

Disruption of Androgen Receptor Function Inhibits Proliferation of Androgen-refractory Prostate Cancer Cells¹

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Abstract

Prostate cancer cells depend on androgens and the androgen receptor (AR) for survival. However, after androgen ablation therapy, tumors relapse to an androgen-refractory state. To determine whether the androgen receptor is critical for proliferation of androgen-refractory prostate cancer cells, we disrupted the activity of the androgen receptor with an antibody and an AR mRNA hammerhead ribozyme in the following cell lines: LNCaP (androgen-sensitive), LNCaP-Rf and LNCaP-C4 (androgen-refractory), and DU-145 (androgen-insensitive). Microinjection of either antibody or ribozyme inhibited proliferation of androgen-refractory cells. These findings demonstrate that the AR is critical for proliferation of androgen-refractory cells, even in the absence of androgens.

Introduction

Prostate cancer is one of the most common malignancies diagnosed in men and represents a significant worldwide health problem. Androgens play a crucial role in the tumorigenesis and progression of prostate cancer, with androgen-regulated gene expression being mediated by the ligand-activated androgen receptor (1). Growth of prostate cancer is initially androgen dependent, and most patients with advanced prostate cancer are treated with androgen ablation therapy to suppress the growth and induce apoptosis of the tumor (2). Although 60–80% of patients initially respond to androgen ablation therapy, the disease eventually progresses to what is defined as androgen-refractory prostate cancer, at which point the tumor is no longer responsive to androgen ablation, and uncontrolled progression of the disease is inevitable. Studies performed with patient specimens have shown that the AR³ is expressed in almost all cancers of the prostate after androgen ablation therapy (3), suggesting that this receptor is important for tumor progression. However, the functional role of the AR under these conditions is unknown.

Androgens and the AR are essential for the normal growth of the prostate gland as well as the growth of prostate cancer. The AR is capable of regulating transcriptional activity in either a positive or negative way. Ligand-dependent activation occurs through binding of androgens to the AR (4), recruitment of various coactivators, and transactivation of genes containing androgen-responsive elements (5). Ligand-independent activation has also been demonstrated, with the AR being regulated by different signaling pathways (6). These studies suggest that the AR can be activated in the absence of androgens. However, the crucial issue remains as to whether the AR itself is critical for proliferation of androgen-refractory prostate cancer cells in the absence of androgens.

Materials and Methods

Cell Lines and Reagents. The following cell lines and corresponding culture media were used. Human prostate cancer cells LNCaP and DU-145 were obtained from American Type Culture Collection (Rockville, MD). LNCaP-C4 cells were obtained from UROCOR, Inc. LNCaP-Rf cells were established by long-term culture of LNCaP cells (approximately >10 weeks) in RPMI 1640 with 10% charcoal-stripped serum supplied by Dr. Haojie Huang (Urology Research Mayo Clinic, Rochester, MN). LNCaP cells were maintained in RPMI 1640 (CELOX Laboratories, Inc., St. Paul, MN) with 10% FBS; DU-145 cells were maintained in DMEM (Life Technologies, Inc.) with 9% FBS, and LNCaP-C4 cells were maintained in T-media (Life Technologies, Inc.) with 5% FBS. All cells were supplemented with penicillin/streptomycin and maintained at 37°C incubator in 5% CO₂.

Antibodies. Monoclonal AR antibody (AR441) at 2 mg/ml and mIgG were purchased from Santa Cruz Biotechnology Laboratories. Monoclonal anti-AR antibody was heat inactivated at 100°C for 5 min when used as a control. FITC or Texas Red-labeled dextran (Molecular probes) was added to microinjected solutions as an indicator of successful microinjection, enabling injected cells to be identified readily by fluorescent microscope. A *trans*-acting hammerhead ribozyme (pHR-2) was provided by Dr. Arun K. Roy (University of Texas Health Science Center, Austin, TX).

Western Blot. Cell lysates were obtained from LNCaP, LNCaP-Rf, LNCaP-C4, and DU-145 cells, and 25 µg of total protein were analyzed by Western blot. Protein extracts were electrophoresed on a 4–12% Tris-glycine gel, and the separated proteins were electrophoretically transferred to nitrocellulose for immunodetection. Membranes were blocked in 5% nonfat dry milk in TBST for 1 h at room temperature and incubated in mouse monoclonal antibody to human AR (Santa Cruz Biotechnology AR441) at a dilution of 1:200 in TBST + 2.5% nonfat dry milk, followed by horseradish peroxidase-conjugated antihuman secondary antibody (Amersham) at a dilution of 1:10,000. Immunoblots were probed with anti-ERK-2 antibody to confirm equal loading.

