Cancer Research

LNCaP Model of Human Prostatic Carcinoma

Julius S. Horoszewicz, Susan S. Leong, Elzbieta Kawinski, et al.

Cancer Res 1983;43:1809-1818.

Updated Version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/43/4/1809

Citing Articles	This article has been cited by 100 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/43/4/1809#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

[CANCER RESEARCH 43, 1809-1818, April 1983]
0008-5472/83/0043$02.00

LNCaP Model of Human Prostatic Carcinoma[1]

Julius S. Horoszewicz,[2] Susan S. Leong, Elzbieta Kawinski, James P. Karr, Hannah Rosenthal, T. Ming Chu, Edwin A. Mirand, and Gerald P. Murphy

Departments of Biological Resources [J. S. H., S. S. L., E. K., E. A. M.], Experimental Surgery [J. P. K., G. P. M.], Genetics and Endocrinology [H. R.], and Diagnostic Immunology and Biochemistry [T. M. C.], Roswell Park Memorial Institute, Buffalo, New York 14263

ABSTRACT

The LNCaP cell line was established from a metastatic lesion of human prostatic adenocarcinoma. The LNCaP cells grow readily *in vitro* (up to 8×10^5 cells/sq cm; doubling time, 60 hr), form clones in semisolid media, are highly resistant to human fibroblast interferon, and show an aneuploid (modal number, 76 to 91) human male karyotype with several marker chromosomes. The malignant properties of LNCaP cells are maintained. Athymic nude mice develop tumors at the injection site (volume-doubling time, 86 hr). Functional differentiation is preserved; both cultures and tumor produce acid phosphatase. High-affinity specific androgen receptors are present in the cytosol and nuclear fractions of cells in culture and in tumors. Estrogen receptors are demonstrable in the cytosol. The model is hormonally responsive. *In vitro,* 5α-dihydrotestosterone modulates cell growth and stimulates acid phosphatase production. *In vivo,* the frequency of tumor development and the mean time of tumor appearance are significantly different for either sex. Male mice develop tumors earlier and at a greater frequency than do females. Hormonal manipulations show that, regardless of sex, the frequency of tumor development correlates with serum androgen levels. The rate of the tumor growth, however, is independent of the gender or hormonal status of the host.

INTRODUCTION

CaP[3] is the second most frequent tumor of males in the United States (41). Unknown etiology, variable pathology, intricate relationship to endocrine factors, and anaplastic progression contribute to the complexity of this disease and confound the investigator. The progress toward establishing effective methods for early detection and successful management of CaP is predicated on both clinical studies and laboratory experimentation with appropriate models. Extensive studies on models of rodent prostate carcinoma (7, 9, 18, 23, 32, 40) have already provided a better understanding of the biology of prostatic neoplasia. These tumors, derived from rat prostate, are readily transplantable, and the tumor cells proliferate *in vitro.* Their value as models is based on the preservation of malignancy, biological and biochemical markers, hormonal responsiveness, and drug sensitivity. Their limitations stem from apparent restrictions imposed on extrapolating data derived from animal models to human neoplasia.

The development of a correspondingly wide array of appropriate models of human origin is hindered by difficulties in sustaining the propagation of human CaP cells outside its natural host. With few exceptions (12, 33), the athymic nude mouse fails to provide satisfactory growth environment for direct CaP xenografts from patients (10, 34). Although human prostate cancer cells grow readily as short-term explant cultures (6, 16, 24, 45), reports on established malignant cell lines with convincing pedigree are rare (14, 20, 27, 42). In addition, some of such cell lines fail to maintain markers characteristic of CaP: *e.g.*, production of secretory human prostatic acid phosphatase (19, 42); organ-specific prostate antigen (30); responsiveness to sex hormones (19, 42); or the presence of the Y chromosome (19).

The subject of this report is the cell line LNCaP which we have isolated from a metastatic lesion of human prostatic cancer (14). The growth properties *in vitro*, clonogenic potential, karyology, androgen responsiveness with its attendant presence of specific receptor molecules, and tumorigenicity in intact and hormonally manipulated athymic nude mice are described. These data extend our knowledge toward a full characterization of a versatile new model suitable to study human prostatic cancer in the laboratory.

MATERIALS AND METHODS

Culture Medium. The LNCaP cell line was originally established (14) and subsequently propagated in RPMI-1640-GA (Grand Island Biological Co., Grand Island, N. Y.) and supplemented with 5% (v/v) heat-inactivated single-lot (No. 300010H) FBS (K.C. Biological, Inc., Lenexa, Kans.). For hormone responsiveness experiments, ZSD (AMF Immuno-Reagents Inc., Seguin, Texas) was utilized in place of FBS. ZSD is a commercially processed serum of bovine origin. The concentration of steroids is reduced by charcoal filtration by approximately 20-fold in comparison with regular FBS. Data provided by the manufacturer for the single lot (No. 050481) of serum used in our experiments indicated the following concentrations: testosterone, 72 pM; 17β-estradiol, 58 pM; cortisol, not detectable; DHT, not determined; cholesterol, 0.52 μM; insulin, 6 μunits/ml; and total protein, 45 mg/ml. The DHT was obtained from Sigma Chemical Co. (St. Louis, Mo.).

Cell Propagation. Confluent monolayers (6 to 8×10^5 cells/sq cm) of LNCaP cells cultivated in 75-sq cm plastic flasks (Falcon Plastics, Oxnard, Calif.) were dispersed with trypsin (0.05%):EDTA (0.02%) solution (Grand Island Biological Co.) and counted. The cells were inoculated into new vessels at 3 to 8×10^4 cells/sq cm in RPMI-1640-GA with 5% (v/v) FBS (0.1 ml/sq cm) and left undisturbed for 48 hr in the incubator (37°, 5% CO_2 atmosphere) to facilitate attachment. The cultures were fed at 5- to 7-day intervals with fresh medium (0.3 ml/sq cm) of the same composition. LNCaP cells from between the 30th to the 55th passage *in vitro* were used to conduct these studies. This corresponds to between 100 and 300 population doublings after the original isolation. The cells are free of *Mycoplasma*.

Cell Counts. An electronic particle counter (Model ZB-1 Coulter Counter) was used to enumerate formalin-fixed nuclei from detergent-

---

[1] This work was supported by USPHS Grant CA 27472 through the National Prostatic Cancer Project.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: CaP, carcinoma of the prostate; RPMI-1640-GA, Roswell Park Memorial Institute Medium 1640 containing additional L-glutamine (1 mM) and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml); FBS, fetal bovine serum; ZSD, Zeta-Sera-D; DHT, 5α-dihydrotestosterone; AP, acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2); HuIFNβ, human interferon β (human fibroblast interferon); RPMI-1640, Roswell Park Memorial Institute Tissue Culture Medium 1640.

Received July 26, 1982; accepted January 6, 1983.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

lysed cells according to the method of Butler *et al.* (4).

Karyology. For cytogenetic analysis, LNCaP cultures were incubated with Colcemid (0.015 μg/ml; 2 hr; Grand Island Biological Co.), harvested, treated with hypotonic solution (0.075 M KCl), fixed with methanol:acetic acid (3:1), and spread on dry slides. The G-banding method (36) of staining was used for chromosome identification and analysis.

Sex Hormone Receptors. Methods used for the preparation of cytosols, nuclear fractions, and measurements of steroid receptors were published (11, 25, 39). Briefly, for androgen receptor determinations, the competition binding assay with methyltrienolone (17β-hydroxy-17-methylestra-4,9,11-trien-3-one) in the presence of triamcinolone acetonide was used (11, 43). Estrogen receptor was quantified by the dextran-coated charcoal assay (25) as well as the sucrose density gradient analysis (39) including nafoxidine as the competitor for the binding of radioactive 17β-estradiol.

Where appropriate, the binding was analyzed using the Scatchard plot method (38). The concentrations of protein (22) and DNA (3, 35) were determined as published.

[$^3H_2$]Methyltrienolone (specific activity, 87 Ci/nmol) and nonradioactive methyltrienolone as well as 17β-[2,4,6,7-^{3}H]estradiol (specific activity, 90 Ci/mmol) were purchased from New England Nuclear (Boston, Mass.). The trizma (free base) and triamcinolone acetonide were obtained from Sigma. Norit A charcoal was purchased from Mathesen, Coleman and Bell Manufacturing Chemists (Norwood, Ohio); dextran (Grade C, M_r 60,000 to 90,000) was obtained from Mann Research Laboratories (New York, N. Y.); and sucrose (RNase free) was purchased from Schwarz/Mann (Orangeburg, N. Y.). Dithiothreitol was obtained from Calbiochem (La Jolla, Calif.), and nafoxidine was a gift from the Upjohn Co., Kalamazoo, Mich.

