

Advances in Brief

Mutations in the Androgen Receptor Gene Are Associated with Progression of Human Prostate Cancer to Androgen Independence

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Abstract

Progression to androgen-independent growth of human prostate cancers may be mediated by alterations in the structure and/or expression of the androgen receptor (AR) gene. To date, mutations in the AR gene have largely been identified in hormone refractory tumors. In this study, single-strand conformational polymorphism analysis and DNA sequencing of the entire AR gene coding region was performed on 25 primary prostate tumors sampled prior to initiation of hormonal (i.e., androgen ablation) therapy. Base changes leading to amino acid substitutions in the AR were identified in 11 (44%) tumors. The presence of AR amino acid substitutions was associated with decreased immunohistochemical staining for AR in tumor cells and the rapid failure of subsequent hormonal therapies. Single-strand conformational polymorphism analysis of exons 2, 3, and 8 of the X-linked hypoxanthine guanine phosphoribosyl transferase (HPRT) gene in the same samples revealed no bandshifts, suggesting that the high frequency of AR gene mutations detected was not a consequence of generalized genetic instability. These data indicate that AR gene mutations occur commonly in advanced prostate cancers prior to endocrine treatment of disease and may contribute to altered androgen responsiveness of the tumors.

Introduction

Prostate cancer is the most frequently diagnosed invasive cancer and the second leading cause of cancer deaths in men in Western countries (1). The predominant form of systemic treatment for patients with metastatic disease is hormonal or androgen ablative therapy (i.e., orchidectomy, luteinizing hormone-releasing hormone analogues, and AR3 antagonists; Ref. 2).

Although an initial response to hormonal therapy is observed in 70–80% of patients with advanced disease, most tumors progress rapidly to androgen-independent growth and only 10–20% of patients are alive 5 years following diagnosis (3). At the present time, the molecular changes in tumor cells that lead to resistance to endocrine treatments and independence of androgens for growth are poorly understood.

The effects of androgens on the development of the normal prostate gland and the growth of prostate tumors are mediated by the AR, which is a member of a superfamily of ligand-activated nuclear transcription factors (4, 5). Proposed mechanisms of progression to androgen-independent growth include loss of AR expression (6, 7), amplification of the AR gene (8), and structural alterations in the receptor protein (9). Other mechanisms that do not involve the AR pathway [e.g., activation of androgen-independent growth factor pathways (10)] have also been proposed.

Immunohistochemical studies have demonstrated expression of AR in primary, advanced, and hormone refractory prostate cancers, suggesting that development of androgen-independence is unlikely to be a consequence of loss of AR expression (11). Indeed, AR gene amplification, which possibly facilitates disease progression by enhancing the growth-promoting effects of androgens at low cellular concentrations, has been demonstrated in a subset of hormone refractory prostate cancers (8). The often rapid progression of advanced prostate cancers from androgen-dependent to androgen-independent growth has led to investigation of structural and/or functional alterations in the AR that could account for the development of resistance to hormonal therapies and disease progression.

Mutations in the AR gene have been identified in a stage B (12) and a small number of advanced (i.e., stage D2) prostate cancers (13–15). In addition, a recent report has demonstrated that 5 of 10 hormone refractory prostate cancer metastases contained mutations in the ligand-binding domain of the AR (16). Where functional analyses of mutated ARs have been performed, broadening of ligand specificity was demonstrated, suggesting that hormone refractory disease may in part be caused by activation of these receptors in the androgen-depleted environment (9, 16). Although previous studies have demonstrated AR gene mutations in hormone refractory disease, their occurrence prior to initiation of androgen ablation treatment has only rarely been reported (12, 17). This finding, while inconsistent with the frequently short-lived responses of prostate tumors to hormonal therapies, may in part be attributed to the examination of small segments of the AR (e.g., the ligand-binding domain) rather than the complete AR coding region.

