Androgen and Glucocorticoid Receptors in the Stroma and Epithelium of Prostatic Hyperplasia and Carcinoma

James L. Mohler, Yeqing Chen, Katherine Hamil, Susan H. Hall, John A. Cidlowski, Elizabeth M. Wilson, Frank S. French, and Madhabananda Sar


ABSTRACT

Differences in stromal and epithelial cell staining for androgen and glucocorticoid receptors (ARs and GRs) were investigated in 20 patients with clinically localized prostatic carcinoma treated by radical prostatectomy. Sections of benign prostatic hyperplasia and prostatic carcinoma from each patient were stained with antibodies to AR and GR using an avidin-biotin peroxidase technique. The specificity of the GR immunoreactivity was established in benign prostatic hyperplasia and prostatic carcinoma by immunohistochemistry using the GR antibody absorbed with synthetic peptide and Western blotting. Nuclear staining intensity and percentage of nuclei stained were summed to obtain AR and GR immunostaining scores. AR staining of prostatic carcinoma epithelial [103 ± 58 (SD)] and stromal (126 ± 48) nuclei was less than in benign prostatic hyperplasia (142 ± 47 and 169 ± 56; paired Student's t tests, P = 0.02 and P = 0.01); however, no difference in staining intensity occurred between stroma and epithelium in either tissue type. GR staining intensely in stromal cell nuclei of benign prostatic hyperplasia (189 ± 50) and prostatic carcinoma (163 ± 60). However, prostatic carcinoma epithelial cells (34 ± 57) had low levels of glucocorticoid receptor staining (P < 10^-7), and benign prostatic hyperplasia epithelium (74 ± 51) was intermediate. In most patients, GR could not be detected in nuclei of prostatic carcinoma epithelial cells but was undiminished in stromal cell nuclei. There was no relationship by multivariate regression analysis between AR or GR staining and age, serum prostate-specific antigen, Gleason grade, or pathological stage. In comparison with AR, the greater variability of GR staining in epithelium versus stroma of prostatic carcinoma warrants further study of GR, particularly in the area of stromal-epithelial interaction.

INTRODUCTION

American men suffered 42,400 deaths and 244,000 new diagnoses of CaP3 in 1995 (1). In spite of the impact of CaP, little is known about its pathogenesis or predictors of response to various therapies. Studies on prostate morphogenesis, growth, and function have focused upon androgenic regulation of epithelial cells. However, recent studies indicate that at least some responses of prostatic epithelial cells to hormonal stimuli may be the consequence of growth factors, morphogens, or inductors produced in neighboring stromal cells (2–5). In view of the potential importance of stromal-epithelial interactions during prostatic development, investigation of hormonal regulation by the stromal compartment may lead to a better understanding of prostatic disease and may provide insight into how abnormal growth processes are modified by hormonal therapy.

Various programs of hormonal therapy have been used for the treatment of patients with CaP since the pioneering work of Huggins and Hodges (6). Approximately 80% of patients with metastatic CaP respond to androgen deprivation with an extension of life expectancy from approximately 9 to 43 months. A quantitative and/or functional difference of the AR may contribute to clinical response to androgen deprivation therapy (7, 8). However, Sadi et al. (9) found no correlation between the proportion of epithelial cells staining positive for AR and interval to progression in 10 patients. Quantitative analysis in a larger group of patients disclosed that heterogeneity of AR staining among nuclei from individual tumors correlated with response (10). Similar to prostatic development, androgen-mediated growth of CaP may occur via paracrine factors produced by the stroma. The AR status of the epithelial compartment in early CaP may provide an incomplete description of disordered growth control.

Approximately 20% of patients with androgen-independent CaP respond to aminoglutethimide or ketoconazole and corticosteroids (11–13). Responses have been attributed to the elimination of adrenal androgens (14). Antibodies specific for the GR have been developed recently (15) but, to our knowledge, they have not been applied to studies of prostatic tissue.