Microinjection Technique. For microinjection experiments, cells (10⁴) were grown on CELLocate coverslips (Eppendorf Scientific, Inc., Hamburg, Germany), 175 µm². Optimal injections were obtained with microneedles freshly prepared from borosilicate glass capillaries (1.0-mm outer diameter; 0.78-mm inner diameter) using a Flamingo/Brown micropipette puller P-97 (Sutter Instruments, Novato, CA), with a tip diameter of approximately 0.3–0.5 µm, and loading micropipettes were pulled manually. Antibodies (2 mg/ml), heat-inactivated antibodies (2 mg/ml), pHR-2 ribozyme (50 µg/ml), and pcDNA3 (50 µg/ml) were dialyzed in microinjection buffer [10 mM KH₂PO₄ (pH 7.2) and 75 mM KCl]. For control injections, the anti-AR antibody was heat inactivated by incubating 5 min at 100°C. Texas Red dextran (red) or FITC 488 (hydrazide sodium salt; 200 mM KCl) was added to all microinjection solutions. This served as an indicator for successful microinjection and enabled the identification of injected cells by confocal microscopy after functional assays were performed. Cells were allowed to recover 4–6 h at 37°C in a 5% CO₂ incubator before a subsequent manipulation. Microinjections were performed using a microscope stage. Cells were pressure injected using an Eppendorf 5242 and Micromanipulator 5150 on a Zeiss Axiovert inverted microscope, with an optimized program adjusted for injection pressure (*P_i*, 79 mm Hg), compression pressure (*P_c*, 15 mm Hg), and time (*T*, 0.3 s). Images were obtained using an intensified charged coupled device camera (Hamamatsu) attached to a Zeiss Axiovert 35 microscope (Carl Zeiss, Inc., Oberkochen, Germany).

Received 10/18/01; accepted 1/2/02.

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¹ Supported in part by the T. J. Martell Foundation and National Cancer Institute Grants CA15083, DK60920, and CA91956.

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³ The abbreviations used are: AR, androgen receptor; PSA, prostate-specific antigen.

Cell Proliferation Assay. LNCaP, LNCaP-Rf, LNCaP-C4, and DU-145 cells (10^4) were grown on CELLocate Eppendorf coverslips prior to microinjections. Cell numbers were counted every 24 h after microinjection. Microinjected cells were identified by Texas Red dextran (red) or FITC 488 (green) fluorescence using confocal microscope.

Confocal Microscopy. Injected cells were analyzed with the laser scanning microscope LSM 10 (Carl Zeiss, Inc.). Argon ion and HeNe lasers were used to excite FITC and Texas red fluorescence, respectively.

Immunofluorescence. Cells on coverslips (Eppendorf Scientific, Inc., Hamburg, Germany) were washed briefly in PBS and fixed for 5 min in cold methanol. Blocking was 20 min at room temperature in 10% normal goat serum in PBS, followed by incubation for 1 h at room temperature with polyclonal PSA antibody (DAKO) at 1:2000 in 1.5% goat serum in PBS. Secondary antibody was Alexa Fluor 488 goat antirabbit IgG conjugate (Molecular Probes, Inc.) at 2 μ g/ml in 1.5% goat serum in PBS for 45 min at room temperature. Coverslips were then washed in three changes of PBS and mounted in ProLong (Molecular Probes, Inc.).

Results and Discussion

To delineate a function for the AR in androgen-refractory prostate cancer, we used an androgen-sensitive human prostate cancer cell line LNCaP, two androgen-refractory sublines LNCaP-Rf and LNCaP-C4, and an androgen-insensitive prostate cancer cell line DU-145. Western blot analysis showed a band consistent with the expression of AR in the first three cell lines, whereas AR protein expression was absent in the androgen-independent cell line DU-145 (Fig. 1).

To examine the role of the AR in androgen-refractory prostate cancer cells, we microinjected reagents that could block the function of the AR. Previous studies have demonstrated that antibodies microinjected into mammalian cells preserve their specificity and are non-toxic; therefore, antibodies can be used to inhibit the function of the antigen against which they are directed (7). Our overall strategy was to use a monoclonal antibody against the COOH-terminal domain of the AR, microinjected directly into the nucleus of these four cell lines under similar conditions. The monoclonal anti-AR antibody was coinjected with Texas Red Dextran-lysine fixable, which allowed us to monitor and identify the microinjected cells for up to 9 days by confocal laser scanning microscopy (LSM510-Carl Zeiss micro-

