Treatment-Dependent Androgen Receptor Mutations in Prostate Cancer Exploit Multiple Mechanisms to Evade Therapy

Mara P. Steinkamp,1 Orla A. O’Mahony,1 Michele Brogle,1 Haniya Rehman,1 Elizabeth W. LaPensee,1 Saravana Dhanasekaran,3 Matthias D. Hofer,2 Rainer Kuefer,5 Arul Chinnaiyan,4 Mark A. Rubin,6 Kenneth J. Pienta,1 and Diane M. Robins1

Departments of 1Human Genetics, Pathology, and Medicine and Urology, University of Michigan Medical School, Ann Arbor, Michigan; 2Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; 3Department of Urology, University of Ulm, Ulm, Germany; and 4Department of Pathology, Weill Cornell Medical College, New York, New York

Abstract

Mutations in the androgen receptor (AR) that enable activation by antiandrogens occur in hormone-refractory prostate cancer, suggesting that mutant ARs are selected by treatment. To validate this hypothesis, we compared AR variants in metastases obtained by rapid autopsy of patients treated with flutamide or bicalutamide, or by excision of lymph node metastases from hormone-naive patients. AR mutations occurred at low levels in all specimens, reflecting genetic heterogeneity of prostate cancer. Base changes recurring in multiple samples or multiple times per sample were considered putative selected mutations. Of 26 recurring missense mutations, most in the NH2-terminal domain (NTD) occurred in multiple tumors, whereas those in the ligand binding domain (LBD) were case specific. Hormone-naive tumors had few recurring mutations and none in the LBD. Several AR variants were assessed for mechanisms that might underlie treatment resistance. Selection was evident for the promiscuous receptor AR-V716M, which dominated three metastases from one flutamide-treated patient. For the inactive cytoplasmically restricted splice variant AR23, coexpression with AR enhanced ligand response, supporting a decoy function. A novel NTD mutation, W435L, in a motif involved in intramolecular interaction influenced promoter-selective, cell-dependent transactivation. AR-E255K, mutated in a domain that interacts with an E3 ubiquitin ligase, led to increased protein stability and nuclear localization in the absence of ligand. Thus, treatment with antiandrogens selects for gain-of-function AR mutations with altered stability, promoter preference, or ligand specificity. These processes reveal multiple targets for effective therapies regardless of AR mutation. [Cancer Res 2009;69(10):4434–42]

Introduction

Tumors arise through the accumulation of somatic mutations that allow uncontrolled growth and lead to general genomic instability and acquisition of random mutations (1). This creates a heterogeneous tumor population that is able to adapt to changes in environment (2). In the case of prostate cancer, this “mutator phenotype” may contribute to the relatively rapid development of treatment resistance.

Because prostate cancer is initially androgen responsive, standard treatment uses combined androgen blockade: reduction of androgen synthesis and direct antagonism of the androgen receptor (AR) with antiandrogens (3). Therapy ultimately fails, indicated by increasing prostate-specific antigen (PSA) levels and recurrent tumor growth (4). Despite castrate androgen levels, AR is still highly expressed and active in hormone-refractory tumors, implying a switch to alternative mechanisms of activation (5). Among mechanisms proposed for AR activity at no or low hormone levels are AR gene amplification, increased coactivator expression, activation by growth factors, and selection of somatic AR mutations (6). Therapy-specific selection of AR mutants may underlie antiandrogen withdrawal syndrome in which tumors regress on treatment cessation (7, 8), and may explain why tumors resistant to one antagonist may respond favorably to another (9, 10).

Many AR mutations have been reported in prostate cancer, but their prevalence and influence on disease progression are unclear due to few comprehensive sequencing studies, variable treatment regimens, and limited access to high-quality samples. Many previous studies focused on the ligand binding domain (LBD), although recent examinations of the entire AR coding region have identified NH2-terminal domain (NTD) mutations as well (11–13). Apart from the T878A mutation that is reported in about one third of hormone-refractory tumors (10, 14), most mutations seem to be rare (15).