AP. AP activity was determined by the method of Babson and Phillips (1) using 3 mM α-naphthyl acid phosphate sodium salt as the substrate, 0.02% Fast Red B salt (5-nitro-2-aminomethoxybenzene diazotate) as the color developer and 6 mM α-naphthol as the standard. All these reagents were obtained from Sigma.

HuIFNβ. The highly purified (specific activity, 2×10^7 reference units/mg protein) HuIFNβ used in these studies was produced in our laboratory for clinical trials (21). Interferon assays were carried out using published methods (13, 15). The International reference standard of human fibroblast interferon (G-023-902-527; Research Resources Branch, NIH) was included in each assay for calibration, and titers were expressed in reference units as geometric means of triplicate tests.

Athymic Nude Mice. A breeding nude mouse colony has been maintained in our laboratory since 1976 under pathogen-limited conditions. The animals bear a *nu/nu* genotype on super Swiss and C3H background, and their life span is in excess of 12 months.

Tumor Induction. Tumors were induced in athymic nude mice (6 to 8 weeks old) by injection of cultured LNCaP cells. The cells were dispersed by trypsin, washed (twice) in serum-free medium RPMI-1640 (10 min centrifugation, 200 × *g*), resuspended (2×10^7 cells/ml, same medium), and injected (0.2 ml) s.c. between the shoulder blades of each mouse. The tumors were measured with calipers (2 perpendicular diameters), and the tumor volume was calculated using the formula $W^2 \times L/2$ cu mm [*W*, shorter diameter; *L*, longer diameter (mm)].

Hormonal Manipulations. Castration or ovariectomy were performed 3 days before inoculation of LNCaP cells. Pellets, containing either 2 mg testosterone propionate or 2 mg 17β-estradiol, with cholesterol, Emcompress (Edward Mendel, Carmel, N. Y.), and magnesium stearate as excipients were prepared by the Roswell Park Memorial Institute Drug Formulation and Development Laboratory and implanted s.c. 3 days before inoculation of LNCaP cells. Both hormones were purchased from Sigma.

RESULTS

Studies *in Vitro*

LNCaP Cell Cultures. The LNCaP cells form monolayers which are only weakly attached to the surface of plastic culture vessels. The cultures require gentle handling at all times because the cells could be easily dislodged by tapping, shaking, or pipeting. The reattachment of dissociated cells is slow, and only about 70% of cells from the original inoculum adhered to the growth surface within 40 hr. Varying the concentration of FBS between 1 and 15% (v/v) does not measurably affect this process (data not shown). The low anchoring potential is also responsible for the 10 to 20% cell loss during media changes in long-term experiments. When cell density in excess of 8×10^5 cells/sq cm is reached, the cell sheet tends to detach spontaneously. High concentrations of LNCaP cells, dispersed by routine trypsinization or by mechanical manipulations, form clumps which are difficult to dissociate or count reliably. Accurate cell counts can be obtained after lysing the cells and counting the free nuclei (4). To initiate growth in new cultures, a broad range of inocula (from 5×10^3 to 1×10^5 cells/sq cm) could be used (Chart 1).

Serum Requirement. Cultures of human cells *in vitro* require serum supplement for growth. The optimal concentration varies for different cells and is usually lower for cells derived from tumors than from normal tissues. To characterize further the LNCaP cells *in vitro*, their growth in RPMI-1640-GA supplemented with different concentrations of FBS [from 0.01 to 15% (v/v)] was measured. The results (Chart 2) show that LNCaP cells can proliferate in the presence of a broad range (0.1 to 15%) of FBS concentrations. Maximal growth was obtained using media supplemented with 2.5% to 15% FBS. Under these culture conditions, the mean population-doubling time was calculated to be approximately 60 hr. Lowering the serum concentration resulted in slower growth. At a FBS concentration of 0.01%, the number of cells in the culture slowly declined. For maintenance of cell stocks and experiments *in vitro*, 5% (v/v) FBS was routinely added to medium RPMI-1640-GA.

Cloning. Single malignant cells are capable of colonial growth in semisolid media. Table 1 shows that LNCaP cells have anchorage-independent proliferation and can be cloned easily and with good efficiency. From plates inoculated with the lowest number of cells, 13 individual clones were isolated, propagated, and cryopreserved (10% dimethyl sulfoxide, liquid N_2 storage) for future studies.

Karyology. The LNCaP cells were examined as to their karyological characteristics at 12 and 32 months after initial isolation, *i.e.*, after approximately 100 and over 300 population doublings *in vitro*, respectively. At both points in time, the LNCaP cells

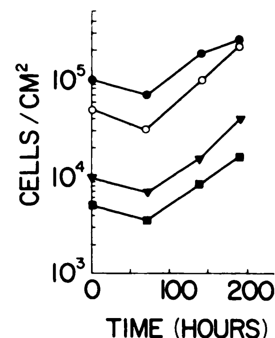

Chart 1. *In vitro* growth of LNCaP cells from inocula of different concentrations. Dispersed LNCaP cells were seeded in tissue culture multiwell plates (6 wells/plate; 9.62 sq cm/well) (Linbro, Hamden, Conn.) at 5×10^3 (■), 1×10^4 (▼), 5×10^4 (○), and 1×10^5 (●) cells/sq cm and grown (37°, 5% CO_2) in RPMI-1640-GA with 5% (v/v) FBS. Cell counts (in triplicate from 3 wells) were carried out after 3, 6, and 8 days without media change. The number of cells per sq cm is plotted against time in hr after seeding.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

exhibited grossly aneuploid human male karyotype with several marker chromosomes present. At 32 months, the number of chromosomes ranged from 33 to 91 with a modal number of 76

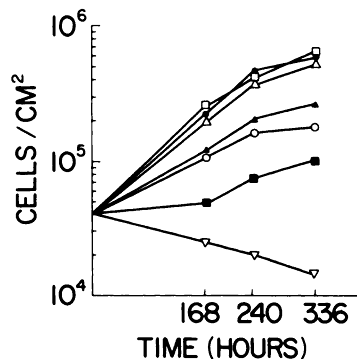

Chart 2. Growth of LNCaP cells *in vitro* in the presence of different concentrations of FBS. Dispersed LNCaP cells were seeded at 4×10^4 cells/sq cm in 6-well plates and grown (37°, 5% CO_2) in RPMI-1640-GA with different concentrations of FBS. Cell counts (in triplicate from 3 wells) were carried out after 7, 10, and 14 days without medium change. The number of cells/sq cm is plotted against time in hr after seeding. FBS concentrations (%, v/v): ▽, 0.01; ■, 0.1; ○, 0.5; ▲, 1.0; △, 2.5; □, 7.5; ●, 15.

Table 1

Colonial growth of LNCaP cells in semisolid agar

Freshly trypsinized LNCaP cells (1.0 ml) in RPMI-1640-GA supplemented with 5% (v/v) FBS were mixed with an equal volume of the same medium containing 0.6% agar (40°). The dispersed cells were plated (in triplicate) over underlays (2.0 ml) of the same medium solidified with 0.5% agar in 50-mm plastic Petri dishes (Falcon Plastics). The plates were examined microscopically over a grid with 1-mm squares to count and to map any clumps containing 2 or more cells. After 3 weeks of incubation (37°, 5% CO_2), colonies derived from single cells were counted.

	% of total no. of cells plated	
No. of cells/plate	Cloning efficiency	Clumping rate
1×10^5	TMTC[a]	TMTC
1×10^4	TMTC	TMTC
5×10^3	15.0	3.6
1×10^3	12.0	1.8
5×10^2	10.4	0.8

[a] TMTC, too many to count.

to 91 (in 26 of 46 metaphases counted) (Table 2; Fig. 1). Some marker chromosomes were present consistently: m_1, long submetacentric marker chromosome of unknown origin; m_4 and m_5, identified as t(1;15); m_6 and m_7, identified as 10q−. The last 4 (m_4 to m_7) were usually present in 2 copies. Other chromosome abnormalities have been observed with lesser regularity.

