Establishment of Two Human Prostate Cancer Cell Lines Derived from a Single Bone Metastasis

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ABSTRACT

Human prostate cancer cell lines are particularly difficult to establish, and most existing cell lines do not exhibit features commonly seen in human prostate cancer. Most available models either grow only in vivo as xenografts or are androgen insensitive and fail to express prostate-specific antigen (PSA). The lack of functionally relevant model systems of advanced prostate cancer has limited prostate cancer research and therapy development. Of 30 processed samples derived from patients with prostate cancer, we established two cell lines (MDA PCa 2a and MDA PCa 2b) that express PSA and androgen receptor, grow in vitro, and are androgen sensitive. Cells from these lines produced tumors in nude mice when injected either s.c. or orthotopically (intraprostatic). Both cell lines were established from a bone metastasis of a patient whose cancer was exhibiting androgen-independent growth. Although both were derived from two samples of the same specimen, they have different genetic features (as assessed by karyotype analysis) and different phenotypes (e.g., morphology and growth rate). It is likely that they are distinct clones isolated by the use of different culture procedures and reflect the genetic heterogeneity of the tumor. These new cell lines are the first available derived from a bone metastasis of an androgen-independent prostate adenocarcinoma that grow both in vivo and in vitro and have retained PSA expression and androgen sensitivity. They therefore constitute important model systems to address critical questions related to the androgen-independent growth of human prostate cancer and to the complex process of bone metastasis.

INTRODUCTION

Prostate cancer is the most frequently diagnosed and the second most deadly cancer in men (1). Although localized prostate cancer may be cured by radical prostatectomy or radiation therapy, the prospect of cure diminishes significantly when prostate cancer cells escape beyond the confines of the gland (2, 3). Patients with regional or distant metastases are usually treated with androgen deprivation therapy. Although most patients initially respond to this therapy, given sufficient time it is believed that all eventually relapse with androgen-independent disease. The androgen-independent phenotype of human prostate cancer is defined in the clinic as the ability of prostate cancer cells to grow despite castrate plasma concentrations of testosterone (2–4). No current standard therapy consistently confers a significant survival benefit to patients who develop androgen-independent prostate cancer (2–4).

The lack of functionally relevant in vitro models has been one of the major obstacles to advancing our understanding of the molecular mechanisms of this disease as well as to developing effective therapies. Most available human prostate cancer models either grow only in vivo as xenografts (5–9) or (with the exception of the LNCaP cell line: Ref. 10) lack some of the common features of clinical prostate cancer (androgen sensitivity and expression of PSA: Refs. 5–9).

The purpose of this work was to develop model systems of advanced prostate cancer. Our specific objectives were: (a) to screen human prostate cancer tissue samples derived from multiple sites for their growth in vitro and in vivo; (b) to optimize their growth conditions; and (c) to characterize the cells grown in vitro. We processed 30 tissue samples derived from primary prostate cancer of various histological grades and from prostate cancer tissue derived from different metastatic sites. Initial attachment and cell growth were obtained in cells from nine specimens; of these only two were established as cell lines (MDA PCa 2a and MDA PCa 2b). The cell lines were derived from a bone metastasis of a patient whose cancer was clinically progressing despite a castrate concentration of testosterone and resulting in spinal cord compression requiring a laminectomy. Both lines express PSA and AR, grow in vivo and in vitro, and are androgen sensitive. These are the first cell lines available derived from a bone metastasis of prostate cancer that express PSA and are androgen responsive. The lines are also the second and third human prostate cancer cell lines to produce PSA and are androgen sensitive. Our cell lines have now grown in vitro
2494 New Prostate Cancer Cell Lines

In Vitro Cell Growth was obtained from different areas of the primary tumors or metastatic lesions were obtained for tissue culture. Tumor samples ranged from 1.0 to 0.5 cm in the largest dimensions. A tissue section immediately adjacent to that submitted for culture was obtained for detailed histopathological analysis and determination of percentage of tumor in the sample.

