Advances in Brief

The Androgen Receptor Status of Neuroendocrine Cells in Human Benign and Malignant Prostatic Tissue


Departments of Urology [S. Y. N., A. T. K. C., P. A. A.] and Pathology, [P. A. d. S., R. A. M.], the University of Rochester Medical Center, Rochester, New York 14642; and the Ben May Institute, the Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois 60637 [R. A. H., S. L.]

Abstract

Neuroendocrine (NE) cells containing neurosecretory granules, rich in various peptide hormones and biogenic amines, are components of the human prostate epithelium and prostatic adenocarcinomas. Neuroendocrine differentiation in prostatic adenocarcinomas has been associated with a poor prognosis and, following androgen withdrawal therapy, tumor cell populations have been observed to become enriched with NE cells. We assessed androgen receptor (AR) expression in NE cells in benign and malignant prostatic tissue using double-labeling immunocytochemistry with validated monoclonal antibodies to the AR and to chromogranin A (a generic NE marker). Neuroendocrine cells in benign and malignant prostatic tissue generally showed nuclear staining with AR. Some distinct AR-negative nuclei were observed in normal NE cells. In prostatic adenocarcinomas with extensive NE differentiation, a subpopulation of AR-negative NE cells was demonstrated. In conclusion, benign and malignant prostatic tissue contain both AR-positive and AR-negative NE cells that may have significance in regards to androgen-independent tumor growth and tumor progression.

Introduction

NE cells (also known as amine precursor uptake decarboxylase or endocrine-paracrine cells) constitute, in addition to the basal and secretory exocrine cells, a third population of highly specialized epithelial cells in the prostate gland (1). These NE cells contain, and most likely secrete, serotonin and a variety of peptides (1). Although little is known of the function of NE cells, they probably play a role in the growth and differentiation of the prostate and the regulation of prostatic secretion through a variety of mechanisms and cell types (1). Chromogranin A is a secretory granule matrix glycoprotein found throughout the NE system as well as in prostatic NE cells, and it is a useful marker for NE differentiation (2, 3). Neuroendocrine differentiation in prostatic carcinoma has recently gained considerable recognition (1), particularly in light of the suggestion that tumors exhibiting marked NE differentiation may be selected for by androgen deprivation (2, 4). In fact, recent literature supports a link between NE differentiation, tumor progression, and androgen-independent prostatic carcinomas (2, 4, 5). If the NE component of aggressive carcinomas is responsible for resistance to hormonal therapy, it may be that AR is not expressed by these NE cells (1, 2). In order to study the relationship between NE cells and androgens, our laboratory sought to clarify the AR status of NE cells in benign and malignant human prostatic tissues.

Materials and Methods

Patients and Histopathological Techniques. Benign prostatic tissue was obtained at operation from two patients with urinary obstruction undergoing open enucleation. The patients' ages were 57 and 65 years, respectively. In each case, at least five specimens, containing hyperplastic nodules, were taken. Malignant prostatic tissue and adjacent normal prostatic parenchyma were obtained from 10 patients, mean age 66 years and ranging 52–72 years, with clinically localized carcinoma of the prostate treated with radical prostatectomy. None of the patients received any hormonal therapy prior to radical prostatectomy.

In each case histopathological diagnosis of the prostatic tissue specimens (hematoxylin and eosin slides) and the immunohistochemically stained sections were reviewed by two independent observers. Tissues were fixed using the Amex method (5) in order to best preserve antigens for immunostaining.

Antigens and Antibodies. The monoclonal antibody AN1-15 which binds to the AR has been described previously (7). This antibody was raised against a fusion protein, produced in bacteria, containing 242 amino acids of the AR amino terminal domain adjacent to the DNA-binding domain (8). This antibody appears to recognize an epitope immediately adjacent to the receptor DNA-binding domain (8). Immunoprecipitation assays have verified that this antibody is specific to AR complexes either prepared from rat ventral prostate or synthesized by transcription of rat and human AR complementary DNAs and translation of the AR mRNA product. Immunoprecipitation of glucocorticoid receptor complexes of rat liver, estrogen receptor complexes of rat breast tumor MCF-7 cells, and progesterin receptor complexes were not significant.

Commercially available murine monoclonal antibodies to chromogranin A (LK2H10) from the Hybritech Corporation were used for double immunostaining.