We reported previously (16) methodology for sensitive and specific detection of AR in frozen sections of fresh human prostate tissue using the polyclonal antibody AR-52 (17) and the avidin-biotin peroxidase method. Epithelial cell nuclei stained less intensively and more heterogeneously for AR in CaP than BPH. In the present study, we used antibodies specific for both...
AR and GR to compare intensity of staining of epithelium and stroma in BPH and CaP.

MATERIALS AND METHODS

Patients. Tissue was obtained in the operating room from 20 patients [63 ± 8 (SD) years old] treated by radical prostatectomy for clinically localized CaP (Table 1). Serum prostate-specific antigen (Hybritech) was 12.3 ± 7.5 ng/ml. Pathological Gleason grade (19) was 6.7 ± 1.0 and CaP proved organ-confined in 11 patients (55%), specimen-confined in 3 patients (15%), extended beyond surgical margins in 4 patients (20%), and metastasized to obturator lymph nodes in 2 patients (10%).

Table 1 Raw data: Patient characteristics and immunohistochemistry scores

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^a B, black; W, white; PSA, prostate-specific antigen (Hybritech); OC, organ-confined; CP, capsular penetration; SV, seminal vesicle invasion; M, margin positive; N, nodes positive; AR, androgen receptor; GR, glucocorticoid receptor; Epi, epithelium; Str, stroma.

Table 2 Steroid Receptor Intensity Scoring. The immunohistochemical technique has been described previously (16). Briefly, frozen sections were air dried and fixed with PBS containing 4% paraformaldehyde, 10% sucrose, and 0.1 M sodium phosphate (pH 7.2). Sections were transferred into 2% hydrogen peroxide in PBS for 5 min, washed, and permeabilized in 0.2% Triton X-100 for 10 min. Non-specific staining was blocked with 2% normal goat serum. Sections were incubated with AR-52 (17) at an IgG protein concentration of 5 μg/ml or GR-57 (15) at a purified IgG protein concentration of 2 μg/ml in a moisture chamber for 16–18 h at 4°C. Sections were exposed at room temperature to biotinylated goat antirabbit IgG secondary antibody (3.75 μg/ml; 1:400 dilution; Vector Labs Inc., Burlingame, CA) for 60 min, reacted with avidin-biotin peroxidase (ABC; Vector Labs, Inc.), diluted 1:400 for 60 min, and exposed for 10 min with a solution containing 75 mg diaminobenzidine (Aldrich Chemical Co., Milwaukee, WI) and 8.5 μl fresh 30% hydrogen peroxide in 100 ml 0.05 μl Tris (pH 7.6). Negative controls included preimmune serum in place of AR and GR for each patient, AR and GR absorbed with synthetic peptide specific for each receptor protein in each run, and AR^-1 and GR^-1 stained spleen for each run. Positive controls were rat liver present on each section as adhesive that stains uniformly and intensely for both AR and GR. To control for variability in immunohistochemical preparation, all tissue sections including controls were stained for AR in a single “run,” and all sections were stained for GR in a second “run.” Finally, all tissue preparation and immunohistochemical staining was performed by one highly trained technician (Y. C.).

Steroid Receptor Intensity Scoring. All sections of BPH and CaP from each patient were graded by one highly trained technician (Y. C.) for intensity of AR and GR staining in
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Fig. 1  Frozen sections of BPH were prepared for steroid receptor immunohistochemistry (reduced from ×800) and stained with H&E (A), AR-52 against the AR (B), GR-57 against the GR (C), or GR-57 competed with synthetic peptide antigen comprising 22 amino acids of the GR (D).