Collection and Initial Processing of Tissue Specimens

Aseptically removed specimens were kept in cold (4°C) sterile tubes with HEPES-buffered saline (11) until processed. The specimens were mechanically disaggregated with sterile crossed scalpels to obtain pieces of about 1 mm³. Many of the samples disaggregated into small clumps of tumor cells in the washing medium as they were cut. In these cases, subsequent enzymatic disaggregation of the tissue was not necessary. In other cases, after cutting, the tissue was resuspended in five volumes of digestion solution [Collagenase Sigma type 1 (40 units/ml of growth medium); Sigma Chemical Co., St. Louis, MO] and incubated at 37°C. The length of the incubation varied from 30 min to overnight, depending on the degree of fibrosis of the specimen. Effective disaggregation of the tissue was monitored over time by gently moving the flask.

Culture of Tissue Specimens

Every tissue specimen to be cultured was incubated at least three different media [HITES (12), BRFF-HPC1, and PFMR4–1A (11)]. HITES medium is modified RPMI 1640 (GIBCO BRL, Gaithersburg, MD) with hydrocortisone, insulin, transferrin, and selenium. (Estradiol was not included in our formulation.) BRFF-HPC1 medium (Biological Research Facility and Facility, Inc., Jamsville, MD) has a very rich formulation and includes cholera toxin, DHT, insulin, EGF, and hydrocortisone. The formulation for PFMR4–1A (kindly provided by Dr. Donna Peehl) includes cholera toxin, DHT, insulin, EGF, and hydrocortisone (11). When enough tissue was available, we cultured the isolated cells with and without serum. In most cases, the presence of serum was necessary to obtain initial attachment and cell growth. Collagen-coated dishes were also used to encourage cell attachment and growth (11). Daily monitoring of the cultures allowed us to alter culture conditions as necessary.

In Vitro Cell Growth

To determine population doubling times of the MDA PCa 2a (passage 30) and MDA PCa 2b (passage 30) cell lines, cells were seeded onto 6-well plates at a density of 4 × 10⁴ cells/well in BRFF-HPC1 medium with 20% FBS and in F12K(+) medium supplemented with 20% FBS. F12K(+) is F12K (Life Technologies, Inc., Gaithersburg, MD) supplemented with 25 ng/ml cholera toxin, 10 ng/ml EGF, 40 nm phosphoethanolamine, 10 μg/ml hydrocortisone, 45 nm selenous acid, and 5 mg/ml insulin. The sources and preparation of these supplements have been described previously (11). The cultures were harvested at days 7 and 14, and the cells were counted using a hemocytometer. Population doublings were calculated as the average of three independent cell counts derived from different wells. The anchorage-independent colony-forming assay in semisolid medium was performed as described previously (13).

Cell Growth Induction by Androgens

The kinetics of growth induction of the MDA PCa 2a and MDA PCa 2b cell lines by DHT was determined by the crystal violet mitogenic assay (14, 15). Cells were seeded on 24-well plates at a density of 10⁴ cells/well and allowed to attach for 48 h in F12K(+) with 20% FBS. On the third day, designated as day 0, the cultures were washed twice in PBS and cultured for 7 or 14 days in F12K(+) with 15% charcoal-stripped FBS and 10⁻¹² m to 10⁻⁶ m DHT (Sigma). The medium was changed on days 3, 7, and 10. Each concentration of DHT was assayed in triplicate. The mitogenic activity of DHT was determined by the uptake and elution of crystal violet dye by the cells in each well (14, 15). A small aliquot of eluted dye was placed in a 96-well plate, and the absorbance of each well at 560 nm was measured with a Titerek Multiscan TCC/340 (Flow Laboratories, McLean, VA). Control experiments demonstrated that the absorbance was directly proportional to the number of cells in each well.

