Detection of Discrete Androgen Receptor Epitopes in Prostate Cancer by Immunostaining: Measurement by Color Video Image Analysis


ABSTRACT

To determine whether multiple features of immunohistochemical staining of the androgen receptor (AR) in prostate cancer could reliably predict androgen dependence, tumor biopsy specimens from 30 patients (stages A-D) were stained using anti-peptide antibodies to the amino- and carboxy-terminals of the AR. Measurements were made of the mean area and total amount (i.e., integrated optical density) of AR staining in at least 20 fields per section using a color video image analysis system, and the mean intensity of AR staining per cell and the percentage of AR positive tumor cells were derived. Video image analysis measurement identified quantitative differences in AR staining between the two antibodies, suggesting that this approach may provide a means of identifying receptor variants in prostate tumors. The AR staining measurements were analyzed by discriminant function analysis to assign individual cases to good and poor clinical outcome groups. AR staining features measured with a single antibody (e.g., amino-terminal) were sufficient to predict outcome following hormonal therapy in stage D2 patients (predictive value, 1.0), whereas all features of AR staining measured with both antibodies were required for the entire patient group (predictive value, 0.97). The principal discriminant in both patient groups contributing to the correct assignment of outcome was the mean intensity of AR staining per cell. These findings suggest that AR staining features measured by video image analysis have the potential to predict outcome in prostate cancer.

INTRODUCTION

Prostate cancer is now the most frequently diagnosed invasive cancer and the second most common cause of cancer death in men in Western societies (1, 2). At present, the majority of prostate cancer patients are diagnosed with either locally invasive or, more frequently, disseminated disease (3). Current forms of treatment for patients with disseminated disease may prolong survival but are essentially only palliative (4, 5). While an initial response is observed in about 70% of patients with advanced disease following primary endocrine ablation, most patients relapse within 3 years and only about 20% survive for 5 years (4). The rapid progression of prostate cancer following failure of primary hormone therapy is attributed to androgen-independent tumor growth. In the androgen-resistant sublines of the R-3327 Dunning rat prostate adenocarcinoma and the androgen-insensitive human prostate cancer cell lines, PC-3 and DU145, androgen independence is associated with a loss or a decrease in AR mRNA and protein levels (6, 7). It is not known whether a change in the expression of the AR contributes to the progression of human prostate cancers to an androgen-independent state.

It would be a considerable advantage to be able to offer patients with advanced prostate cancer an alternative form of management without waiting for evidence of relapse to hormonal therapy. In contrast to breast cancer where biochemical (i.e., radioligand-binding) determinations of estrogen and progesterone receptor levels have been useful in predicting the response of individual tumors to hormonal therapy (8), the predictive value of AR measurements in prostate cancer is questionable (9). Radioligand binding to prostate tumor homogenates does not discriminate between the AR content of malignant versus stromal or benign epithelial cells (10, 11). More recently, with the availability of antibodies to the AR (12–15), it has been possible to use immunohistochemical approaches to circumvent this problem of AR expression in nonmalignant cells present in the prostate biopsy tissue while, at the same time, provide some information regarding the micro-heterogeneity of AR expression within the prostate tumor cell population (14–23).

In the present study, we have taken advantage of improvements in computer-aided color VIA techniques (24, 25) to measure AR staining features of tumor cells in a test cohort of 30 human prostate cancers. The specific aims of this study were: (a) to determine whether it is possible to distinguish between functional (i.e., wild-type) and potentially nonfunctional (e.g., truncated) forms of the AR in prostate tumors using antibodies directed against the NH2-terminus and the COOH-terminus of the receptor (15); and (b) to determine whether VIA measurements of AR staining using these antibodies are predictive for tumor progression (all patients) or outcome following hormonal therapy (stage D2 cases only).

