Reducing the Agonist Activity of Antiandrogens by a Dominant-negative Androgen Receptor Coregulator ARA70 in Prostate Cancer Cells*

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Although the progression of prostate cancer initially is dependent on androgens, tumor progression to an androgen-independent growth eventually occurs in most of patients treated with androgen ablation and/or antiandrogen therapy. After the initial response, antiandrogens lose their efficacy and eventually act as agonists to promote androgen receptor (AR)-mediated growth of prostate cancer cells. An aberrant regulation of AR activity, presumably by AR coregulators, may contribute to this acquired agonist activity of antiandrogens. Using an in vitro mutagenesis and a double-negative selection in yeast two-hybrid screening, we have identified a dominant-negative AR coregulator ARA70 (dARA70N), which can inhibit AR transcriptional activity by inactivating the normal function of ARA70 in the LNCaP cells. Whereas ARA70 in oligomeric form interacts with AR and enhances its transcriptional activity, dARA70N lacks AR interaction and might retain the ability to form a non-functional heteromer with ARA70 and interrupt AR transcriptional activity without a change in AR protein itself. The addition of dARA70N reduces the agonist activity and rescues the normal function of antiandrogens in prostate cancer cells. RNA-interference-mediated silencing of ARA70 gene further confirms these observations. Taken together, these findings indicate that ARA70 may contribute to the acquired agonist activity of antiandrogens and plays an important role in making prostate cancer cells resistant to androgen ablation and/or antiandrogen therapy. ARA70 may, thus, be a critical target for developing therapeutic agents against AR-mediated progression of prostate cancer.

Prostate cancer still remains the most frequently diagnosed malignancy in aging men, and each year about 31,500 men in the United States lose their lives to this malignancy (1). Androgens and the androgen receptor (AR) play key roles in this malignancy, and since 1941 androgen ablation has been the cornerstone of treatment in patients with locally advanced or metastatic prostate cancer. Androgen deprivation, as achieved by surgical or chemical castration, usually results in a favorable clinical response and a dramatic regression of prostate tumor (2–3). Although 80–90% of patients initially respond to this therapy, tumors gradually become resistant, and eventually most of the patients develop androgen-independent progression of the disease. Although castration alone causes a 95% reduction in serum testosterone levels, the concentration of 5α-dihydrotestosterone (DHT) does not fall below 60%, because of the presence of circulating adrenal androgens, which are effectively converted to DHT in the prostate tissue (4–5). Thus, prostate DHT levels still remain relatively high even after castration. To overcome this effect, ablation therapy has been supplemented with antiandrogens that can block remaining androgen action. This is called maximal ablation therapy (6–7), and recently it has become the predominant form of treatment for advanced prostate cancer patients. Although originally designed to produce a complete blockade of AR-mediated growth, this therapy also failed to deliver a significant improvement in 5-year survival (8). After an initial clinical response, most often cancer progression relapses and continues in the presence of androgen ablation and/or antiandrogens. It has been postulated that during advanced stages, antiandrogens lose their efficacy as antagonists and instead function as agonists to promote AR-mediated growth of prostate cancer cells (9–11). In some cases, patients treated with antiandrogens ultimately suffer from withdrawal syndrome whereby antiandrogens become androgenic. This results in an agonist effect with an increase of prostate-specific antigen (PSA), a marker for monitoring the progress of prostate cancer (12). However, the mechanism of this transition, which promotes AR function, is largely unknown. Because the majority of androgen-independent tumors and metastases retain a functional AR signaling pathway (13–14), molecules or proteins other than AR itself have been postulated to play some roles in this transition stage. AR coregulators that are able to enhance AR activity could be one such candidate.

One of the AR coregulators, ARA70, has been characterized as having the capacity to enhance AR transcriptional activity in response not only to testicular or normally weak adrenal androgens but also to the antiandrogens hydroxyflutamide (HF) and 17β-estradiol (E2) in prostate cancer cells (15–21). Using CV-1 cells, Zhou et al. (22) also show that ARA70 could induce E2-mediated AR transactivation. In contrast, Gao et al. (23) report that E2 has little induction on AR transactivation in hydroxyflutamide; wt, wild type; AD, activation domain; RNAi, RNA-interference.
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the absence or presence of ARA70 in CV-1 cells. The reasons for these discrepancies using the same CV-1 cells yet having different results remain unclear. However, using HeLa, PC3, and TSU-Prl1 cells, other laboratories also demonstrate that ARA70 could induce E2-mediated AR activity (24–26).