In the present study, the frequency of mutations in the entire AR coding region in advanced primary prostate cancers was examined using the PCR and SSCP analysis. The presence of base substitutions was confirmed by sequencing of cloned...
PCR-amplified fragments exhibiting SSCP bands of reproducibly altered mobility. Expression of AR protein was evaluated using quantitative immunohistochemistry and video image analysis. Our results indicate that mutations in the AR gene are present in advanced prostate cancers prior to initiation of treatment and are associated with reduced AR immunostaining in tumor cells and the rapid failure of subsequent androgen ablative therapy.

**Materials and Methods**

**Prostate Tissues and Cell Lines.** Twenty-five primary prostate tumors and 12 BPH specimens were obtained from patients undergoing transurethral resections of the prostate performed for acute urinary obstruction. The presence of carcinoma or BPH in the tissue blocks was confirmed by pathological assessment on hematoxylin and eosin-stained paraffin sections (Department of Pathology, Flinders Medical Center, Bedford Park, Australia). All tumor specimens were obtained from patients prior to initiation of hormonal therapy. Tumor stage was determined according to the Modified Whitmore Jewett System (18). The cohort consisted of 9 stage C and 16 stage D2 tumors.

Following surgery and commencement of hormonal therapy (orchidectomy and/or androgen receptor antagonists), an initial decrease in serum PSA levels was observed in all patients. Serum PSA measurements were determined by the Department of Clinical Biochemistry at Flinders Medical Center using a solid-phase, two-site immunoenzymatic assay (Tandem-E PSA; Hybritech, Inc., San Diego, CA) as described previously (19). The normal range of serum PSA levels in healthy men is 0–4 ng/ml for this assay. In this study, serum PSA levels were categorized into three groups: category 1, levels <4 ng/ml; category 2, levels between 4 and 10 ng/ml; and category 3, levels >10 ng/ml. Patients were followed clinically with serum PSA measurements and bone and computed tomographic scans as required. Clinical follow-up and survival data of 3–7 years were available for 20 of the patients. Five patients were lost to follow-up. Tumor response was assessed by standard clinical and biochemical features (i.e., sustained reduction in serum PSA measurements, improvement in bone and computerized tomographic scans, and survival).

Outcome groups were defined at 2 years following initiation of hormonal therapy as follows: (a) response—consisted of those patients with stable disease (i.e., no clinical evidence of disease progression, with PSA levels remaining in the original posthormonal therapy category), or improving disease status (i.e., clinical evidence of decreasing tumor mass and a fall in PSA category) or (b) failure—consisted of those patients with progressive disease (i.e., increasing tumor mass with or without a change in PSA category) and/or those who died from prostatic carcinoma within the first 2 years following initiation of hormonal therapy.

The human prostate cancer cell lines PC-3 and LNCaP were obtained from the American Type Culture Collection (Rockville, MD) and maintained in culture as previously described (6).

**Amplification of Genomic DNA.** Genomic DNA was isolated from frozen or paraffin-embedded prostate specimens, peripheral blood lymphocytes from normal volunteers, and the
LNCaP and PC-3 prostate cancer cell lines according to published procedures (6, 20). In the case of tumors, DNA was extracted from formalin-fixed paraffin-embedded sections of the prostate samples. The size and integrity of all PCR products were confirmed on 2% agarose gels.

SSCP Analysis. SSCP analysis was performed using a modification of the procedure of Orita et al. (26). Radiolabeled PCR products were diluted 1:10 in loading buffer that contained 50 mM potassium phosphate buffer (pH 7.4), 6% polyethylene glycol, 2% sodium dodecyl sulfate (SDS), 0.05% xylene cyanol blue- and 0.05% bromophenol blue. Samples were denatured at 100°C for 10 min, snap frozen on dry ice, and thawed on wet ice before electrophoresis in nondenaturing 4.5% polyacrylamide gels. Parameters for SSCP analysis were optimized according to Hayashi (24) for each amplified fragment of genomic DNA. Mobility shifts identified by SSCP analysis were confirmed on two independent PCR-amplified fragments of genomic DNA to ensure maximum sensitivity for detection of mutations (i.e., gels contained 0–10% glycerol and were run at 10°C, 22°C, or 30°C). A nondenatured aliquot of the amplification reaction was also electrophoresed with the denatured sample to determine the mobility of the double-stranded DNA. Following electrophoresis, gels were transferred onto Whatman filter paper, dried under vacuum, and imaged by Phosphor Imager and Image Quant software (Molecular Dynamics, Sunnyvale, CA). Mobility shifts identified by SSCP analysis were confirmed using an independent PCR amplification reaction.

