown). PC-3T cells, incubated in FBS, showed a 35%...
reduction in PTHrP mRNA levels as assessed by RT-PCR, compared with the parental cell line, PC-3, also incubated in FBS (Fig. 2A). Although PC-3T cells cultured in SFBS showed an increase PTHrP mRNA levels by 24%, no change in the level of PTHrP mRNA expression was observed in PC-3 cells cultured in the same media (data not shown). The addition of 100 nM DHT reduced PTHrP mRNA production to a level comparable with FBS-treated PC-3T cells, which was subsequently reversed on coincubation of DHT with 1 μM FLU (Fig. 2A). Similar results were observed when Northern blot analysis was performed on the experimental groups. PTHrP mRNA levels were 40% higher in PC-3 cells than in androgen-responsive PC-3T cells. Removal of androgens from the culture media of PC-3T cells caused PTHrP mRNA levels to increase by 30%, followed by a substantial reduction in PTHrP mRNA levels in cells treated with DHT. Furthermore, PTHrP expression levels became comparable with those observed in PC-3T cells cultured in SFBS when treated with a combination of DHT and FLU (Fig. 2B).

Effect of androgens on production of protein levels of PTHrP

An immunocytochemical assay specific for PTHrP (1–34) showed strong staining in the cellular nucleus and cytoplasm of PC-3 cells. The PTHRP-specific color staining was quantitated, and results clearly indicated that PTHrP protein levels were 45% greater in PC-3 cells than in PC-3T cells (Fig. 3, A and B). To further confirm these results, conditioned culture media were collected from PC-3 and PC-3T cells and 50 μg total protein analyzed by Western blot analysis. PTHrP protein levels were significantly higher in PC-3 cells, compared with PC-3T cells (Fig. 3C). PTHrP protein levels produced by control and experimental cells were also determined by an IRMA that measures PTHrP (1–86) secreted into

![Graphs showing effects of androgens on PTHrP gene and protein expression](https://example.com/graphs.png)
the conditioned media. No significant difference in PTHrP secretion was observed in PC-3 cells cultured with SFBS, compared with those with FBS (Fig. 4A). PC-3 cells produced 60% more PTHrP than the protein levels produced by androgen-sensitive PC-3T cells. As observed for the mRNA levels, protein levels produced were increased by 35% by incubating the cells in media containing SFBS. This increase in PTHrP production, inhibited when PC-3T cells were treated with 100 nM DHT for 12 h, was restored when cells were treated with DHT in combination with FLU (Fig. 4B).

The specificity of the effect of androgen on PTHrP production was further evaluated by treating PC-3T cells with different concentrations of DHT. IRMA measuring PTHrP (1–86) secreted into conditioned media indicated that DHT, at increasing concentrations from 1 to 100 nM, inhibited PTHrP production in a dose-dependent manner (Fig. 5).

Androgen regulation of PTHrP promoter activity

The effect of androgens on PTHrP promoter activity was studied using the rat Leydig tumor cell line, H-500, that produces high levels of PTHrP and contains endogenous androgen receptor. The transient transfection of pGL3 vector containing the rat PTHrP promoter upstream of the luciferase reporter gene allowed measurement of promoter activity in response to androgen treatment. The empty pGL3 vector, not containing the PTHrP promoter, was used as a control, and the relative luciferase activity measured was calculated as fold increase from the empty vector. The highest PTHrP promoter activity was observed for experimental cells incubated in SFBS in the absence of androgens. H-500 cells treated with FBS or SFBS + 100 nM DHT resulted in a 35% reduction in PTHrP promoter activity. On the other hand, coinubation of DHT-treated PC-3T cells with the androgen receptor antagonist FLU reversed the observed reduction in promoter activity and restored it to levels similar to those of PC-3T cells cultured with SFBS (Fig. 6).

Effect of androgens on tumor growth in vivo and tumoral PTHrP mRNA and protein levels
Subcutaneous inoculation of the wild-type PC-3 cells into the right flank of 6-wk-old male BALB/c nu/nu mice resulted in the development of palpable tumors by wk 2 post tumor cell inoculation. In contrast, development of primary tumors was significantly delayed when the animals were...
inoculated with PC-3T cells, in which palpable tumors were only detected at wk 4, post tumor inoculation. Furthermore, the tumor volumes recorded, on wk 8, was 50% less than that for PC-3 tumors. To evaluate the effects of androgen ablation on PC-3T tumor development, PC-3T cells were injected into castrated animals. Castrated nude mice developed palpable tumors only detected at wk 4, post tumor inoculation. Furthermore, the tumor volumes recorded, on wk 8, was 50% less than that for PC-3 tumors.