HER2/Neu, a member of the epidermal growth factor family of receptor tyrosine kinases, has been reported to activate the AR-signaling pathway in the absence of androgens and to "super-potentize" AR signaling in the presence of low levels of androgens (27–28). The mechanisms of this modulation of AR signaling by HER2/Neu could involve the phosphorylation of AR, which could then enhance the interaction between AR and ARA70. All these results suggested that the interaction between AR and ARA70 could play important roles for the pro-

Experimental Procedures

Chemicals and Plasmids—E2, DHT, and hydroxylamine were purchased from Sigma, and HF was a gift from Schering. pGAD10-ARA70N fused with GAL4 activation domain (AD) was the clone originally identified from the cDNA library. pAS2-AR containing the C terminus of the AR ligand-binding domain from wild-type (wt) AR fused to GAL4 DNA binding domain, pGAL0-AR containing AR ligand binding domain fused with GAL4 DNA binding domain, and pGALVP16-ARA70 or dARA70N fused to the AD of VP16 were constructed as previously described (16). pSG5-AR, pSG5-ARA55, pSG5-ARA70, pSG5-ARA54, and pSG5-ARC1 were constructed as previously described (10, 15, 18). pSG5-dARA70N was constructed by subcloning EcoRI fragment from yeast vector pGAD10-ARA70N (N-terminal ARA70, amino acids 1–401) into pSG5 expression vector. AR antibody NH27 was from Dr. Mizokami, ARA70 antibody was from Pharmingen, and the PSA antibody was obtained from DAKO.

Generation of Mutant ARA70N Library—The ARA70N mutant library was made by hydroxylamine-mediated mutagenesis (29). About 100 μg of the plasmid DNA, pGAD10-ARA70N, was incubated in 5 ml of 1 M hydroxylamine for 1 h at 70 °C. The plasmid DNA was extracted with phenol-chloroform (1:1) and recovered with ethanol precipitation.

Yeast Two-Hybrid System—The hydroxylamine-mutated pGAD10-ARA70N library was transformed into Y190 yeast cells expressing AR (previously transformed with pAS2-AR). The transformants were plated on synthetic dropout medium lacking tryptophan and leucine in the presence of 100 μM DTT. A pool of white colonies on synthetic dropout medium lacking leucine in the presence of 100 nM DHT. A pool of white colonies indicating no dominant-negative interaction was further transformed into yeast strain Y190 expressing mutants was then cloned into BS/U6 plasmid after enzyme digestion. The selection of coding sequences was determined empirically by blast search to avoid any significant sequence homology with other genes. Using primer extension, we first generated the double-stranded DNA with an Apal (blunt) and EcoRI site, which was then cloned into BS/U6 plasmid after enzyme digestion. The construction of RNai-B and RNai-C were also done in a similar manner.

Immunoprecipitation of Overexpressed Proteins and Immunoblotting—The cell lysates from COS-1 cells transfected with expression plasmids or control vector were sonicated 2 × 10 s, followed by 10 min of centrifugation. Equal amounts (500 μg) of total proteins were incubated for luciferase (Luc) activity using the Dual Luciferase assay kit (Promega). Identification of a Dominant-negative ARA70 Mutant—The strategy for the identification of dominant-negative mutants was based on the assumption that the ARA70 mutant, which loses its interaction with AR, might inhibit AR transcriptional activity by inactivating the normal function of the wt ARA70, presumably via a heteromer formation. Using hydroxylamine-mediated mutagenesis, a library of ARA70 mutants was generated. Because the N-terminal ARA70 is responsible for interaction and enhancement of AR transactivation, we used ARA70 N-terminal domain (amino acids 1–401) to generate the library of ARA70 mutants. Using a yeast two-hybrid system, the mutated library was screened for those mutants that did not interact with AR. The screening system was further extended to screen out those mutants that did not interact with AR. The screening system was further extended to screen out those mutants that did not interact with AR. The screening system was further extended to screen out those non-interacting mutants that could effectively interfere with the interaction between ARA70 and AR in a dominant-negative fashion. A pool of potential dominant-negative mutants was, thus, generated by this double negative selection in yeast two-hybrid assay.