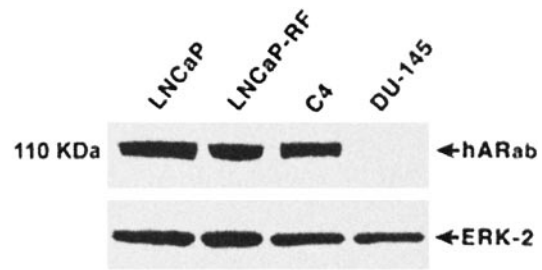
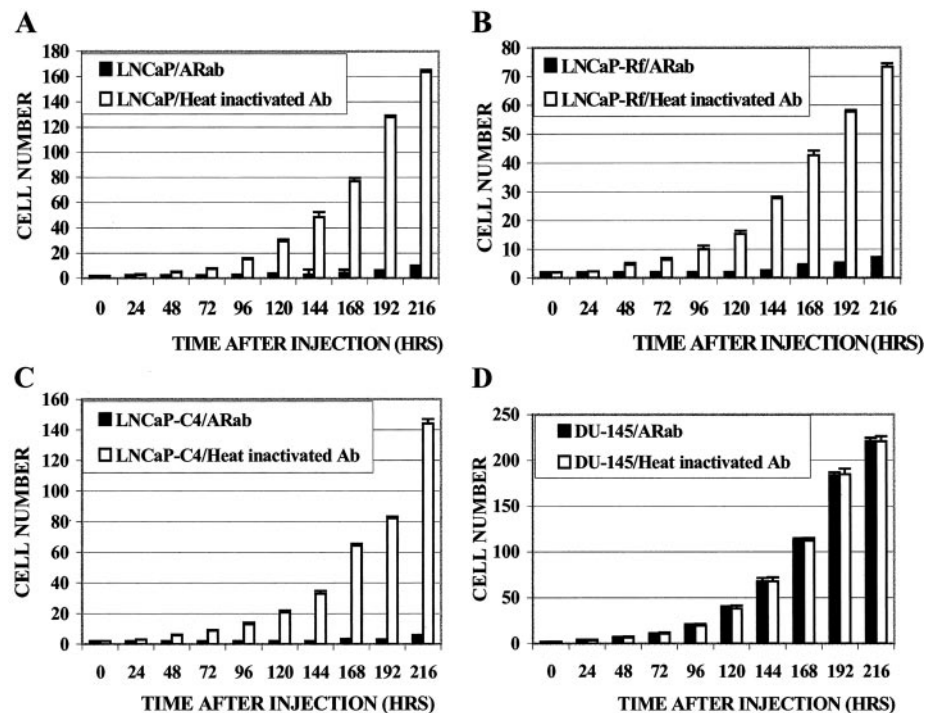


Fig. 1. Expression of AR protein in prostate cancer cells. Cell lysates were obtained from LNCaP, LNCaP-Rf, LNCaP-C4, and DU-145 cells, and 25 μ g of total protein were analyzed by Western blot. Protein extracts were electrophoresed on a 4–12% Tris-glycine gel, and the separated proteins were electrophoretically transferred to nitrocellulose for immunodetection. The membrane was blocked in 5% nonfat dry milk in TBST for 1 h at room temperature and incubated in mouse monoclonal antibody to human AR (Santa Cruz Biotechnology AR441) at a dilution of 1:200 in TBST + 2.5% nonfat dry milk, followed by horseradish peroxidase-conjugated antimouse secondary antibody (Amersham) at a dilution of 1:10,000. Immunoblots were reprobed with anti-ERK-2 antibody to confirm equal loading.

scope). Nuclear microinjection of AR antibody (2 mg/ml) markedly reduced proliferation in LNCaP, LNCaP-Rf, and LNCaP-C4 cells (Fig. 2, A–C). Significant morphological changes in LNCaP, LNCaP-Rf, and LNCaP-C4 cells were observed after 30 h of AR antibody microinjection that persisted for up to 9 days (Fig. 3). These cells developed dendrite-like cytoplasmic extensions reaching ~ 100 μ m (range, 60–200 μ m) compared with the control cells (injected with heat-inactivated AR antibody), which exhibited no morphological changes. LNCaP-C4 androgen-refractory prostate cancer cells are dramatically elongated, with prominent processes (three or more) growing in different directions from the cytoplasm. In contrast, nuclear microinjection of androgen-refractory antibody in DU-145 cells had no effect on either proliferation or cell morphology (Fig. 3D). These data correlated with the expression of AR protein in these cell lines as determined by Western blot analysis. To assure antibody specificity, we microinjected both mouse IgG or heat-inactivated AR antibodies, as controls, into the parallel groups of cells. None of the cells showed a response to injected control antibodies, demonstrating

Fig. 2. Effects of microinjection of monoclonal anti-AR antibody into PCa cells. Cells were grown ($10^4/35 \times 10$ -mm Falcon tissue culture dishes) on CELLocate Eppendorf coverslips. A monoclonal anti-AR antibody (2 mg/ml) was injected into the nucleus of LNCaP (A), LNCaP-Rf (B), LNCaP-C4 (C), and DU-145 (D) cells under similar conditions. A heat-inactivated AR antibody was microinjected as a control in these four cell lines. Cell number was determined every 24 h up to 216 h after microinjection. Cells were monitored by using a Confocal microscope. Bars, SE of three independent experiments.