Tumorigenicity of MDA PCa 2 Cells in a Nude Mouse Assay

Six- to 8-week-old male athymic (nude) mice (BALB/c strain; Charles River Laboratory, Wilmington, MA) were used for the tumorigenicity assay of MDA PCa 2 cells. The animals were housed aseptically in the Athymic Animal Facility of the M. D. Anderson Cancer Center and manipulated under surgical aseptic conditions in a laminar flow hood. Cells to be injected into the mice were trypsinized, washed, counted, and resuspended at 4–5 × 10⁶ cells/100 μl of growth medium for the s.c. injection. To perform the intraprostastic injection, 1–2 × 10⁶ MDA PCa 2a cells were suspended in 20–30 μl of growth medium.

s.c. Injection. Four mice were each injected s.c. with 5 × 10⁶ MDA PCa 2a cells/site at each of four sites. Two sites were injected with Matrigel (1:1 v/v; Collaborative Biomedical Products/Becton Dickinson, Bedford, MA) and two with growth medium. Six mice were each injected s.c. with 4 × 10⁶ MDA PCa 2b cells at two sites. Three mice received cell injections with Matrigel (1:1 v/v) and three with growth medium.

Intraprostatic Injection. Fourteen mice were injected with 2 × 10⁶ MDA PCa 2a cells into the prostate (20–30 μl). Mice were anesthetized with methoxyflurane, and a lower midline incision was made. Tumor cell suspensions were injected into the dorsal prostatic lobes using a 30-gauge needle with a 1-ml disposable syringe and a calibrated push-button-controlled dispensing device (Hamilton Syringe Company, Reno, NV). The abdominal wound was closed in one layer with wound clips (Autoclip; Clay Adams, Parsippany, NY).

Surgical Castration of Male Mice

The animals were anesthetized with methoxyflurane under sterile conditions. A midline or bilateral scrotal incision was made. Aseptically removed specimens were kept in cold (4°C) sterile tubes with HEPES-buffered saline (11) until processed. The specimens were mechanically disaggregated with sterile crossed scalpels to obtain pieces of about 1 mm³. Many of the samples disaggregated into small clumps of tumor cells in the washing medium as they were cut. In these cases, subsequent enzymatic disaggregation of the tissue was not necessary. In other cases, after cutting, the tissue was resuspended in five volumes of digestion solution [Collagenase Sigma type 1 (40 units/ml of growth medium); Sigma Chemical Co., St. Louis, MO] and incubated at 37°C. The length of the incubation varied from 30 min to overnight, depending on the degree of fibrosis of the specimen. Effective disaggregation of the tissue was monitored over time by gently moving the flask.
made to expose the testicles. The testis were pulled away from the surrounding connective tissue until the spermatic cord was visible. The cord was ligated 1–2 cm above the junction of the cord and the testis. The cord was subsequently cut, the testis was removed, and the scrotum was closed with wound clips (Autoclip; Clay Adams) or nonabsorbable sutures. The inhalation agent was removed before closure of the scrotum to reduce the recovery period. Each animal was observed until it was able to flee or defend itself. No postanesthesia analgesics were given. The incision site was examined 24 h after the procedure.

PSA Blood Levels
Blood from the mice was obtained at regular intervals from a small incision in the main tail vein. Serum was separated from the blood, and PSA was measured using a dual-reactive enzymatic immunoassay kit with a lower limit of sensitivity of 0.4 ng/ml (Hybritech, Inc., San Diego, CA).

Chromosome Preparation and Giemsa Banding
Freshly fed (24 h earlier) and approximately 80% confluent cell cultures were used for chromosome preparations. Prior to harvest, cells were treated with Colcemid (final concentration, 0.04 μg/ml) for 30 min at 37°C. After centrifugation, the cell pellet was treated with a 0.06 M KCl hypotonic solution for 20 min and fixed in acetic acid-methanol (1:3 by volume). Slides were prepared following the routine air-drying procedure. Optimally aged slides were used for the induction of Giemsa banding (16). At least 15 metaphase plates from each cell line harvests were photographed using the Genetiscan (PSI, Inc., Houston, TX) and four metaphases from each sample were cut and karyotyped in detail.

