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Androgen Receptor Modulation in Benign Human Prostatic Tissue and Prostatic Adenocarcinoma During Neoadjuvant Endocrine Combination Therapy

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ABSTRACT: Modulation of androgen receptor (AR) expression during neoadjuvant endocrine therapy in human prostates of patients with localized prostate cancer was investigated by immunohistochemistry. In 8 of 15 untreated prostatectomy specimens, the majority of prostatic glandular cells displayed nuclear immunostaining for AR, whereas only 1 of 26 pretreated cases displayed a similar glandular AR expression. Expression of AR in the prostatic stromal compartment of nontreated cases proved to be quite heterogeneous, since 4 of the 15 (27%) examined specimens did not show stromal AR expression. After preoperative neoadjuvant therapy, this value was 68%, although this difference did not reach statistical significance. Prostatectomy specimens of the treated patients contained carcinomas with a higher Gleason score than those of untreated patients. AR expression in carcinomas of treated patients was diminished ($P = 0.05$), which may be attributed to their relatively lower differentiation grade. The data strongly suggest that neoadjuvant hormone therapy reduces AR expression by nonneoplastic prostatic glandular cells and carcinoma cells by a selective, but incomplete, elimination of AR-positive cells.

KEY WORDS: **human prostate, endocrine combination therapy, androgen receptor, LHRH agonist, antiandrogen**

INTRODUCTION

Several studies have demonstrated the presence of androgen receptors (AR) in the human prostate, both in the secretory epithelial cells of the prostatic glands and in fibroblasts and smooth muscle cells [1–3]. In the basal cells of the human and rat prostatic glands, expression of AR is low or not detectable [2–4], although some basal cells may express high levels of AR [5].

Some studies have described the modulation of AR expression in epithelial cells of male rodent accessory sex glands during the manipulation of androgen levels. Thus, short-term castration of rats was reported to result in a decrease of AR expression in prostatic epithelial cells [4,6]. The study by Takeda et al. [6] also showed a rapid restoration of AR expression in prostatic epithelial cells by administration of androgens to castrated rats. Other studies, however, described a modest increase in AR expression after short-term androgen ablation in rat ventral prostate [7], or a differential regulation in the separate rat prostate lobes [8]. Due to the heterogeneity of expression of AR in stromal cells of the rat prostate, effects of androgen manipulation on stromal cell expression of AR have not been reported.

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A ligand-binding study on tissue homogenates of hyperplastic prostatic tissue of men treated for 3 months with a luteinizing hormone-releasing hormone (LHRH) agonist indicated that the nuclear content of AR was significantly lower than that in prostatic tissue of untreated men [9]. The availability of a well-characterized monoclonal antibody directed against human AR [2] enabled us to visualize separately the expression of AR protein in the human prostatic epithelial and the stromal constituents, as well as in adenocarcinoma cells during long-term androgen blockade achieved by combined administration of the antiandrogen flutamide and an LHRH agonist. This neoadjuvant endocrine therapy blocks the production of testicular androgens and interferes with the effects of androgens synthesized locally from precursor adrenal steroids, especially dehydroepiandrosterone and its sulphate [10,11]. Previous histopathological studies have shown marked effects of this treatment on the morphology of normal human prostatic glands and prostatic carcinomas [12]. Demonstration of modulation of AR expression in human prostatic tissue may give insight into the role of AR in benign and malignant processes of the prostate.

MATERIALS AND METHODS

Patient Material

Tissue specimens of approximately 1 cm^3 were obtained from patients who had undergone a radical prostatectomy for localized, nonmetastasized prostatic adenocarcinoma at the Hôtel-Dieu de Québec, Québec City, Québec, Canada. Tissue samples were taken from the prostatectomy specimen immediately after surgery. The samples were frozen in liquid nitrogen, embedded in Tissue-Tec, and stored at −80°C until use.

The study comprised specimens from 48 patients who entered into a prospective randomized clinical trial investigating the advantage of 3 months of neoadjuvant endocrine therapy over omission of preoperative treatment [13]. Samples of 6 cases in this series were excluded due to poor tissue preservation. All patients received an LHRH agonist and the nonsteroidal antiandrogen drug flutamide for 3 months. This therapy was maintained until surgery, and led to a significant decrease in tumor size [12,13]. Tumors were graded according to the Gleason grading system, revealing a considerable difference in tumor grade between pretreatment and nontreated prostatectomy specimens (Fig. 1). In frozen tissue samples of 7 nontreated patients and 4 treated patients, residual tumor was present. Frozen tissue specimens of 2 treated patients containing residual tumor were additionally included in order to study AR expression in prostatic carcinoma after 3 months of combination endocrine therapy.