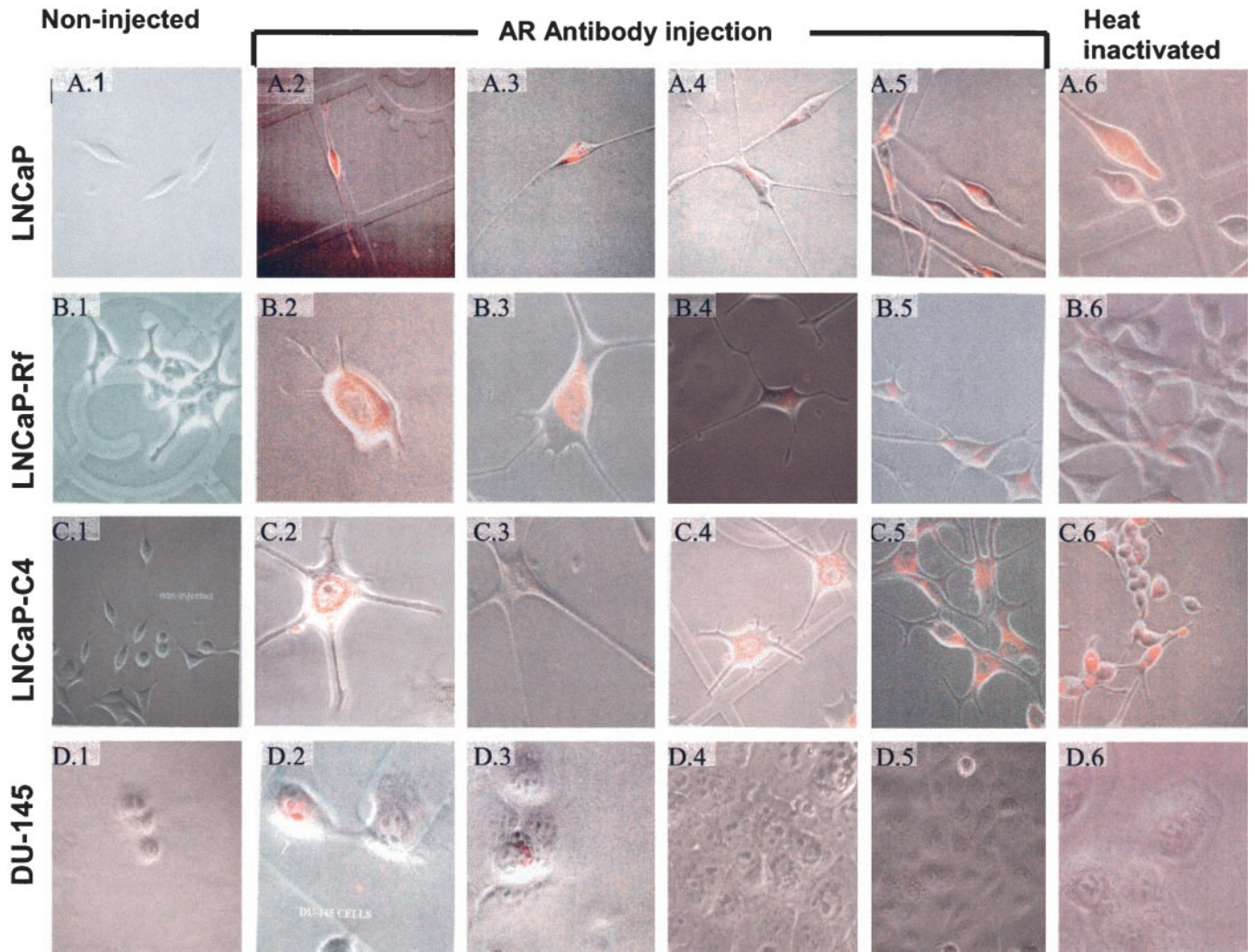


Fig. 3. Morphological changes in prostate cancer cells after microinjection with AR antibody. *Top row*, A.1 to A.6 (LNCaP); *second row*, B.1 to B.6 (LNCaP-Rf); *third row*, C.1 to C.6 (LNCaP-C4); *bottom row*, D.1 to D.6 (DU-145) cells. *Column 1*, noninjected cells (differential interference contrast images). *Columns 2–5*, cells microinjected with AR antibody and Texas red Dextran (red). *Column 6*, cells injected with heat-inactivated antibody and Texas red Dextran (red). *Rows A.2 to A.6*, note spindle morphology and long extensions growing from the cytoplasm at 48 h (A.2), 72 h (A.3 and A.5), and 120 h (A.4) after microinjection of AR antibody. Similar morphological changes were observed after the injection of a single cell (A.2 and A.3) or a group of cells (A.5). No morphological changes were observed after injection of heat-inactivated antibody after 48 h (A.6). *Rows B.2 to B.6*, note the marked enlargement of the nuclei and the processes growing from the cytoplasm after 48 h (B.2), 72 h (B.3), and 120 h (B.4). Similar morphological changes were observed in groups of cells microinjected with AR antibody after 72 h (B.5). No morphological changes were observed in cells injected with heat-inactivated antibody (B.6). *Rows C.2 to C.6*, note the stellate morphology and marked prominent nucleus and the elongated processes growing from the cytoplasm at 48 h (C.2) and 72 h (C.3) after AR antibody microinjection. Observe dendrite-like formations after AR antibody in a single injected cell (C.2 and C.3), in a group of two cells (C.4), or in a group of eight cells (C.5). No morphological changes were detected after microinjection of heat-inactivated antibody (C.6). *Rows D.2 to D.6*, note the prototypical round cell that exhibits no morphological changes after 24 h (D.2), 48 h (D.3), 96 h (D.4), and 120 h (D.6) after microinjection of AR antibody or heat-inactivated antibody (D.6). Data shown are representative of 10 independent experiments.