Sex Hormone Receptors in Cultured LNCaP Cells. Specific androgen- and estrogen-binding proteins were reported in normal and neoplastic human prostatic tissues (8, 11, 28, 43). To determine if these macromolecules are present in the LNCaP cells maintained *in vitro*, androgen receptor in both the cytosol and the nuclear extract and estrogen receptor in the cytosol were measured. Table 3 shows that both specific androgen and estrogen receptors are present in the cytosol. Nuclear androgen receptor was also detected, but at a relatively lower concentration. Scatchard plot analysis (Chart 3) of the results indicated the presence of a separate single class of specific, high-affinity, low-capacity, and saturable androgen-binding molecules.

DHT Modulation of Cell Growth and AP Production. Human prostatic epithelial cells *in vivo* are responsive to male sex hormones. Therefore, both the cell proliferation and the AP production in LNCaP cultures in media supplemented with DHT were studied. The DHT concentrations tested (between 1 nM and 1 µM) include and exceed the normal range of DHT levels found in the serum of healthy males (0.8 to 4.1 nM) (37). Simultaneous and parallel experiments were conducted using media supplemented with 5% (v/v) of either ZSD or FBS. ZSD was used because it is depleted of steroid hormones by activated charcoal during the manufacturing process. The LNCaP cells in RPMI-1640-GA supplemented with 5% (v/v) ZSD grew at a slower rate with a mean population-doubling time of approximately 144 hr as compared with the 60 hr for cells cultivated in medium with 5% (v/v) FBS. ZSD, however, does not contain apparent inhibitors of LNCaP cell division, since its addition (up to 10% v/v) to media already containing FBS (at 5% or 10% v/v) did not diminish the growth rate of the cultures (data not shown).

Table 2

Chromosome number distribution of LNCaP cells after 12 and 32 months of growth in vitro

The chromosome number was obtained from photographs of stained (trypsin-Giemsa) specimens of cells arrested in metaphase (see also Fig. 1).

	Distribution of cells with following numbers of chromosomes																Total No. of cells counted
	33	40	41	45	46	47	48	49	54	57	60	64	69	70	72	76–94 and above	
12 mos. *in vitro*					3	2	1		1	1		1				15	24
32 mos. *in vitro*	1	2	1	4	4			1			2		1	2	2	26	46

Table 3

Androgen and estrogen receptors in cultured LNCaP cells

For each of 2 experiments shown, 2×10^8 LNCaP cells were mechanically removed from culture vessels, centrifuged (200 × *g*, 10 min, 0°), and washed twice in ice-cold serum-free RPMI-1640. The cell pellets were rapidly frozen and stored in liquid N_2 until used (within 30 days). Competition binding assays of cytosol and nuclear extracts by the dextran-coated charcoal procedure were used to determine androgen and estrogen receptor content. K_d values were derived from Scatchard plots. Protein was measured according to the method of Lowry *et al.* (22) and DNA according to Richards' modification (35) of the method of Burton (3).

	Cells		Androgen receptor					Estrogen receptor (in cytosol)		
			In cytosol			In nuclear extract				
Experiment	mg DNA/g cells	mg cytosol protein/g cells	fmol/mg DNA	fmol/mg cytosol protein	K_d (nM)	(fmol/mg DNA)	K_d (nM)	fmol/mg DNA	fmol/mg cytosol protein	K_d (nM)
1	5.5	31.5	870	153	1.4[a]	ND[b]	ND	250	44	4.7
2	3.6	36.5	2160	266	0.9	309	1.3	585	72	5.2

[a] For Scatchard plot, see Chart 3.
[b] ND, not determined.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

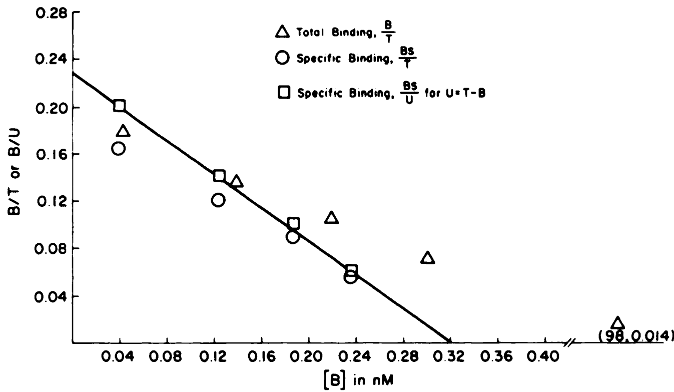

Chart 3. Scatchard plot analysis of androgen binding (*B*) in the cytosol from cultured LNCaP cells. Duplicate cytosol samples (200 µl, 3.1 mg protein per ml) were incubated (22 hr, 4°) with [$^3H_2$]methyltrienolone (50 µl, serial dilutions from 1 to 16.2 nM) in the presence and in the absence of nonradioactive methyltrienolone (508 nM). The mixtures contained also triamcinolone acetonide (1000-fold excess). Protein bound radioactivity was determined using the dextran-coated charcoal technique, and the fraction specifically bound was calculated. The calculated K_d is 1.4 nm.

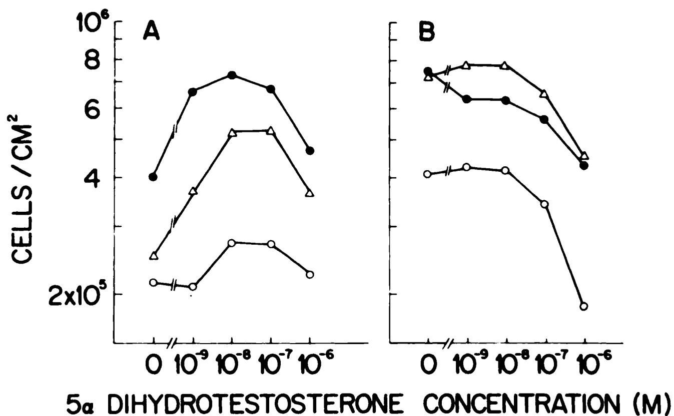

Chart 4. Effect of DHT on proliferation of LNCaP cells in culture. LNCaP cells were cultured for 20 days (37°, 5% CO_2) in medium RPMI-1640-GA with 5% (v/v) ZSD and then seeded in tissue culture 6-well plates (9.62 sq cm/plate) at 1 × 10^5 cells/sq cm in the same medium (0.1 ml/sq cm). Following attachment (48 hr), the medium was changed (0.2 ml/sq cm): one half of the cultures (A) were fed medium RPMI-1640-GA with 5% (v/v) ZSD, and the other half (*B*) were fed with 5% (v/v) FBS. At this time, DHT solutions (0.1 ml) in RPMI-1640-GA were added to each plate to obtain DHT concentrations indicated on the *abscissa*. Controls received 0.1 ml of a 0.2% solution of solvent mixture (ethanol:propylene glycol, 9:1) in which stock DHT solution (10 mM) was prepared, resulting in final ethanol concentrations of 0.07 mg/ml final propylene glycol concentrations of 0.01 mg/ml. Media and hormone were replaced after 5 and 8 days. Cell counts (in triplicate from 3 wells) were carried out at 120 hr (○), 192 hr △, and 312 hr (●) after DHT addition. Media harvested at the time of cell counting were used to measure AP activity (see Chart 5).

The addition of DHT to growth medium has a distinct modulating influence on the proliferation of LNCaP cells (Chart 4). The orderly and dose-dependent curves obtained demonstrated 2 apparently different effects of DHT depending on which serum (ZSD or FBS) was present in the medium: (*a*) the addition of DHT to media supplemented with ZSD stimulated cell proliferation at all concentrations tested, with an optimum of about 10 nM (Chart 4*A*); and (*b*) DHT addition to media supplemented with FBS resulted in a dose-dependent suppression of cell proliferation (Chart 4*B*).

The AP activity in the LNCaP culture medium is also influenced by the addition of DHT. DHT was found to have potent stimulatory effects over the entire range of the tested concentrations (Chart 5) regardless of which serum, ZSD or FBS, was used.

