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Widespread distribution of nuclear androgen receptors in the basal cell layer of the normal and hyperplastic human prostate*

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Summary. The role of the basal cell layer in organogenesis, epithelial renewal and development of benign prostatic disorders is largely unknown. The objective of the present study was to investigate whether or not basal cells express the nuclear androgen receptor (AR). Computer-assisted image analyses of immunohistochemical double stainings were performed to localize AR and basal-cell-specific cytokeratins in identical sections. The results showed that the basal cells express nuclear AR widely in normal and hyperplastic conditions. When compared with the staining intensities detected in secretory luminal cells, the receptor was most frequently expressed at lower levels in basal cells, which may exhibit strong AR immunoreactivity focally. Basal cells with increased AR expression were most frequently detected in hyperplastic lesions including post-atrophic and atypical hyperplasia. The presence of nuclear receptors for both androgens and oestrogens or progestins in basal cells may indicate that these cells are targets of the hormonal imbalance which has frequently been implicated in the aetiology of benign prostatic hyperplasia.

Key words: Prostate – Hyperplasia – Androgen receptor – Basal cells

Introduction

The normal and hyperplastic prostate epithelium is composed of two different cell types that clearly differ in their ultrastructural features and differential expression of cytokeratins (Wernert et al. 1987; Hedrich and Epstein 1989). The luminal or glandular cells are polar and highly differentiated cells that are functionally active in the synthesis and elaboration of prostatic secretion (Brandes 1966; Dahl et al. 1973; Fickinger 1974). In contrast, the basal cells are undifferentiated epithelial cells whose function remains largely unknown, although a role of putative stem cells has frequently been suggested (Fickinger 1971; Dermer 1978; Bazer 1980; Trump et al. 1981; Heatfield 1982; Merchant et al. 1983). There is also increased evidence that both cell types undergo different hormonal regulation. The secretory epithelium is composed of androgen-dependent cells as documented by their sensitivity to androgen withdrawal and the presence of nuclear androgen receptors (AR) (Isaacs and Coffey 1989; Masai et al. 1990; Ruizeveld de Winter et al. 1990). Conversely, the basal cells proliferate under oestrogen therapy or androgen ablation and express nuclear receptors for oestrogen and progestins (Wernert et al. 1988; Mobbs and Lin 1990). To date, no data are available which link the two cell types unequivocally in a precursor-progeny relationship or in a functional sense (English et al. 1987; Evans and Chandler 1987).

The objective of the present study was to investigate whether or not basal cells may express nuclear AR. For this purpose immunohistochemical double stainings were performed to demonstrate AR and basal-cell-specific cytokeratins in identical sections of normal and hyperplastic prostate tissue. The presence of AR in basal cells was confirmed by analysing the results of immunohistochemical double staining using computer-assisted image processing.

Materials and methods

The material comprised ten prostatectomy specimens resected for prostate cancer and five cystoprostatectomies which were removed for bladder cancer. Several tissue samples were collected from non-neoplastic lesions which were quick frozen in liquid isopenthane at −80° C until sectioning. Several sections of 4–6 µm thickness were cut, air-dried for 30 min, fixed in paraformaldehyde (4%) for 10 min and stained immediately. The histological examination revealed 12 areas of nodular hyperplasia, 8 areas of normal acini, atrophic glands ($n=8$), basal cell hyperplasia ($n=4$), postatrophic hyperplasia ($n=3$) and atypical adenomatous hyperplasia ($n=3$).

The mouse monoclonal antibody F 39.4.1 directed against the 110,000 kDa human AR was provided by Sanbio (Uden, The Neth-