that the microinjection process *per se* had no effect on proliferation in prostate cancer cells. These results suggest that the presence of AR antibody in LNCaP, LNCaP-Rf, and LNCaP-C4 cells was sufficient to inhibit proliferation for up to 8 days. Cells started to proliferate again between 7 and 8 days; this duration most likely reflects metabolism of the injected antibody.

To investigate whether the AR antibody inhibited the functional activity of the AR, we monitored the expression of the androgen-responsive protein, PSA. PSA is regulated by androgens at the transcriptional level (8). In the absence or low concentration of androgens, PSA was expressed in the two androgen-refractory prostate cancer cell lines, LNCaP-Rf and LNCaP-C4, suggesting that the AR is functional. LNCaP, LNCaP-Rf, and LNCaP-C4 cells were microinjected with AR antibody or control antibody, and PSA expression was monitored by immunostaining after 24–96 h (Table 1). Microinjection of AR antibody, but not control antibodies, significantly reduced PSA expression between 72 and 96 h (Fig. 4; Tables 2 and 3). On the basis

of these results, we conclude that the AR has a functional role in androgen-refractory prostate cancer cells.

To confirm the above results, we used a *trans*-acting hammerhead ribozyme (9) directed to the AR mRNA (pHR-2) to block expression of the AR. This ribozyme is capable of reducing or inhibiting the production of AR protein and proliferation of LNCaP cells (10). Cells were microinjected with either pHR-2 plasmid DNA or empty vector pcDNA3 and coinjected with FITC (green fluorescent dye), which allowed us to monitor and identify the microinjected cells for up to 9 days (Fig. 5). After microinjection of the ribozyme, proliferation of LNCaP, LNCaP-Rf, and LNCaP-C4 cells (Fig. 5, A–C) was arrested for up to 7 days, after which time proliferation resumed. The ribozyme had no effect on DU-145 cells (Fig. 5D). Moreover, microinjection of the vector had no effect on any of these cells. Control experiments were performed by assessing AR expression with immunocytochemistry in LNCaP cells that were microinjected with pHR-2 ribozyme. We