DNA Sequencing. Regions of the AR gene exhibiting a reproducible mobility shift on SSCP analysis were amplified in independent PCR reactions and subcloned using the TA Cloning kit (Invitrogen, San Diego, CA). The sense and antisense strands of 3–10 resultant clones were sequenced with a fmol Sequencing kit (Promega, Madison, WI) and [γ-32P]dATP labeled M-13 forward or reverse primers according to the manufacturers’ protocols. The products of the sequencing reactions were electrophoresed on denaturing polyacrylamide gels, dried, and visualized with a Phosphor Imager. Alterations in the AR gene sequence were confirmed on two independent PCR-amplified DNA fragments and from sequence derived from both sense and antisense DNA strands. Repetition of all PCR-SSCP analyses and confirmation of the DNA sequencing results on multiple clones indicates that mutations detected did not occur as a result of misincorporation of bases by Taq DNA polymerase.

AR Immunohistochemistry. Five-μm frozen and paraffin sections of the prostate samples were stained with polyclonal
antiseras to either the amino (U402) or carboxyl (R489) termini of the human AR using an avidin-biotin immunoperoxidase method as previously described (19, 27, 28). AR immunostaining in paraffin sections was enhanced using an antigen unmasking procedure as previously described (29). Paraffin sections were mounted onto Histogrip-coated microscope slides (Zymed Laboratories, Inc., San Francisco, CA) and microwaved in 0.01 M citrate buffer (U402: pH 6.0, 8 min; R489: pH 6.5, 18 min) before immunohistochemical staining. Immunoreactive products were visualized with the chromogen 3′3′-diaminobenzidine tetrahydrochloride, and the area and intensity of staining were measured by computer-assisted color video image analysis using the Video Pro 32 system (Leading Edge Pty Ltd., Adelaide SA, Australia) according to established procedures (19, 28). The areas of unstained (A1) and positively stained nuclei (A2) and the intensity of AR immunostaining (i.e., the integrated optical density) were measured for at least 15 fields in both the glandular regions of BPH tissues and in sections containing predominantly carcinoma in the case of prostate cancer specimens. From these measurements, the level of AR staining (i.e., MIOD [MIOD = integrated optical density/(A1 + A2)]) was calculated.

Statistical Analysis. All data were expressed as mean ± SE. Immunostaining parameters and clinical outcome of patients were analyzed using a paired t test. The relationship between the presence of amino acid substitutions in the AR and response to hormonal therapy was analyzed using a χ² test. Significance was established at P < 0.05.

Results

PCR-SSCP Analysis of the AR Gene in Prostate Cancer Specimens. SSCP analysis of PCR products encompassing the entire coding region of the AR gene resulted in the identification of reproducible band shifts corresponding to variant DNA conformers in 13 of 25 primary human prostate tumor samples. No mobility shifts were identified using DNA derived from peripheral blood lymphocytes of six normal individuals (data not shown). The human prostate cancer cell line LNCaP, which is known to have an A to G base transition at codon 868 in exon 8 of the AR gene (30), consistently exhibited a band of altered mobility following PCR-SSCP analysis and was used as a control for this exon (Fig. 2a). None of the AR gene exon 8 DNA fragments amplified from the 25 prostate tumor samples exhibited a mobility shift similar to that seen for the LNCaP cell line. Typical examples of the SSCP shifts for exons 1, 5, and 8 of the AR gene amplified from the prostate tumors are shown in Fig. 2a-d. At least one normal DNA conformer was observed with variant DNA conformers in the SSCP analysis (Fig. 2a-d), suggesting the presence of both wild-type and mutant AR gene sequences within the prostate tumor specimens.

