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Androgen receptor status in endocrine-paracrine cell types of the normal, hyperplastic, and neoplastic human prostate

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Abstract. Neuroendocrine differentiation is a frequent occurrence in common prostatic adenocarcinomas and may have prognostic implications in prostatic malignancies. In the present study, we used immunohistochemical double label methods to evaluate the nuclear androgen receptor (AR) status in endocrine-paracrine (EP) cells of normal, hyperplastic, and neoplastic prostate including tumours that recurred after hormonal and radiation therapy. In normal and hyperplastic glands, EP cells characterized by the panendocrine marker chromogranin A (Chr A) did not reveal AR-positivity. This may indicate that prostatic EP cells represent an androgen-indepenent cell population whose regulatory functions are not influenced by circulating androgens. Unequivocal co-expression of Chr A and AR was very rarely detected in subsets of endocrine differentiated tumour cells in treated and untreated specimens. The widespread absence of nuclear AR in neuroendocrine tumour cells suggests that this phenotype belongs to those cell clones in prostate cancer which are initially androgen-independent and refractory to hormonal therapy.

Key words: Androgen receptor – Chromogranin A – Double label methods – Prostate – Prostate cancer

Introduction

Prostatic epithelium contains a large number of endocrine-paracrine (EP)-cells that have become recognized as a third epithelial cell type along with secretory luminal and basal cells (Di Sant'Agnese and de Mesy Jensen 1984; Di Sant'Agnese et al. 1985; Abrahamsson et al. 1986; Di Sant'Agnese 1992). The nuclear androgen receptor (AR) is expressed predominantly in secretory luminal cells (Masai et al. 1990; Ruizeveld de Winter et al. 1990; Bonkhoff and Remberger 1993) and was also detected recently in basal cell types (Bonkhoff and Remberger 1993). The AR status in prostatic EP-cells is currently unknown. Neuroendocrine (NE) differentiation occurs to a variable degree in virtually all prostatic adenocarcinomas and may correlate with a high histological grade and poor prognosis (Abrahamsson et al. 1987; Dauge and Delmas 1987; Di Sant'Agnese 1988; Bonkhoff et al. 1991; Di Sant'Agnese 1992). Although formal proof is still lacking, there is some evidence to suggest that neoplastic cells of the endocrine phenotype are androgen-unresponsive and thus refractory to hormonal therapy (Di Sant'Agnese 1988, 1992).

To obtain more insight in the fundamental implication of NE-differentiation in the prostate and prostatic malignancies we evaluated the AR-status in endocrine cell types. Immunohistochemical double staining was performed for simultaneous demonstration of nuclear AR and the panendocrine marker chromogranin A (Chr A) in prostatic tissue and prostatic adenocarcinomas showing variable degree of NE-differentiation.

Materials and methods

The material comprised 11 total prostatectomies resected for prostate cancer and 18 transurethral resections for urinary flow obstruction including 8 carcinomas following hormonal and radiation therapy. The material from prostatectomy specimens and palliative transurethral resections were available as formalin-fixed, paraffin-embedded blocks and as fresh tissue samples which were quick-frozen in liquid isopentane at −80° C until sectioning. Histological evaluation revealed 22 areas of nodular hyperplasia, 13 areas of normal acini and 19 pluriform adenocarcinomas. The cancerous lesions included anaplastic, cribriform and glandular grade III ($n=14$) and 5 grade II tumours graded according to Böcking (1981).

A mouse monoclonal antibody F39.4.1 directed against the 110 kDa human AR was provided by Sanbio (Uden, Netherlands). Specificity of F39.4.1 for the human AR was established by immunoprecipitation, immunocomplex density-gradient centrifuga-

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tion and immunohistochemistry on human prostate tissue (Zegers et al. 1991). The immunohistochemical demonstration of AR in frozen sections was performed by the peroxidase antiperoxidase (PAP)-method (Dako, Hamburg, Germany), as described in detail previously (Bonkhoff and Remberger 1993). In formalin-fixed, paraffin-embedded tissue, the slides were pretreated with microwave irradiation (600 W for 5 min and 450 W for 5 min) prior to the application of the primary antibody. Detection was achieved by a biotinylated rabbit-to-mouse antibody (Dako) and the streptavidin-biotin-complex (Dako) using the 3-3-diaminobenzidine (DAB)-nickel complex (Bonkhoff et al. 1991) as chromagen. Negative controls were performed by omitting the primary antibody which showed no immunostaining.