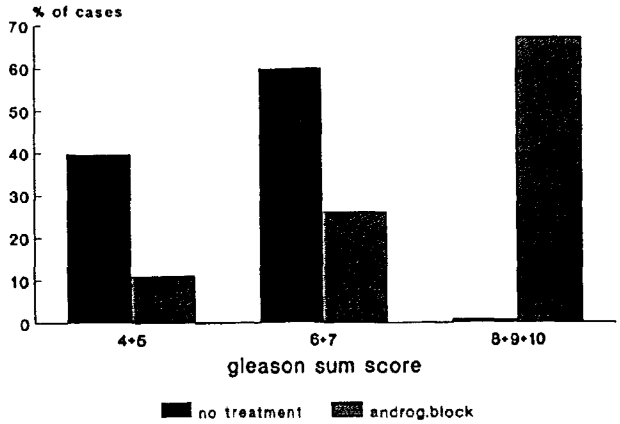

Fig. 1. Tumor grades after androgen blockade. Gleason grading scores of prostatic carcinomas in pretreated and untreated prostatectomy specimens. Treatment consisted of 3 months of neoadjuvant combination therapy.

Immunohistochemistry

Cryostat sections of 5-μm thickness were air-dried and fixed for 5 min in 10% formalin, rinsed in phosphate-buffered saline (PBS), and subsequently transferred to chilled methanol (−20°C). After the incubation in methanol, the sections were dehydrated in chilled acetone for 2 min. Next, the sections were pretreated with 5% nonimmune rabbit serum diluted in PBS. Monoclonal antibody F39.4 (Sanbio, Uden, the Netherlands) was reacted with the tissue overnight at 4°C, and visualization of antibody binding was achieved with an indirect peroxidase anti-peroxidase method as described previously [2]. The substrate was 3-3-diamino-benzidine (Sigma, Deisenhofen, Germany) with 0.03% H_2O_2. Nuclear counterstaining was done with Mayer's hematoxylin. Negative controls consisted of slides that were reacted with PBS instead of the primary antibody, followed by the indirect peroxidase anti-peroxidase method. No nuclear or cytoplasmic staining was observed in the control specimens.

The semiquantitative analysis of immunohistochemical staining for AR was done blindly by two of us (T.H.v.d.K., B.T.). Only nuclear immunoreactivity was observed. Percentage of immunopositive cells was scored as none, <10%, between 10–50%, and >50%. Results of semiquantitative analysis of stromal and glandular AR expression in specimens of treated and nontreated patients were compared with an Exact Trend Test (EGRET package, Statistics and Epide-

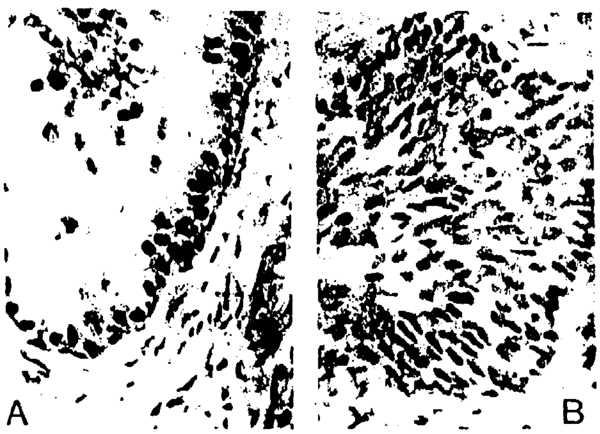

Fig. 2. Immunohistochemical staining with monoclonal antibody F39.4 of prostatic tissue. **A:** Prostatic tissue specimen from a patient not submitting to endocrine therapy. Nuclei of secretory epithelial cells are intensely stained, as well as a proportion of the surrounding stromal cells. **B:** Prostatic tissue specimen from a patient who received 3 months of combination therapy prior to surgery. Note the basal cell hyperplasia, with the rare presence of intensely stained nuclei. Nuclear counterstaining with hematoxylin, × 100.