* This study is dedicated to Professor Dhom on the occasion of his 70th birthday

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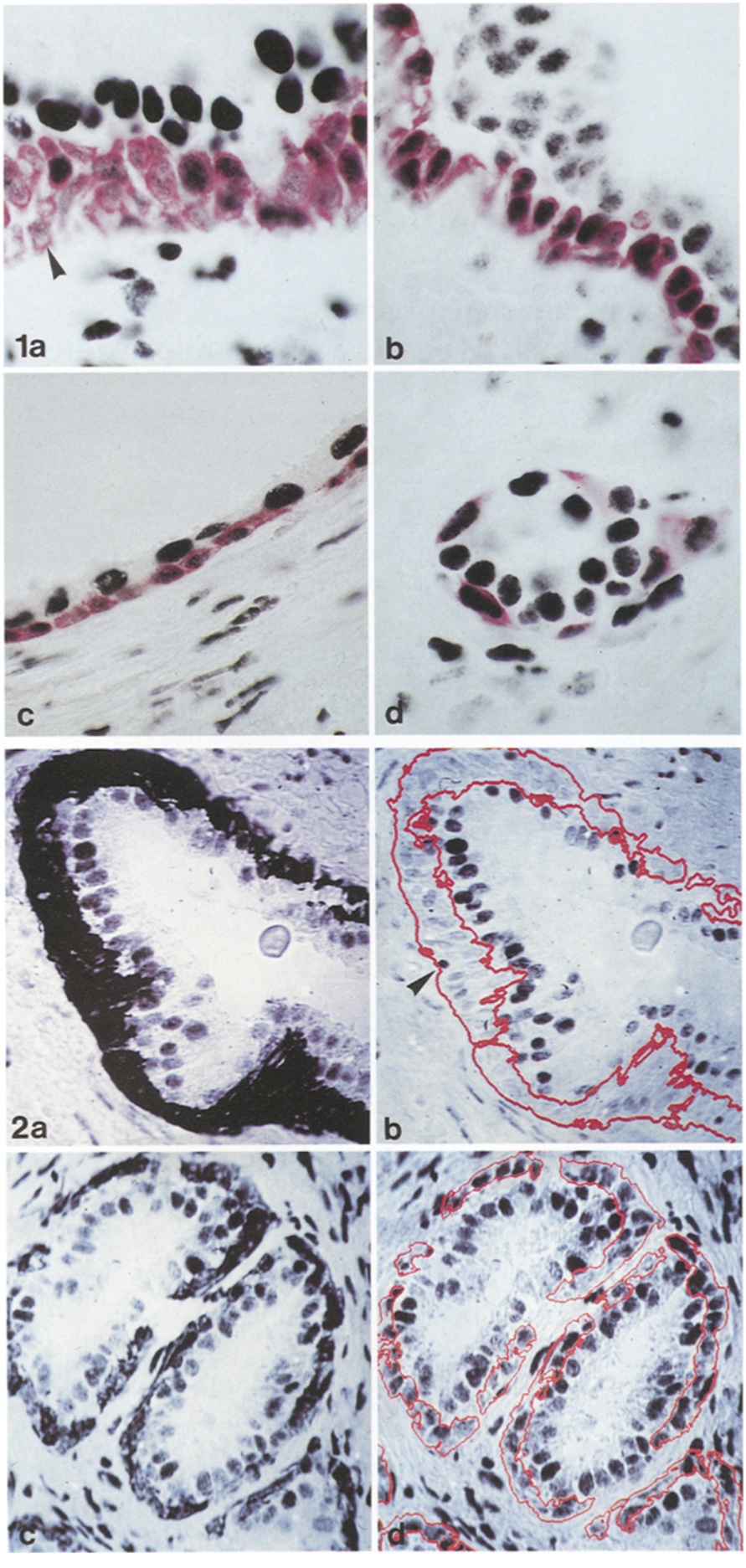

Fig. 1a–d. Simultaneous immunohistochemical demonstration of nuclear androgen receptors (AR) (*black*) and basal-cell-specific cytokeratins (*red*). ×400. **a** Normal gland. When compared with secretory luminal cells, most basal cells show decreased reactivity for nuclear AR (*arrow*). Note strong nuclear positivities in some basal cells. **b** Hyperplastic gland. The AR is more reactive in basal cells than in the secretory epithelium. **c** Atrophy. AR reactivity is detected in both cell compartments. **d** Post-atrophic hyperplasia. Note the discontinuous pattern of basal-cell-specific cytokeratins. Strong receptor expression is detected in both cell compartments