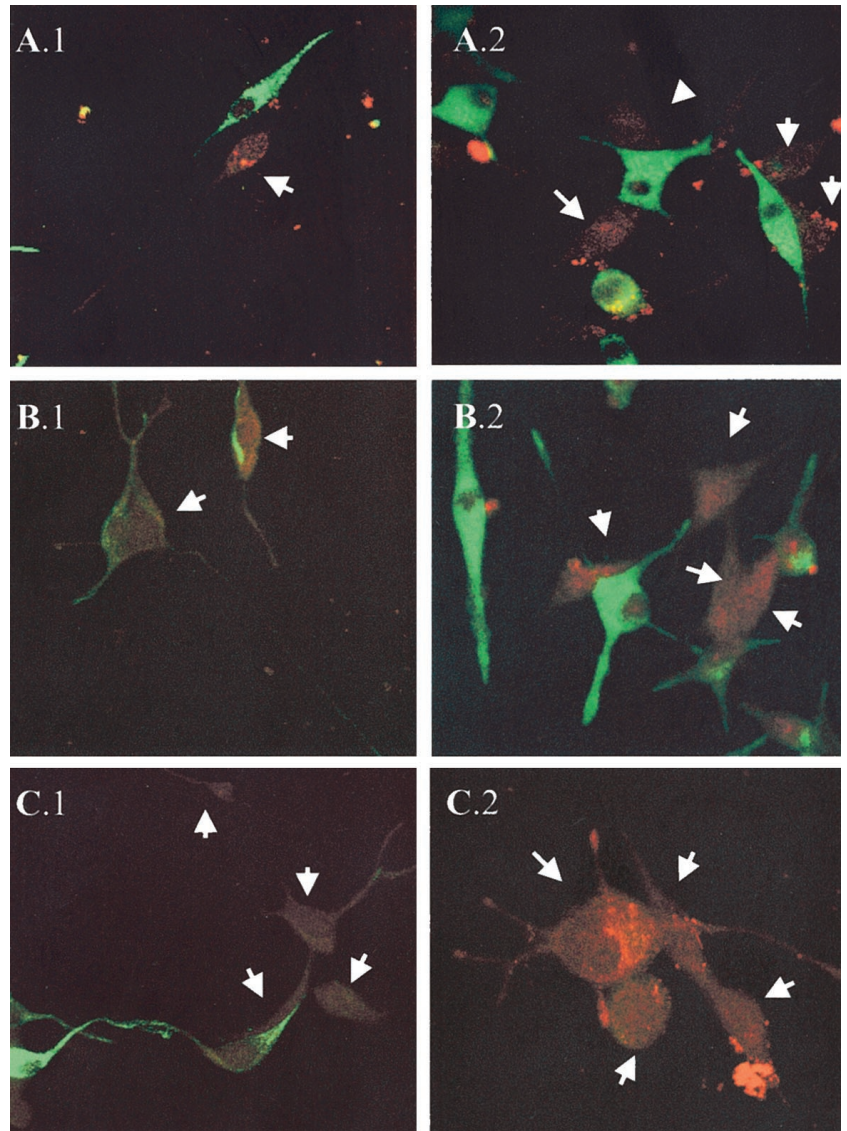


Fig. 4. Subcellular distribution of PSA expression after microinjection of mAR antibody in PCa cells. LNCaP (A), LNCaP-Rf (B), and LNCaP-C4 (C) cells were cultured on coverslips under similar conditions. Cells were microinjected with AR antibody and immunostained at 24, 48, 72, and 96 h after microinjection. Images are merged showing microinjected cells with AR antibody (Texas Red) and immunostained after 72 h (A.1, B.1, and C.1) to 96 h (A.2, B.2, and C.2) of microinjection. Cells were fixed for 5 min in cold methanol. Cells were blocked with 10% goat serum in PBS for 20 min at room temperature. Primary antibody was polyclonal PSA (DAKO) at 1:2000 in 1.5% goat serum in PBS. Secondary antibody was Alexa Fluor 488 (green) goat antirabbit IgG conjugate (Molecular Probes) in 1.5% goat serum in PBS. Fluorescent confocal images were collected using sequential line excitation filters at 488 and 568 nm. Emission filter sets used were 505 to 550 nm for PSA detection (green) and 585 nm for AR detection (Texas Red) for microinjected cells. Cells microinjected with AR antibody (arrowheads) show a marked decrease in PSA expression (green) after 96 h. Note PSA expression (green) in cells noninjected as compared with cells injected with AR antibody (red).

Table 1 Time course of PSA expression in prostate cancer cells microinjected with monoclonal AR antibody

Laser scanning confocal microscopy was used to visualize PSA expression in microinjected cells. Prostate cancer cells were injected with AR antibody or heat-inactivated antibody. Cells were fixed 24, 48, 72, and 96 h after injection and stained to estimate PSA expression.

Time (h)	PSA expression			
	24	48	72	96
LNCaP/AR antibody	++++ ^a	+++	+	----
LNCaP/heat inactivated	++++	++++	++++	++++
LNCaP-RF/AR antibody	++++	++	+	----
LNCaP-RF/heat inactivated	++++	++++	++++	++++
LNCaP-C4/AR antibody	++++	++	+	----
LNCaP-C4/heat inactivated	++++	++++	++++	++++

^a +++++, 100% PSA expression; +++, 75%; ++, 50%; +, 25%; ----, no PSA expression.

found that the AR expression was dramatically reduced in those cells that receive the ribozyme, whereas there was no change in the AR expression after microinjection of the control vector (data not shown). Thus, we conclude that the ribozyme was effective in knocking out the expression of the AR.

Several mechanisms have been proposed whereby the AR signal transduction pathway, which could be activated after androgen abla-

tion therapy, may contribute to the development of androgen-refractory prostate cancer:

(a) One mechanism is through amplification of the AR gene. Although the AR gene is rarely amplified in primary prostate cancer, it

Table 2 Effect of AR antibody injection on PSA expression in prostate cancer cells

Cell line	No. of cells injected with AR antibody	% of cells injected with AR antibody after 96 h in which expression of PSA was detected
LNCaP	120	11%
LNCaP-Rf	120	8%
LNCaP-C4	120	2%

Table 3 Effect of heat-inactivated antibody injection on PSA expression in prostate cancer cells

Cell line	No. of cells injected with heat-inactivated antibody	% of cells injected with heat-inactivated antibody after 96 h in which expression of PSA was detected
LNCaP	120	100%
LNCaP-Rf	120	100%
LNCaP-C4	120	100%