Resistance to Interferon. Interferons are glycoproteins with antiviral as well as antiproliferative activities. Both activities are readily demonstrable on a wide range of normal and neoplastic cell types. Since interferons are being tested as potential therapeutic agents in a variety of human tumors, including prostatic neoplasia, the *in vitro* susceptibility of LNCaP cells to HuIFNβ was measured. HuIFNβ, at concentrations as high as 10,000 reference units/ml, was found to have only a negligible effect on the proliferation of LNCaP cells during the 7-day period of exposure (Table 4). The presence of interferon did not affect the cell viability or influence the levels of AP production. The interferon levels detected at 24 and 96 hr postaddition (Table 4) indicate that the expected decay of interferon activity in LNCaP cultures is not greater than either the decay observed during incubation in cell-free medium (data not shown) or the decay reported for other cells (13).

To determine whether interferon could induce an antiviral state in LNCaP cells and thus prevent the killing action of vesicular stomatitis virus on these cells, HuIFNβ (10 to 10,000 reference units/ml) was preincubated with the cells for 24 hr. Such treat-

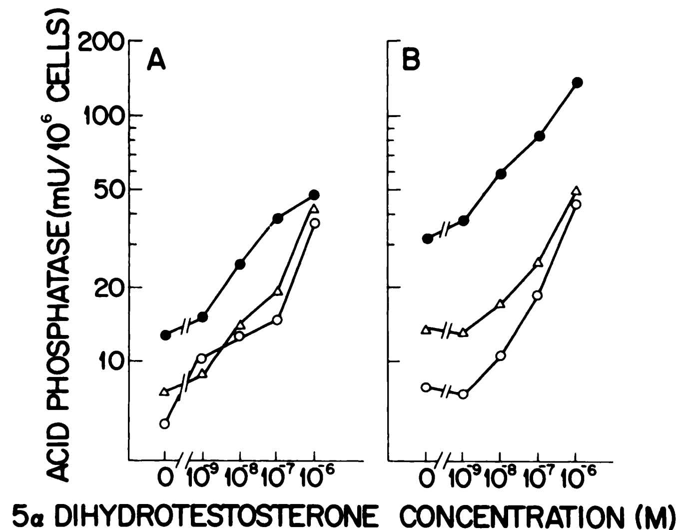

Chart 5. Effect of DHT on the activity of AP in LNCaP culture fluids. AP concentration was measured in triplicate in culture fluids from the same experiment illustrated by Chart 4. *A*, cells in RPMI-1640-GA with 5% (v/v) ZSD; *B*, cells in the same medium with 5% (v/v) FBS. Results are expressed in milliunits (*mU*) of AP per 10^6 cells and are plotted against increasing concentrations of DHT on the *abscissa* 120 hr (○), 192 hr (△), and 312 hr (●) after DHT addition.

Table 4

Effect of HuIFNβ on LNCaP cells

HuIFNβ (0.1 ml) was added to LNCaP cultures (in quadruplicate, 5.8 × 10^4 cells/sq cm, 25-sq cm plastic flasks (Falcon Plastics) at Time 0. The growth medium (5.0 ml) and interferon were replaced at 96 hr. Total cell counts, viability, and AP activity in the culture fluids were determined at 168 hr. Interferon in culture fluids was assayed as published (13).

Interferon (reference units/ml) at			No. of population doublings at 168 hr	Cell viability[a] (%)	AP (milliunits/ 10^6 cells)
0 hr	24 hr	168 hr			
10,000	6,200	870	2.4542	81	5.4
1,000	790	45	2.7962	80	4.0
100	48	<5	2.8048	ND[b]	4.1
None	<5	<5	2.7993	83	4.5

[a] By trypan blue exclusion.
[b] ND, not determined.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

Table 5

LNCaP tumors in male and female athymic nude mice

Mice (6 to 8 weeks old) were given s.c. injections of 4 × 10⁶ cultured LNCaP cells and observed for 12 weeks. Tumors were measured with calipers at 5- to 7-day intervals. Data were combined from 2 to 6 experiments.

	Mean time of tumor appearance (days postinjection)	Tumor incidence (%)	Calculated mean tumor volume-doubling time (hr)
Males	23.79 ± 0.85[a,b]	58[c]	85.5 ± 6.5[d]
Females	33.64 ± 2.02[e]	36[f]	87.7 ± 5.1[d]
Significance of difference	$p < 0.0001$[g]	$p < 0.0005$[h]	Not significant[g]

[a] $n = 28$.
[b] Mean ± S.E.
[c] $n = 153$.
[d] $n = 15$.
[e] $n = 58$.
[f] $n = 162$.
[g] Students' t test.
[h] χ^2 test.

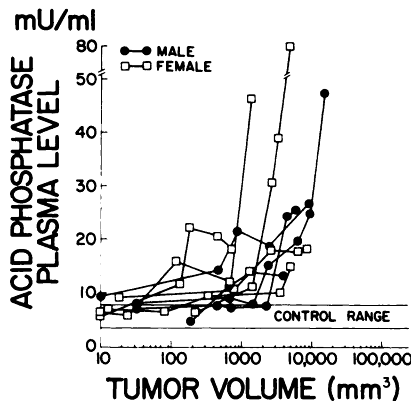

Chart 6. AP activity in the plasma of athymic nude mice with LNCaP tumors. Plasma samples were taken at weekly intervals from 4 male and 5 female mice with LNCaP tumors. AP activity in the plasma was plotted against tumor volume at the time of sampling. Control range [4.1 to 9.4 milliunits (*mU*)/ml] was established on plasma samples from 11 male [6.46 ± 0.54 milliunits/ml] and 9 female [6.09 ± 0.56 milliunits/ml] athymic nude mice without tumors.

ment did not result in protection of the cell sheet from total destruction (within 36 hr) when challenged with vesicular stomatitis virus at 100 times lower multiplicity of infection then used routinely for interferon titrations. This result indicates that LNCaP cells in culture are at least 1 million-fold more resistant than are normal human diploid fibroblasts to the induction of antiviral protection by HuIFNβ.

Studies in Athymic Nude Mice

Inoculum. The frequency of tumor formation following s.c. injection of 3.3 × 10⁶ and 16.5 × 10⁶ cells/animal did not significantly differ: 5 of 10 and 6 of 10 animals, respectively, developed tumors. No tumors grew after injection of 0.65 × 10⁶ cells (10 mice) for up to 4 months of observation. For further studies, a tumor-inducing inoculum of 4 × 10⁶ cells, injected s.c. in 0.2 ml, was chosen.

Tumor Formation and Growth. Nude mice given injections of LNCaP cells grown *in vitro* developed at the injection site poorly differentiated adenocarcinomas (14). Palpable tumors appeared between 14 and 56 days postinoculation. Once formed, the tumors grew rapidly (Chart 7). During the exponential phase of

Table 6

AP activity in the plasma and LNCaP tumors from nude mice

Four athymic nude mice with LNCaP tumors were bled, the plasma was collected, and AP activity was measured according to the method of Babson and Phillips (1). The tumors were dissected, weighed, and homogenized (ice bath; Sorvall Omni-Mixer with microattachment; 6000 rpm; 3 min) in acetate buffer (0.02 M; pH 5.0; 3 ml/g tumor) containing Tween 80 (0.01%). AP and protein were measured in the cytosol (supernatant after centrifugation of the homogenate at 105,000 *g* for 60 min) (1, 22).

				Cytosol AP	
Mouse	Sex	Tumor wt (g)	Plasma AP (milliunits/ml)	Total milliunits	milliunits/mg protein
1	Female	6.7	506	23,879	107
2		1.05	39	3,791	193
3	Male	6.4	18	12,115	34
4		6.4	26	15,577	39

Table 7

LNCaP tumor development in intact and hormonally manipulated athymic nude mice

Mice (6 to 8 weeks old) were castrated, ovariectomized, or implanted with hormone (2-mg) pellets 3 days before s.c. injection with 4 × 10⁶ cultured LNCaP cells. Data from 2 experiments were combined: Experiment 1 involved Groups A, B, C, F, G, and H; Experiment 2 involved Groups A, B, D, E, F, G, I, and J (see also Chart 7).