Protein Extraction and Western Blot
Protein extracts from cells growing in monolayer were obtained using TRIPA buffer and following standard procedures (17). Cell lysates were centrifuged at 100,000 X g for 45 min at 4°C. The supernatants were normalized for protein content, and 50–100 μg of protein per lane (depending on the molecular weight of the protein to be detected) was fractionated on 7.5–12% SDS polyacrylamide. Gels were blotted to a Hybond-ECL nitrocellulose membrane (Amersham Corp., Arlington Heights, IL), which was probed and washed following the instructions for the enhanced chemiluminescence Western blotting detection system (Amersham Corp.).

Statistical Methods
The relationship between tumor size and PSA production before and after castration was examined using the analysis of covariance (18).

RESULTS
In 19 months, we processed 30 tissue samples, including specimens derived from primary prostate cancer of various histological grades, as well as prostate cancer tissue derived from different metastatic sites. Initial attachment and cell growth were obtained in nine specimens, but only two human prostate cancer cell lines, MDA PCa 2a and MDA PCa 2b, have been successfully established. These new cell lines, both obtained from one patient, express PSA and AR, grow in vivo and in vitro, and are androgen sensitive.

Clinical History
The patient was a 63-year-old black male who presented with impending T9 cord compression at an outside hospital in September 1993. He underwent spinal decompression and tumor resection followed by bilateral orchiectomy. Pathological examination showed a poorly differentiated adenocarcinoma. He came to M. D. Anderson Cancer Center in October 1993 with a near-complete neurological recovery and good pain control.

He did well until June 1995, when he again showed evidence of progression with a rising PSA in the presence of castrated plasma levels of testosterone (14 ng/dl). He was initiated on treatment consisting of suramin and doxorubicin. A lesion at T12 continued to grow during treatment and was encroaching on the spinal cord, causing significant right flank pain. In August 1995, he underwent elective resection of a T12 paraspinal mass. At that time, his PSA was 114 ng/ml. Pathological examination revealed metastatic adenocarcinoma. The tumor showed strong staining for PSA. No staining for chromogranin was present. The resected tumor fragments measured in aggregate 12 × 12 × 5 cm. Two different areas of grossly viable tumor were sampled for tissue culture studies. The control sections for both samples showed histologically similar carcinoma.

The patient received postoperative radiation therapy but continued to deteriorate clinically. Palliative estramustine and etoposide were given without benefit. He developed pulmonary lymphangitic spread of tumor in his lungs and suffered from significant bone marrow suppression. He died of complications from his cancer in December 1995. His PSA at the time of death was 1158 ng/ml.

Initial Isolation of Epithelial Cells and in Vitro Growth
Two samples from different areas of the tumor resected on August 1995 were used for culture. They were processed according to the procedure described in "Materials and Methods" and digested with collagenase (Sigma type 1, 40 units/ml in growth media) at 37°C for 3 h.

We used different culture conditions in the two tumor samples to isolate the epithelial cells from contaminant stromal cells and to obtain in vitro growth. The two conditions that were successful are detailed below.

Isolation of Cells Growing in Suspension. MDA PCa 2a cells were initially maintained in HITES with 10% FBS for 1 month. Epithelial cells did not attach under those conditions and, therefore, were effectively separated from fibroblasts. After 1 month, the nonadherent cells were transferred to a collagen-coated dish with BRFF-HPC1 medium and 10% FBS. After transfer to that medium, the cells grew as monolayers.

Use of Low Percentage Serum in the Growth Medium to Favor Epithelial Growth over Fibroblasts. MDA PCa 2b cells were grown as a monolayer in the presence of fibroblasts in BRFF-HPC1 supplemented with 2% FBS for 2 months until the fibroblasts died, at which time the FBS was increased to 10%.

The MDA PCa 2 cell lines have sustained continuous in vitro growth as monolayer cultures since their initial isolation.
2496 New Prostate Cancer Cell Lines

Fig. 1 Phase-contrast micrographs of human prostate cancer cell lines. A, MDA PCa 2a passage 21; B, MDA PCa 2b passage 15. Note that MDA PCa 2a cells are larger and, unlike MDA PCa 2b, form tight intercellular junctions.