We then subcloned these potential dominant-negative mutants into a mammalian expression vector, pSG5, and assayed their dominant-negative effects in COS-1 cells that do not express ARA70 or AR. We co-transfected ARA70N mutants and...
ARA70N along with AR and a mouse mammary tumor virus (MMTV)-Luc reporter gene. After 24 h of androgen treatment, cells were harvested and assayed for Luc activity as a function of AR transcriptional activity. One select ARA70 mutant showed up to 90% inhibition of ARA70-enhanced AR transactivation in a dose-dependent manner (Fig. 1a). When MMTV-Luc was replaced by PSA-Luc, containing a natural PSA promoter, or by a synthetic (ARE)4-Luc, similar results were obtained (Fig. 1b and c). The inhibition was specific for ARA70, since the mutant did not affect AR transactivation by other related AR coactivators including ARA54, ARA55, or SRC-1 (Fig. 2A). Also, the inhibition was relatively selective for AR, since the effect on other related nuclear receptors including glucocorticoid receptor (GR) and progesterone receptor (PR) was not significant in PC-3 prostate cancer cells that express endogenous ARA70 (Fig. 2B). These results suggested that mutant ARA70N functionally inactivates ARA70 and thereby inhibits ARA70-enhanced AR transactivation in the cells. We selected this mutant as dominant-negative ARA70 mutant and designated it as dARA70N. Sequence analysis revealed a single point mutation (C to T transition) resulting in a proline to serine substitution at amino acid 308 of ARA70N. This inhibition was further confirmed in two other prostate cancer cell lines, PC-3(AR)2 and LNCaP, which express endogenous ARA70 (Fig. 2B). The PC-3(AR)2 cells express endogenous ARA70, dARA70N significantly inhibited AR transcriptional activity. This inhibition was further confirmed in two other prostate cancer cell lines, PC-3(AR)3 and LNCaP, which express endogenous AR as well as ARA70 (Fig. 3a, b and c, respectively). The PC-3(AR)3 is a stably expressed AR cell line derived from parental PC-3 cells, and the LNCaP has a mutated yet functional AR, which was also found in human prostate tumors. When MMTV-Luc was replaced by PSA-Luc or by a synthetic (ARE)1-Luc, similar results were obtained (data not shown).

To further determine the suppression effect of dARA70N in endogenous AR target gene, we analyzed the expression of PSA that is widely used as a marker for monitoring prostate cancer progression (32–33). We transfected dARA70N or ARA70N into LNCaP cells, treated them for 24 h with DHT or vehicle, and then harvested the cells and assayed for PSA expression using Western blot analysis. The LNCaP cells express PSA in response to androgen (Fig. 3B, lane 2), which was significantly inhibited by transfection of dARA70N (lanes 4 and 5). Because LNCaP cells express relatively higher endogenous ARA70, dARA70N significantly inhibited AR transcriptional activity.
transfection of an additional ARA70N slightly increased PSA levels in these cells (lane 3). It is possible that the activation of the AR target gene (PSA) may be already optimal or saturated by those higher expressed endogenous ARA70s and ARs in the cells. The expression of transfected ARA70N and dARA70N has been shown in Fig. 3c. 

The inhibition was independent of any change of the expression of AR or ARA70, since dARA70N did not influence the expression of the AR or ARA70 protein levels in the cells (Fig. 3B, b and c, respectively). Because dARA70N selectively inactivates endogenous wtARA70 in the cells, we did not expect the level of AR protein to change. The fact that dARA70N inhibited the expression of AR target gene (PSA) without a change in AR protein itself may represent a very significant finding in this study. It not only indicates the role of ARA70 in AR transcriptional activity but also that AR functional activity could be regulated by modulating the expression of AR coactivator, ARA70, in the cells. Taken together, these findings thus offer strong evidence for the potential roles of ARA70 on the AR-mediated progression of prostate cancer.