				Statistically significant difference[b] at	
Experimental groups	Plasma testosterone (ng/ml)[a]	Animals with tumors/animals inoculated	% with tumors	$p < 0.01$ from Group	$p < 0.05$ from Group
Males					
A. Normal Controls	1.9	18/29	62	B, G	F
B. Castrated	<0.1	7/32	22	A, H	C
C. Castrated + testosterone[c]	0.45	8/14	57		B
D. Castrated + estradiol[d]	ND[e]	7/14	50		
E. Normal + estradiol[d]	ND	7/14	50		
Females					
F. Normal controls	<0.1	10/30	33	H	A
G. Ovariectomized	<0.1	7/28	25	A, H	
H. Ovariectomized + testosterone[c]	0.49	13/15	87	B, F, G	I
I. Ovariectomized + estradiol[d]	ND	6/14	43		H
J. Normal + estradiol[d]	ND	8/15	53		

[a] Measured by radioimmunoassay (Bioscience Laboratories, Van Nuys, Calif.) on plasma samples pooled from 10 animals.
[b] χ^2 test.
[c] Animals implanted with 2-mg testosterone propionate pellets.
[d] Animals implanted with 2-mg 17β-estradiol pellets.
[e] ND, not determined.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

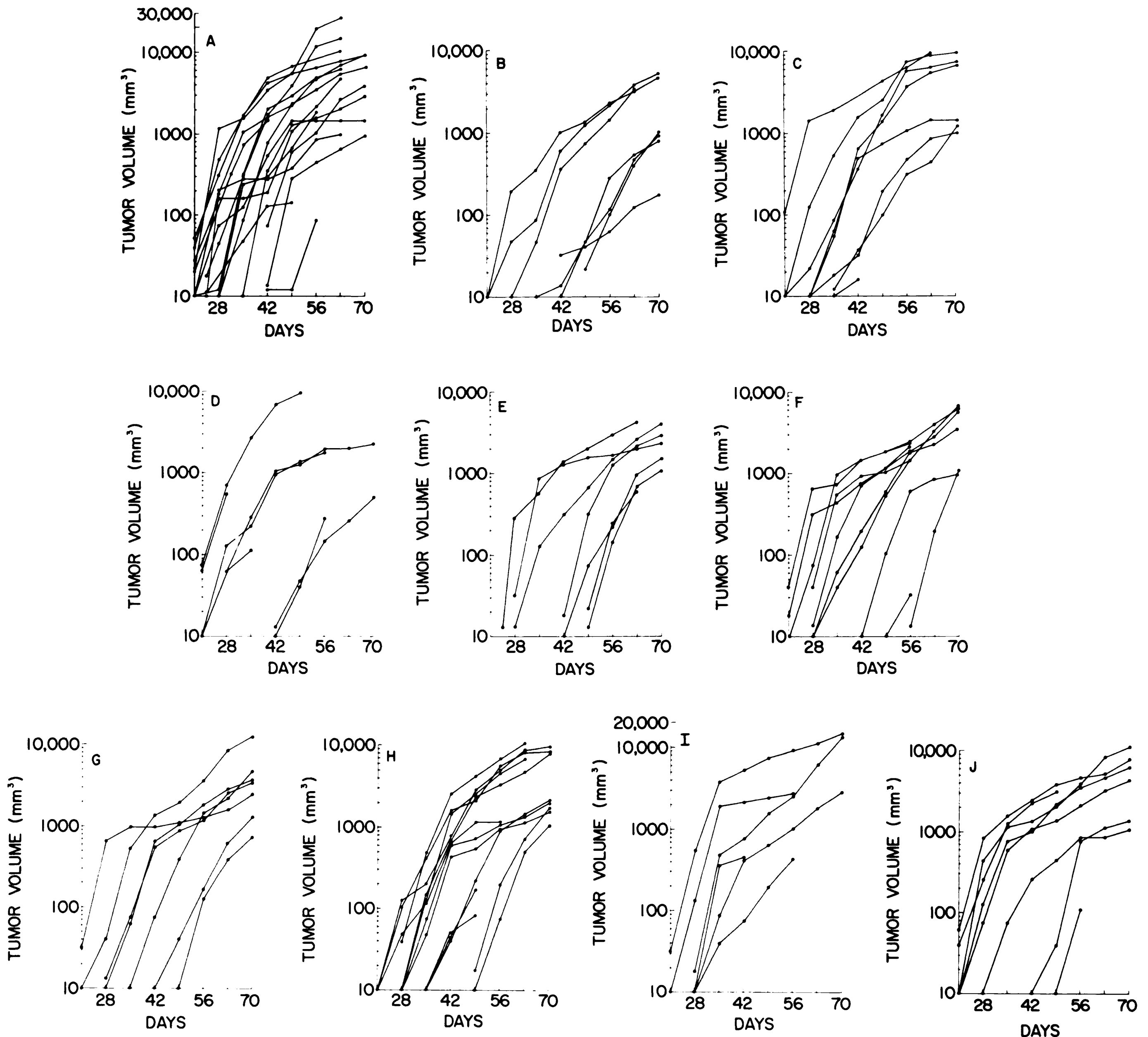

Chart 7. Kinetics of LNCaP tumor growth in intact and hormonally manipulated athymic nude mice. Experimental details were as described for Table 7. The tumor volume (*ordinate*) in cu mm was calculated from measurements of tumor diameters with calipers at the indicated times (*abscissa*) after injection of LNCaP cells. *A*, control intact males; *B*, castrated males; *C*, castrated males implanted with testosterone propionate (2-mg) pellets; *D*, castrated males implanted with 17β-estradiol (2-mg) pellets; *E*, males implanted with 17β-estradiol (2-mg) pellets; *F*, control intact females; *G*, ovariectomized females; *H*, ovariectomized females implanted with testosterone propionate (2-mg) pellets; *I*, ovariectomized females implanted with 17β-estradiol (2-mg) pellets; *J*, females implanted with 17β-estradiol (2-mg) pellets.

tumor enlargement, the calculated mean tumor volume-doubling time was about 86 hr (Table 5), which is only 1.5 times longer than the mean population-doubling time of LNCaP cells grown *in vitro*. Within 6 weeks after inoculation, the majority of tumors reached the weight of 1 g. The largest tumor observed weighed 28 g at 9 weeks postinjection. No apparent distal metastases were found upon autopsy and subsequent histological examination of the internal organs.

Tumors in Males *versus* Females. The frequency of tumor development and the mean time of tumor appearance are significantly different for males *versus* females (Table 5). Male mice develop tumors earlier and at a greater frequency than did females. The rate of tumor growth, however, is independent of the gender of the host (Chart 7).

AP. Prostatic cancer in humans is frequently associated with elevation of prostatic acid phosphatase in the plasma. Since prostatic acid phosphatase is produced *in vitro* by LNCaP cells (14), the AP activity in LNCaP tumor cytosol and in the plasma of tumor-bearing animals was measured. In nude mice, the plasma AP level was found to increase concomitantly with the size of the tumors (Chart 6), but a considerable variation was encountered among individual animals. Ninety-six % of animals with tumors over 100 cu mm (33 of 35 males and 16 of 16 females) had plasma AP above 10 milliunits/ml (*i.e.*, higher than the upper range in control mice); however, the magnitude of the plasma AP elevation did not reflect tumor volume on an individual

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

basis. These observations could not be accounted for either by the total content of AP per tumor or by the relative AP activity per mg of cytosol protein. Table 6 shows that a 28-fold difference in plasma AP level was observed between 2 animals (Animals 1 and 3) while the total AP content per tumor differed only by 2-fold in these same animals.

LNCaP Tumors and Hormonal Manipulations. Prostatic cancer in humans, as well as animal models of prostatic neoplasia, have shown responses to hormonal manipulation of the host. In the LNCaP tumor model system, this was examined using 10 groups of athymic nude mice, each with a different hormonal status. Following injection of LNCaP cells, each group of animals was observed for time and incidence of tumor development (Table 7) as well as kinetics of tumor growth (Chart 7). Table 7 shows that, under conditions of androgen deficiency, the percentage of animals which developed tumors was significantly reduced. When the androgen deficiency was corrected by s.c. testosterone propionate implants, the tumor incidence returned to levels similar to those in intact males (Tables 5 and 7). Estrogen treatment did not result in a statistically significant modulation of tumor development. The graphic representations of individual tumor growth in each of the 10 experimental groups (Chart 7, *A* to *J*) illustrate that the rate of increase of tumor volume was essentially independent of the hormonal status of the animals. These results demonstrate that the formation of LNCaP tumors is androgen responsive but that tumor growth is independent of intact gonadal function.

Sex Hormone Receptors in LNCaP Tumors. High-affinity specific androgen receptor (Chart 8) was found in the cytosol from all 38 nude mouse tumors examined (Table 8). No significant differences were observed in receptor content between tumors from intact and hormonally manipulated animals of both sexes (Table 8), regardless of whether the results are expressed as per g of tissue, per mg protein, or per mg DNA. No correlation emerged between the tumor size and the cytosol androgen receptor concentration (Chart 9).