epithelial cells and stromal fibroblasts (0, absent; 1, weak; 2, moderate; or 3, strong), and the percentage of nuclei (0–100%) stained at each intensity level was determined. Total intensity score (0–300) was the summation of the products of each intensity score and its corresponding percentage (16). One hundred cells for each tissue type and steroid receptor stain were selected by 20 random movements of the microscope stage and grading of the five nuclei (AR) or cells (GR) closest to a point superimposed upon the center of the field of view. Images were collected and stored upon videotape to document accuracy of grading. Random sampling of 100 cells assured accurate description of average as well as variation in staining intensity for AR (16) and for GR (data not shown). Student’s t tests were used to compare the 20 paired specimens of BPH and CaP obtained from the same radical prostatectomy specimens and stromal-epithelial differences within patients. Multivariate regression analyses were performed to test for dependence of staining scores upon patients’ ages, serum prostate-specific antigens, sum Gleason grades, and clinical and pathological stages.

Specificity of GR Antibody in Prostate Tissue. Since GR-57 has not been used previously in prostate tissue, its specificity for GR was investigated in BPH and CaP by two methods: (a) GR-57 was competed with the NH2-terminal domain peptide (amino acids 346–367) of the human GR synthesized as described previously and used as the antigen to raise the polyclonal GR-57 antibody in rabbits (15). Tissue sections were prepared and stained as described above except that synthetic peptide was added to GR-57 at a concentration ranging from 2 to 10 μg/ml (1–5 times the concentration of GR-57); (b) frozen specimens of histologically confirmed BPH and CaP were pulverized and solubilized in 8 m urea, incubated 1 h at 0–4°C, and centrifuged to pellet insoluble residue. Seventy-five μg of each tissue extract were subjected to denaturing electrophoresis in 8.0% polyacrylamide gels and transferred to Immobilon P (Millipore Corp., Bedford, MA; Refs. 2, 20). GR was detected using the ECL chemoluminescence (Amersham Corp., Arlington Heights, IL) Western detection protocol, except that the primary antibody was diluted at 1:3000 (0.3 mg/ml) and the secondary antibody was diluted 1:10000. The primary antibodies used were preimmune serum, GR-57, and GR-57 preincubated with a 1:1 concentration of the synthetic peptide described above (15).

RESULTS

Frozen sections of BPH and CaP treated with control preimmune serum stained negligibly. In BPH (Fig. 1), AR staining was confined to the nucleus, whereas GR staining occurred to some extent in cytoplasm but was more intense
within the nucleus. Specificity of GR-57 for GR in prostate tissue was demonstrated immunohistochemically when staining was progressively decreased upon the addition of 2, 4, and 6 μg/ml of synthetic peptide. GR-57 staining was abolished by the addition of a 4-fold (Fig. 1D) and 5-fold concentration of synthetic peptide. Western blotting of tissue extracts of BPH and CaP revealed no detectable immunoreactivity when preimmune serum was substituted for OR-57. OR-57 detected protein of Mr 97,000 that corresponds to the molecular weight of human OR used to generate GR-57. Right, molecular weight markers.

A representative example of AR immunohistochemistry (Fig. 3, A and B) demonstrates the relationships between benign and malignant tissues. Malignant epithelial (staining intensity score, 80) and stromal (staining intensity score, 50) cell nuclei stain similarly. However, benign epithelial (staining intensity score, 150) and stromal (staining intensity score, 110) cells stain more intensely for AR protein. In the group of twenty patients, the mean AR intensity scores in CaP epithelial (103 ± 58) and stromal (126 ± 48) nuclei were less than those in BPH (142 ± 47 and 169 ± 56; \( P < 0.02 \) and 0.01, respectively). No significant difference occurred between stroma and epithelium in either tissue type.