(24 mo). MDA PCa 2a is in passage 56, and MDA PCa 2b is in passage 50. MDA PCa 2a and MDA PCa 2b are propagated in either BRFF-HPC1 with 20% FBS or F12K(+) with 20% FBS. Several vials of each cell line (5 × 10^6 cells/vial) have been frozen at every third passage. Recovery of frozen cells after 6 months was good; 60–70% of cells were viable and grew. The cells are free of Mycoplasma as assessed by PCR amplification of tissue culture supernatant using genus-specific PCR primers (19).

MDA PCa 2 cells were originally derived from different areas of the same tumor specimen and have some elements of morphology in common; they grow in clumps, form layers, and never reach confluence. However, as shown in Fig. 1, there are some morphological differences between the two cell lines. MDA PCa 2a cells are bigger, and unlike MDA PCa 2b, form tight intercellular junctions as seen under light microscopy.

The population doubling times also differed in the two cell lines. For MDA PCa 2a growing in BRFF-HPC1 with 20% FBS, the population doubling time was 82 h at 7 and 14 days. When grown in F12K(+) with 20% FBS, the doubling time was 87–93 h at 7 and 14 days. For MDA PCa 2b cells growing in BRFF-HPC1 with 20% FBS, the doubling time was 42–53 h at 7 and 14 days; growing in F12K(+) with 20% FBS, it was 61–73 h at 7 and 14 days. The difference in growth rates and the morphological differences described above suggest that the two cells lines originated from different clones within the tumor isolated by the different culture methods.

Clonal Growth Assay in Soft Agarose

Both MDA PCa 2a and MDA PCa 2b formed colonies in soft agarose. Their colony-forming efficiency was 2–4%, which is within the expected range for human cancer cell lines.

Karyotype Analysis of MDA PCa 2a and MDA PCa 2b

Giemsa-banded karyotype analysis was performed on these two cell lines before and after growth in nude mice. Before injection in nude mice, MDA PCa 2a at passages 6 and 18 contained between 49 and 94 chromosomes, with a mode at 63. There were five consistent clonal marker chromosomes present in this cell line (M1–M5), tentatively identified as: M1, del(3q); M2, t(5q;7q;15q); M3, del(8p); M4, t(5q;7q;14q); and M5, del(17p12). A typical Y chromosome was present in most metaphases, and some cells had more than one copy of the Y chromosome. A karyotype showing marker chromosomes and other anomalies is shown in Fig. 2a. MDA PCa 2b at passage 10 contained between 44 and 92 chromosomes, with a mode at 47. This cell line had seven clonal marker chromosomes, including the five that were found in MDA PCa 2a (Fig. 2b). The two new marker chromosomes were tentatively identified as m1, del(2p) and m2, t(4;?). A typical Y chromosome was absent in some cells but not in others.

To characterize the cells that produced tumors in nude mice, tumor samples from mice were minced, processed with collagenase, and grown in vitro for 3–6 weeks prior to harvesting for karyotype analysis. This analysis showed human cells with the same characteristic markers found in the original cell lines.

Regulation of MDA PCa 2 Cell Growth by Androgens in Vitro

To determine if the MDA PCa 2 cells were androgen sensitive, we evaluated the in vitro mitogenic effects of DHT in charcoal-stripped FBS. As shown in Fig. 3, peak responses were seen with 1 × 10^-7 M DHT after 14 days in MDA PCa 2a (P < 0.001) and after 7 days in MDA PCa 2b cells (P < 0.001), when compared with controls grown in hormone-free medium.

Expression of AR and PSA in MDA PCa 2 Cells

Expression of AR and PSA was examined by Western blot analysis of MDA PCa 2 cell lysates. The human prostate cancer cell line LNCaP was used as a positive control for AR and PSA expression (5, 10, 20, 21); the androgen-insensitive cell lines PC3 and DU145 served as negative controls (5, 20, 21). As shown in Fig. 4, both AR and PSA are expressed in each of the MDA PCa 2 cell lines. These results are in agreement with the androgen-sensitive phenotype of MDA PCa 2 cells described in the previous section.
Fig. 2  Giemsa-banded karyotypes of human prostate cancer cell lines. A, MDA PCa 2a at passage 6; B, MDA PCa 2b at passage 10. M, markers (M1 to M5) common to both cell lines. m, unique markers m1 and m2, present only in MDA PCa 2b. Both types of marker chromosomes from an additional metaphase spread are shown at the bottom of each karyotype. Note the presence of two copies of chromosome Y in MDA PCa 2a and an apparent absence of a typical chromosome Y in this cell of the MDA PCa 2b. Unidentified markers (UM) present in both cell lines are also shown.