To demonstrate NE-cell types we used the mouse monoclonal antibody against Chr A (Dako) which was detected by the avidin-biotin-complex method (Dako) and DAB as chromagen. Double labelling techniques for sequential immunohistochemical demonstration of Chr A were previously described (Bonkhoff et al. 1991).

The extent of NE differentiation in carcinomas was graded on a scale of + to + + + : weak (+): few scattered Chr A-positive cells; moderate (+ +): up to 10 areas with numerous Chr A-positive cells/microscopic field using the ×40 objective; strong (+ + +): over 10 areas with numerous Chr A-positive cells/microscopic field using the ×40 objective.

Results

In freshly dissected, formalin-fixed and paraffin-embedded material the immunolocalization of nuclear AR by the F31.3.1 antibody yielded similar results as those obtained in corresponding frozen tissue (Figs. 1, 2). Only slided showing intense staining reaction were considered for the comparative evaluation of nuclear AR in endocrine and exocrine cell types.

In normal and hyperplastic glands, the nuclear AR was expressed predominantly in secretory luminal cell types and stromal nodules, whereas the basal cell layer showed weak receptor immunoreactivity. Chr A-positive cells were irregularly distributed throughout the ducts and acini and were sometimes arranged in small clusters, as observed in prostatic ducts and hyperplastic foci. In contrast with other epithelial cell types, which showed widespread distribution of nuclear AR, all EP-cells characterized by Chr A did not reveal detectable AR-reactivities, either in formalin-fixed material or in frozen sections (Fig. 1).

The prostatic adenocarcinomas investigated showed a variable degree of NE-differentiation. Untreated specimens included 2 cases with weak (+), 3 cases with moderate (+ +) and 6 cases with strong (+ + +) NE-differentiation. In carcinomas that relapsed after orchiectomy and prostatic radiation, we found one case with weak (+), 3 cases with moderate (+ +) and 4 cases with strong (+ + +) endocrine features. Irrespective of the extent of NE-differentiation, all carcinomas investigated showed widespread nuclear positivity for AR in non-endocrine (Chr A-negative) tumour cells (Fig. 2). Conversely, unequivocal coexpression of AR and Chr A in identical cells were very rarely detected in subsets of tumour cells (Fig. 2a). In both treated and untreated specimens, virtually all endocrine differentiated cells were receptor-negative (Figs. 2). The same observations were made in frozen sections (Fig. 2b).

Discussion

Among the three epithelial cell types encountered in the prostatic epithelium (secretory, basal and EP-cells), the secretory luminal phenotype requires continuous support by androgens for its maintenance (Aumüller 1983; English et al. 1987; Isaacs and Coffey 1989). Recent data on the immunolocalization of AR in the prostatic epithelium indicated that basal cells may also be androgen-responsive (Bonkhoff and Remberger 1993).

The results of the present study show that prostatic EP-cells characterized by the panendocrine marker Chr A do not reveal detectable AR-immunoreactivity. The consistent absence of the receptor clearly suggests that EP-cells represent an androgen-independent population in the normal and hyperplastic prostate. These cells may function by endocrine, paracrine, neurocrine and lumencrine mechanisms and play an important role in the homeostatic regulation of the gland (Di Sant'Agnese 1992). Recent data using double labelling methods for Chr A and the proliferation-associated Ki-67 antigen indicated that EP-cells may be involved in controlling cell proliferation through a paracrine mechanism (Bonkhoff et al. 1991). Although the biological significance of prostatic EP-cells is not yet fully understood, there is now some evidence to suggest that their regulatory functions are not influenced by androgens.