The group of 20 patients exhibited two types of GR staining. In four patients, malignant and benign tissues stained similarly, and stromal cells stained more intensely than epithelial cells. For example, patient 17 (Fig. 3, C and D) showed GR staining intensity scores for epithelium and stroma of 140 and 255, respectively, for BPH and 180 and 235, respectively, for CaP. In 16 patients, GR staining of malignant epithelial cells was absent or barely visible, whereas staining remained intense in the remaining cell types. For example, patient 3 (Fig. 3, E and F) showed no GR staining in neoplastic epithelial cells, but staining intensity scores ranged from 120 to 200 in stroma of neoplastic areas and epithelial and stromal cells of benign areas. Among all 20 patients, GR stained intensely in stromal cells of BPH (189 ± 50) and CaP (163 ± 60). However, CaP epithelial cells (34 ± 57) had very low levels of GR staining (\( P < 10^{-3} \)), and BPH epithelium (74 ± 51) was intermediate.

Data on immunohistochemical staining of AR and GR receptors for each type of tissue subtype are presented in simplified form in Fig. 4. The greatest change in steroid receptor expression, the decrease in GR staining observed in epithelial cells in CaP, would be even more significant within the subgroup of 16 patients in whom GR could not be detected at all or was barely visible, although the GR staining intensity was undiminished in CaP stromal cells or benign tissue.

Multivariate regression analyses showed no relationship between AR or GR staining and patients’ ages, serum prostate-specific antigens, sum Gleason grades, and clinical and pathological stages. For example, sum Gleason grade did not correlate with epithelial (\( r = -0.29 \), \( P = 0.22 \)) or stromal (\( r = -0.10 \), \( P = 0.69 \)) AR staining. Similarly, differences between AR and GR staining in stroma versus epithelium in BPH and CaP or epithelium in BPH versus CaP were not related to clinical parameters. For example, sum Gleason grade did not correlate with differences in AR staining between benign and neoplastic epithelium (\( r = -0.20 \), \( P = 0.39 \)) or between neoplastic stroma and epithelium (\( r = 0.05 \), \( P = 0.83 \)). Among all relationships examined, no clinical factor was responsible for greater than 8% of the variation observed in steroid receptor staining.

**DISCUSSION**

Steroid receptors may have a role in the development, progression, and treatment failure of human prostatic carcinomas. Point mutations have been described in the AR steroid binding domain in the human LNCaP cell line (21, 22) and but occur rarely in clinically localized (23) and androgen-independent metastatic CaP (24). Previous investigations of AR content in benign and malignant prostatic tissue have demonstrated immunohistochemical staining of AR predominantly in the glandular epithelial cells (8, 16, 25–27). The recent demonstration of the modulation of the epithelial compartment by the stroma (4) led us to investigate whether the AR content of the stroma might govern the biological behavior of the epithelium since epithelial AR content appears not to. We found no quantitative differences in AR staining between stromal and epithelial nuclei in either BPH or CaP. However, the abundance of AR in the stromal and epithelial compartments suggests that, in addition to direct actions on epithelial cells, hormonal-dependent carcinogenesis and progression may be regulated through stromal-epithelial interactions, as occurs during embryological development of the prostate (5).
The relationship between CaP and BPH could be minimized by quantitative analysis of AR staining (correlation previously in a smaller group of 10 patients significant inverse intensity of AR staining (16). Masai et al. demonstrated that CaP exhibits decreased intensity of nuclear staining that is heterogeneous from gland to gland and even from cell to cell when compared to BPH. We found decreased intensity and similar heterogeneity of AR staining within the stromal compartment that was comparable to that observed within the epithelial compartment in CaP. The intensity scores for AR staining in this study were diminished compared to our prior report. Neoplastic nuclei stained with an intensity score of 103 ± 58 versus 166 ± 69 in the previous work, and benign nuclei scored 142 ± 47 versus 246 ± 41 previously. However, the same relationship between CaP and BPH was maintained; CaP nuclei stained with 67% of the intensity score and BPH nuclei with 72% of the intensity score. These differences emphasize the need for internal controls when comparing immunohistochemical studies from different laboratories and even from different investigators within the same laboratory. Interobserver variation could be minimized by quantitative analysis of AR staining intensity as performed by Sadi and Barrack (10) using cytophotometry of immunoperoxidase-reacted sections.

