Androgen Causes Growth Suppression and Reversion of Androgen-Independent Prostate Cancer Xenografts to an Androgen-Stimulated Phenotype in Athymic Mice

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Abstract

Most prostate cancer patients develop androgen-independent recurrent prostate tumors a few years after androgen ablation therapy. No therapy, however, has been shown to substantially extend survival in these patients. Previously, we reported that androgen suppresses the growth of androgen-independent LNCaP prostate tumor cells both in vitro and in vivo. In cell culture, androgen receptor (AR)-rich androgen-independent LNCaP 104-R1 cells adapt to growth suppression by androgen and then their growth is androgen stimulated. Because maintaining androgen dependency of prostate tumor cells would prolong the usefulness of androgen ablation therapy, we determined if androgen-independent prostate tumors would revert to an androgen-stimulated phenotype in vivo upon androgen treatment. Growth of the LNCaP 104-R1 tumors was suppressed by androgen, but tumors then adapted to suppression by androgen and growth became androgen stimulated. Tumor AR and prostate-specific antigen mRNA and protein were initially high in 104-R1 tumors but decreased during adaptation. Subsequent removal of androgen decreased the serum prostate-specific antigen level further and stopped the growth of the adapted tumors. Because androgen caused growth suppression and then reversion of androgen-independent tumors to an androgen-stimulated phenotype and because the growth of androgen-stimulated tumors could be restrained by androgen ablation, these results suggest a novel therapy for AR-positive androgen-independent prostate cancer. (Cancer Res 2005; 65(6): 2082-4)

Introduction

Androgen ablation therapy causes regression of primary and metastatic androgen-dependent prostate cancer (1). However, most patients develop androgen-independent tumors 1 to 3 years after androgen ablation therapy. As a model to study progression from androgen dependence to androgen independence, we derived an androgen-independent human prostate cancer cell line, LNCaP 104-R1, from androgen-dependent LNCaP 104-S cells after long-term androgen deprivation (2). The growth of 104-R1 cells in vitro does not require androgen but it is instead suppressed by physiologic concentrations of androgen. Suppression by androgen is due in part to down-regulation of c-myc and accumulation of the cell cycle inhibitor p27Kip1 that causes cell cycle G1 arrest (2–4). 104-R1 cells adapt to the growth suppression and eventually R1Ad cells emerge. Growth of R1Ad cells is stimulated by androgen (3). Androgen receptor (AR) protein levels increase during the progression from androgen-dependent LNCaP 104-S cells to androgen-independent LNCaP 104-R1 cells, but decreases during the transition from 104-R1 to R1Ad cells (3). Here we investigate if androgen-independent prostate tumors would revert to an androgen-stimulated phenotype in vivo upon androgen treatment.

Materials and Methods

Animals. Experiments involving mice were approved by the University of Chicago Institutional Animal Care and Use Committee. Male BALB/c nu/nu mice (National Cancer Institute, Frederick, MD) were injected s.c. in both flanks with \(1 \times 10^6\) LNCaP 104-R1 cells suspended in 0.5 mL Matrigel (BD Biosciences, Franklin Lakes, NJ) 14 days after castration. Tumors were measured weekly using calipers and volume was calculated using the formula volume = length \(\times\) width \(\times\) height \(\times\) 0.52 (5).

Prostate-Specific Antigen and Testosterone Assay. Blood was collected from the orbital sinus of mice and serum was prepared from blood and stored at –80°C. Prostate-specific antigen (PSA) and testosterone were measured in mice sera using ACTIVE PSA ELA and ACTIVE Testosterone ELA kit (Diagnostic System Laboratories, Inc, Webster, TX).

Western Blot Analysis. Samples were prepared from tissue homogenized in 2× Laemml buffer as described (3). AR and PSA protein expression was determined by using antibodies to AR (AN-21; ref. 3) and PSA (DAKO, Glostrup, Denmark).