**Dominant-negative ARA70N Reduces the Agonist Activity of Antiandrogens in Prostate Cancer Cells**—Treatment of prostate cancer patients with maximal ablation therapy, a combination of surgical or chemical castration with antiandrogens such as bicalutamide or HF, still has limited success. Although initially effective, most patients fail to respond to this therapy beyond 1–2 years. Prostate tumors become resistant and continue to grow in the presence of low levels of androgens. Moreover, antiandrogens given to antagonize residual androgen action lose their efficacy as antagonists and eventually function as agonists to promote AR-mediated growth of prostate cancer cells. Using transient transfection and reporter gene assays we demonstrated that overexpression of dARA70N helped to abolish the agonist activity and rescued the antagonist effect of antiandrogens or other non-androgenic molecules in prostate cancer cells. We designed experimental conditions that closely mimicked conditions of maximum androgen ablation therapy. Prostate cancer patients may have minimal serum concentrations of androgens (0.1 nM DHT) and a relatively high level of antiandrogens (1–5 μM) during maximal ablation therapy. LNCaP cells were transfected with dARA70N or ARA70N, and after 24 h of androgen/antiandrogen treatment in the above conditions, cells were harvested and assayed for AR transcriptional activity. As depicted in Fig. 4A, HF and E2 increased AR activity, functioning as agonists in ARA70-expressed LNCaP cells (lanes 6, 8, 13, and 15). In the presence of 0.1 nM DHT, these molecules failed to antagonize or block androgen actions in the cells (lanes 7, 9, 14, and 16). However, overexpression of dARA70N reduced this agonist activity (lanes 10 and 17) and effectively rescued the antagonist effect of these antiandrogens in LNCaP cells (lanes 11 and 18). This rescue by the dARA70N was well reflected in the dimin-
ished expression of PSA in the cells as measured by Western blot analysis. As depicted in Fig. 4B, both E2 and HF by themselves increased PSA expression (lanes 5 and 11). In conditions of maximal androgen ablation therapy, these drugs increased (instead of decreased) PSA expression in the cells (lanes 6 and 12). Although LNCaP cells express endogenous ARA70, transfection of ARA70N further increases (lanes 7, 8, 13, and 14), whereas dARA70N significantly diminished PSA expression in the cells (lanes 9–10 and 15–16). Taken together, these findings demonstrate that AR expression is essential for PSA induction, and an addition of dARA70N will inactivate endogenous ARA70 function, resulting in antiandrogens having less agonist activity in the LNCaP cells.

RNAi-mediated Silencing of ARA70 Gene Inhibits AR Transactivation and Reduces the Agonist Activity of Antiandrogens in Prostate Cancer Cells—To further confirm these roles of ARA70 on the AR transactivation and on the agonist activity of antiandrogens, we investigated the effect of RNAi-mediated ARA70 gene silencing in LNCaP cells. We constructed three RNAi constructs (Fig. 5A) as described under “Experimental Procedures.” Although all three constructs showed inhibitory effects, the construct A was most effective in inhibiting exogenously expressed ARA70 and AR transactivation in COS-1 cells (data not shown). To see whether the construct A could silence endogenous ARA70 and thereby could inhibit endogenous AR function, we transfected it into PC-3(AR)2 cells, which express both ARA70 and AR. As depicted in Fig. 5B, RNAi-A construct inhibited endogenous AR function in a dose-dependent manner (lanes 4–5). Western blot analysis using cell lysates from PC-3(AR)2 cells transfected with RNAi-A confirmed the silencing of the ARA70 gene in the cells (Fig. 5C, upper panel, lanes 3–4). The silencing was specific for ARA70 only, since the expression of a related AR coactivator, ARA55, or AR protein itself was not effected by this RNAi construct (middle and lower panel, respectively).

To test whether the reducing ability of dARA70N could be reproducible by RNAi-mediated silencing of ARA70 gene, we transfected LNCaP cells with RNAi-A and measured the expression of PSA after 48-h treatments of androgen and/or antiandrogens in conditions of androgen therapy as described earlier. As depicted in Fig. 5D, transfection of RNAi significantly reduced the expression of PSA as stimulated by DHT (lanes 1–4). Although E2 and HF were supposed to antagonize AR function, resulting in the decrease of target gene expression, in these cells both E2 and HF actually increased expression of the AR target gene PSA (lanes 5, 6, 9, and 10). However, transfection of RNAi-A significantly reduced the agonist activity of these antiandrogens and thereby inhibited the expression of PSA both in the absence and presence of DHT (lanes 7, 8, 11, and 12). These findings are consistent with our previous findings using dARA70N. Taken together, our study clearly demonstrated that the agonist activity of antiandrogens can be reduced by inactivation of wtARA70, indicating a role of ARA70 in the acquired agonist activity of antiandrogens in LNCaP prostate cancer cells.