In the present study of 20 patients, neither AR content nor heterogeneity correlated with Gleason grade. We reported previously in a smaller group of 10 patients significant inverse correlation (r = -0.77, P = 0.009) between Gleason grade and intensity of AR staining (16). Masai et al. (29) also reported an inverse correlation between Gleason grade and percentage of "strongly stained" cancer cells. The different relationship between grade and AR staining may be explained by the small numbers of cases studied or by the absence of a relationship between tumor grade and response to endocrine therapy (30).

GR belongs to the family of ligand-activated, transcriptional regulators that convey endocrine information to target cells. Many of the physiological effects of steroid hormones are mediated through selective interaction of steroid receptor complexes with enhancer-like elements in the promoter and intron regions of steroid-responsive genes (31, 32). GR, like AR, contains a unique amino terminal variable region that includes a transactivation domain that is important for the regulation of gene expression (33, 34). The central DNA-binding domain contains zinc fingers crucial for the specific interaction of receptor with DNA sequences that contain glucocorticoid regulatory elements (35, 36). Finally, the carboxyl terminus of the GR contains a ligand binding domain involved in the process of receptor activation.

A specific role for the GR receptor in CaP remains unknown. Once CaP becomes androgen independent, responses to secondary treatment that may be mediated through GR are unusual but do occur. Medical adrenalectomy using aminoglutethimide or ketoconazole produced subjective improvement in 33, 48, and 60% of 58, 40 and 20 patients with androgen-independent CaP (37–39). A more critical test of the effect of these two drugs was conducted using the objective response criteria devised by the U.S. National Prostate Cancer Project. Two groups of investigators studied prospectively previously castrated men with progressive metastatic CaP treated with aminogluthethimide. Worgul et al. (11) found one complete response, four partial responses, and six men with stable disease among 25 patients; Drago et al. (12) found one complete re-

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*Fig. 3* Patient 19 was representative of AR immunohistochemistry. BPH reduced from ×800 showed AR intensity scores of 150 for epithelium and 110 for stroma (A); CaP reduced from ×400 showed AR intensity scores of 80 for epithelium and 50 from stroma (B). Patient 17 was representative of four cases of GR immunohistochemistry, where BPH and CaP epithelial staining was similar but less intense than stromal staining. BPH reduced from ×400 showed GR intensity scores of 140 for epithelium and 255 for stroma (C); CaP reduced from ×400 showed GR intensity scores of 180 for epithelium and 235 for stroma (D). Patient 3 was representative of 16 cases where GR staining of epithelial cells was absent or nearly absent but CaP stroma and BPH epithelium and stroma stained similarly. BPH reduced from ×800 showed GR intensity scores of 120 for epithelium and 200 from stroma (E); CaP reduced from ×800 showed GR intensity scores of 0 for epithelium and 120 for stroma (F).
The relative decrease in GR in CaP epithelium is intriguing and could result from loss of a differentiated cell function. However, we found no relationship between GR content or heterogeneity and sum Gleason grade. In clinically localized CaP, cell maintenance and growth may depend upon androgens and glucocorticoids. Once the patient has been androgen deprived, GR may retain a role in transcriptional regulation. Glucocorticoid could influence androgen-independent CaP through GR interactions with hormone response elements in androgen-regulated genes (46). Glucocorticoids may enhance the actions of androgens on certain genes through positive transcriptional effects mediated by hormone response elements. Simple response elements, 15-bp partial palindromes, mediate transactivation by both AR and GR. Alternatively, glucocorticoids might inhibit androgen-specific transactivation mediated by complex response elements such as the intron element of the \( M_f \), 20,000 protein gene (47) that is transcriptionally unresponsive to glucocorticoid, although it binds GR. The differential loss of the GR in the epithelial and stromal compartments of CaP is an interesting phenomenon that should be investigated further.

## REFERENCES

Androgen and glucocorticoid receptors in the stroma and epithelium of prostatic hyperplasia and carcinoma.

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