MATERIALS AND METHODS

Patient Cohort. All patients accrued into this prospective study were referred to the combined Urology Services of Flinders Medical Centre (Bedford Park, SA, Australia) and the Repatriation General Hospital (Daw Park, SA, Australia) between March 1989 and September 1991. Patients presented with symptoms of acute urinary obstruction and were diagnosed with carcinoma of the prostate following pathological assessment of transurethral resection tissue. The only exclusion criteria were: (a) lack of frozen biopsy tissue with pathological evidence of carcinoma; and (b) loss of patients to follow-up by the combined Urology Services. Patient details, including disease stage according to the Modified Whitmore Jewett system (26), are shown in Table 1.

Patient Outcome Groups. Patients were allocated to two outcome groups based on clinical and biochemical features (i.e., serum PSA levels, bone and computerized tomography scans, and survival). Serum PSA levels were measured routinely by the Department of Clinical Biochemistry at Flinders Medical Centre using a solid phase, two-site immunoenzymatic assay (Tandem-E PSA; Hybritech, Inc., San Diego, CA). For this assay, the normal range of serum PSA levels in healthy men is 0 to 4 ng/ml. In this study, serum PSA levels were categorized into three groups: category 1, levels less than 4 ng/ml; category 2, levels between 4 and 10 ng/ml; and category 3, levels greater than 10 ng/ml.

The actual outcome groups were defined as follows. Group A, the good outcome group, consisted of patients with stable or improving disease. Stable cases showed no clinical evidence of progression, and the PSA levels remained in the original categories. Improving disease cases showed clinical evidence of decreasing tumor mass and a fall in PSA category. Group B, the poor outcome...
Table 1 Clinical stage and treatment details for the 30 prostate cancer patients included in this study

<table>
<thead>
<tr>
<th>Stage</th>
<th>Treatment</th>
<th>Radial prostatectomy</th>
<th>Radiotherapy</th>
<th>Orchietomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2/B1</td>
<td>4</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>B2</td>
<td>12</td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Patients were staged according to the Modified Whitmore Jewett System (26).

1 Localized disease; serum PSA level within the normal range (i.e., <4 ng/ml); tumor-negative regional lymph nodes.

2 Clinically localized to the prostate; elevated serum PSA level (>4 ng/ml); negative bone scan.

3 Local spread; elevated serum PSA level (>10 ng/ml); negative bone scan.

4 Metastatic disease; elevated serum PSA level (~10 ng/ml); positive bone scan ± positive CT scan for lymph nodes.

The group, consisted of progressive disease cases or those who died from prostatic carcinoma. Progressive disease cases showed an increase in tumor mass or the development of metastases with or without a change in serum PSA levels. A rise in PSA category (i.e., either from category 1 to 2 or 3, or from category 2 to 3) over a 6-month period was also used as an indicator of disease progression.

The median (range) values for patient age and duration of follow-up for the test cohort were: Group A, 77 (66—85) years old, 37 (28—56) months follow-up; and Group B, 70 (65—83) years old, 29 (16—53) months follow-up. The comparable values for the duration of follow-up for the subgroup of 16 stage D2 patients were: Group A, 35 (28—46) months; and Group B, 29 (16—39) months.

Tissues and Tissue Processing. Immediately after resection, randomly selected fragments of prostatic tissue were embedded in Tissue-Tek OCT compound (Miles Scientific, Naperville, IL), frozen, and stored at −80°C until processed. Serial 5-μm frozen sections were cut using a Leitz cryostat at −20°C, thawed, and mounted onto poly-L-lysine (Sigma Chemical Co., St. Louis, MO) coated slides and immediately fixed in 4% formaldehyde (Unilab, Ajax Chemicals, Sydney, Australia) in PBS (pH 7.3) for 15 min at room temperature (20°C).

Tissue sections were made permeable in methanol containing 0.3% hydrogen peroxide (H2O2) for 5 min followed by acetone for 1 min, both at −20°C. The sections were washed in two changes of PBS and stored at −20°C in specimen storage medium (8.6% w/v sucrose, 14% w/v magnesium chloride, and 50% v/v glycerol in PBS) until stained. The first and final sections of each series were stained with a standard laboratory hematoxylin and eosin stain for confirmation of the histological diagnosis by a pathologist (J. M. S.).