Immunohistochemistry. Androgen receptor and chromogranin A were localized in the same tissue sections via double-immunostaining using streptavidin horseradish peroxidase enzyme conjugate and streptavidin-alkaline phosphatase enzyme conjugate as follows. Tissue was deparaffinized using consecutive rinses of xylene, acetone, and phosphate-buffered saline. The slides were overlaid with normal rabbit serum (1:20), incubated at 25°C for 20 min, and drained. Each slide was incubated overnight at 4°C with AR antibody AN1-15 (University of Chicago; 1:30). After each step the tissue was rinsed in phosphate-buffered saline. A biotinylated rabbit anti-rat IgG antibody (1:200) and alkaline phosphatase enzyme conjugate were used for double immunostaining. The slides were covered with Naphthol AS-BI phosphate and naphthol AS-BI phosphate enzyme conjugate, incubated at 25°C for 30 min at 25°C, respectively. Using chromogen 3-amino-9-ethylcarbazole the immune complexes were visualized. Next, following distilled water rinse and phosphate-buffered saline, horse serum was added for 20 min at 25°C. Chromogranin A antibody (from Hybritech Corp.) (1:20,000) was then incubated overnight at 4°C. A horse anti-mouse IgG antibody (1:200) and alkaline phosphatase enzyme conjugate (1:50) were added and incubated for 30 min at 25°C, respectively. Using chromogen 3-amino-9-ethylcarbazole the immune complexes were visualized. Next, following distilled water rinse and phosphate-buffered saline, tissue slides were incubated at 25°C for 30 min at 25°C. Chromogranin A antibody (from Hybritech Corp.) (1:20,000) was then incubated overnight at 4°C. A horse anti-mouse IgG antibody (1:200) and alkaline phosphatase enzyme conjugate (1:50) were added and incubated for 30 min at 25°C, respectively. Finally, Naphthol fast blue chromogen was applied for 30 min at 25°C. The chromogen was prepared by mixing 2 mg of Naphthol AS-MX phosphate, 0.2 ml dimethyldimethanide, 9.8 ml 0.1 M Tris buffer (pH 8.2), and 10 µl 1 M levamisole with 10 mg fast blue BB salt, and this was filtered. A final rinse in tap water was performed and the slides were coverslipped. Primary antibodies were diluted with 0.5% phosphate-buffered saline.
validation. Liquid-phase absorption controls were performed to validate immunohistochemical staining using the oligopeptide Leu–Asp–Ser–Thr–Arg–Asp–His–Val–Leu–Pro–Ile–Asp–Tyr–Tyr–Phe–Pro–Pro–Gln–Lys–Thr–Cys (8). The oligopeptide is included in the amino acids sequence of the AR amino terminal domain used to raise the antibodies (7). Monoclonal antibody AN1-15 was reconstituted and diluted to a concentration of 14 μg/ml. This was incubated with oligopeptide at 28 μg/ml at 4°C for 48 h. The antigen-antibody mixture was then applied in place of the primary antibody to Amex-processed prostate in the same manner as described in “Immunohistochemistry.” Antibody AN1-15 controls were also repeated. The AN1-15 slides showed good staining while slides incubated with the antigen-antibody complex showed complete absence of staining in Amex-processed prostate specimens.

With monoclonal/monoclonal combinations where the primary antibodies are of the same species, cross-reactivity is a theoretical possibility. In any double-staining system there is also the possibility that the streptavidin enzyme conjugate from the second step will react with the biotinylated secondary of the first step. The probable saturation of biotin sites and tight binding of streptavidin to biotin render this unlikely. If the antigens of interest are in different cellular compartments (nuclear versus cytoplasmic) or in distinct and different cell types, any significant cross-reactivity should be readily apparent and we have seen none.

Further validation included staining of Amex-processed human spleen sections which were negative for antibody AN1-15 as previously reported (9). Results

Double immunostaining was performed on multiple sections of Amex-processed human prostate from two cases of benign prostatic hyperplasia and multiple sections of 10 cases of carcinoma of the prostate. Immunostaining with the monoclonal antibody AN1-15 to the AR and murine monoclonal antibodies to chromogranin A (LK2H10) was performed on separate sections as well. The use of Amex-processed sections allows for excellent immunostaining (similar to frozen section), better preservation of tissue morphology, and easier handling of tissue and blocks.

Immunostaining of the Androgen Receptor. In all sections AR was uniformly distributed among the nuclei of secretory epithelial cells (nuclei stained orange-red). In the basal cell layer of the epithelium, the majority of cells did not show any nuclear staining for the AR. The AR expression in the nuclei of stromal cells was evident but more variable. The overall background was negligible (see Fig. 1).

Immunostaining of Neuroendocrine Cells. Chromogranin A immunoreactive cells were identified in every section of prostate in the epithelial lining of the acini and the ducts. Although sporadic in distribution, numerous areas had multiple NE cells with typical dendritic processes.

Localization of Androgen Receptor in Neuroendocrine Cells in Benign Prostatic Tissues. Fig. 2 depicts a cluster of double-immunostained NE cells, (cells with blue cytoplasm) with predominately AR-positive nuclei in benign prostatic tissue. Normal NE cells generally showed nuclear staining with AR similar to other secretory epithelial cells. An occasional NE cell showed a distinct lack of AR staining despite staining of other adjacent epithelial cells. The classic appearance of a NE cell in the basal portion of a prostatic duct with dendritic processes is depicted positive with nuclear staining for the AR (see Fig. 3).