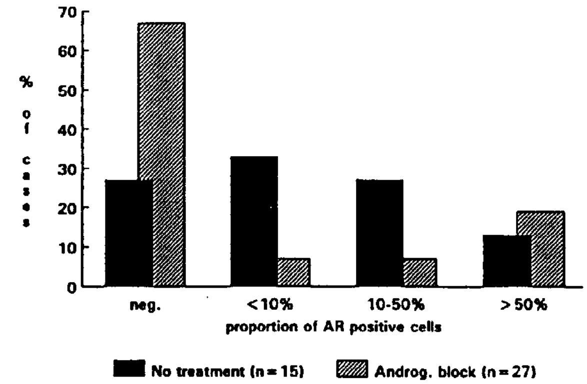

Fig. 3. Modulation of AR expression in prostatic stroma. Effect of 3 months of neoadjuvant combination therapy.

miology Research Corporation and Cytel Software Corporation, Cambridge, MA). Owing to the small number of cases with prostate cancer, the Exact Association Test was applied to compare the distribution of AR positivity in treated and nontreated cases.

RESULTS

Expression of AR as defined by immunostaining with monoclonal antibody F39.4 was observed in prostates of untreated patients both in the glandular and stromal compartments in a pattern similar to that described previously [3]. Both in normal and hyperplastic prostatic glands, some heterogeneity of the nuclear staining of the secretory epithelial cells was present. However, in most cases >50% of the glandular epithelial cells showed a moderate-to-intense immunostaining reaction. In the basal cells surrounding the secretory epithelial cells of the normal and hyperplastic prostatic glands, AR expression was generally not detectable (Fig. 2A). A strong variation of stromal immunoreactivity among different specimens of the nontreated patients was noted. The fraction of cases lacking stromal immunoreactivity for AR increased to 68% as compared to 27% in the untreated group (Fig. 3). Statistical analysis revealed that the difference in stromal AR expression between the two groups was not significant ($P = 0.217$).

Prostatic tissue specimens derived from 26 patients treated with endocrine combination therapy revealed microscopically the presence of atrophic and proliferative changes of the prostatic glands. Glands with the aspect of basal cell hyperplasia, as described previously [12], were often present (Fig. 2B). Combination endocrine therapy was accompanied by a significantly ($P < 0.001$) diminished immunostaining of the glandular cell components (Fig. 4). In only one prostatic tissue sample of 26 treated patients was the majority of epithelial cells immunoreactive, while in another 3 cases 10–50% of epithelial cells were positive.

The number of freshly frozen tissue specimens containing prostatic adenocarcinoma was rather limited, particularly in those obtained from patients submitting to prior endocrine therapy, since the tumor was not detectable grossly. The series of treated adenocarcinomas was therefore expanded with an additional 2 cases. A just-significant difference in AR expression pattern was observed between the nontreated and pretreated groups ($P = 0.048$). In 10 of 14 untreated cases, the majority of tumor cells displayed nuclear AR expression, whereas only 1 of the examined 6 pretreated cases did so (Fig. 5). Apparently, exposure to combined androgen ablation therapy parallels an increase in heterogeneity, as well as a decrease in AR expression in tumor cells. This may be attributed to the observed lower differentiation grade of the tumors in the pretreated prostatectomy specimens, in comparison to those of untreated patients (Fig. 1).

DISCUSSION

In this study, monoclonal antibody F39.4 was used to visualize AR expression in prostatic tissues from patients not exposed to prior endocrine therapy and from patients submitting for about 3 months to an endocrine combination therapy prior to surgery. Previous studies have indicated that ligand-binding of

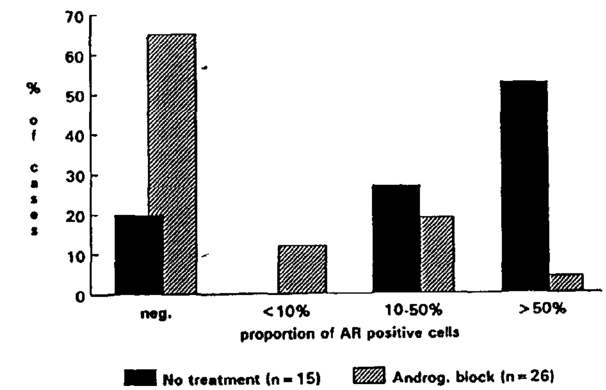

Fig. 4. Modulation of AR in prostatic glands. Effect of 3 months of neoadjuvant combination therapy.