Studies in mouse prostate cancer models, wherein treatment is experimentally controlled, add compelling evidence for treatment selection. In the transgenic adenocarcinoma of mouse prostate (TRAMP) model, intact versus castrate hormonal status selects for AR mutations in different domains (16). Our lab recently identified mutations in tumors from TRAMP mice expressing a “humanized” AR (17). Mutations in AR were frequent but at low levels, generally comprising 10% or less of the tumor RNA. Examination of recurring alterations identified ones distinct between flutamide- and bicalutamide-treated mice, as well as clustered mutations shared among groups. Characterization of select mutants revealed altered AR function, including differential activation of androgen-responsive promoters.

Here we extend this analysis to a set of high-quality patient samples with detailed treatment records from the University of Michigan Specialized Program of Research Excellence (SPORE) in Prostate Cancer. To determine whether antiandrogens impose treatment-specific selection pressure, AR mutations were compared from flutamide- and bicalutamide-treated samples.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Diane M. Robins, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109-5618. Phone: 734-764-4563; Fax: 734-763-3784; E-mail: drrobins@umich.edu.

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Materials and Methods

Patient samples. RNAs from metastases of patients treated with bicalutamide or flutamide were obtained from the University of Michigan Specialized Program of Research Excellence (SPORE) in Prostate Cancer rapid autopsy program; tissue was procured as described (18). Biopsies of treatment-naïve lymph node metastases were obtained from the University Hospital in Ulm, Germany as part of the UM SPORE-Ulm Collaborative Clinical Case Procurement Program (19).

Mutation identification. One microgram of RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen) with 0.5 μg oligo(dT) (Invitrogen) and was replaced with standard DMEM or RPMI + 2.5% charcoal-stripped NuSerum. Cells were transfected with Fugene 6 reagent for 4–6 h. Cells were harvested 48 h posttransfection into 1 × Passive Lysis Buffer (Promega). Luciferase activity was measured using the Dual Luciferase Reporter Assay System (Promega) on a Veritas Microplate LuminoMeter (Turner Biosystems, Inc.).

Immunoblotting. CV-1 cells were seeded at 4 × 10⁴ per 60-mm dish and fed phenol red-free medium + 1μmol/L R1881, 4 h before transfecting as above with 100 ng receptor (wtAR or mutant) and 1.5 μg vector (pCMV5). Twenty-four hours later, cells were rinsed in cold 1× PBS, harvested in 100 μl radioimmunoprecipitation assay (RIPA) buffer + protease inhibitors, lysed at 4°C for 10 min, and centrifuged at 4°C for 10 min. Protein was quantified by the DC Protein Assay (Bio-Rad). Twenty micrograms of protein were run on 5% stacking/8% separating SDS-polyacrylamide gels and transferred to nitrocellulose. The blot was probed with antibody to the AR NH2 terminus (N20, Santa Cruz Biotechnology; 1:500) and horseradish peroxidase–conjugated ECL anti-rabbit IgG (GE Healthcare; 1:5,000) for 45 min. Bands were detected with ECL Western blotting reagents (Pierce Biotechnology).

Cytokine and lactacystin treatments. CV-1 cells were transfected with wtAR or AR-E255K as above. After 24 h, cells were rinsed with PBS and incubated in medium containing 30 μmol/L cycloheximide (Sigma) +/− 1nmol/L R1881 for times indicated, or treated with 10 μmol/L lactacystin (Cayman Chemical) for 18 h. At indicated times, cells were lysed in RIPA buffer plus inhibitors as above. Immunoblot bands were quantitated by densitometry using ImageJ (National Center for Biotechnology Information). AR levels were normalized to β-tubulin and percent protein was determined relative to amount at time 0 (100%).