Although the concentration of the nuclear androgen receptor was low [118 ± 23 (S.E.) fmol/mg DNA and 453 ± 92 fmol/g of tumor], it was detected in 12 of 38 tumors examined. Nonquantifiable trace amounts of nuclear androgen receptor were found in 20 additional tumors. In the remaining 6 tumors, no nuclear androgen receptor was detected.

Estrogen receptor (Table 9) was present in the cytosol from all 14 tumors examined from nude mice (intact, castrated, or ovariectomized). The specific and high-affinity (K_d from 1.2 to 5.5 nM) macromolecules sedimented in the 8S region using the sucrose density gradient centrifugation procedure.

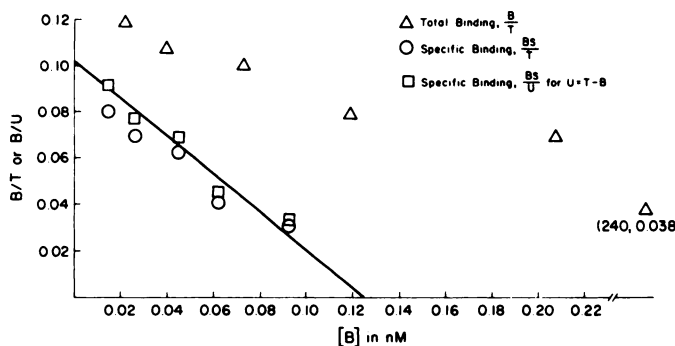

Chart 8. Scatchard plot analysis of androgen binding (*B*) in the cytosol prepared from a LNCaP tumor in a male athymic nude mouse. Duplicate cytosol samples (200 µl, 2.3 mg protein per ml) were incubated (22 hr, 4°) with [$^3H_2$]methyltrienolone (50 µl, serial dilutions from 1 to 16.2 nM) in the presence and in the absence of nonradioactive methyltrienolone (508 nM). The mixtures contained also triamcinolone acetonide in 1000-fold excess. Protein bound radioactivity was determined using the dextran-coated charcoal technique, and the fraction specifically bound was calculated. The calculated K_d is 1.2 nM.

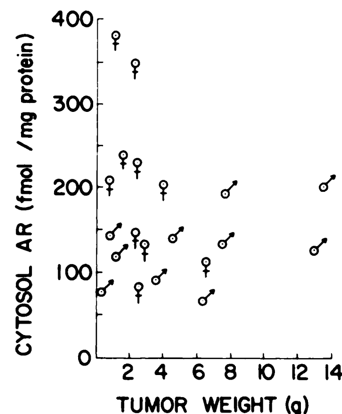

Chart 9. Cytosol androgen receptor (*AR*) in LNCaP tumors of different weights. Tumors induced in male and female athymic nude mice after injection of cultured LNCaP cells were removed at 3 to 10 weeks postinoculation and weighed (*abscissa*). The concentration of the androgen receptor in the cytosol was measured according to the method of Hicks and Walsh (11); protein was measured according to the method of Lowry *et al.* (22) (*ordinate*).

Table 8

Cytosol androgen receptor in LNCaP tumors from athymic nude mice

Tumors were induced by s.c. injection of 4 × 10^{6} cultured LNCaP cells in 6- to 8-week-old nude mice. Ovariectomy, castration and s.c. implants with 2-mg testosterone propionate pellets were performed 3 days before injection of LNCaP cells. Ten weeks after inoculation, the tumors were removed and stored in liquid N_2. Androgen receptor in the cytosol prepared from individual tumors was measured by the competition binding assay with methyltrienolone according to the method of Hicks and Walsh (11). Protein was measured according to the method of Lowry *et al.* (22); DNA was measured according to Richards' modification (35) of the method of Burton (3).

Tumor host	No. of tumors	Cytosol protein content (mg/g tumor)	DNA content (mg/g tumor)	Cytosol androgen receptor concentration			K_d (nM)
				fmol/mg protein	fmol/mg DNA	fmol/g tumor	
Male	10	50.6 ± 5.3[a]	4.2 ± 0.37	129 ± 16[b]	1,682 ± 236	6,965 ± 1,227	1.04 ± 0.16
Castrated male	4	60.9 ± 1.3	4.0 ± 0.05	126 ± 16	1,930 ± 240	7,683 ± 975	0.85 ± 0.03
Castrated male with testosterone implant	5	52.0 ± 3.6	3.3 ± 0.19	117 ± 12	1,844 ± 144	6,054 ± 631	0.71 ± 0.04
Female	10	52.7 ± 4.2	4.6 ± 0.33	208 ± 31[b]	2,709 ± 818	11,196 ± 2,245	1.44 ± 0.22
Ovariectomized	2	77.1	4.5	194	3,320	14,960	0.90
female		56.0	4.4	125	1,590	7,000	0.60
Ovariectomized female with testosterone implant	7	40.8 ± 1.6	3.1 ± 0.16	177 ± 21	2,284 ± 212	7,171 ± 820	0.65 ± 0.004

[a] Mean ± S.E.
[b] Significant difference analyzed by Students' t test at $0.05 > p > 0.02$. None of the remaining differences between groups is significant at $p < 0.05$.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

Table 9

Cytosol estrogen receptors in LNCaP tumors from athymic nude mice

Tumors were induced by s.c. injection of 4×10^6 cultured LNCaP cells in 6- to 8-week-old nude mice. Ovariectomy or castration was performed 3 days before injection of LNCaP cells. Ten weeks after inoculation, the tumors were removed and stored in liquid N_2. Estrogen receptor in the cytosol prepared from individual tumors was measured both by the competition binding assay with DCC[a] (25) and by the SDGC analysis (39) with nafoxidine as the competitor.

Receptor concentration (fmol/mg cytosol protein)							
Males				Females			
Intact		Castrated		Intact		Ovariectomized	
SDGC	DCC	SDGC	DCC	SDGC	DCC	SDGC	DCC
1.9	7.7	7.0	51.3	5.2	34.4	ND	2.6
2.7	14.3	ND	14.8	5.9	60.2	ND	6.9
2.0	21.0	ND	14.4	4.8	26.5		
4.9	22.9		19.5	2.8	23.3		

[a] DCC, dextran-coated charcoal after 18 hr incubation; SDGC, sucrose density gradient centrifugation after 4-hr incubation (8S peak); ND, not determined.

DISCUSSION

This report characterizes the cell line LNCaP and demonstrates its utility as a model for laboratory studies on human prostatic cancer *in vitro* and in the athymic nude mice.

The LNCaP cells established from human CaP (14) could be readily propagated in the laboratory by routine cell culture methods if appropriate measures are taken to prevent the weakly attached cells from being dislodged from the plastic growth surface. High growth saturation densities of monolayers, adequacy of low serum concentrations to promote cell division, anchorage-independent proliferation in semisolid media, and excellent cloning efficiency of the LNCaP cells are consistent with the generally recognized properties of neoplastic cells *in vitro*.

The LNCaP cells are aneuploid. They have a full complement of human chromosomes, including the Y-chromosome as well as several marker chromosomes. Their karyotype is clearly different from that of HeLa cells which in the past were frequently mistaken for some established malignant cell lines (29). The continuously maintained high degree of polyploidy, as well as the major karyological characteristics in LNCaP cultures, examined at 20 months or approximately 200 population doublings apart, suggest that the selection pressure from growth conditions *in vitro* has not eliminated the apparent heterogeneity of the LNCaP cells.

Evidence that the LNCaP cell line originated from human prostatic cancer tissue is provided by: their morphology (14); preservation of functional differentiation; and maintenance of malignant properties in the athymic nude mice. Organ-specific glycoproteins, such as human prostatic acid phosphatase (14) and prostatic antigen (30), are present in cultured cells, in cell culture fluids, in tumors induced in the nude mice, and in the plasma of tumor-bearing animals.