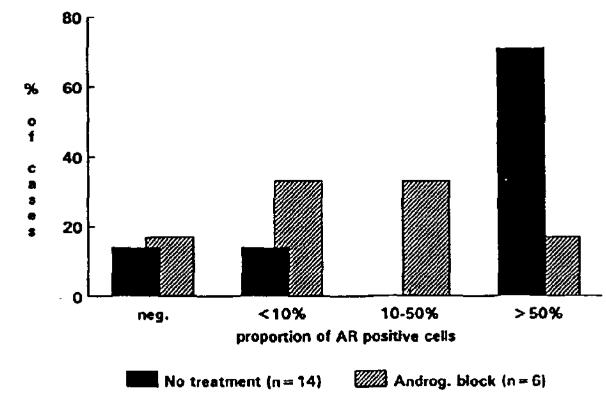

Fig. 5. Effect of 3 months of neoadjuvant combination therapy on AR expression in prostatic carcinomas.

the AR does not influence the interaction of F39.4 with AR [14,15]. Therefore, the changes in immunostaining of prostatic tissue observed during 3 months of endocrine combination therapy are believed to reflect true changes in AR expression, i.e., they are not a consequence of ligand-induced transformation of AR.

In earlier studies, it was shown that monotherapy with LHRH agonists [16], as well as endocrine combination therapy [12], particularly affects the glandular component of benign human prostatic tissue. Prostatic glands become atrophic with or without concomitant basal cell hyperplasia. In glands of the normal and hyperplastic human prostate, AR expression is largely confined to secretory epithelial cells [2,3]. Therefore, the observed loss of AR expression by the epithelial cells may be considered a consequence of the androgen blockade-induced disappearance of the secretory cell population. Similarly, in dogs it was noted that at 4 weeks after castration or treatment with a LHRH agonist, the secretory epithelial cell layer of the prostate largely disappears [17,18]. Transmission electron-microscopy demonstrated that a small subset of regressed epithelial cells containing secretory granules remains after this period of androgen withdrawal. Upon androgen reconstitution, this cell population of regressed secretory epithelial cells contributes to the recovery of the secretory epithelial compartment of dog prostatic glands by proliferation and differentiation [18,19]. Most likely, the persistent population of AR-positive epithelial cells observed in this study after 3 months of combination endocrine therapy represents the human counterpart of the regressed secretory epithelial cells of the castrated dog prostate. The demonstration of prostate-specific antigen (PSA)-positive glandular cells in benign prostatic glands of similarly pretreated patients [12] is in line with the view that a subset of secretory (PSA-positive) cells persists in the human prostate after androgen withdrawal. Basal cell prominence and hyperplasia commonly observed following combination therapy may be explained in part by the fact that basal cells usually do not express AR and therefore are insensitive to androgen blockade.

In prostatectomy specimens from nontreated patients, a moderate-to-intense, but heterogeneous, expression of AR is observed in the stromal cells. Ultrasonographic studies revealed a decrease in prostatic volume as a consequence of androgen ablation therapy [20]. This reduction in prostatic size in hyperplastic prostates was largely attributed to a decrease of the epithelial compartment [9,16]. Microscopically, the stromal compartment of the human prostate seems not to be affected by the androgen blockade. Our results suggest that a considerable fraction of stromal cells is not sensitive to androgen ablation with respect to AR expression. Although a tendency of downregulation of AR expression in the stromal compartment after total androgen blockade was observed (Fig. 3), a conclusion could not be reached with confidence due to the considerable heterogeneity of stromal AR expression in the nontreated group. Autologous regulation of AR expression has been demonstrated to occur in nonepithelial cells, e.g., in primary cultures of human genital skin fibroblasts and in an osteosarcoma cell line [21].

The increased heterogeneity of AR expression observed in prostatic adenocarcinoma cells during the regression phase under androgen ablation therapy might be attributed to the predominance of poorly differentiated carcinomas in this group, as compared to the untreated carcinomas. Some previous studies have also described reduced AR expression in poorly differentiated carcinomas of the prostate [1,22]. Nevertheless, progressive endocrine therapy-resistant prostatic carcinomas generally display a uniformly high AR expression [22,23].

From the data obtained in this study, it can be hypothesized that androgen ablation therapy eventually leads to the incomplete elimination of AR-positive nonneoplastic epithelial cells and tumor cells. These residual AR-positive cells seem insensitive to downregulation of AR in an environment largely depleted of androgens. Apparently, androgen ablation does not induce programmed cell death in this AR-positive cell population. It remains to be established whether AR is functionally involved in protection against cell death during androgen ablation therapy.

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