**Dominant-negative ARA70N Forms a Non-functional Heteromer with wtARA70**—The dARA70N was identified on the assumption that the non-interacting ARA70 mutant might form a non-functional heteromer with wtARA70, which may or may not interact with AR, but interrupt the AR transcriptional activity. We used a mammalian two-hybrid assay to further verify this phenomenon. The dARA70N, ARA70N, and AR were fused to GAL4 DNA binding domain or VP16 AD, and plasmids were transfected in COS-1 cells. The interactions between expressed proteins were analyzed using pG5-Luc reporter gene. As shown in Fig. 6A, compared with ARA70N, the dARA70N lost its ability to interact with AR (lanes 4 versus 5), which is consistent with our yeast two-hybrid screening results. However, transfection of the cells with an un-fused dARA70N did not prevent wtARA70N interaction with AR (lane 6). In Fig. 7A, ARA70 could interact with itself, presumably forming a homodimer or oligomer, both in the presence and absence of DHT (lanes 3–4). The dARA70N could also form a dimer (or heteromer) with wtARA70N both in the presence and absence of DHT (lanes 6–7), and could disrupt ARA70–ARA70 interaction (lane 5).

To further verify these interactions, we did co-immunoprecipitation assays using cell lysates transfected with AR, His- and/or FLAG-tagged ARA70N, or dARA70N. In Fig. 6B, we immunoprecipitated cell lysates with AR antibody or control IgG and detected the presence of ARA70 or AR using His and AR antibody, respectively. ARA70N physically interacted with AR (lane 2), whereas dARA70N lost its interaction with AR
Transfection of cells with un-fused dARA70N resulted in the same extent of AR-ARA70 interaction, indicating that dARA70 did not prevent wtARA70 interactions with AR (lane 4). In Fig. 7B, cell lysates were immunoprecipitated with FLAG antibody or control IgG and then the presence of ARA70N, dARA70N, or ARA55 was detected using His antibody. ARA70 could interact with itself (lane 2) as well as with dARA70N (lane 4) but not with another related AR coactivator, ARA55 (lane 6). Transfection of cells with an un-fused dARA70N disrupted ARA70-ARA70 interaction (lane 3), further indicating that dARA70N could form a heteromer with ARA70. Taken together, our mammalian two-hybrid and co-immunoprecipitation data suggested that an addition of dARA70N seemed to form a non-functional heteromer with wtARA70 (as dARA70N-ARA70), which then might be able to interact with AR (dARA70N-ARA70-AR complex), resulting in the diminished AR transcriptional activity.

Aberrant Phosphorylation of the Dominant-negative Mutant (dARA70N) at Ser-308 Contributes to the Inhibitory Effect of dARA70N—Sequence analysis revealed a single point mutation from proline to serine at amino acid 308 of dARA70N (P308S), which generated a potential consensus site for phosphorylation of the dominant-negative mutant by ataxia telangiectasia mutated kinase. To test whether the mutation resulted in an increased phosphorylation of the dARA70N, we transfected COS-1 cells with ARA70N, dARA70N, or an alanine-substituted ARA70N (P308A). We then immunoprecipitated cell lysates with ARA70 antibody and detected the phosphorylated ARA70 by using phosphoserine antibody. As depicted in Fig. 8A, compared with ARA70N, dARA70N underwent increased serine phosphorylation, whereas an alanine substitution did not cause any significant change in the phosphorylation status. To explore the possibility that serine phosphorylation might contribute to the inhibitory effect of dARA70N, we created the phospho-mimetic aspartate mutation (P308D) that should mimic the effects of phosphorylated protein (34–35). As shown in Fig. 8B, transfection of P308D exhibited an inhibitory effect on AR transactivation, whereas P308A showed an enhancement of AR activity that is similar to...
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AR transcriptional activity. A, equal amounts (500 μg) of cell lysates from COS-1 cells transfected with ARA70N, dARA70N (P308S), or alanine-substituted ARA70N (P308A) were immunoprecipitated by ARA70 antibody, and the phosphorylated ARA70 was detected by phosphoserine antibody as described under “Experimental Procedures.” B, LNCaP cells were transfected with pSG5 vector, ARA70N, dARA70N (P308S), and alanine- or aspartate-substituted ARA70N (P308A or P308D). After 24-h ligand treatment, cells were harvested and assayed for AR transcriptional activity as described. Values represent the mean ± S.D. of at least three independent determinations and are presented as the ratio of MMTV-Luc versus Renilla Luc activity for each sample.