Immunocytochemistry. PC-3 cells (4 × 10⁴) were seeded onto four-chamber slides and transfected with 100 ng receptor in phenol red–free RPMI + 10% charcoal-stripped Nuserum. Twenty-four hours later, cells were fed fresh medium +/− 1 nmol/L R1881 and incubated for 24 h. Cells were rinsed in ice-cold PBS, fixed on ice in 4% paraformaldehyde for 5 min, permeabilized in 0.1% Triton X-100/PBS for 10 min, blocked in 5% heat-inactivated goat serum (Invitrogen) in 0.1% Triton X-100/PBS for 1 h, and then incubated in AR N20 (Santa Cruz Biotechnology; 1:500) overnight and with FC-conjugated goat anti-rabbit antibody (1:1,000) for 1 h. Cells were mounted with Prolong Gold plus 4,6-diamidino-2-phenylindole (Invitrogen). Images were captured using an Olympus BX-51 microscope with an Olympus DP-70 high-resolution digital camera.

Results

Identification of AR mutations in prostate cancer metastases. To examine directly whether AR mutations differ between treated and untreated tumors, whether mutation frequency increases following antiandrogen treatment, and whether different antiandrogens select for distinct mutations, the AR coding region was sequenced from prostate cancer metastases collected in the University of Michigan Rapid Autopsy Program (18). Because secondary hormone therapy is often used after relapse, only 8 of 30 patients met the criterion of treatment with only one antiandrogen—never treated with flutamide and never with bicalutamide (Table 1). AR from three hormone-naive lymph node metastases from patients at the University of Ulm Hospital (Ulm, Germany) was sequenced for comparison (19). RNAs from all samples were reverse transcribed, and the entire AR coding region amplified, subcloned, and sequenced; mutations were compared within and between groups. Sequencing the equivalent of 20 full-length AR mRNAs per metastasis (10 from two independent RT reactions) identified 280 single

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1 http://www.androgenndb.mcgill.ca/

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nucleotide changes in 191 codons. The average alteration rate within the population was 4.1 base changes/10,000 bp, comparable to the rate observed in h/mAR-TRAMP tumors using the same method (17). For that study, baseline error due to sequence peculiarities [e.g., variability in CAG (Q) and GGN (G) tracts and high GC content overall] and methods (e.g., RT and subcloning error) was established by sequencing Ar RNA from mouse testis. Testis samples carried 2.2 base changes/10,000 bp, indicating that about half the differences in tumor samples by this approach are likely somatic mutations. A similar error level was reported in comparable studies using RT and PCR amplification (24).

Of the total base alterations, 160 were missense, with 10% in the polyQ and G-tracts, and 69 were silent mutations, 30% of which were in the polymorphic G-tract. A breakdown of mutation types per treatment group is available (Supplementary Table). There were no significant differences between treatment groups in total number or types of mutations. Mutations in the NTD (amino acids 1–535) were overrepresented relative to AR length, accounting for 73% of mutations from all groups (excluding the polyamino acid tracts). Most mutations were present in one or two clones per sample, or 5% to 10% of the RNA population, similar to mutation frequencies in mouse (16, 17). Because it is difficult to distinguish between true mutations that occur in a single clone and methodologic errors, analysis was restricted to mutations that occurred in multiple clones.

**Treatment-specific patterns of recurring mutations.** Mutations that provide a growth advantage are likely to be more common within the tumor. Mutations occurred more than once in 36 codons, either in multiple cases (24 codons; Fig. 1A) or in multiple clones within a tumor (17 codons; Fig. 1B). Recurring missense mutations include those that alter a codon to different amino acids [e.g., V716M (Fig. 1B)]. A silent change at E213 is a known polymorphism (25), occurring in half the differences in tumor samples by this approach are likely somatic mutations. A similar error level was reported in comparable studies using RT and PCR amplification (24).

**Multiple metastases from one patient express only AR-V716M.** The mutation V716M was present in all 20 clones sequenced from the lung metastasis of flutamide-treated patient D (Table 1). Ten silent mutations recurred, six of which were in the G-tract. A silent change at E213 is a known polymorphism (25), occurring in all 20 clones of three samples, but also in four and seven clones from two other samples.

