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

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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 obtained by rapid autopsy of patients treated with antiandrogens. 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 of prostate cancer. 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.

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 N-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 (SPRORE) in Prostate Cancer. To determine whether antiandrogens impose treatment-specific selection pressure, AR mutations were compared from flutamide-treated, bicalutamide-treated, and hormone-naive patients.

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

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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 Cooperative 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) in a 20-μL reaction. Two reverse transcription (RT) reactions were done per sample to control for error. The entire AR coding region was PCR amplified in five fragments using the primers listed below. Twenty-five-microliter reactions contained 2.5 units of Platinum Taq DNA polymerase (Invitrogen), 2 μL Buffer and 1 μL 6X enhancer (supplied by the manufacturer), 1.5 mmol/L MgSO4, 0.3 mmol/L deoxynucleotide triphosphates, 0.5 μmol/L each primer, and 1 to 3 μL of RT reaction. Primer pairs are as follows. AR1 forward, position 1074: 5′-CGGGTGAAGGGAGGTAGG-TG-3′; AR1 reverse, position 1732: 5′-CTTGGCCTGGATAAGTCCT-3′; AR2 forward, position 1689: 5′-AACTCTTCCACGACAAGC-3′; AR2 reverse, position 2448: 5′-CAGTGGATGACCCGGGTG-3′; AR3 forward, position 2412: 5′-TCAC-TCTGGACACACTCTTCCACA-3′; AR3 reverse, position 2693: 5′-GGCCCGGACCTTCCGATGCGACA-3′; AR4 forward, position 2639: 5′-GGTCGGACCA-GTGTCGCCATAC-3′; AR4 reverse, position 3399: 5′-TCCCTGGAGTTGACAT-TGGTG-3′; AR5 forward, position 3312: 5′-GACCAAGTGGCTGTCATT-CACA-3′; AR5 reverse, position 3982: 5′-GAATATCCCCAAAAGCAGCTG-3′.

Products were processed as described (17). Briefly, products were visualized on 1% agarose gels; bands were excised and purified with the QIAex II Gel Extraction Kit (Qiagen). 5′-A overhangs were added by incubation with T4 polynucleotide (Invitrogen) at 70°C for 30 min. Products were ligated into pGEM-T easy (Promega) and transfected into DH5α chemically competent bacteria (Invitrogen). DNA from 20 clones per sample (10 clones per RT reaction) was purified with QIAprep Spin Miniprep columns (Qiagen) and sequenced by the University of Michigan DNA Sequencing Core.

Sequence was compared with the human AR (GenBank accession no. NM_000044) using Sequencher software (version 4.1, Gene Codes), and mutations checked against the Androgen Receptor Gene Mutations Database7 (15).

Mutant AR plasmids. Mutations E255K and W435L were introduced into the pCMV5 hAR expression vector using the QuickChange Site Directed Mutagenesis Kit (Stratagene) and the primers below. DMSO was added to the mutant strand synthesis to prevent Q and G tract contraction. Plasmids Mutagenesis Kit (Stratagene) and the primers below. DMSO was added to the mutant strand synthesis to prevent Q and G tract contraction. Plasmids were ligated into pGEM T easy (Promega) and transfected into DH5α competent bacteria (Invitrogen). DNA from 20 clones per sample (10 clones per RT reaction) was purified with QIAprep Spin Miniprep columns (Qiagen) and sequenced by the University of Michigan DNA Sequencing Core.

Cancer Research

Identifications 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 androgens 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—4 were treated with flutamide and 4 with bicalutamide (Table 1). AR from three hormone-naïve 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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7 http://www.androgendb.mcgill.ca/
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. Most mutations were present in one or two clones per treatment group. 

Mutations in the NTD (amino acids 1–535) were overrepresented relative to AR length, accounting for 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.** Muta-

tions 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 residues (away from wild type, e.g., L194F/R) or to the same new residue (e.g., Q58L). Both types could be functionally significant. All but two missense mutations identified in multiple cases were located in the NTD, with few specific to a single group—half occurred only with antiandrogen treatment and half were shared by treated and untreated patients (Fig. 1A). In contrast, the 13 missense and 2 nonsense mutations present in multiple clones per tumor were case specific and not restricted by domain (Fig. 1B). Ten silent mutations recurred, six of which were in the G-tract. A silent change at E213 is a known polymorphism (25), occurring within an E3 ubiquitin ligase interacting area (27). L874P, also located near codons H875 and T878, which, when mutated, allow flutamide to activate AR (28, 29).