Tumorigenicity of MDA PCa 2 Cells in an Athymic Mouse Assay

**MDA PCA 2a**

The tumorigenicity of MDA PCa 2a was assessed by two methods: s.c. injection and intraprostatic injection in athymic mice.

**s.c. injection.** Seven weeks after injection, six of the eight sites injected with tumor cells and Matrigel had a 47–170-mm³ tumor. Nine weeks after injection, three of eight sites injected with medium (no Matrigel) had tumors larger than 40 mm³. No metastases were detected by macroscopic examination of mice organs at necropsy. Tumor volume was calculated as length × width × height × 0.5236 (the formula for an ellipsoid). PSA blood levels of the tumor-bearing mice were determined at regular intervals and were found to increase over time after s.c. injection of MDA PCa 2a cells. At week 8, PSA blood levels ranged from 40 to 150 ng/ml.

**Intraprostatic injection.** Ten weeks after injection, 10 of 14 mice injected showed increased values of PSA in their blood (24–170 ng/ml). Eleven weeks after injection, 7 of 14 mice that received injections had palpable tumors. Seventeen weeks after injection, the tumors were 200–800 mm³.

**MDA PCa 2b**

The tumorigenicity of MDA PCa 2b cells was assessed through s.c. injection. Six weeks after injection, all six sites injected with Matrigel had tumors ranging in size from 40 to 80 mm³; three of six sites injected with growth medium had small tumors. The mice injected with Matrigel had serum PSA values ranging from 370 to 667 ng/mL, whereas the mice injected without Matrigel had values ranging from 53.4 to 129.5 ng/mL. Eight weeks after injection, all 12 sites injected had tumors. Intraprostatic injections were not done with MDA PCa 2b cells, due to the high rate of tumor induction achieved through s.c. injections. In vivo, MDA PCa 2b grew faster than MDA PCa 2a.

The ability of MDA PCa 2 cells to induce tumors that secrete PSA as a function of tumor size in athymic mice is compelling evidence that these cell lines originated from human prostatic cancer. In addition, these results suggest that this system is a useful model to study human prostate cancer. Expression of PSA is an invaluable marker of prostate cancer progression (2–4), because tumor growth can be monitored by PSA blood levels, and loss of PSA expression occurs only in very late stages during the progression of the disease to a more aggressive phenotype.

**Effect of Castration of Mice Bearing Tumors Produced by MDA PCa 2 Cells**

The effect of castration on the in vivo growth of MDA PCa 2 cells was assessed by monitoring tumor size and PSA blood levels after castration of mice bearing tumors produced by s.c. injection of the cells. Male athymic mice were injected with 4 × 10⁶ cells with medium (no Matrigel). Mice were castrated when their tumors were 40 mm³ or larger. This occurred at 8–1/2 weeks in 3 of 10 mice injected with MDA PCa 2a cells and at 6 weeks in 5 of 9 mice injected with MDA PCa 2b cells. As shown in Figs. 5 and 6, the tumors stopped growing or decreased in size, and PSA blood levels dropped dramatically after castration of the mice injected with either cell line. These results demonstrate that the MDA PCa 2 cell lines are androgen responsive.

**PSA Blood Levels as a Function of Tumor Size**

Four regression lines (MDA PCa 2a before and after castration, and MDA PCa 2b before and after castration) using tumor weight to predict PSA were calculated. Because tumor weight is a measure of cellular mass and PSA is normally
DISCUSSION

We have established two new human prostate cancer cell lines, MDA PCa 2a and MDA PCa 2b. Evidence that these cell lines originated from human prostatic cancer includes the preservation of PSA expression and androgen sensitivity.