Androgen Receptor Immunohistochemistry. Immunohistochemical staining for the AR was performed as previously described (15) using two affinity-purified rabbit anti-peptide antibodies that recognize epitopes in the NH2-terminal 21 amino acids (designated U402) and the COOH-terminal 20 amino acids (designated R489) of the human AR. The antiserum was generously provided by Drs. Carol M. Wilson, Michael J. McPhaul, and Jean D. Wilson, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas Texas.

Color VIA. The immunostained sections were examined using an Olympus BH-2 microscope (×200) coupled to a computer-aided color VIA system (Video Pro 32; Leading Edge P/L, Adelaide, South Australia and Leica Australia P/L). Briefly, images of AR staining were captured using a Panasonic color CCD video camera (model WV-CL700/A) and digitized using a PV 100 multi-media 16-bit color video digitizer card in an Intel 80486 DX processor-based personal computer. The resultant digitized image is displayed on a SVGA monitor in a 640 × 480 pixel variable window with 21-bit resolution. The image windows used by Video Pro 32 system are composed of up to 640 × 442 pixels and are separated into 8-bit brightness and color values for each pixel. The range of grey levels for each pixel is from 0 to 255. The system can define the color to be measured with great precision. The transmitted light intensity was standardized by using a fixed rheostat setting at the microscope light source which was connected to a voltage-stabilized conditioned power line. The stability of light output was measured from time to time using a photometer (Leica) as part of the routine VIA quality control procedures (25).

VIA measurements were confined to the nuclei within sections of tumor tissue. Discrimination between malignant and benign tissue areas was based upon standard histological and cytological features (27). VIA measurements were made of the total area stained brown with DAB for AR and the total amount of AR staining (i.e., the IOD), in each field, for at least 20 fields per section. The exact number of fields measured was determined by the cumulative quotient principle (28) to achieve variance below 5% for the mean area (equivalent to number) of tumor nuclei analyzed. Similar measurements were made for the AR negative, hematoxylin (i.e., blue)-stained nuclei. From these measurements, the following parameters of AR staining and the coefficients of variation of these parameters were derived for each tissue section: (a) the mean area, MA of AR staining per field, which is an estimate of the number of nuclei positively stained; (b) the IOD per field, which is proportional to the total amount of AR staining; (c) the MOD (equals IOD/area of DAB staining), which is a measure of the average concentration of AR in the positively stained nuclei; and (d) the percentage of AR-positive nuclei (i.e., area DAB/area of DAB + area of hematoxylin staining). Repeat immunostaining of tumor sections was reproducible, as assessed by VIA, with coefficients of variation of less than 5% for the MA and IOD measurements with either antibody (i.e., U402 and R489).

Statistical Analysis. The statistical technique of DFA (29) was used to assign cases to the predesignated (i.e., clinical) outcome groups (see above) on the basis of all staining features measured using VIA. Correlation analysis was used to compare the results derived for each AR staining feature obtained for the two antibodies. The statistical significance level (α) was set at a critical value (P) of less than 0.05. Except in one instance, as indicated in the text, the probability for the correlation coefficients was significant (i.e., P < 0.05). DFA was performed using the Minisat statistical package (Windows based version; Minisat, Inc., State college, PA).

RESULTS

Immunohistochemical examination of AR expression in the human prostate cancer biopsy specimens using either the anti-NH2 or the anti-COOH terminal antibody to the human AR revealed a heterogeneous pattern of specific nuclear staining in the majority of tumors examined. Several distinct patterns of AR staining were observed. Pattern 1 consisted of intense, uniform staining in all tumor nuclei with both antibodies (2 of 30). In pattern 2, the majority of tumor nuclei were positively stained by both antibodies but with variable intensity of staining (17 of 30). Pattern 2 is illustrated in Fig. 1 by a poorly differentiated prostate carcinoma stained with the anti-NH2 terminal antibody, U402. Pattern 3 was one of discrete foci of tumor cells with weak or absent AR staining and adjacent tumor cells or apparently benign glands which were more intensely stained (5 of 30). Pattern 4 was similar to pattern 3, but the regions of negatively stained tumor cells were more extensive (6 of 30). A region of a tumor section with no AR staining is illustrated in the inset to Fig. 1. In many specimens, there was a degree of weak cytoplasmic staining, not present in all cells, which possibly is the result of receptor diffusion into the aqueous media during thawing and processing of the sections prior to fixation.