Fig. 2a–d. Evaluation of AR expression in the basal cell layer using computer-assisted image processing. Simultaneous immunohistochemical demonstration of AR and basal-cell-specific cytokeratins (**a, c**) whose localization is indicated by the red contour in the final images (**b, d**). Prostatic gland with slight basal cell hyperplasia (**a, b**). Weak nuclear positivities are detected in basal cells. Compared to the staining intensities in luminal cells, only three basal cells exhibit similar nuclear positivities (*arrow*). Atypical adenomatous hyperplasia (**c, d**). Note the focal absence of cytokeratins. Both cell compartments show strong receptor reactivities. ×366

erlands). Specificity of F 39.4.1 for the human AR was established by immunoprecipitation, immune-complex density-gradient centrifugation and immunohistochemistry on human prostate tissue (Zegers et al. 1991). The antibody was incubated overnight at 4° C in a dilution of 1:10. Detection was achieved by a rabbit anti-mouse antibody (Dakopatts, Hamburg, FRG) and a peroxidase anti-peroxidase (PAP) complex from the mouse (Dakopatts). To intensify the immunoreaction, the linking antibody and the PAP complex were applied once again for 10 min each, as recently described (Bonkhoff et al. 1991). Antigen-antibody-binding complexes were visualized by either 3-3-diaminobenzidine (DAB) (Sigma, Deisenhofen, FRG) or the DAB-nickel complex, as recently described (Bonkhoff et al. 1991). Negative controls were performed by omitting the primary antibody, which showed no immunostaining.

To demonstrate the basal cell layer we used the mouse monoclonal antibody 903, which is specific for higher-molecular-weight cytokeratins 5, 10, 11 (Enzo Diagnostic, Neckargmünd, FRG). This antibody reacts with the basal cell layer but not with luminal secretory cells. The antibody was inducated for 1.5 h at room temperature. Detection was achieved by a rabbit to mouse antibody (Dakopatts), the alkaline phosphatase anti-alkaline phosphatase complex (Dianova, Hamburg, FRG) and New Fuchsin (Sigma) as substrate (Fig. 1). For computer-assisted image processing, the 903 antibody was detected by the biotinylated rabbit to mouse antibody, the avidin biotin complex mouse kit (Dakopatts) and 3-amino-9-ethylcarbazole (AEC) as chromagen (Dakopatts). The double label procedures were described in more detail previously (Bonkhoff et al. 1991).

The results of sequential immunohistochemical demonstrations of AR and cytokeratins were stored as digitized grey images (first object image) using the image analyser IPS (Kontron, Munich, FRG) (Fig. 2a, c). After documentation, the AEC complex of the cytokeratin staining was removed by dehydrating and rehydrating the slides in graded alcohols. The resulting preparation was exactly positioned and stored in a digitized form (second object image). In the first object image, the cytokeratin-specific staining was discriminated and transduced into a binary image. The contour of this binary image was copied into the second object image and overlaid with an arbitrarily chosen colour. In the final image (Fig. 2b, d), the content of the contour represented the localization of basal-cell-specific cytokeratins. The computer-assisted image processing was described in more detail previously (Bonkhoff and Wernert 1989).

Results

Sequential immunohistochemical demonstrations of AR and basal-cell-specific cytokeratins revealed that the basal cell layer widely exhibited nuclear positivities for AR as well as in the normal and various hyperplastic conditions (Fig. 1). The presence of nuclear AR in basal cells was confirmed by analysing the results of double staining by computer-assisted image processing (Fig. 2).

We consistently attempted to compare the immunoreactivities of AR detected in basal cells with those in secretory luminal cells. Although more precise, computer-assisted image processing allows the screening of only relatively small areas. Therefore, we evaluated the differential expression of AR in both cell populations in conventional double label preparations. Compared with staining intensities detected in luminal cells, basal cells most frequently exhibited decreased AR reactivity in normal and hyperplastic glands. In normal acini, only a few basal cells showed similar or increased immunoreactivity compared to the corresponding luminal cells (Figs. 1a, 2b). Atrophic glands focally showed strong receptor positivities in the basal cell layer (Fig. 1c). In nodular hyperplasia, most hyperplastic acini predominantly expressed AR in luminal cells, although some glands were more reactive in the basal cell layer than in the secretory epithelium (Fig. 1b). In post-atrophic and atypical adenomatous hyperplasias, strong immunoreactivities were found in both cell compartments (Figs. 1d, 2d).