**Differences between treatments were most apparent for mutations that occurred multiple times per tumor (Fig. 1B).** Only two of these mutations occurred in hormone-naïve samples, whereas flutamide- and bicalutamide-treated tumors carried eight and seven mutations, respectively. Three antiandrogen-treated tumors carried most of the recurring mutations (Table 1). Length of treatment did not affect frequency of mutations, although power was limited by sample size.

All recurring mutations in the LBD were from antiandrogen-treated tumors, suggesting selection for altered conformation of, or ligand contacts within, the binding pocket. Half of the antiandrogen-treated tumors carried at least one recurring LBD mutation, but none overlapped between the flutamide and bicalutamide groups, indicating distinct selection conferred by each drug. The flutamide-treated group had three missense mutations in the LBD: V716M, L798P, and L874P (Fig. 1B). V716M, discussed below, creates a promiscuous receptor (26). L798P is a novel mutation within an E3 ubiquitin ligase interacting area (27). L874P is a novel mutation within an E3 ubiquitin ligase interacting area (27).

**Multiple metastases from one patient express only AR-V716M.** The mutation V716M was present in all 20 clones sequenced from the lung metastasis of flutamide-treated patient D (Table 1). To rule out a germ-line mutation, 281 bp around V716M were amplified and sequenced from the patient’s normal kidney genomic DNA. Only the wild-type G occurred at position 3261, indicating that the mutation was somatic (Fig. 1C). Additional examination of cDNA and/or genomic DNA from two other metastases from this patient yielded only mutant sequence with no

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**Table 1. Summary of sample information**

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<tr>
<th>Patient no.*</th>
<th>Source of metastasis</th>
<th>Hormone therapy</th>
<th>Mo on therapy</th>
<th>TMPRSS2-ETS gene fusions</th>
<th>Base pair changes</th>
<th>Multiple clones/patient</th>
<th>Multiple patients</th>
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<td>Flu</td>
<td>12</td>
<td>Yes</td>
<td>V716M</td>
<td>ΔQ86, G457D, 69 bp ins</td>
<td></td>
</tr>
<tr>
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<td>Flu</td>
<td>48</td>
<td>Yes</td>
<td>None</td>
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</tr>
<tr>
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<td>Bic</td>
<td>48</td>
<td>No</td>
<td>None</td>
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<td>Bic</td>
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<td>ΔQ86, ΔQ86, 69 bp ins, E666D</td>
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</tr>
<tr>
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<tr>
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<td>N/A</td>
<td>T440I</td>
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<td>0</td>
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<td>None</td>
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<tr>
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</tbody>
</table>

Abbreviations: Flu, flutamide; Bic, bicalutamide; N/A, not applicable (these samples were not examined for gene fusions).

*Patient numbers for antiandrogen-treated patients correspond to the numbers listed in Shah et al. (18).

†TMPRSS2-ETS gene fusion data from Mehra et al. (48).
detectable wild type (Fig. 1C), indicating that a clonal population carrying AR-V716M accounted for all three metastases. No other mutations recurred in this sample. Given that AR-V716M is activated by a wide array of ligands (26), its predominance in this patient’s cancer supports its role in treatment resistance.

The splice variant AR23 was only in antiandrogen-treated cases. A variant generated by the use of a cryptic splice site in intron 2 was identified in one or more clones in five of eight tumors from treated patients, but in none of the hormone-naïve tumors. Alternative splicing inserted 69 bp of intron 2 in frame to add 23 amino acids between the zinc fingers of the DNA binding domain. This variant, AR23, was previously found in androgen insensitivity syndrome due to a mutation upstream of exon 3 that altered splicing (30). Recently, AR23 was identified in a prostate metastasis from a bicalutamide-treated patient (31). AR23 was engineered into an expression plasmid, and its activity assayed after transfection. As also shown by Jagla and colleagues (31), AR23 was incapable of nuclear localization on hormone addition but rather formed cytoplasmic speckles (Fig. 2A) and failed to activate androgen-responsive reporters (Fig. 2B). Previously, AR23 was shown to increase endogenous AR-T878A activity when overexpressed in LNCaP cells (31). In Fig. 2B, AR23 also increased wtAR activation (2-fold greater PSA-luc activity) following coexpression in PC-3 cells. Moreover, in the presence of AR23, wtAR was less inhibited by