VIA was used in this study to measure the area and total amount (i.e., IOD) of AR staining in nuclei of the tumor biopsy specimens and, from these measurements, the mean receptor level per nucleus (i.e., MOD) and the percentage of AR-positive tumor cells were derived. Fig. 2 shows the relationship between the percentage of cells positive for the AR epitope recognized by the anti-NH2 terminal antibody and either: (a) the IOD or (b) the MOD of AR staining for all 30 prostate tumors examined. A poor correlation was obtained in both cases (IODR4892 = 0.43; MODR4892 = 0.26). Similar poor correlations were also obtained using the anti-COOH terminal antibody (IODR4022 = 0.43; MODR4022 = 0.05, P > 0.05). For any given percentage of AR positivity, both the total amount of AR (i.e., IOD) and the average content per cell (i.e., MOD) varied by as much as 6- to 7-fold between different tumor specimens (Fig. 2).
The VIA measurements also identified clear differences in AR staining obtained with the two antibodies. Fig. 3 shows the relationships between the anti-NH₂ and the anti-COOH terminal antibodies for the different parameters of AR staining measured by VIA for all 30 tumor biopsies. Poor correlations between the two antibodies for each parameter were obtained: (a) mean area of AR staining, MA, \( r^2 = 0.2 \); (b) IOD \( r^2 = 0.14 \); (c) MOD \( r^2 = 0.29 \); and (d) percentage of AR positive cells, \( r^2 = 0.30 \). Only in the case of the MOD measurements for both AR antibodies do the cases cluster relatively close to the regression line. The correlations between the primary measurements of AR staining (i.e., MA and IOD) for the two antibodies shown in Fig. 3 identify several possible subpopulations within the 30 cases examined: (a) comparable staining with both antibodies; (b) markedly reduced staining with the anti-COOH terminal antibody relative to the level measured with the anti-NH₂ terminal antibody; (c) markedly reduced staining with the anti-NH₂ terminal antibody relative to the level measured with the anti-COOH terminal antibody; and (d) markedly reduced staining with both antibodies.

The statistical technique of DFA was used to assign the 30 test cases to the predesignated clinical outcome groups on the basis of the VIA measurements of AR staining features. As shown in Tables 2 and 3, the DFA assigned cases to the outcome groups with a high degree of concordance (97%) with the original clinical assessment. Only a single case in the poor outcome group was differently classified (Table 2). The principal discriminant features contributing to the assignment of outcome groups are shown in Table 3. The most powerful contributor to the DFA was the mean concentration of AR in the tumor cells determined by the anti-NH₂ terminal antibody (i.e., AR\(_{U402 MOD}\)). The predictive values (i.e., number of cases correctly assigned to the predesignated outcome groups by DFA/the total number of cases) for the two antibodies, both individually and in combination, are summarized in Table 4. Any single parameter of AR staining in the DFA, e.g., the percentage of AR positive cells, did not enable patient outcome to be accurately predicted, even with the use of two AR antibodies (predictive value, 0.63). Similarly, tumor stage and grade did not accurately predict patient outcome, the predictive value being only 0.63 when both parameters were included together in the DFA (data not shown). When all measurements of AR staining were used in the DFA, patient outcome was correctly assigned for.
Table 2 Comparison of predesignated (i.e., clinical) outcome and outcome assigned by DFA of VIA measurements of all features of AR and hematoxylin staining for all 30 patients

<table>
<thead>
<tr>
<th>Outcome group</th>
<th>Assignment of outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>DFA</td>
</tr>
<tr>
<td>Good</td>
<td>21</td>
</tr>
<tr>
<td>Poor</td>
<td>9</td>
</tr>
</tbody>
</table>