Real-time Quantitative PCR. Tumors were removed from mice and stored in RNAlater (Ambion, Austin, TX) overnight at 4°C before RNA isolation using the Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA). Contaminating DNA was removed using DNeasy (1-DNA-free, Ambion), cDNA was synthesized from total RNA using the Omniscript RT synthesis kit (Qiagen, Valencia, CA). For real-time quantitative PCR, QuantiTect Probe PCR kit (Qiagen) and ABI Prism 7700 cycler (Applied Biosystems) were used in a dual-labeled probe protocol. Samples were run for 40 cycles in triplicate. The following primers were used for AR real-time PCR: forward primer, 5’-CGCCCGGCTATGTTTCT; reverse primer, 5’-TTCGGACACA-CGGCCTGACA; VIC-labeled probe, VIC-5’-TGGATTCCGCATGCA-CAAGTCCCG-3’-TAMRA. Primer and VIC-labeled probe sequences used for PSA real-time quantitative PCR were described by Gelmini et al. (6). AR and PSA transcript levels were normalized to human glyceraldehyde-3-phosphate dehydrogenase transcript levels in each sample. Glyceraldehyde-3-phosphate dehydrogenase transcript levels were determined using the following primers: forward primer, 5’-TCGACAGTCAAGCCTTACCTGAC-CAAGTCCCG-3’-TAMRA. The following primers: forward primer, 5’-TGGATTCCGCATGCA-CAAGTCCCG-3’-TAMRA.

Results and Discussions

LNCaP 104-R1 cells inoculated into mice formed tumors. At day 67, these mice were implanted s.c. with a 20 mg testosterone pellet, whereas no testosterone was given to control mice. Tumors in the testosterone treatment group shrank within a week, whereas all tumors in the control group continued to grow (Fig. 1). When
testosterone pellets were implanted into the remaining control mice, all of these tumors shrank as well. In both groups, regression of 104-R1 tumors lasted for ~35 to 40 days and then tumor growth resumed. The resumption of tumor growth was not because of a decrease in androgen because blood testosterone levels did not change significantly before (43.62 ± 4.05 ng/mL) and after (40.38 ± 10.21 ng/mL) tumor relapse. The length of time required for tumors to adapt to the suppression by androgen and to regrow was not dependent on the size of tumor at the time the androgen pellet was implanted.

To investigate if androgen was now required for the growth of testosterone-adapted (R1Ad) tumors, testosterone pellets were removed from mice bearing adapted R1Ad tumors. The removal of androgen stopped the growth of these R1Ad tumors (Fig. 2), whereas R1Ad tumors in mice implanted with testosterone pellets continued to grow. Tumor volume did not change in mice without testosterone for >60 days. These observations suggest that the growth of the adapted tumors required androgen and androgen deprivation followed by androgen treatment maintained tumors in an androgen-dependent state.

AR expression level may play an important role during prostate cancer progression. Up-regulation of AR expression level in androgen-independent prostate tumor cells was observed in several advanced prostate cancer patients (7). We previously reported that during the progression of androgen-dependent 104-S cells to androgen-independent 104-R1 cells in vitro, AR mRNA and AR protein increased dramatically by >5-fold (2–4). Chen et al. (8) reported that a modest increase in AR mRNA was the only change consistently associated with the development of resistance to androgen ablation therapy, and the increase in AR mRNA and protein was both necessary and sufficient to convert prostate cancer growth from a hormone-sensitive to a hormone-refractory stage. In line with these observations, we found that AR protein and mRNA levels were higher in 104-R1 tumors than in 104-S tumors (Fig. 3A and B). All R1Ad tumors had lower AR protein and mRNA levels than 104-R1 tumors. AR down-regulation may release androgen-independent prostate tumors from suppression by androgen.