DNA Sequencing of the AR Gene. DNA sequencing (Fig. 3) of cloned PCR products derived from independent amplification reactions using DNA isolated from specimens yielding variant DNA conformers on SSCP analysis resulted in the identification of base changes in the AR gene, which were confined to 13 of the 25 prostate tumor samples (Fig. 1 and Table 2). AR gene sequences were confirmed in three or more clones sequenced in both sense and antisense orientations. At least one wild-type AR gene sequence was identified in each sample. Eleven (44%) of the tumors contained missense mutations; 4 of these tumors contained base changes, resulting in more than one amino acid substitution in the AR, and a nonsense mutation was identified in exon 6 of tumor p346 (in addition to a missense mutation in exon 1; Table 2). The only sequence
 alterations in the AR gene identified in two tumors were a silent base change in exon 1 (p396) and base substitutions in three separate introns within close proximity to the intron/exon splice junctions (p383). Eleven silent base changes in the AR coding region and seven base substitutions in the intronic sequence were also identified in the subgroup of tumors with missense mutations (data not shown). No base changes in the AR gene were indicated in the remaining 12 tumors by SSCP analysis.

One tumor (p408) contained a normal AR gene sequence with the exception of a low number (n = 13) of CAG trinucleotide repeats in the glutamine homopolymeric region of exon 1. Analysis of this region of the AR gene in tumor p408 using a sample containing >50% nonmalignant cells (lymphocytes, smooth muscle cells) demonstrated the presence of a single PCR product, suggesting that the germline DNA of this patient also contained 13 CAG repeats. With the exception of tumor sample p259 with 30 CAG repeats in exon 1 of the AR gene, the remaining tumor specimens contained 19–26 CAG repeats.

**PCR-SSCP Analysis of the AR Gene in BPH Specimens.** PCR-SSCP analysis of exons 1–8 of the AR gene in 12 BPH specimens identified band shifts in two samples (data not shown). In a single patient (p617), an A–C base transition in codon 628 that resulted in the substitution of a threonine residue for a lysine, and a silent mutation in codon 699 were identified in exon 4 of the AR gene. A single silent base change in codon 203 was found in exon 1 of sample p635. SSCP analysis of the AR gene coding region detected only bands of similar mobility to control DNA in the remaining 10 BPH samples (data not shown).

**PCR-SSCP Analysis of the HPRT Gene in Prostate Cancer and BPH Specimens.** The 25 prostate cancer and 12 BPH specimens examined using SSCP analysis exhibited bands with identical mobilities to control DNAs for exons 2, 3, and 8 of the X-linked HPRT gene (data not shown). DNA derived from the human prostate cancer cell line PC-3 exhibited a PCR-SSCP band shift in exon 8 of the HPRT gene, while LNCaP DNA showed the wild-type pattern for all three exons analyzed (data not shown).

**Immunocytochemical Analysis of AR Protein in Prostate Cancer and BPH Specimens.** The level of AR immunostaining (MIOD) in the 25 prostate cancers determined using an anti-NH2 terminal antibody (U402) ranged between 0 and 57 pixel density units, with a mean (±SE) of 28 (±4). In contrast, the MIOD of AR staining in the BPH specimens ranged between 12 and 50 pixel density units, with a mean value of 39 (±3), which was significantly greater than the MIOD of AR staining in the tumors (P < 0.05). AR immunostaining (MIOD) of prostate cancer specimens identified as having an amino acid substitution in the receptor (21 ± 6) was found to be significantly lower than that in prostate cancers without amino acid substitutions (33 ± 6; P = 0.03).