FIG. 4. Effects of androgens on the production of immunoreactive PTHrP in PC-3 and PC-3T cells. Cells were grown to 70% confluence in RPMI media containing FBS and incubated overnight in serum-free conditions. Cells were then treated for 12 h in media containing FBS, SFBS, or SFBS with 100 nM DHT alone or in combination with 1 µM FLU. PTHrP (1–86) secreted in conditioned media was determined using IRMA as described in Materials and Methods. Results are expressed as picograms of PTHrP (1–86) per 10^6 cells per 12 h. Bars represent the means ± SEM of three different experiments. *, Significant differences in PTHrP values from control PC-3 cells (P < 0.05). **, Significant differences in PTHrP values from control PC-3T cells in FBS (P < 0.05).

FIG. 5. Effects of DHT on PTHrP production in PC-3T cells. PC-3T cells were grown to 70% confluence in RPMI media containing FBS and incubated overnight in serum-free conditions. Cells were then treated for 12 h in media containing SFBS with different concentrations of DHT (1, 10, 100 nM). PTHrP (1–86) secreted in conditioned media was determined using IRMA as described in Materials and Methods. Results are expressed as picograms of PTHrP (1–86) per 10^6 cells per 12 h. Bars represent the means ± SEM of three different experiments. *, Significant differences in PTHrP values from control PC-3T cells in SFBS alone (P < 0.05).

FIG. 6. Effects of androgens on PTHrP promoter activity. Rat Leydig tumor cells H-500 were grown to 50–70% confluency. Cells were transiently cotransfected with pGL3 vector containing the rat PTHrP promoter upstream of a luciferase reporter gene and with β-gal vector. Cells were incubated in FBS for 24 h and then incubated in serum-free conditions for 24 h. Subsequently cells were treated with media containing FBS, SFBS, or SFBS and DHT (100 nM) alone or in combination with FLU (1 µM). Luciferase activity was quantified using a luminometer, and results were normalized for transfection efficiency as measured by β-gal reaction. Values represent fold increase in luciferase activity, compared with control pGL3 vector containing no PTHrP promoter. Results represent means ± SEM of three different experiments. *, Significant differences from control H-500 cells in FBS (P < 0.05).
tumors by wk 2, and, more importantly, throughout the study these tumor volumes were significantly larger than those observed in noncastrated animals inoculated with PC-3T cells (Fig. 7).

The primary tumors were removed, and RT-PCR was performed on the RNA isolated to measure the relative PTHrP mRNA levels (Fig. 8). PC-3T tumors showed lower levels of PTHrP mRNA expression, compared with PC-3 tumors. Moreover, the tumoral level of PTHrP mRNA was significantly higher in castrated animals bearing PC-3T tumors than in noncastrated animals (Fig. 8).

An immunohistochemical reaction specific for PTHrP (1–34) was performed on tumor sections obtained from each of the three animal groups. Intense color staining of PC-3 tumor cells was observed and quantitated. PC-3T tumor sections exhibited a greater than 60% reduction in PTHrP immunostaining, compared with PC-3 tumor cells. PC-3T tumor sections isolated from castrated animals showed a 45% increase in staining specific for PTHrP, compared with noncastrated animals (Fig. 9).

### Discussion

Normal and hyperplastic prostate cells require androgens for their survival and proliferation, whereas androgen-insensitive malignant prostate carcinoma cells exhibit a reduced androgen requirement for survival (11, 30, 31). Androgen ablation and antiandrogen hormonal therapies initially benefit early-stage prostate cancer patients (32, 33). However, the success of these therapies is largely dependent on the hormone-sensitive status of the malignant cells. The prostate tumor is composed of nonuniform subpopulations of cells possessing different hormonal responsive abilities (34). The removal of endogenous circulating androgens and the blocking of androgen receptor with androgen receptor antagonist prevents the growth and survival of hormone-dependent prostate cancer cells resulting in an initial tumor mass reduction. However, the proliferative capacity of androgen-insensitive tumoral cellular subpopulations will not be inhibited. The onset of cancer progression results in the primary tumor being composed predominantly of androgen-insensitive malignant cells that are no longer affected by the androgen deprivation (32). In the later stages of prostate

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**FIG. 7.** Effect of androgens on the growth of human prostate tumor cells in nude mice. PC-3 and PC-3T cells (1 × 10⁶ cells in 20% matrigel) were injected sc in the right flank of nu/nu BALB/c male mice. Additionally, PC-3T cells were injected into castrated male animals. Tumors were measured weekly using caliper, and tumor volumes were calculated according to the equation (length × width²)/2. Results represent means ± SEM of three different experiments with six animals in each group. *, Significant differences in tumor growth from control animals inoculated with PC-3 cells. **, Significant differences in tumor growth from noncastrated animals inoculated with PC-3T cells (P < 0.05).

**FIG. 8.** Effect of androgens on PTHrP gene expression in tumors. Total cellular RNA extracted from tumors of normal male animal BALB/c nu/nu mice inoculated with PC-3 and PC-3T cells and from tumors of castrated animals inoculated with PC-3T cells. Levels of PTHrP and GAPDH mRNA were determined by RT-PCR as described in Materials and Methods (upper panel). Results are expressed as the ratio of PTHrP to GAPDH mRNA (lower panel). Bars represent the means ± SEM of three different experiments. *, Significant differences in the ratios from control PC-3 tumors (P < 0.05).
cancer, hormone therapy becomes ineffective, and other forms of treatment must be used.