The subgroup of patients with advanced disease (i.e., stage D2) was analyzed independently using DFA to determine the ability of the AR staining features to predict outcome to androgen withdrawal therapy. The percentage of AR positive cells was a weak contributor to the DFA (Table 5), and when analyzed alone, was not an accurate indicator of response to hormonal therapy (Table 6). The VIA measurements of all AR staining features determined using the anti-NH2 terminal antibody were sufficient to independently assign all stage D2 cases to the designated outcome groups (predictive value, 1.0; Table 6). The predictive power of the DFA classification using the VIA measurements of all AR staining features determined with the anti-COOH terminal antibody was only marginally weaker (predictive value, 0.94). For both antibodies, the mean concentration of AR in the tumor cells (i.e., AR_U402 MOD and AR_R489 MOD) was the principal feature of the AR staining contributing to the DFA (Table 5).

Table 3 DFA for all patients

<table>
<thead>
<tr>
<th>Measured features</th>
<th>Contribution to DFA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR_U402^ MOD</td>
<td>54.4</td>
</tr>
<tr>
<td>AR_U402 % positive nuclei</td>
<td>14.7</td>
</tr>
<tr>
<td>AR_R489^ % positive nuclei</td>
<td>13.7</td>
</tr>
<tr>
<td>Hematoxylin_U402 MOD</td>
<td>11.1</td>
</tr>
<tr>
<td>Hematoxylin_R489 MOD</td>
<td>5.3</td>
</tr>
<tr>
<td>All other features</td>
<td>0.8</td>
</tr>
</tbody>
</table>

^ AR_U402, anti-NH2 terminal androgen receptor antibody.
MOD, mean optical density.
^ AR_R489, anti-COOH terminal androgen receptor antibody.
^ Includes MA, IOD, and the coefficients of variation of all measurements for the AR (both antibodies) and hematoxylin-stained tumor nuclei.
ANDROGEN RECEPTOR EXPRESSION IN PROSTATE CANCER

Table 4 Summary of DFA for all staining features

<table>
<thead>
<tr>
<th>Antibody</th>
<th>% AR + ve nuclei</th>
<th>AR features</th>
<th>All features</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARU402</td>
<td>0.50</td>
<td>0.63</td>
<td>0.67</td>
</tr>
<tr>
<td>AR489</td>
<td>0.50</td>
<td>0.77</td>
<td>0.87</td>
</tr>
<tr>
<td>ARU402 + AR489</td>
<td>0.63</td>
<td>0.77</td>
<td>0.97</td>
</tr>
</tbody>
</table>

- Includes MA, IOD, MOD, the percentage of AR positive nuclei, and the coefficient of variation for these measurements.
- Includes MA, IOD, MOD, the percentage of AR positive nuclei, and the coefficient of variation for these measurements.
- Includes MA, IOD, MOD, the percentage of AR positive nuclei, and the coefficient of variation for these measurements.
- Includes MA, IOD, MOD, the percentage of AR positive nuclei, and the coefficient of variation for these measurements.

Table 5 DFA for stage D2

<table>
<thead>
<tr>
<th>Measured features</th>
<th>Contribution to DFA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARU402 MOD</td>
<td>54.5</td>
</tr>
<tr>
<td>AR489 MOD</td>
<td>39.5</td>
</tr>
<tr>
<td>ARU402 % positive nuclei</td>
<td>3.4</td>
</tr>
<tr>
<td>AR489 % positive nuclei</td>
<td>1.1</td>
</tr>
<tr>
<td>All other features</td>
<td>1.2</td>
</tr>
</tbody>
</table>

- Includes ARU402, anti-NH2 terminal androgen receptor antibody.
- Includes AR489, anti-COOH terminal androgen receptor antibody.

Table 6 Summary of DFA for all AR staining features

<table>
<thead>
<tr>
<th>Antibody</th>
<th>% AR + ve nuclei</th>
<th>AR features</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARU402</td>
<td>0.69</td>
<td>1.00</td>
</tr>
<tr>
<td>AR489</td>
<td>0.57</td>
<td>0.94</td>
</tr>
</tbody>
</table>

- Includes MA, IOD, MOD, the percentage of AR positive nuclei, and the coefficient of variation for these measurements.
- Includes ARU402, anti-NH2 terminal androgen receptor antibody.
- Includes AR489, anti-COOH terminal androgen receptor antibody.