**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 28 (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**

<table>
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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 patients</th>
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<td>Flu</td>
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<td>Yes</td>
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<td>Flu</td>
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<tr>
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</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...
hydroxyflutamide 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 prostate-specific transgene, highlighting the oncogenic potential of AR (16, 35). The novel mutations W435L and E255K were engineered into expression vectors for functional characterization.

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 TGTTCT 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 efficacy 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.

**Figure 2.** Splice variant AR23 has altered subcellular localization and enhances wild-type AR (wtAR) activity. A, punctate cytoplasmic localization of AR23. AR23 transfected into PC-3 cells shows diffuse cytoplasmic localization without ligand (top) like wtAR (not shown), but forms cytoplasmic puncta with 10 nmol/L R1881 (middle) unlike wtAR nuclear localization (bottom). AR detection used AR N20 and FITC-conjugated secondary antibody. B, transactivation of wtAR, AR23, or 1:1 wtAR/AR23 (4 ng each) with 200 ng PSA-luc and 100 ng promoterless renilla in PC-3 cells. Cells were harvested 24 h after agonist or antagonist treatment (HOF, hydroxyflutamide; Bic, bicalutamide), and luciferase activity was assayed. Average normalized values of three independent trials are presented as percent wtAR transactivation at 1 nmol/L R1881. C, transrepression of NF-κB activity in CV-1 cells. wtAR or AR23 was transfected with the NF-κB reporter pBVκ-luc, and NF-κB was activated with 12-0-tetradecanoylphorbol-13-acetate (TPA). wtAR reduced activation to 20% of vector alone with 10 nmol/L R1881 + 1 nmol/L TPA; with AR23, NF-κB activity remained 80% of control. Bars, SE. *, P < 0.05 (Student’s t test).
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-naive patients, providing evidence for genetic heterogeneity and a “mutator phenotype” in prostate cancer (1). Very few mutations in the hormone-naive 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 ΔQ86 are common in both mice and men regardless of treatment. In both species, there are fewer recurring mutations in hormone-naïve 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

**Figure 4.** AR-E255K has increased stability and ligand-independent nuclear localization. A, AR degradation following cycloheximide treatment. wtAR or AR-E255K plasmid (100 ng) was transfected into CV-1 cells, which were treated after 24 h with 30 μmol/L cycloheximide. Cells were harvested at times indicated, and 20 μg of total protein were electrophoresed (left). AR bands from scanned immunoblots were quantified using ImageJ, and values normalized to the amount of protein at time 0 (100%). The log10 of the percentage was plotted versus time for wtAR and AR-E255K without hormone (right). Half-life was calculated as log10 of 50% based on the linear regression. AR-E255K shows a longer half-life (t1/2 = 12.5 h) than wtAR (t1/2 = 5.2 h). Full gels are in Supplementary Data. B, proteasome inhibition with lactacystin increased unliganded wtAR, but not AR-E255K, levels. CV-1 cells were transfected as above, treated after 24 h with 10 μmol/L lactacystin, harvested 18 h later, and immunoblotted. C, following transfection into PC-3 cells, wtAR was largely cytoplasmic without hormone (top) whereas most cells with AR-E255K showed more nuclear staining (bottom). Color images and composite are in Supplementary Data. AR was detected as for Fig. 2. Percentages of cells with cytoplasmic to nuclear AR fluorescence are graphed as follows: C, exclusively cytoplasmic; C > N, cytoplasmic greater than nuclear; N > C, nuclear greater; N, exclusively nuclear. n, number of cells counted for all three trials. Columns, mean percentages; bars, SE. D, AR-E255K showed increased transactivation of PSA-luc in RWPE cells compared with wtAR. Columns, average percent wtAR activation of three trials; bars, SE. **, P < 0.005 (Student’s t test).
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 occurred 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 androgen-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-dependent activity while increasing ligand-independent function via greater mimicry of coactivator interactions. Alternatively, W435L may affect AR stability via altered exposure of FQNLF, 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 FxxLF function, as supported by greater N-C interaction shown in the mammalian two-hybrid assay.

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.

Acknowledgments

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References


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 AR-E255K is similar to wild type in transfection, overexpression may mask relevant differences. The analogous mAR-E231G shows modest differences in transfection but is oncogenic as a prostate-specific transgene (35).

Treatment-Selected AR Mutations in Prostate Cancer


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