Functional differentiation, consistent with human prostatic epithelial derivation, is also reflected by the responsiveness of the LNCaP cells to androgens. The cellular proliferation *in vitro* is modulated in a dose-dependent manner by the presence of DHT in the culture medium. In addition, DHT has an enhancing influence on AP production by the LNCaP cultures. This effect is apparently independent of the mitotic stimulation induced by DHT. Under conditions when DHT suppresses cell growth, the level of secretory AP in medium continues to rise. These observations suggest that the influence of androgen on cell division and AP synthesis may follow different regulatory pathways in human CaP. Recently, Hudson (16) reported an increase in the level of AP when short-term primary cultures of human CaP were maintained in media with DHT or testosterone. The detection of a specific, high-affinity, low-capacity, saturable androgen receptor in the cytosol and nuclear extracts from both cultured LNCaP cells, as well as in nude mice tumors, provides the molecular basis for the observed androgen responsiveness *in vitro* and *in vivo*. LNCaP cells also contain specific estrogen receptor in the cytosol, a finding consistent with the previously described presence of estrogen receptor in normal and malignant human prostatic epithelium (28).

The presence of complete FBS in culture medium was found to prevent the demonstration of DHT growth-stimulatory effects in LNCaP cells. Such effects became apparent when steroid-depleted, charcoal-treated serum, *e.g.*, ZSD, is used as the growth medium supplement. The reasons for this are unclear, but these findings imply the existence of a significant interplay between androgens and charcoal-removable serum components in the regulation of CaP cell proliferation. Several conflicting observations (19, 24, 42, 44) on the effects of androgens on the *in vitro* growth of normal and malignant human prostatic epithelium are perhaps due, in part, to the use of a variety of complete mammalian sera as medium supplement.

Malignant properties and hormonal responsiveness *in vivo* of the LNCaP cells are maintained. After injection of the uncloned LNCaP cells into nude mice, the time and frequency of tumor development is favored by androgens. However, once the tumors are established, their growth rates are similar, regardless of the gender or hormonal manipulation of the animals suggesting that the LNCaP cultures may consist of cells which are heterogeneous in their responsiveness to sex hormones. This is supported by published evidence (17) that in the castrated rats the progression of the Dunning prostatic adenocarcinoma to an androgen-independent state is due to the basic heterogeneity of the original inoculum and a rapid selection process *in vivo*. Future experiments with single-cell-derived clones could resolve this issue.

The resistance of the LNCaP cell lines to the antiproliferative as well as the antiviral effects of HuIFNβ implies, but does not prove conclusively, the existence of a defect in the cell receptor for type I interferon (2). Such defects are rare among human cells (5). It may therefore be of value to assess, before large-scale clinical trials with interferon are undertaken in this disease, whether LNCaP cells are unique in this respect or whether interferon resistance is a common property of human CaP.

Recent observations (unpublished) in our laboratory on the sensitivity of LNCaP tumors in nude mice to chemotherapeutic regimens currently used in the treatment of CaP suggest the probable utility of this model for screening new drugs or combinations of drugs for potential activity against prostatic neoplasia.

The complexity of CaP requires several model systems available to the investigator to study this disease (26). The well-characterized LNCaP cells should find its proper place in research aimed at finding relevant answers concerning etiology, genetic stability, hormonal regulation, immunological properties, and the detection (30, 31) and therapy of human prostatic carcinoma.

ACKNOWLEDGMENTS

The authors thank Dr. Avery A. Sandberg and Dr. Zenon Gibas for helpful advice and discussion in karyological studies; Darlene Denzien, Sharon Mingus, Dr. Robert

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

Drury, and Joyce Romano for excellent technical assistance; Helen Biro for careful maintenance of the nude mice colony; and Lisa Barone for typing the manuscript.

REFERENCES

1. Babson, A. L., and Phillips, G. E. An improved acid phosphatase procedure. Clin. Chim. Acta, *13:* 264-265, 1966.
2. Branca, A. A., and Baglioni, C. Evidence that types I and II interferons have different receptors. Nature (Lond.), *294:* 768-770, 1981.
3. Burton, K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J., *62:* 315-323, 1956.
4. Butler, W. B., Kelsey, W. H., and Goran, N. Effects of serum and insulin on the sensitivity of the human breast cancer cell line MCF-7 to estrogen and antiestrogens. Cancer Res., *41:* 81-88, 1981.
5. Chen, H. Y., Sato, T., Fusa, A., Kuwata, T., and Content, J. Resistance to interferon of a human adenocarcinoma cell line, HEC-1, and its sensitivity to natural killer cell action. J. Gen. Virol., *52:* 177-181, 1981.
6. Clark, S. M., and Merchant, D. J. Primary cultures of human prostatic epithelial cells from transurethral resection specimens. Prostate, *1:* 87-94, 1980.
7. Coffey, D. S., and Isaacs, J. T. Requirements for an idealized animal model of prostatic cancer. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 379-391. New York: Alan R. Liss, Inc., 1980.
8. Dahlberg, E., Snochowski, M., and Gustafsson, J. A. Comparison of the R-3327H rat prostatic adenocarcinoma to human benign prostatic hyperplasia and metastatic carcinoma of the prostate with regard to steroid hormone receptors. Prostate, *1:* 61-70, 1980.
9. Drago, J. R., Goldman, L. B., and Maurer, R. E. The Nb rat prostatic adenocarcinoma model system. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 265-291. New York: Alan R. Liss, Inc., 1980.
10. Gittes, R. F. The nude mouse—its use as tumor bearing model of the prostate. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 31-37. New York: Alan R. Liss, Inc., 1980.
11. Hicks, L. L., and Walsh, P. C. A microassay for the measurement of androgen receptor. Steroids, *33:* 389-406, 1979.
12. Hoehn, W., Schroeder, F. H., Riemann, J. F., Joebsis, A. C., and Hermanek, P. Human prostatic adenocarcinoma: some characteristics of a serially transplantable line in nude mice (PC 82). Prostate, *1:* 95-104, 1980.
13. Horoszewicz, J. S., Leong, S. S., and Carter, W. A. Noncycling tumor cells are sensitive targets for the antiproliferative activity of human interferon. Science (Wash. D. C.), *206:* 1091-1093, 1979.
14. Horoszewicz, J. S., Leong, S. S., Chu, T. M., Wajsman, Z. L., Friedman, M., Papsidero, L., Kim, J., Chai, L. S., Kakati, S., Arya, S. K., and Sandberg, A. A. The LNCaP cell line—a new model for studies on human prostatic carcinoma. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 115-132. New York: Alan R. Liss, Inc., 1980.
15. Horoszewicz, J. S., Leong, S. S., Ito, M., DiBerardino, L., and Carter, W. A. Aging *in vitro* and large scale interferon production by 15 new strains of human diploid fibroblasts. Infect. Immun., *19:* 720-726, 1978.
16. Hudson, R. W. The effects of androgens and estrogens on human prostatic cells in culture. Can. J. Physiol. Pharmacol., *59:* 949-956, 1981.
17. Isaacs, J. T., and Coffey, D. S. Adaptation *versus* selection as the mechanism responsible for the relapse of prostatic cancer to androgen ablation therapy as studied in the Dunning R-3327-H adenocarcinoma. Cancer Res., *41:* 5070-5075, 1981.
18. Isaacs, J. T., Weissman, R. M., Coffey, D. S., and Scott, W. W. Concepts in prostatic cancer biology: Dunning R-3327H, HI and AT tumors. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 311-323. New York: Alan R. Liss, Inc., 1980.
19. Kaighn, M. E., Lechner, J. F., Babcock, M. S., Marnell, M., Ohnuki, Y., and Narayan, K. S. The Pasadena cell lines. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 85-109. New York: Alan R. Liss, Inc., 1980.
20. Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F., and Jones, L. W. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest. Urol., *17:* 16-23, 1979.
21. Leong, S. S., and Horoszewicz, J. S. Production and preparation of human fibroblast interferon for clinical trials. Methods Enzymol., *78:* 87-101, 1981.
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265-275, 1951.
23. Lubaroff, D. M., Canfield, L., and Reynolds, C. W. The Dunning tumors. *In:* G. P. Murphy (ed.), Models and Prostate Cancer, pp. 243-263. New York: Alan R. Liss, Inc., 1980.
24. Malinin, I. T., Claflin, A. J., Block, N. L., and Brown, A. L. Establishment of primary cell cultures from normal and neoplastic human prostatic gland tissue. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 161-180. New York: Alan R. Liss, Inc., 1980.
25. McGuire, W. L. Quantitation of estrogen receptor in mammary carcinoma. Methods Enzymol., *28:* 248-254, 1975.
26. Merchant, D. J. Requirements of *in vitro* model systems. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 3-7. New York: Alan R. Liss, Inc., 1980.
27. Mickey, D. D., Stone, K. R., Wunderli, H., Mickey, G. H., Vollmer, R. T., and Paulson, D. F. Heterotransplantation of a human prostatic adenocarcinoma cell line into nude mice. Cancer Res., *37:* 4049-4058, 1977.
28. Morphy, J. B., Emmott, R. C., Hicks, L. L., and Walsh, P. C. Estrogen receptors in the human prostate, seminal vesicle, epididymis, testis, and genital skin: a marker for estrogen responsive tissues. J. Clin. Endocrinol. Metab., *50:* 938-948, 1980.
29. Nelson-Rees, W. A., Daniels, D. W., and Flandermeyer, R. R. Cross-contamination of cells in culture. Science (Wash. D. C.), *212:* 446-452, 1981.
30. Papsidero, L. D., Kuriyama, M., Wang, M. C., Horoszewicz, J. S., Leong, S. S., Valenzuela, L., Murphy, G. P., and Chu, T. M. Prostate antigen: a marker for human prostate epithelial cells. J. Natl. Cancer Inst., *66:* 37-42, 1981.
31. Papsidero, L. D., Wojcieszyn, J. W., Horoszewicz, J. S., Leong, S. S., Murphy, G. P., and Chu, T. M. Isolation of prostatic acid phosphatase-binding immunoglobulin from human sera and its potential for use as a tumor-localizing reagent. Cancer Res., *40:* 3032-3035, 1980.
32. Pollard, M.: The Pollard tumors. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 293-302. New York: Alan R. Liss, Inc., 1980.
33. Reid, L. M., Minato, N., Gresser, I., Holland, J., Kadish, A., and Bloom, B. R. Influence of anti-mouse interferon serum on the growth and metastasis of tumor cells persistently infected with virus and of human prostatic tumors in athymic nude mice. Proc. Natl. Acad. Sci. U. S. A., *78:* 1171-1175, 1981.
34. Reid, L. M., and Shin, S. Transplantation of heterologous endocrine tumor cells in nude mice. *In:* J. Fogh and B. C. Giovanella (eds.), The Nude Mouse in Experimental and Clinical Research, pp. 313-351. New York: Academic Press, Inc., 1978.
35. Richards, G. M. Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Anal. Biochem., *57:* 369-376, 1974.
36. Sandberg, A. A. The Chromosomes in Human Cancer and Leukemia, pp. 98-117. Amsterdam: Elsevier/North Holland Biomedical Press, 1980.
37. Saroff, J., Kirdani, R. Y., Chu, T. M., Wajsman, Z., and Murphy, G. P. Measurements of prolactin and androgens in patients with prostatic diseases. Oncology (Basel), *37:* 46-52, 1980.
38. Scatchard, G. The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci., *51:* 660-672, 1949.
39. Schneider, S. L., and Dao, T. L. Effect of Ca^{++} and salt on forms of estradiol cytoplasmic receptor in human neoplastic breast tissue. Cancer Res., *37:* 382-387, 1977.
40. Shain, S., McCullough, B., and Segaloff, A. Spontaneous adenocarcinoma of the ventral prostate of the aged A × C rats. J. Natl. Cancer Inst., *55:* 177-180, 1975.
41. Silverberg, E. Cancer statistics. CA, *27:* 26-41, 1977.
42. Stone, K. R., Mickey, D. D., Wunderli, H., Mickey, G. H, and Paulson, D. F. Isolation of a human prostate carcinoma cell line (DU145). Int. J. Cancer, *21:* 274-281, 1978.
43. Walsh, P. C., and Hicks, L. L. Characterization and measurements of androgen receptors in human prostatic tissue. *In:* G. P. Murphy and A. A. Sandberg (eds.) Prostate Cancer and Hormone Receptors, pp. 51-63. New York: Alan R. Liss, Inc., 1979.
44. Webber, M. M. Growth and maintenance of normal prostatic epithelium *in vitro*—A human cell model. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 181-216. New York: Alan R. Liss, Inc., 1980.
45. Webber, M. M., Stonington, O. G., and Poche, P. A. Epithelial outgrowth from suspension cultures of human prostatic tissue. In Vitro (Rockville), *10:* 196-205, 1974.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research