DISCUSSION

Previous inability to demonstrate a relationship between AR content measured by radioligand binding assays and response to hormonal therapy in patients with metastatic prostate cancer has been attributed to the diversity of cell types in tumor biopsy specimens (10, 11). The cloning of the human AR and the subsequent development of antibodies have permitted the use of immunohistochemical methods to evaluate the androgen-responsiveness of human prostate cancers. With the exception of a recent report by Sadi and Barrack (23) using a combination of immunohistochemistry and video image analysis to determine the relative staining intensity of the AR in 17 patients with stage D prostate cancer, AR staining intensity has not been objectively measured. Previous studies have been restricted to the measurement of the percentage of AR positive tumor cells (16, 18, 19) and/or the subjective (i.e., observer-dependent) assessment of AR staining intensity in human prostate cancer tissues (17, 20—22). Neither method of assessing AR staining, either alone or in combination, demonstrated a relationship between AR expression in prostate tumor biopsies and response to endocrine therapy. Using objective VIA measurements, the present study has confirmed the earlier findings for the percentage of AR positive cells. Indeed, our study has shown that the percentage of AR positive tumor cells is not a good indicator of either the total amount of AR present in a tumor biopsy or the mean amount of AR per tumor cell, from which we infer that this staining feature may be an unsuitable index of the androgen responsiveness of a prostate cancer. We have shown that the principal parameter for the purpose of assigning cases to the predesignated clinical outcome groups was the mean amount of AR per tumor nucleus (MOD). In the study of Sadi and Barrack (23), mean AR staining intensity was not significantly different among the 17 stage D tumors examined, but the variance of staining intensity in their study was significantly greater in the poor responders to hormonal therapy than in the good responders. While a statistical difference can be detected in our study between outcome groups using the Mann-Whitney test to analyze individual parameters of AR staining (data not shown), as in the case of Sadi and Barrack (23), the large overlap of values limits the clinical usefulness of that approach. The technique of DFA, as used in this study, incorporates all measurements of AR staining in the assignment of individuals into previously defined groups (e.g., good or bad outcome following hormonal treatment). In a previous study of the prognostic value of nuclear shape variables in advanced prostate cancer (30), the Mann-Whitney test was able to define differences between outcome groups (i.e., response to hormonal therapy), but there was considerable overlap of individual values diminishing the clinical usefulness of this statistical approach. DFA in fact showed that it was not possible to assign individual stage D2 patients to outcome groups using nuclear shape features (predictive value, 0.65) to a clinically useful degree.

In the present study, the AR staining features determined by either the anti-NH2 or anti-COOH terminal antibody were almost equally effective in correctly allocating the 16 stage D2 patients to the designated outcome groups. For both antibodies, the mean concentration of AR in the tumor cells (MOD) was the principal but not exclusive parameter contributing to the DFA, suggesting that this feature of AR staining may evolve as a good indicator of response to hormonal therapy in future studies. Staining parameters derived from the anti-COOH terminal antibody measurements were more valuable in correctly assigning the entire test group, which included all clinical stages, to the predesignated outcome groups than those obtained with the anti-NH2 terminal antibody. Assignment was best for the 30 cases using a combination of all nuclear staining features measured with the two AR antibodies and the hematoxylin-only stained cells. This observation suggests that the hematoxylin counterstaining may detect a feature(s) of the AR-deficient tumor cell nuclei, such as aneuploidy or variation in numbers of nucleolar organizer regions, which may be related to tumor progression but, when analyzed independently, are not good predictors of patient outcome (30—32).