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**Figure 1.** Recurring AR mutations from prostate cancer metastases. **A,** mutations found in multiple cases. For codons carrying mutations to different amino acids, both changes are shown. **B,** mutations in multiple clones per sample. Only ΔQ86 was shared among groups. AR domains and repeats are boxed. Mutations above the map are silent or nonsense; mutations below are missense. Codon color indicates treatment group. Q, polyglutamine tract; NTD, NH2-terminal domain; G, polyglycine tract; DBD, DNA binding domain; H, hinge region; LBD, ligand binding domain. **C,** V716M was the only AR sequence in three metastases from patient 28 but did not occur in normal kidney. Electropherograms (left to right): amplified cDNA clone from metastasis 1 with G3261A (numbering from GenBank NM_000044) resulting in V716M; wild-type sequence from normal kidney genomic DNA; cDNA and genomic DNA of metastasis 2. Green arrow, mutation; black arrow, wild-type base.
hydroxylflutamide or bicalutamide. This effect was not specific to AR because transactivation by NF-κB and activator protein-1 also increased with AR23 (31), and AR23 reduced glucocorticoid receptor inhibition by RU-486, which antagonizes both receptors (Supplementary Data). AR23 could not transrepress activated NF-κB-induced transcription, unlike wtAR (Fig. 2C; ref. 32). Thus, cytoplasmic activity of AR23 broadly, but not universally, influences nuclear activities.

**Novel mutations in the AR NTD in conserved functional motifs.** The largely unstructured NTD contains two activation functions (AF1 and AF5) that bind coactivators and are critical for AR activity (33). The NTD directs intramolecular amino-carboxy (N-C) interactions via FxxLF and WxxLF motifs that stabilize ligand-bound AR. In this study, 14 of 19 mutations in the NTD fell into four regions: the polymorphic Q-tract, the COOH-terminus of Hsp70-Interacting Protein (CHIP) interaction domain, the WxxLF motif, and the end of AF5 involved in coactivator interactions (Fig. 1; ref. 34). Mutations in the CHIP interacting domain were previously discovered in TRAMP: AR-E231G causes cancer as a selective element (Fig. 3). This promoter-specific effect was also cell type dependent because there was minimal effect in PC-3 cells.

To probe W435L action further, we used a mammalian two-hybrid system in which the ability of the AR NTD (fused to the VP16 binding domain) is assessed by luciferase activity driven by Gal4 selective elements (Fig. 3). This promoter-specific effect was also cell type dependent because there was minimal effect in PC-3 cells.

**W435L alters an AR N-C interaction motif.** The mutation W435L was identified in one clone each of two antiandrogen-treated patients. Its position within the WxxLF motif suggests that this mutation may influence N-C interactions. To determine the effect on transactivation, AR-W435L was cotransfected into CV-1 fibroblasts, immortalized prostate RWPE cells, and prostate cancer PC-3 cells along with varied reporters. Androgen-responsive elements are generally either canonical inverted repeats of a TGTTCCT half-site that bind multiple steroid receptors (e.g., HRE3) or direct repeats that are weaker but AR selective (e.g., HRE2; ref. 36). Natural promoters often contain both element types as well as binding sites for other factors. AR-W435L increased transactivation preferentially for mouse mammary tumor virus (MMTV) in CV-1 cells and PSA in RWPE, likely due to greater efficiency on AR-selective elements (Fig. 3). This promoter-specific effect was also cell type dependent because there was minimal effect in PC-3 cells.

To probe W435L action further, we used a mammalian two-hybrid system in which the ability of the AR NTD (fused to the VP16 activation domain) to bind the LBD (fused to the Gal4 DNA binding domain) is assessed by luciferase activity driven by Gal4 DNA elements (20). When W435L was introduced into the NTD-VP16 fusion, activity was more than 50% greater than for wtAR, confirming that this mutation enhances N-C interaction (Fig. 3D).