Clinical follow-up data were available for 20 of the 25 patient samples used in these studies. Of the 20 patients, 12 responded to hormonal therapy and 8 progressed rapidly to hormone refractory disease. Immunohistochemical analysis of prostate cancer cells in these specimens demonstrated that the mean AR level (±SEM) in responders to hormonal manipulation [32 (± 6) pixel density units] was not statistically different from AR staining in cancers that progressed on hormonal therapy in <2 years (21 ± 7 pixel density units), although this result

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**Table 2** Summary of mutations identified in the AR gene in 25 human prostate tumors

<table>
<thead>
<tr>
<th>Tumour Exon</th>
<th>Mutation</th>
<th>Consequence</th>
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<tbody>
<tr>
<td>Missense</td>
<td>p205 1</td>
<td>ATG-ACG</td>
</tr>
<tr>
<td></td>
<td>p245 1</td>
<td>CTC-CCC</td>
</tr>
<tr>
<td></td>
<td>p259 1</td>
<td>CCA-TCA</td>
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<tr>
<td></td>
<td>p332 6</td>
<td>TGG-TGA</td>
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<tr>
<td></td>
<td>p346 6</td>
<td>AGC-AAC</td>
</tr>
<tr>
<td></td>
<td>p405 1</td>
<td>AAA-AGA</td>
</tr>
<tr>
<td></td>
<td>p413 6</td>
<td>AGC-AAC</td>
</tr>
<tr>
<td></td>
<td>p424 4</td>
<td>CAG-CCG</td>
</tr>
<tr>
<td></td>
<td>p405 6</td>
<td>TCT-CCT</td>
</tr>
<tr>
<td>Nonsense</td>
<td>p346 6</td>
<td>TGG-TGA</td>
</tr>
</tbody>
</table>

 a Amino acid positions according to Tilley et al. (5).
Fig. 4 AR immunostaining of prostate tumor p346. Top panel, paraffin section showing positive nuclear staining in the majority of tumor cells with the anti-NH$_2$ terminal AR antibody U402. A few stromal cells are also positively stained for AR. Bottom panel, absence of AR staining in the same tumor sample using the anti-COOH terminal receptor antibody R489. Inset, AR immunoreactivity in the glandular epithelial cells of a BPH specimen (positive control) stained with antibody R489 at the same time as tumor p346. ×250.

approached significance ($P = 0.051$; data not shown). Six of the eight tumors that progressed rapidly following initiation of hormonal therapy contained amino acid substitutions in the AR gene. In contrast, only 3 of 12 tumors that responded to androgen ablation therapy ≥2 years contained mutations in the AR gene which resulted in amino acid substitutions in the receptor. [An additional tumor in this latter group, p346, contained a premature termination codon in codon 794 of the ligand-binding domain which was likely to have resulted in a nonfunctional AR (see below).] The potential clinical importance of amino acid substitutions in the AR in untreated primary tumors was demonstrated by a significant correlation between their presence and the rapid failure of subsequent hormonal therapies ($\chi^2 = 4.85; P < 0.015$).

Although receptor protein was detected immunohistochemically in the majority of prostate cancer specimens containing mutations in the AR gene, in most samples it was not possible to determine the proportion of tumor cells expressing mutant AR. In a single tumor (p346), a G–A transition in codon 794 in exon 6 of the AR resulted in the introduction of a premature termination codon (Fig. 1 and Table 2). Expression of a truncated AR protein in this tumor was suggested by the positive immunoreactivity of the tumor cells when stained with an antiserum (U402) directed against the NH$_2$ terminus of the AR
and the absence of staining of tumor cells with antisera (R489) directed against the COOH terminus of the receptor (Fig. 4).