Discussion

The basal cell layer is an integral part of the prostate epithelium, but its role in organogenesis and its pathophysiological significance in benign prostatic hyperplasia are largely unknown. The potential stem cell function of basal cells in the development of the prostatic epithelium has been suggested frequently (Fickinger 1971; Dermer 1978; Bazer 1980; Trump et al. 1981; Heatfield 1982; Merchant et al. 1983), but not invariably accepted (English et al. 1987; Evans and Chandler 1987; Aumüller 1983). There are several lines of evidence indicating that basal and glandular cells are independent and separate entities which undergo different hormonal regulation. Androgen ablation promotes atrophy or loss of glandular cells, whereas basal cells are maintained (English et al. 1987; Isaacs and Coffey 1989). Furthermore, both cell types express different steroid receptors. Oestrogen and progesterone receptors are localized solely in the basal cell layer, whereas AR was exclusively found in luminal cells (Wernert et al. 1988; Mobbs and Lin 1990; Ruizeveld de Winter et al. 1990; Zegers et al. 1991).

According to our results, the basal cell layer widely expresses nuclear AR in the normal and various hyperplastic conditions. The discrepancy between our results and those of other authors (Ruizeveld de Winter et al. 1990; Zegers et al. 1991) is most probably related to differences in staining procedures. Furthermore, we performed immunohistochemical double staining to identify AR reactivities in basal cells, which may be difficult to distinguish from adjacent luminal or stromal cells in cryostat sections. When compared with the staining intensities detected in secretory luminal cells, the receptor was most frequently expressed at lower levels in basal cells. Nevertheless, similar or increased staining intensities were noted in subsets of basal cells which were most frequently observed in areas of nodular hyperplasia, atypical adenomatous and post-atrophic hyperplasia.

The presence of AR in basal cells does not necessarily imply that they are androgen-responsive. Experimental data from orchiectomized rats indicated that most of the basal cells are androgen-independent (English et al. 1987). Thus, the larger population of basal cells expressing AR at low levels are most likely androgen-independent because the pertinent receptor is probably down-regulated and does not mediate androgen-effects. However, subsets of basal cells apparently over-express their receptors and thus may become androgen-responsive.

This, in turn, may induce differentiating processes into secretory luminal cells.

In the aging male, an imbalance of oestrogens and androgens has often been cited as a possible cause of benign prostatic hyperplasia (for review, see Walsh 1989). The anti-gonadotrophic effect of progestins would also modulate the androgenic influence on the prostate, but progestational anti-androgens also interact directly with the AR (Nicholson et al. 1986). Nuclear receptors for both oestrogen and progesterone are only expressed in the basal cell layer as well as in normal and hyperplastic conditions (Wernert et al. 1988; Mobbs and Lin 1990). Together with the findings of the present study, it seems likely that oestrogens, progestins and androgens act upon the same cells. Considering that most of basal cells are insensitive to androgenic stimulation in normal conditions, one may speculate that oestrogens or progestins balance the androgen effect on basal cells by down-regulating the pertinent receptor. Alternatively, the age-related imbalance of steroid hormones may result in an up-regulation of AR in basal cells which, in turn, may increase their propensity to differentiate into secretory luminal cells.

On the basis of this hypothetical pathway of differentiation we may speculate that the abnormal growth of the secretory epithelium is at least in part due to an increase in the total number of androgen-responsive basal cells. Accordingly, the altered hormonal control of basal cells may represent one of the multi-step processes by which benign prostatic hyperplasia develops from normal prostate.

Irrespective of possible explanations, the presence of AR in the basal cell layer may shed new light on the functional implications of basal cells in organogenesis and development of benign disorders of the human prostate.

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