To characterize the androgenic response of prostate tumors at different stages of the progression, we assayed the protein and mRNA level of PSA, the most common marker used for detecting prostate cancer growth in patients. Removal of testosterone pellets decreased both PSA protein and mRNA expression dramatically in adapted R1Ad tumors (Fig. 3A and C). Implantation of mice with 104-R1 tumors with a testosterone pellet initially increased the serum PSA level, but as the growth of 104-R1 tumors was suppressed by testosterone and the R1Ad tumors developed, the serum PSA level decreased probably due to the lower level of AR in R1Ad tumors. Removal of the testosterone pellet from these mice bearing R1Ad tumors decreased the serum PSA level further (Fig. 3D), suggesting that PSA expression in R1Ad tumors was stimulated by androgen as well. Because the serum PSA level is reported to be proportional to tumor volume (9), we calculated PSA density (serum PSA concentration divided by tumor volume) and found that PSA densities correlated very well with serum PSA levels (Fig. 3D).

The serum PSA level in mice bearing androgen-independent 104-R1 tumors without a testosterone pellet implant was 15-fold higher than mice with androgen-dependent 104-S tumors (Fig. 3D). This may be similar to the clinical situation where androgen-independent prostate cancer develops in patients after androgen deprivation therapy. Often, the serum levels of PSA in these patients increase several fold although the serum testosterone levels are very low.

Based on the assumption that androgen-dependent and androgen-independent tumor cells coexist in prostate cancer patients and that suppression of androgen-dependent tumor cells can accelerate the growth of independent tumor cells, intermittent androgen replacement therapy has been tested in recent years as a potential therapy for prostate cancer. Akakura et al. (10) hypothesized that the replacement of androgens at the end of a period of androgen ablation might result in the regeneration of androgen-dependent tumor cells with potential to respond to successive rounds of androgen withdrawal and replacement, forestalling tumor progression and thus prolonging the usefulness of androgen-deprivation therapy. Growth of advanced prostate tumors was delayed in some patients in clinical trials using this
approach (11). Stopping androgen-ablation therapy after tumor relapse will increase endogenous androgen levels in blood, and according to our studies, this elevation of androgen may then suppress the growth of androgen-independent prostate tumor cells. The increase in endogenous androgen levels may cause the reversion of androgen-independent prostate tumor cells to an androgen-stimulated phenotype, as shown in this study, and subsequent therapy involving androgen-ablation should retard the growth of androgen-stimulated tumor cells.

We have shown that, in athymic mice, testosterone causes tumor regression and reversion of the androgen-independent LNCaP 104-R1 tumors to androgen-stimulated R1Ad tumors. Furthermore, the growth of R1Ad tumors can be stopped by removal of androgen. The fact that androgen can cause the reversion of androgen-independent tumors to an androgen-stimulated phenotype in athymic mice suggests a novel therapy for AR-positive androgen-independent prostate cancer. It is likely that such a therapy is applicable to other cancers, such as breast cancer, where after failing hormone depletion therapy and becoming hormone-independent, the cancer cells may respond to hormone suppression and reversion as we described in this report.

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Figure 3. AR and PSA expression in tumor xenografts. Tumor and serum samples were obtained from group of mice described in the legends of Figs. 1 and 2. (A) PSA, AR, and actin protein levels in 104-S tumors (in intact mice), 104-R1-T tumors (Fig. 1, day 78, control group), R1Ad + T tumors (Fig. 1, day 136, treatment group), and R1Ad-T (Fig. 2;14 days after testosterone pellet removed) were assayed by Western blot. Expression level of AR mRNA (B) and PSA mRNA (C) in tumors analyzed in A were determined by real-time quantitative PCR. (D) Serum PSA level and PSA density (see text) of mice with 104-S tumors, 104-R1-T tumors (Fig. 1, day 67, control group), 104-R1 + T tumors (Fig. 1, day 73, treatment group), R1Ad + T tumors (Fig. 1, day 125, treatment group), R1Ad-T tumors (6 days after testosterone pellet removed in Fig. 2). White columns, PSA values; black columns, PSA density values. Values are the mean for 4 to 10 samples.

References

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