Discussion

Mutations in the AR gene that alter the amino acid sequence of the receptor were identified in a high proportion (44%) of 25 primary prostate tumors sampled prior to the commencement of hormonal therapy. In contrast to mutations previously identified in the AIS, which are germline, those identified in the present study in prostate cancers appear to be somatic mutations, since both wild-type and mutant DNA sequences were detected. Base changes resulting in amino acid substitutions were found in exons 1, 2, 4, and 6–8 of the AR gene. Amino acid alterations in the ligand-binding domain of the AR have been described previously in hormone refractory prostate cancers (9, 16). With the exception of the study by Schoenberg et al. (31), where the polymorphic CAG repeat region of the AR was analyzed, and a single report of a mutation at codon 340 of the AR in human prostate cancer (17), previous studies have not analyzed exon 1 of the AR which encompasses 58% of the coding region of the gene. In the present study, 50% of amino acid substitutions identified in the AR were within exon 1. In view of a recent report demonstrating that residues 1–485 of exon 1 are required for full wild-type AR activity (32), it is likely that at least seven of the eight exon 1 amino acid substitutions located within this region of the exon will have significant effects on androgen or antiandrogen action in the prostate tumors.

The relatively high frequency of mutations detected in advanced prostate cancers in this study suggests that AR gene mutations may be involved in disease progression. The recent identification of AR gene mutations in 50% of hormone refractory prostate cancers also supports this association (16). Advanced malignancy, including prostatic cancer, is often associated with tumor cell aneuploidy. Previous studies have documented the increased frequency of mutations in exons 3 and 8 of the X-linked HPRT gene in spontaneous and chemically induced tumors (33). The location of mutations in the HPRT gene is associated with sequences susceptible to mutation (i.e., “hot spots” in exons 3 and 8), rather than specific selection for genotype or phenotype (33). However, the absence of mutations in exons 2, 3, and 8 of the HPRT gene in the same DNA samples used for the analysis of the AR gene suggests that the relatively high frequency of AR gene mutations detected in this study is not due to generalized genetic instability in prostate tumors or instability of the X chromosome. This finding and the association between the presence of mutations in the AR gene in the primary tumor and the rapid failure of subsequent hormonal therapy suggest that specific mechanisms for mutation of the AR gene are involved in the progression of human prostate cancers.

Amino acid substitutions in two exons of the AR were detected in four of the tumors in the present study. These findings may be the result of two populations of cells within tumor specimens that each contain a single amino acid substitution or a single population of cells with two amino acid substitutions. Where base changes occurred in different PCR-amplified fragments, these two alternatives could not be distinguished. Multiple amino acid substitutions in the DNA-binding domain of the AR gene have been documented previously in a patient with AIS (34), in the ligand-binding domain of the AR in an androgen-independent prostate cancer metastasis (16), and in a latent prostatic cancer (35). Similarly, multiple amino acid substitutions have been described in the 5α-reductase type 2 gene in cases of 5α-reductase deficiency (36) and in the p53 gene in an individual breast tumor (37). In vitro studies of the functional consequences of two amino acid substitutions in codons 595 and 615 of the AR in a patient with incomplete AIS demonstrated that the alteration at codon 595 was able to partially restore DNA-binding activity to mutant AR that contained an inactivating amino acid substitution at codon 615 (34). Thus, the functional consequences of more than one alteration in the coding region of the AR in vivo will most likely be determined by the extent of the interaction between the amino acids involved with other receptor accessory proteins that are required for ligand-binding and/or transcriptional activity.

Although AR gene mutations have not been described previously in BPH, base changes resulting in two amino acid substitutions in the AR gene in 1 of 12 clinical BPH specimens were detected in the present study. Functional consequences of the amino acid substitutions in the BPH tissues have not yet been determined. Histopathological examination of specimens confirmed the diagnosis of BPH, and the patient has remained asymptomatic >2 years following transurethral prostatic resection. Although it is not possible to determine the cell types or proportion of cells containing AR gene mutations identified in this study, previous studies have detected foci of cancer cells in up to 8% of surgical BPH specimens (38). Therefore, the presence of cancer cells containing AR gene mutations in the tissues provided for SSCP and DNA sequence analysis or the existence of benign hyperplastic prostate (epithelial or stromal) cells containing AR gene mutations cannot be eliminated in the present study.