MDA PCa 2 cells constitute an important model system for the study of the clinically defined androgen-independent growth of human prostate cancer. These cell lines were derived from a patient with prostate cancer who relapsed two years after surgical castration. At the time of relapse, the tumor from which the cells were derived was actively growing, producing symptoms that necessitated surgery. This objective evidence of disease progression in the presence of castrate plasma levels of testosterone (14 ng/dl) is the clinical definition of androgen-independent growth used at the University of Texas M. D. Anderson Cancer Center and indicates the presence of an androgen-independent tumor (4, 22).

Recurrent prostate cancer is a spectrum of diseases that differ in clinical presentation and prognosis (2-4). A recent report proposes a classification of prostate cancers as hormone-naïve, androgen-independent/hormone-sensitive, or hormone-independent based on their hormone sensitivity (4). Androgen-independent/hormone-sensitive cells are categorized as androgen-independent because they grow in the presence of castrate plasma levels of testosterone, but their growth rate responds to other hormonal manipulations (e.g., adrenal androgen blockade and antiandrogens). The importance of this distinction is that it is generally recognized that a subpopulation of patients who have relapses after one hormone treatment may still respond to a second hormone treatment (4). The MDA PCa 2 cells, which are derived from an androgen-independent tumor but are sensitive to androgens in vitro and in vivo, are presumed to represent the androgen-independent/hormone-sensitive tumor type.

To our knowledge, the LNCaP cell line is the only other existing human prostate cancer cell line that is androgen sensitive. LNCaP was derived from a metastatic site (supraclavicular lymph node) of a patient with an androgen-independent prostatic adenocarcinoma (10). In contrast to MDA PCa 2a and 2b,
the LNCaP cell line is only weakly tumorigenic when injected s.c. into male nude mice, but the tumorigenicity can be increased by coinoculation with Matrigel or fibroblasts (23). The complex role of androgens in prostate cancer progression has been highlighted in a recent publication (24). In that report, an androgen-repressed human prostate cancer cell line derived from a patient with androgen-independent prostate cancer was characterized. Those findings imply that a subpopulation of cells is inhibited by androgens. The authors suggest that the androgen-repressed phenotype may represent late events in prostate cancer progression (24).

A clinical hallmark of prostate cancer progression to the androgen-independent state is the development of osseous metastases; up to 84% of patients have bone metastases at autopsy. This is the only malignancy to consistently produce osteoblastic lesions (2, 23, 25). The marked clinical affinity that prostate cancer has for bone is responsible for much of the clinical morbidity that is a result of this cancer. Our cell lines may serve as a useful model of the complex process of prostate cancer metastasis and progression within bone. The availability of this new model system is essential for the study of these interactions. The marker chromosomes characteristic of these prostate cancer cell lines contain regions that have been identified by allelotyping studies and comparative genomic hybridization analysis as sites of common allelic gains or losses in clinical prostate cancer specimens, i.e., 8p (26-28), 7q (27, 29-32), 5q (27, 28), 3q (27), 2q (27), and 17p (22, 27). Of particular interest is the presence of an interstitial or intercalary deletion of 17p12, with preservation of 17p11 and 17p13. This may suggest the presence of an additional tumor suppressor gene in 17p along with p53, which is located at 17p13.1 (22). It is also of interest that Cher et al. (27) found an increased frequency of allelic gains in the 4q25-28 region among African-Americans, because the MDA PCa 2 cells which were derived from an African-American patient have three to four copies of chromosome 4, reflecting amplification of the 4q25-28 region. A marker chromosome involving chromosome 4 was also present in the MDA PCa 2b cell line.

The MDA PCa 2 cell lines, although obtained from different samples of the same specimen, have unique genetic features (as assessed by karyotype analysis) and phenotypes (different morphology and different growth rate). It is likely that these two cell lines are distinct clones that reflect the genetic heterogeneity of the tumor and will provide important model systems to cellular interaction that may participate in prostate cancer progression within bone.

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N M Navone, M Olive, M Ozen, et al.


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