**Mutations in the conserved CHIP interacting domain.** Two mutations from treated patients, A253V and E255K, lie adjacent to the most highly conserved region of the NTD where interaction with CHIP, an E3-ubiquitin ligase, promotes AR degradation (37). To determine whether E255K enhances AR stability, CV-1 cells transfected with wtAR or AR-E255K were treated with cycloheximide to inhibit protein synthesis; cells were harvested at indicated times thereafter to detect protein degradation. R1881 greatly stabilized both mutant and wild-type AR proteins as expected (Fig. 4A). However, AR-E255K half-life was extended compared with wtAR, particularly in the absence of ligand (12.5 versus 5.2 hours, respectively). E255K migrated slower than wtAR, which may be due to differential protein modification.
To explore whether E255K stabilization was influenced by the 20S proteasome, cells were treated with the proteasome inhibitor lactacystin. Without ligand, proteasome inhibition increased wtAR steady-state levels as expected (38). However, AR-E255K levels were unaffected, indicating that proteasome activity has little effect on this mutant (Fig. 4B).

Because both the proteasome and chaperones are implicated in nuclear transit, AR-E255K localization was examined by immunocytochemistry. Without R1881, wtAR was mostly cytoplasmic as expected, but AR-E255K showed significant nuclear localization (Fig. 4C). Tallying the localization in cells showed skewing of AR-E255K to the nucleus without ligand compared with wtAR (Fig. 4C).

AR-E255K induced reporter gene expression similarly to wtAR in CV-1 and PC-3 cells with no increased activity without androgen or with added coactivators ARA70 and SRC-1 (data not shown). However, in RWPE cells, AR-E255K increased transactivation of PSA-luc 2.5-fold relative to wtAR (Fig. 4D). This may be due to host cofactor differences, as well as somewhat greater activity on canonical elements like HRE3. Thus, AR-E255K exhibited increased stability, substantial nuclear localization without ligand, and cell type–dependent differential promoter activation.

**Discussion**

This study reveals a low level of mutation throughout the AR coding region in metastases from antiandrogen-treated as well as hormone-naïve patients, providing evidence for genetic heterogeneity and a “mutator phenotype” in prostate cancer (1). Very few mutations in the hormone-naïve samples occur in multiple clones per case, suggesting that most provide little growth advantage and may be random “passenger” mutations. However, antiandrogen treatment leads to more mutations in greater abundance, suggesting that treatment selects for a subset of AR mutations within this diverse population.

Examination of recurring mutations within and between samples indicates specific codons that may provide a selective advantage during cancer progression. Remarkably, mutations recurring in multiple samples are mostly in the NTD and are shared across...
treatment groups. This emphasizes the broad function of the NTD in growth factor and coactivator interactions and in receptor stability, and suggests that some AR variants provide general growth advantages regardless of treatment. In contrast, all missense mutations in the LBD are case specific and are only found in antiandrogen-treated patients, evidencing their likely selection by treatment. Further, the lack of overlap in mutations between bicalutamide and flutamide treatments suggests that these antagonists select for distinct variants.

Although the patient samples are fewer than the 40 tumors we examined from h/mAR-TRAMP mice (17) and are metastases rather than primary tumors, similarities emerge. Overall mutation frequency is comparable, although there are more mutations present in multiple clones per human sample, likely reflecting the clonal nature of metastases and extended time with disease. Q58L and DQ86 are common in both mice and men regardless of treatment. In both species, there are fewer recurring mutations in hormone-naive tumors, substantiating selection pressure of therapy.

Mutations also occur in similar domains in human and mouse ARs, particularly following flutamide treatment. Mutations in flutamide-treated tumors occur in two regions important for ligand specificity: the highly conserved signature sequence (i.e., mAR-W719C, hAR-V716M) and the distal region where some mutations allow promiscuous ligand recognition (i.e., mAR-P893S, hAR-L874P; refs. 17, 39). Whereas this study did not find the common T878A variant, L874P may act similarly (40), perhaps displacing the T878 residue that extends into the ligand pocket, thus accommodating the larger hydroxyflutamide.