The quantitative differences in AR staining measured with the anti-NH2 and anti-COOH terminal antibodies possibly explain why the predictive power of the DFA is increased by using a combination of staining features obtained with both antibodies. The differences in AR staining with the two antibodies are most apparent when the mean area or IOD measurements are examined. These parameters reflect the number of AR-positive tumor cells and the total amount of AR, respectively, in the tumor biopsy specimen and identify three possible subpopulations which are not revealed by the derived parameters of staining. The main category, showing concordance of AR staining with the anti-NH2 and anti-COOH terminal antibodies, is that expected for staining with two AR antibodies when used under optimized conditions for immunohistochemistry (e.g., antibody excess) and the structure of the receptor in the tumor samples is normal. This was the sole category identified in similar studies of human benign prostatic hyperplasia2 and breast cancer6 tissues stained for AR using the two antibodies. Collectively, these observations suggest that the two groups of prostate tumors identified as having a disproportionate amount of total AR staining with the two antibodies may be of physiological significance.

3 M. A. Grimbableston and W. D. Tilley, unpublished observations.
4 R. E. Hall and W. D. Tilley, unpublished observations.
While the reason for the existence of the quantitative differences in AR staining with the two antibodies in the prostate tumors is not clear, the differences may reflect tumor-associated changes which are important in progression. There is evidence that some prostate cancer cells which continue to grow after initiation of anti-androgen therapy retain the expression of the AR. For example, AR expression was observed in 80% of prostatic tumor cells in 13 of 17 patients at varying intervals after the commencement of adjuvant ablation therapy (19). The intensity of staining, however, was not measured. Similarly, AR expression is retained by androgen-independent mouse mammary tumors (33). These observations suggest that mechanisms other than the loss of AR expression are involved in the progression of prostate cancer to an androgen-independent state. A possible explanation is the presence of a mutation in the AR gene in a subpopulation of tumor cells which results in aberrant regulation of growth by steroids. In the case of inherited forms of androgen insensitivity, subtle changes in the structure of the AR gene may have a profound effect on receptor function and thus result in major phenotypic abnormalities during male sexual development (34). Similar mutations in the AR gene may be important in tumor progression and the development of androgen independence in prostate cancer. For example, a mutation in exon 8 of the AR gene in the androgen-responsive human prostate cancer cell line, LNCaP, results in an altered specificity of the AR molecule for steroid binding and allows stimulation of cell growth by estrogens, progesterones, and anti-androgens in addition to androgens (35). A mutation in the AR gene has been reported in an early stage (i.e., stage B) primary human prostate cancer (1 of 26 patients, Ref. 36) and in two tumors in independent studies of endocrine-resistant, metastatic (i.e., stage D2) disease (1 of 7 patients, Ref. 37; 1 of 9 patients, Ref. 38). Similarly, estrogen receptor variants have been described in human breast cancer tissues (8), and more recently, the development of male breast cancer has been linked to mutations in the DNA binding domain of the human AR (39).

While the frequency and clinical significance of AR mutations in relation to tumor progression in prostate cancer remains to be determined, it is possible that mutations in the AR could explain some of the differences in AR expression detected by VIA with the two antibodies in this study. For example, the presence of AR mutations similar to those reported in individuals with either the partial or complete form of the androgen insensitivity syndrome, which can result in NH2-terminal or COOH-terminal truncations of the receptor (34, 40), may contribute to the differences in staining intensity described in this study. The recent report (41) of both wild-type and a functionally impaired M, 87,000 form of the AR which is truncated at the NH2-terminus in normal genital skin fibroblasts raises the possibility that multiple forms of the AR might be present in both normal and malignant androgen target tissues. An altered ratio of the wild-type to an NH2-terminal truncated form of the AR in prostate cancer is one possible mechanism currently being examined to explain the increased level of AR staining measured in four of the prostate tumors with the anti-COOH-terminal antibody relative to the level measured with the anti-NH2-terminal antibody.

In summary, objective assessment of AR staining in prostate cancer tissues using VIA in combination with DFA is potentially a powerful method for predicting disease progression and/or response to hormonal therapy. The present study has been performed on a relatively small trial group of prostate cancer patients, and clearly it is necessary that a larger prospective study is conducted to confirm our findings. An important aspect of the current study has been the measurement of AR staining with two antibodies since this has provided a strategic method to identify prostate tumors containing AR which may be structurally abnormal. Further investigation of the basis of the quantitative differences in the levels of AR staining observed with the two AR antibodies may provide new insights into the role of the AR in the progression of prostate cancer.

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