Identification of androgen-responsive or androgen-independent cell clones in tissue sections would be very useful in the prognostic evaluation of prostatic cancer. Data obtained from AR-binding assays and immunohistochemical investigations of the receptor do not allow a prediction of the response to androgen withdrawal therapy and the time of progression to androgen independence (Gorelic et al. 1987; Kwast et al. 1991; Sadi et al. 1991). In fact, tumour biopsies from patients with clinical androgen-independent disease may reveal strong AR-positivity (Kwast et al. 1991). Thus, identification of nuclear androgen receptor does not imply that these cells are necessarily androgen-responsive. Conversely, absence of nuclear AR may be strongly suggestive for an androgen-independent status. In the present study, we evaluated the differential expression of nuclear AR in NE and exocrine cell types in common prostatic carcinomas. The great majority of endocrine cell types lack AR-reactivity in both untreated and treated carcinomas. Conversely, all carcinomas investigated showed widespread receptor positivities in exocrine (Chr A-negative) cell types. The latter findings basically agree with recent data on the AR-status in primary and treated carcinomas reported by other investigators (Ruizeveld de Winter et al. 1990; Kwast et al. 1991).

The absence of detectable AR in Chr A-positive tumour cells clearly suggests that endocrine differentiated cell types belong to those cell clones in prostate cancer which are initially androgen-independent and refractory to hormonal therapy. NE-differentiation in prostate cancer generally portends a high histological grade and a poorer prognosis of the tumour (Dauge and Delmas 1987; Di Sant'Agnese 1988, 1992). Thus, the androgen-insensitive state of the endocrine phenotype may account

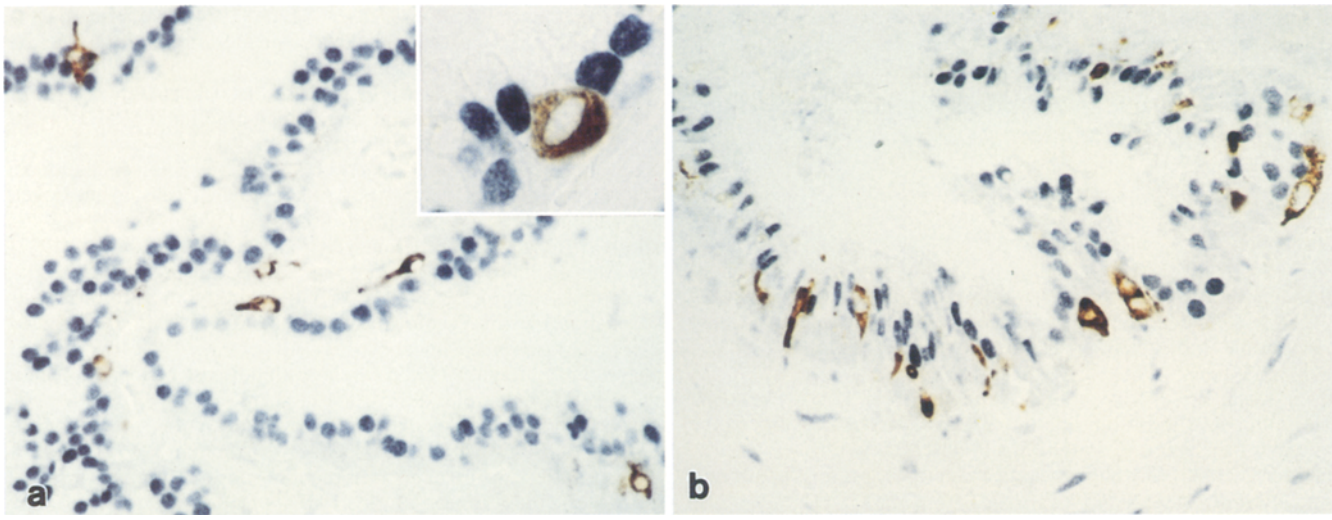

Fig. 1. Normal ducts (**a**, ×200, insert ×1000) and hyperplastic gland (**b**, ×1000). Chromogranin A-(Chr A)positive cells (*brown*) lack androgen receptor (AR)-reactivity (*black*)