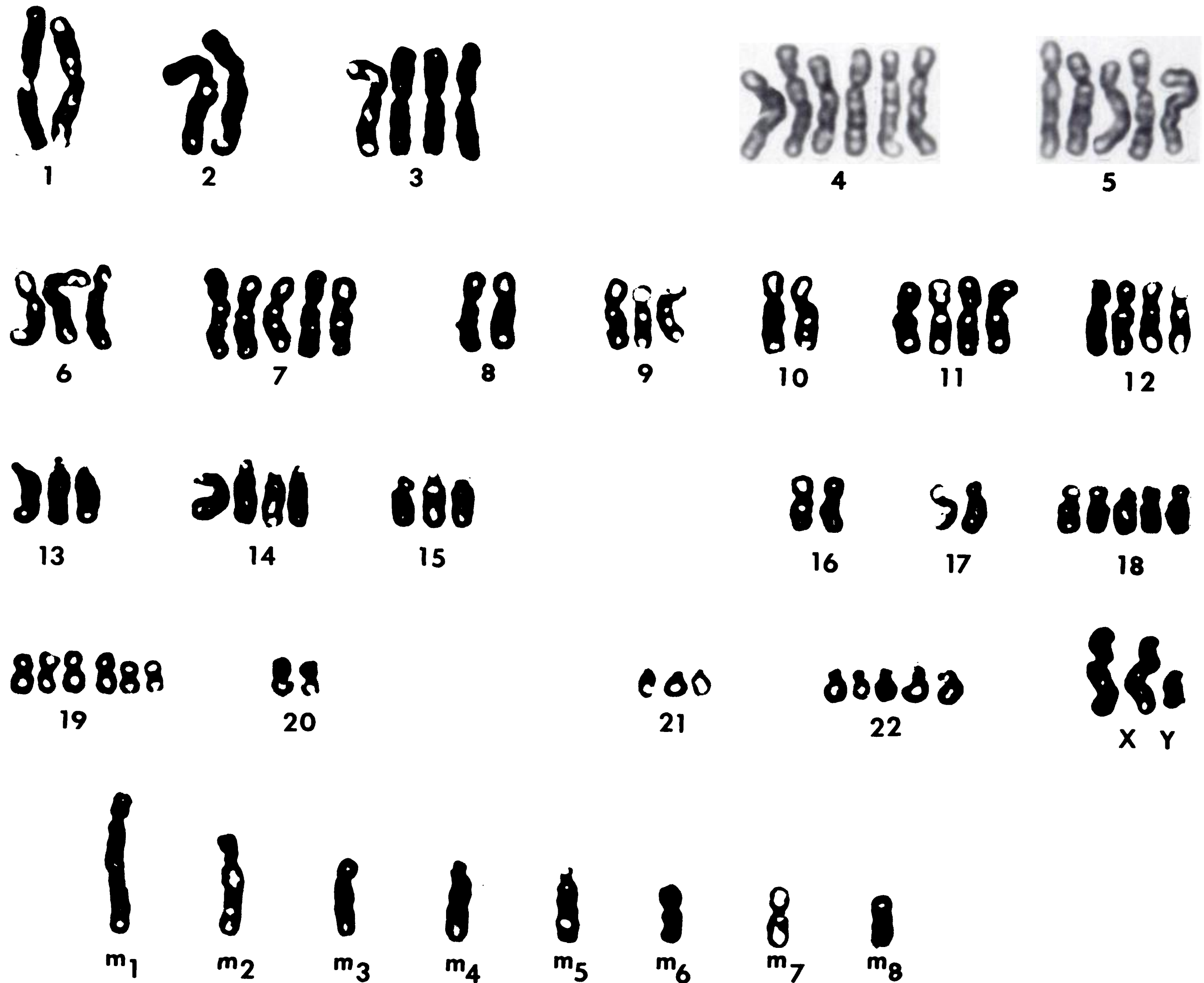

Fig. 1. G-banded karyotype of a LNCaP cell from a culture maintained for 32 months *in vitro* after isolation. One set of normal human chromosomes is present in addition to multiple copies and marker chromosomes (m_1 to m_8) for a total of 88 chromosomes.

Downloaded from cancerres.aacrjournals.org on February 21, 2013
Copyright © 1983 American Association for Cancer Research