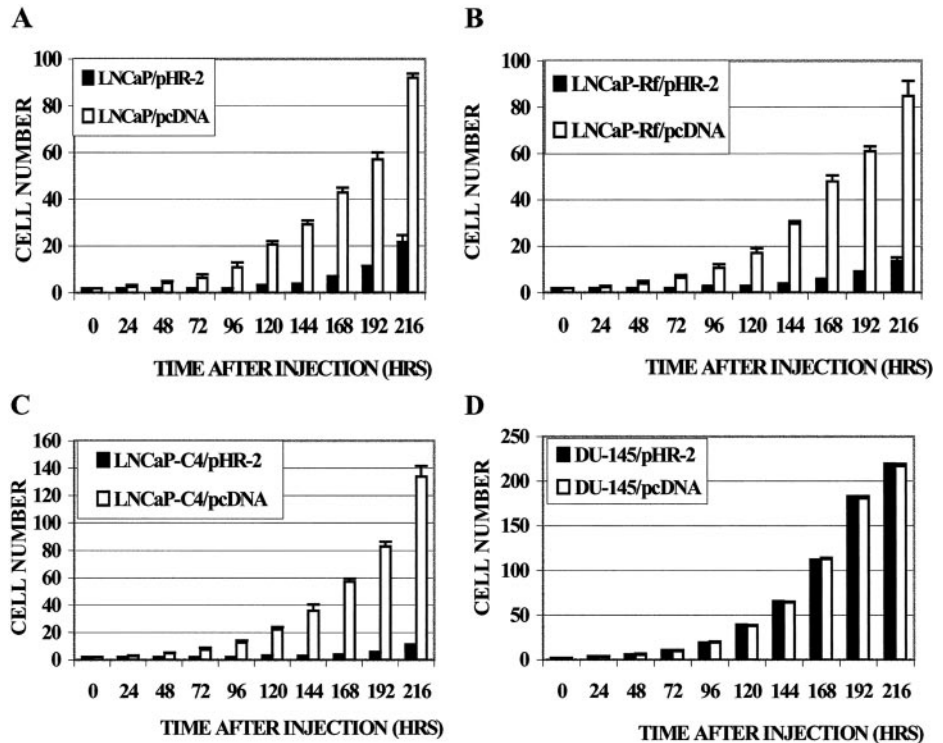


Fig. 5. Effects of microinjection of HR-2 hammerhead ribozyme into HRPca cells. Cells were grown ($10^4/35 \times 10$ -mm Falcon tissue culture dishes) CELLocate Eppendorf coverslips. LNCaP (A), LNCaP-Rf (B), LNCaP-C4 (C), and DU-145 (D) cells were microinjected with AR mRNA hammerhead ribozyme under similar conditions with their respective control (pCDNA empty vector). Cell number was determined every 24 h up to 216 h after microinjection, as shown. Cells were monitored by using a confocal microscope. Bars, SE of three independent experiments.

is amplified in up to 30% of androgen-refractory prostate cancer cases (11).

(b) There are frequent mutations in the AR in androgen-refractory prostate cancer tumors (12), which can expand the ligand-binding specificity of the AR, thus allowing it to bind other steroids and adrenal androgens (13), as well as antiandrogens (hydroxyflutamide, nilutamide, and bicalutamide; Ref. 14). The mutated AR may allow cells to be more responsive to other steroids. Evidence of AR mutations has been found in tumors of patients who have failed hormonal therapy (15).

(c) A third potential pathway is the activation of the AR by various growth factors and cytokines (16–19), such as epidermal growth factor, human epidermal growth factor receptor-2, keratinocyte growth factor, insulin-like growth factor-1, luteinizing hormone-releasing hormone, and neuropeptides through protein kinase A signaling pathways (20). Furthermore, the participation of AR coactivators on transcription can stimulate transcription of the AR in the presence of low levels of androgens or other steroids (21) and activate the cellular pathways downstream of the AR (22).

Our results demonstrate by two independent techniques that the AR is critical for androgen-refractory prostate tumor cell proliferation. We demonstrate that the specific down-regulation of AR with anti-AR antibody or ribozyme results in androgen-refractory prostate tumor cell growth inhibition and decline of PSA expression. This is the first report on inhibition of proliferation of androgen-refractory prostate cancer cells by direct inactivation of the AR function. In conclusion, our findings demonstrate a direct connection between the AR and proliferation in androgen-refractory prostate cancer cells. These data provide evidence that the AR is functional in androgen-refractory prostate cancer and strongly suggest that the AR may be critical for the development of androgen-refractory prostate cancer. This offers the potential for new approaches for the management of androgen-refractory prostate cancer. Additional studies are needed to delineate the various pathways through which the AR may initiate these effects.

Acknowledgments

We thank Dr. Charles Young for advice and discussion and Jim Tarara for excellent technical advice and support.