In contrast to AIS where mutations in the AR gene usually result in loss of ligand-binding and/or AR function (39, 40), the mutations described thus far in prostate cancers appear to cause a broadening of ligand specificity and/or activation of AR by estrogens, progestins, adrenal androgens, or antiandrogens (e.g., flutamide) in addition to testicular androgens (9, 16). As such, these mutant ARs may contribute to the proliferation of prostate tumors in an androgen-depleted environment (e.g., following orchiectomy) or during antiandrogen therapy. The flutamide withdrawal syndrome (41), in which tumor growth appears to be paradoxically driven by the antiandrogen flutamide, may also be explained by the presence of mutant ARs in prostate tumor cells which are transcriptionally activated rather than inhibited by flutamide similar to the mutant AR expressed in the LNCaP cell line.

The exon 1 polymorphic CAG repeat region in the AR gene of patient p408, a white Caucasian male, contained 13 glutamine residues. In population studies of normal individuals, 11–31 CAG repeats have been reported (42). The most commonly observed CAG repeat frequency in white Caucasian men was 21 (range, 15–31), whereas the repeat number in black American males was significantly lower (i.e., n = 18; range, 11–29). In vitro studies indicate that elimination of the CAG tract of the AR results in increased transcriptional activity of the receptor (43). In contrast, expansion of the CAG repeat region, as observed in Kennedy’s disease, results in partial loss of AR func-
tion (44). Based on these studies, the increased risk of developing prostate cancer in black American males has been proposed to be related to a reduced frequency of CAG repeats in this population (42). Similarly, it is feasible that the low number of glutamine residues in patient p408 may have contributed to the development of his prostate cancer. A reduction in CAG repeat number in exon 1 of the AR from 24 to 18 has been reported previously in the tumor of a prostate cancer patient with metastatic disease who exhibited a paradoxical agonist response to flutamide treatment (31). In that study, however, the entire AR coding region was not analyzed to eliminate the possibility of an amino acid substitution in the hormone-binding domain, which could account for the agonist action of flutamide.

The increased detection of AR gene mutations in prostate tumors as compared to BPH specimens suggests that tumor cells contain the receptor mutations. However, with the exception of a single tumor (p346), where evidence of a truncated AR protein was detectable in the majority of tumor cells by differential staining with AR antibodies directed against the NH₂ and COOH termini of the receptor, the proportion of cells (malignant and/or nonmalignant) expressing mutant ARs could not be deduced using the current methodology. AR protein expression in tumor cells ranged from almost negative to strongly positive. Although no causal relationship has been established between the presence of AR gene mutations identified in the present study and reduced AR expression in the tumors, significantly lower levels of immunoreactive AR were observed in prostate tumors containing amino acid substitutions in the AR. Similar findings have been reported in individuals with AIS where mutations in the AR were associated with decreased expression of AR protein (45). Conversely, reduced cellular AR protein levels have also been documented following ligand-binding and receptor activation (46). Therefore, it is feasible that decreased AR levels in prostate cancer specimens containing mutant receptors may reflect ligand-mediated activation rather than altered transcription, translation, or stability of the mutant AR. Nevertheless, the findings of the present study suggest that the androgen responsiveness of prostate tumors may be determined by both the functional consequences of the AR gene mutations and the cellular levels of receptor expression. This conclusion is supported by the association of amino acid substitutions in the AR and reduced receptor immunostaining with the rapid failure of hormonal therapy.

The present study indicates that AR gene mutations exist in a subset of advanced stage primary prostate tumors prior to initiation of hormonal therapy, and that within a cohort of 20 of these patients able to be followed up clinically, the presence of AR gene mutations was associated with failure of subsequent hormonal therapy. These findings support the hypothesis that the often rapid onset of androgen-independent progression of prostate cancer may arise due to selective outgrowth of existing cells with mutant AR rather than the acquisition of new mutations following the initiation of treatment. Future quantitation of the proportion of cells expressing mutant ARs within prostatic tumors prior to initiation of therapy and in new metastatic tumors arising following failure of hormonal treatment will confirm whether prostate cancer progression is at least in part due to selective outgrowth of cells expressing AR gene mutations.

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