ARA70N, indicating that phosphorylation at Ser-308 may have a role in the inhibitory effect of the dARA70N. Surprisingly, the inhibition by P308D was stronger than the original mutant dARA70N (P308S). The aspartate-substituted dARA70N (P308D) thus seemed to serve as a better dominant-negative mutant. These observations indicated that an aberrant phosphorylation of dARA70N might play a role in the inhibition of AR transcriptional activity in the cells.

DISCUSSION

Androgens and AR play key roles in the development of the prostate gland as well as the prostate cancer. Androgen ablation supplemented with antiandrogens has been a predominant form of treatment for the locally advanced or metastatic prostate cancer. Although initially effective, most often cancer progression relapses, tumors continue to grow in the presence of androgen ablation and/or antiandrogens, and eventually growth becomes androgen-independent. In this stage, antiandrogens given to antagonize AR function behave as agonists to promote AR-mediated growth of prostate cancer cells. The exact mechanism for the development of this acquired agonist activity of antiandrogens in promoting AR function is largely unknown. However, because tumor growth still mostly depends on a functional AR signaling pathway, it is widely speculated that biological macromolecules, including AR coregulators that can modulate AR function and/or specificity, may have some roles in such transition process. One of the AR coregulators, ARA70, has been characterized as having the capacity to modulate AR specificity in response to agonists and antagonists in prostate cancer cells. ARA70 interacts with a ligand-bound AR and enhances its transcriptional activity not only in response to androgens but also in response to antiandrogens and other non-androgenic molecules (10–11). We investigated whether interruption or modulation of ARA70-AR interaction could lead to ablation of the agonist activity of both androgens and antiandrogens, which may have potential implications in AR-mediated prostate cancer progression.

Using an in vitro mutagenesis and a double-negative selection in yeast-two hybrid screening, we have identified a dominant-negative AR coregulator, dARA70N, that inhibits ARA70-enhanced AR transcriptional activity by inactivating the normal function of ARA70 in prostate cancer cells. A single point mutation (C to T transition) generates a proline to serine substitution at amino acid 308, transforming a potent AR coregulator (ARA70) into a strong inhibitor (P308S) of AR transcriptional activity. In an oligomeric form ARA70 interacts with AR and enhances its transcriptional activity. The dominant-negative mutant, lacking AR interaction, retains the ability to form a non-functional heteromer with ARA70 and interrupts AR transcriptional activity without any change in AR protein itself. An aberrant phosphorylation of the dARA70N mutant may contribute to this inhibitory effect on AR transcription. An aspartate substitution (P308D) results in a stronger inhibitor and can serve as a better dominant-negative mutant. Overexpression of the dominant-negative mutant either in the form of Ser-308 or Asp-308 abolishes the agonist activity of antiandrogens or other non-androgenic molecules in LNCaP prostate cancer cells.

The dARA70N is, thus, able to block AR transactivation in the absence of DHT, the normal ligand, and other ligands or antiandrogens that act on the mutant AR in LNCaP cells. Surprisingly, however, it blocks all of them to a similar extent, and there is no differential effect of dARA70N on DHT, HF, or E2. ARA70 may have broadened the mutant AR specificity without reducing its affinity for DHT, so that optimal AR transactivation is possible in the presence of any one of these ligands. Further study is needed to verify such a phenomenon. However, the ability of dARA70N to reduce the agonist activity of antiandrogens in conditions similar to androgen ablation therapy and a demonstration of similar results by RNAi-mediated silencing of ARA70 gene strengthen the roles of ARA70 in the AR-mediated prostate cancer progression, particularly in the acquired agonist activity of antiandrogens in LNCaP cells.

Previously, a number of studies have reported a role of AR mutations in the modulation of AR specificity in prostate cancer cells (36–39). The LNCaP cells used in this study express a mutant AR (T877A), which is also found in prostate cancer patients that can change the AR specificity. The dARA70N inhibition of the agonist activity of antiandrogens in this cell line clearly indicates a probable dominant role of ARA70 over AR mutations in the process. Apart from mutant AR, the expression of ARA70 may provide prostate cancer cells a selective growth advantage during antiandrogen therapy. ARA70, thus, offers a possibility for developing new therapeutic agents that can reduce the agonist activity and restore the normal function of antiandrogens in prostate cancer patients. The dominant-negative mutant (Ser-308 or Asp-308) itself may be evaluated as a gene therapeutic reagent. However, further study will be needed to determine whether reducing the agonist effects of antiandrogens by the dominant-negative ARA70N mutant will be sufficiently effective to suppress the prostate cancer growth in vivo.

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