Localization of Androgen Receptor in Neuroendocrine Cells in Carcinoma of the Prostate. All 10 adenocarcinomas of the prostate showed focal NE differentiation and the majority of NE tumor cells stained positively for AR, i.e., approximately 95% of the total NE tumor cell population. In three prostatic carcinomas with more extensive NE differentiation, a subpopulation of the NE tumor cells showed no nuclear staining for AR. Fig. 4 depicts a region of carcinoma with
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Discussion

In the present study we used a validated monoclonal antibody (AN1-15) that appears to recognize an epitope immediately adjacent to the DNA-binding domain of the AR (8). Immunostaining of the AR in AMEX-fixed prostatic tissue was superior to frozen section results previously reported in the literature. Specifically, our sections demonstrated better preservation of tissue morphology and a more distinct staining of the nuclei with negligible background. As reported by others (10, 11), the AR was present in the nuclei of the secretory epithelial cells and mainly absent in the basal cell layer. The AR status of the neuroendocrine cells in the prostate or in other NE organ systems, however, has not been described previously. The presence of hormone-dependent and -independent NE cell populations was proposed in a recent study, whereby human prostatic acinar NE cells appeared at puberty while ductal and urethral cells were present throughout the life cycle (12). In addition, we found a marked increase in acinar NE cells in the guinea pig prostate with advancing age whereas those cells in the ducts and urethra remain constant (13). Our present results confirm previous observations that NE cells in benign prostatic tissue are predominantly AR positive. Furthermore, the findings of a subpopulation of AR-negative NE cells supports the concept of a hormone-independent NE cell population. The molecular mechanisms which drive induction and switch off AR expression in the various cell types of the prostate are completely unknown. The AR expression in various types of NE cells and their distribution with respect to zonal anatomy warrant further investigation.

The functional role of prostatic NE cells is unknown. The morphology of these cells, their secretory products, their strategic location, and their similarity to the much better characterized NE cells in other organ systems strongly suggest that these cells are involved in the regulation of growth and differentiation as well as homeostatic regulation of the exocrine secretory process (1, 2). Bilateral stromal-epithelial interactions appear to mediate the action of androgens on epithelial growth with the prostatic epithelium converting testosterone to dihydrotestosterone (via 5α-reductase activity). Through their secretory products, the prostatic NE cells could act as the intermediaries of these stromal-epithelial interactions. Furthermore, serotonin, the most common bioactive product expressed by prostatic NE cells, may be directly involved in the regulation of the AR. Recently, dopamine has been demonstrated to activate steroid hormone receptors in a ligand-independent manner (14), raising the possibility that other biogenic amines, such as serotonin, possess similar activity with the AR.

Immunohistochemical analysis of the expression of the AR in prostate tumor tissue showed that the majority of NE cells were AR positive. Our findings parallels other reports in that essentially all cells in well and moderately differentiated tumor compartments contained AR (11, 15). On the other hand, a subpopulation of NE tumor cells were AR negative in three moderately to poorly differentiated prostatic adenocarcinomas. A highly variable expression of AR in undifferentiated tumor compartments has been reported previously (11, 16, 17). The demonstration of AR-negative NE cells in both benign and malignant prostatic tissue supports the concept that NE differentiation may be involved in androgen-insensitive prostatic adenocarcinomas (1, 2, 4, 5). In fact, the degree of NE differentiation in prostatic adenocarcinomas has been associated with a poor prognosis which may be based on resistance to hormonal manipulation therapy and direct or indirect growth factor activity of neurosecretory products (2, 4, 5). Furthermore, following long-term androgen ablative therapy, tumor cell populations have been observed to become enriched with NE cells (2). A recent report of elevated serum chromogranin A levels in patients with hormone-refractory, metastatic prostate cancer supports the concept that these tumors contain an elevated population of NE cells and that they are not androgen dependent (4).

At present, knowledge of the mechanism of androgen-independent growth of prostate cancer is limited. Traditional theory related to the development of androgen independence of prostate cancer implicates the overgrowth by an androgen-independent subpopulation which is selectively enhanced (18). An important factor affecting the phenotype of prostatic cancer cell subpopulations is the hormonal milieu (17). Because the expression of the NE phenotype is not suppressed by androgen ablation (2, 4, 5), it is possible that loss of AR in cells with NE differentiation or a clonal propagation of AR-negative NE cells may lead to androgen insensitivity either by an overgrowth of these cells or by the unregulated secretion of neurosecretory growth products. In order to further assess the possible role of AR in prostatic NE cells, quantitative and statistical analyses are needed to determine whether the proportion of AR-positive and AR-negative NE cells is significantly different in normal, hyperplastic, and malignant prostatic tissue as well as various degrees of tumor differentiation. Finally, using antibodies to AR and NE markers, a morphometric and quantitative image analysis may clarify whether androgen- ablative therapy favors the NE phenotype in prostatic carcinomas.

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References


Fig. 4. Double immunostaining of androgen receptor and chromogranin A in carcinoma of the prostate. This is a region of carcinoma with focal NE differentiation. Note the malignant appearance of the NE cells and the presence of androgen receptor-negative nuclei in several of the NE cells (arrows) X 124.
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