PC-3 cells representing late-stage prostate carcinoma exhibit a high proliferation rate in vitro and in vivo. The stable transfection of a functional, human androgen receptor caused cellular proliferation to be substantially reduced in PC-3T cells (25). When tested in vivo, athymic nude mice inoculated with hormone-responsive PC-3T cells and castrated animals inoculated with PC-3T cells (A). The NC was performed by the omission of the primary antibody specific to PTHrP (1–34). Immunohistochemical activity was quantified as described in Materials and Methods (B). Quantification is presented as the summary total gray demonstrating a positive immunohistochemical reaction. Four animals were present in each group, and three tumor sections were analyzed for each animal. Results represent means ± SEM of three different experiments. *, Significant differences in PTHrP values from control PC-3 tumors (P < 0.05).

FIG. 9. Effect of androgens on PTHrP production by tumors. Immunohistochemistry for PTHrP was performed, as described in Materials and Methods, in tumors sections of animals inoculated with PC-3 or PC-3T cells and castrated animals inoculated with PC-3T cells (A). The NC was performed by the omission of the primary antibody specific to PTHrP (1–34). Immunohistochemical activity was quantified as described in Materials and Methods (B). Quantification is presented as the summary total gray demonstrating a positive immunohistochemical reaction. Four animals were present in each group, and three tumor sections were analyzed for each animal. Results represent means ± SEM of three different experiments. *, Significant differences in PTHrP values from control PC-3 tumors (P < 0.05).

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PC-3 cells representing late-stage prostate carcinoma exhibit a high proliferation rate in vitro and in vivo. The stable transfection of a functional, human androgen receptor caused cellular proliferation to be substantially reduced in PC-3T cells (25). When tested in vivo, athymic nude mice inoculated with hormone-responsive PC-3T cells developed significantly smaller tumors and exhibited a later onset of tumor development, compared with animals receiving wild-type hormone-insensitive PC-3 cells. In this system, androgens were found to inhibit cellular growth as opposed to androgens promoting the growth of prostatic malignant cells. This paradox has also been observed in a breast cancer cell system in which contrary to what was expected, estrogens were found to inhibit cellular proliferation of late-stage human breast cancer. The S30 cells, transfected with a functional estrogen receptor, grow more slowly than the parental cells that are estrogen nonresponsive, MDA-MB 231 cells (35). However, these studies, although demonstrating a different growth pattern of hormone-responsive tumor cells, did not examine the effect of hormonal status of tumor cells on the expression of growth factors and proteases that in turn can lead to altered tumor growth. This inhibition in cellular growth could be mediated by the suppression of PTHrP. In other reports, PTHrP has been found to stimulate growth of chondrocytes and keratinocytes (36, 37).
Higher PTHrP expression levels have been associated with late-stage prostate cancer cells, compared with normal prostate cells (1), and in our studies PC-3T cells were found to express on average 40% less PTHrP, compared with the parental cells PC-3. The effect of androgens on PTHrP production have been further confirmed by treating cells in the absence of androgens and then with the addition of the active androgen metabolite DHT. PTHrP production was inhibited in a dose-dependent manner by increasing concentrations of DHT in this human prostate cancer cell system. To demonstrate the effects of androgen on PTHrP promoter activity, we used H-500 cells that express the PTHrP receptor and high levels of PTHrP. Additionally, the ability of androgens to regulate PTHrP production has previously been demonstrated in these cells, thus providing an optimal setting to characterize PTHrP promoter activity. In these studies, the full-length rat PTHrP promoter was used. Removal of androgens by incubating in SFBS enhanced promoter activity, whereas DHT treatment led to inhibition of this activity. Future in-depth promoter analysis studies are needed to identify a putative negative androgen response element within the 5' untranslated region of the PTHrP gene.

Putative AUUU-rich destabilizing motifs have also been identified in the 5' untranslated region of the PTHrP gene (38, 39) and posttranscriptional stabilization of the PTHrP gene following exposure to serum has been observed in osteosarcoma cells, smooth muscle cells, and human keratinocytes (20, 40). Additional studies of the effect of androgens on the stability of the PTHrP gene are therefore required to identify the presence of any potential posttranscriptional regulation of the PTHrP gene (20, 38–40). Together, these studies would help clarify the mechanism of the inhibitory effect of DHT on PTHrP regulation and provide new strategies to treat PTHrP overproduction.

Our findings therefore could have important implications for the role of PTHrP in prostate cancer progression and new directions in the future treatment of both androgen-dependent and androgen-independent prostate cancer. Hormonal therapies in combination with other anticancer therapies may prove to provide the best strategy for the blockade of prostate cancer progression and metastasis and reduction of the morbidity and mortality of this prevalent disease (41).

Acknowledgments

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