References

- Mora, G. R., and Tindall, D. J. Ligand dependent and independent activation of the androgen receptor. *In: L. W. Chung, W. B. Isaacs, and J. W. Simons (eds.), Prostate Cancer: Biology, Genetics, and the New Therapeutics*, pp. 219–239. Totowa, NJ: Humana Press, 2000.
- Denmeade, S. R., Lin, X. S., and Isaacs, J. T. Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. *Prostate*, 28: 251–265, 1996.
- Van der Kwast, T. H., Schalken, J., Ruizeveld de Winter, J. A., Van Vroonhoven, C. C., Mulder, E., and Boersma, W. Androgen receptors in endocrine therapy-resistant human prostate cancer. *Int. J. Cancer*, 48: 189–193, 1993.
- Yong, E. U., Lim, J., Qi, W., Ong, V., and Mifsud, A. Molecular basis of androgen receptor diseases. *Trends in molecular medicine. Ann. Med.*, 32: 15–22, 2000.
- Jenster, G. The role of the androgen receptor in the development and progression of prostate cancer. *Semin. Oncol.*, 26: 407–426, 1999.
- Sadar, M. D., Hussain, M., and Bruchoovsky, N. Prostate cancer: molecular biology of early progression to androgen independent. *Endocr. Relat. Cancer*, 6: 487–502, 1999.
- Antman, K. H., and Livingston, D. M. Intracellular neutralization of SV40 tumor antigen following microinjection of specific antibodies. *Cell*, 19: 627–635, 1980.
- Young, C. Y., Montgomery, B. T., Andrews, P. E., Qui, S. D., and Tindall, D. J. Hormonal regulation of prostate-specific antigen messenger RNA in human prostatic adenocarcinoma cell line LNCaP. *Cancer Res.*, 51: 3748–3752, 1991.
- Uhlenbeck, O. C., Dahm, S. C., Ruffner, D. E., and Fedor, M. J. Structure and mechanism of the hammerhead ribozyme self-cleaving domain. *Nucleic Acids Symp. Ser.*, 21: 95–96, 1989.
- Chen, S., Song, C. H. S., Lavrovsky, Y., Bi, B., Vellanoweth, R., Charterjee, B., and Roy, A. K. Catalytic cleavage of the androgen receptor messenger RNA and functional inhibition of androgen receptor activity by a hammerhead ribozyme. *Mol. Endocrinol.*, 12: 1558–1566, 1998.
- Koivisto, P., Kononen, J., Palmberg, C., Tammela, T., Hyytinen, E., Isola, J., Trapman, J., Cleutjens, K., Noordzij, A., Visakorpi, T., and Kallioniemi, O. P. Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. *Cancer Res.*, 57: 314–319, 1997.
- Visakorpi, T., Hyytinen, E., Tanner, M., Keinänen, R., Palmberg, C., Palotie, A., Tammela, T., Isola, J., and Kallioniemi, O. P. *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. *Nat. Genet.*, 9: 401–406, 1995.
- Wallen, M. J., Linja, M., Kaartinen, K., Schleutker, J., and Visakorpi, T. Androgen receptor gene mutations in hormone-refractory prostate cancer. *J. Pathol.*, 189: 559–563, 1999.
- Culig, Z., Hobbisch, A., Cronauer, M. V., Cato, A. C. B., Hittmair, A., Radmayr, C., Eberle, J., Bartsch, G., and Klockler, H. Mutant androgen receptor detected in an

- advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. *Mol. Endocrinol.*, *7*: 1541–1550, 1993.
15. Crawford, E. D., Eissenberg, M., McLeod, D., Spaulding, J. T., Benson, B. A., Davis, M. A., and Goodman, P. A. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. *N. Engl. J. Med.*, *17*: 419–424, 1989.
 16. Suzuki, H., Akakura, K., Komiya, A., Aida, S., and Shimazaki, J. Codon 877 mutation in the androgen receptor gene in advanced prostate cancer: relation to anti-androgen withdrawal syndrome. *Prostate*, *29*: 153–158, 1996.
 17. Culig, Z., Hobisch, A., Cronauer, M. V., Radmayr, C., Trapman, J., Hittmair, A., Bartsch, G., and Klocker, H. Androgen receptor activation in prostate tumor cell lines by insulin-like growth factor-1, keratinocyte growth factor, and epidermal growth factor. *Cancer Res.*, *54*: 5474–5478, 1994.
 18. Jenster, G. Ligand-independent activation of the androgen receptor in prostate cancer by growth factors and cytokines. *J. Pathol.*, *191*: 227–228, 2000.
 19. Craft, N., Shostak, Y., Carey, M., and Sawyers, C. L. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nature (Lond.)*, *5*: 280–285, 1999.
 20. Sadar, M. D. Androgen independent induction of prostate-specific antigen gene expression via cross-talk between the androgen receptor and protein kinase A signal transduction pathways. *J. Biol. Chem.*, *274*: 7777–7783, 1999.
 21. Horwitz, K. B., Jackson, T. A., Bain, D. L., Richer, J. K., Takimoto, G. S., and Tung, L. Nuclear receptor coactivators and corepressors. *Mol. Endocrinol.*, *10*: 1167–1177, 1996.
 22. Koivisto, P. A., and Rantala, I. Amplification of AR is associated with p53 mutation in hormone refractory recurrent prostate cancer. *J. Pathol.*, *187*: 237–241, 1999.

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Cancer Res 2002;62:1008-1013.

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