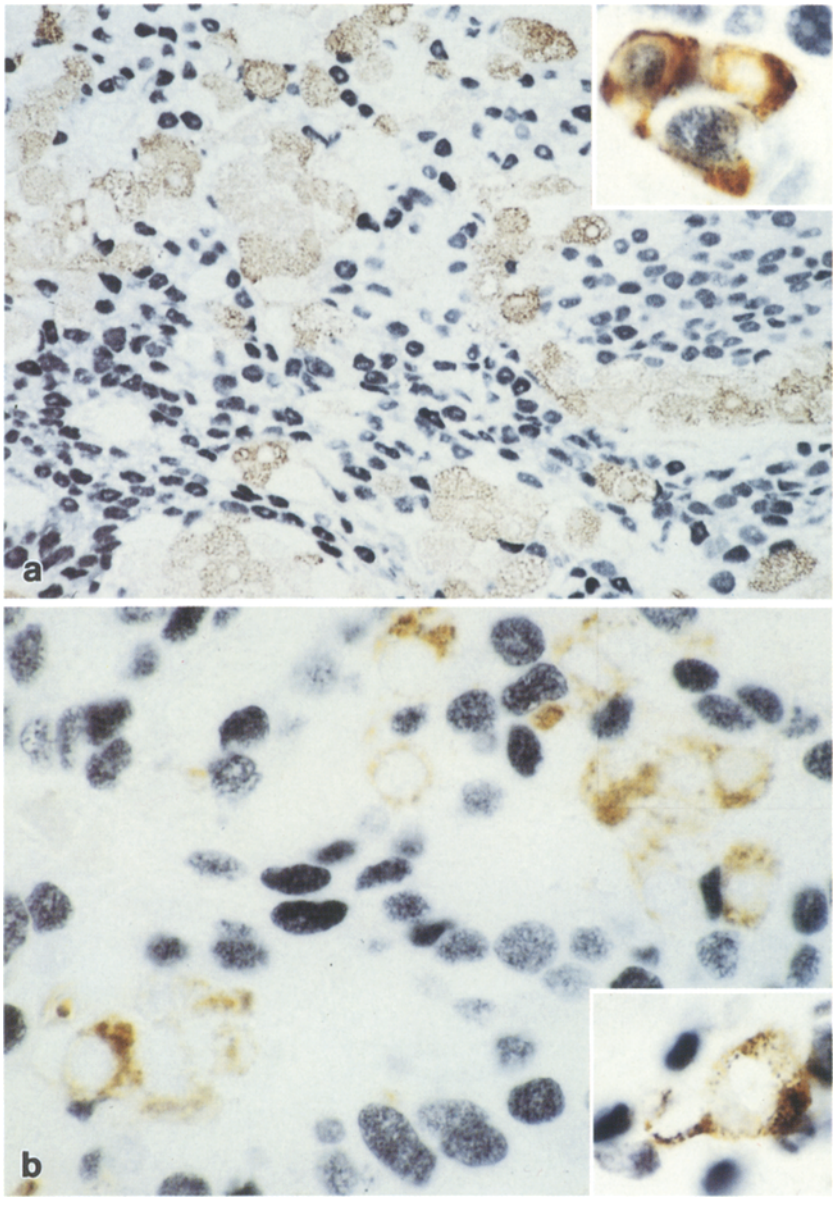

Fig. 2. Treated (**a**, ×200) and untreated (**b**, ×1000) carcinomas with numerous Chr A-positive tumour cells. In contrast to non-endocrine cell types, virtually all Chr A-positive cells lack AR in formalin-fixed and frozen sections (insert **b**, ×1000). Unequivocal co-expression of Chr A and AR in identical cells is rarely detected (insert **a**, ×1000)

for the aggressive behaviour of prostatic malignancies with increased NE-features.

Screening for neuroendocrine differentiation may therefore be valuable in the prognostic and therapeutic evaluation of prostate cancer.

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