The capacity of LBD mutations to affect disease progression is highlighted by the dominance of AR-V716M in three metastases examined from one flutamide-treated patient. We infer that V716M arose either within the primary tumor or early in metastatic invasion. This sample had no other recurring mutations, suggesting that an effective variant reduces the selective value of other mutations. Interestingly, this patient survived much longer than the other cases. In eight patients, this was the only case of fixation of
an AR mutation, indicating that this is a relatively rare event; most cancers may instead have subsets of cells with different mutations, each providing a similar growth advantage.

Only one LBD mutation recurring following bicalutamide treatment, perhaps because a single residue change is unlikely to be sufficient to accommodate this bulkier antagonist in a manner compatible with agonism. Only mutation of W742 has been shown to allow bicalutamide to activate AR (41). The single recurring LBD mutation in a bicalutamide-treated patient, R761K, is at a residue commonly mutated in castrated h/mAR-TRAMP mice (17), implying the mechanism is not partial agonism.

Not only mutants but also splice variants may be subject to treatment selection, as shown recently for variant ARs that lack LBDs in hormone-refractory prostate cancer (42). The AR23 splice variant found in antiandrogen-treated patients here may be present in cells along with wtAR, but has effects on other nuclear factors as well. Although itself inactive, AR23 increases wtAR transactivation when coexpressed. Cytoplasmic aggregates of liganded AR23 may sequester antiandrogens or interacting partners or participate in intracellular signaling via intact NTD and LBD domains, allowing wtAR to function (31). Alternatively, AR23 in an unfolded state, suggested by aggregation, may compromise the cell chaperone system, allowing AR and other proteins to evade degradation for generally enhanced activity. This decay function may be valuable against treatment because AR23 is absent in untreated patients.

The W435L mutation increases transactivation of AR-selective promoters in some cells. This contrasts with the h/mAR-TRAMP mutant AR-R753Q, which functions on canonical but not selective elements (17). Selection for differential promoter usage may change over the course of disease and incorporate multiple mechanisms. The effect of W435L might also vary with disease stage or cell type. Recently, the WxxLF motif has been implicated in ligand-independent AR activation (43). Mutation to LxxLF could weaken normal competition with the FxxLF motif, thus increasing ligand-independent activity while increasing ligand-independent function via greater mimicry of coactivator interactions. Alternatively, W435L may affect AR stability via altered exposure of FQNL, which helps target AR to the proteasome (44). Because steady-state levels of AR-W435L seem to be unaffected, it is more likely that W435L affects transcription and coactivator interactions, either directly or via influence on FxLxF function, as supported by greater N-C interaction shown in the mammalian two-hybrid assay.

Mutation of the highly conserved CHIP interaction domain in both murine and human tumors underscores the importance of this region and illustrates the utility of mouse models for obtaining clinically relevant insights. E255K stabilizes AR and increases nuclear localization in the absence of hormone. This may have a similar effect to AR amplification, seen often in metastatic prostate cancer (45). Increased AR levels may enhance response to low ligand concentrations, increase ligand-independent activation, or promote agonism of antiandrogens (46). Although transactivation by ER-E255K is similar to wild type in transfection, overexpression may mask relevant differences. The analogous mAR-E251G shows modest differences in transfection but is oncogenic as a prostate-specific transgene (35).

In summary, this study identified a greater number of recurring mutations in metastases from treated versus untreated patients. Furthermore, the variety of mutations identified indicates that antagonist treatment does not select for a few common mutations but instead selects for numerous rare mutations, many of which may affect AR function and might be overlooked using bulk sequencing methods. Combining the novel mutations identified here with those from previous studies highlights AR domains within which mutations share a similar phenotype (47). These mutations affect diverse AR processes beyond transcriptional potency, including cell localization, stability, and promoter selectivity. Better understanding of these processes may present new targets for therapies that obviate the ability of AR to evade antiandrogen treatment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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No potential conflicts of interest were disclosed.

References


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