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Usefulness of immunoperoxidase staining with high-molecular-weight cytokeratin in the differential diagnosis of small-acinar lesions of the prostate gland

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Summary. There are a number of benign, small-acinar lesions in the prostate gland that may be difficult to differentiate from small-acinar adenocarcinoma. An important diagnostic criterion in this differentiation is the loss of the basal layer in small-acinar adenocarcinoma and its preservation in benign conditions. A monoclonal antibody to high-molecular-weight cytokeratins (34βE12) has been shown to stain these basal cells preferentially. To assess the usefulness of this antibody in distinguishing benign from malignant small-acinar lesions, we examined 21 cases of small-acinar adenocarcinoma and 47 examples of benign lesions, which included atypical adenomatous hyperplasia, atrophy, post-sclerotic hyperplasia, basal cell hyperplasia, and fibroepithelial nodule. Positive staining with 34βE12 was seen in 13/13 cases of atypical adenomatous hyperplasia, although in some cases the staining was weak and focal. Positivity with 34βE12 was also demonstrated in all other benign lesions studied. All 21 cases of small-acinar adenocarcinoma showed no reactivity with 34βE12. The results suggest that 34βE12 is of value in distinguishing between well-differentiated, small-acinar prostatic adenocarcinoma and its mimics. However, care is needed in interpretation of staining in formalin-fixed material due to the variable reactivity, particularly in cases of atypical adenomatous hyperplasia.

Key words: Prostatic basal cells – Prostatic adenocarcinoma – High-molecular-weight cytokeratin

Introduction

The diagnosis of well-differentiated adenocarcinoma of the prostate may be extremely difficult in routine histological material. This is important as prostatic adenocarcinoma is the second-most common malignancy in adult males and a major cause of death in middle-aged and elderly men (Silverberg 1986). It is frequently well-differentiated and has a small-acinar growth pattern. A number of benign lesions mimic this small-acinar pattern and therefore must be differentiated from adenocarcinoma. An important diagnostic criterion in this differentiation is the selective loss of the basal cell layer in adenocarcinoma (Totten et al. 1953; Mostofi et al. 1980; Gleason 1985; Kovi 1985; Murphy 1989). Recently it has been noted that an antibody to high-molecular-weight cytokeratins preferentially stains this basal cell layer (Brawer et al. 1989; Nagle et al. 1987).

The purpose of our study was to determine whether this antibody would have practical usefulness in distinguishing benign from malignant small-acinar lesions of the prostate.

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Materials and methods

Sixty-eight cases were selected from the surgical pathology files at Victoria Hospital, London, Ontario. All material was formalin-fixed and paraffin-embedded and included tissue from radical prostatectomy specimens, transurethral resections, and needle biopsies. Cases were selected on the basis of their being classical examples of the various entities described. Cases in which the diagnosis was not certain on histological criteria were excluded.

Twenty-one cases of small-acinar adenocarcinoma were examined, together with 16 cases of atrophy, 13 cases of atypical adenomatous hyperplasia, 5 cases of post-sclerotic hyperplasia, 11 cases of basal cell hyperplasia, and 2 examples of fibroepithelial nodule.

Antibodies utilized in the study were anti-high-molecular-weight cytokeratin (34βE12, Enzo Biochemicals, New York, NY), anti-cytokeratin (AE1/AE3, Hybritech Boehringer-Mannheim, Indianapolis, Ind.), anti-prostate specific antigen (PSA, Dako Corp., Santa Barbara, Calif.) and anti-prostatic acid phosphatase (PAP, Dako Corp.).

All tissue was deparaffinized with xylene and rehydrated in decreasing concentrations of alcohol. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in absolute methanol for 7 min. All reactions were carried out at room temperature. Immunoreactivity with cytokeratin antibodies was enhanced by predigestion with 0.4% pepsin at 37° C for 17 min. Immunohistochemical staining was performed using the avidin-biotin complex immunoperoxidase method (Hsu et al. 1981).

Results

All normal and hyperplastic prostatic glands within the tissue examined stained with 34βE12. Reactivity was confined to the basal cell layer and showed variability in intensity from area to area. In most regions the basal cell layer was continuous but focal areas of disruption were present (Fig. 1).

The cases of atrophy were characterized by the presence of small glands arranged in a lobular fashion around a central duct with most having a sclerotic tissue reaction most prominent around the ducts. Individual acini were lined by flattened epithelial cells with hyperchromatic nuclei and scant cytoplasm. Basal cells were difficult or impossible to visualize in most. In all 16 cases, staining with 34βE12 highlighted a continuous flattened layer of basal cells (Fig. 2).

The 5 cases of post-sclerotic hyperplasia showed a central duct with marked sclerosis of the surrounding stroma. In contrast to usual atrophy, however, there was a proliferation of small acini, which appeared in some areas to bud off the ducts. The acinar-lining cells varied from flattened to cuboidal with some having hyperchromatic nuclei. Occasional cells had small nucleoli. Basal cells were visible in some glands but inapparent in others. All 5 cases showed strong reactivity of basal cells with the 34βE12 antibody (Fig. 3).

The 11 cases of basal cell hyperplasia showed nodular aggregates of small glands filled with basaloid cells. Individual cells had round-to-oval nuclei with a diffuse chromatin pattern and scattered, small nucleoli. In some cases lumina remained while in others the acini were completely filled by the basal cells. Staining with 34βE12 was in general weakly positive in all instances, although focal intense positivity was seen (Fig. 4).

Fibroepithelial nodule is a term applied by Sesterhenn and Mostofi (1988) to a distinctive small-gland proliferation in the prostate, which has also been reported under the terms "sclerosing adenosis" and "adenomatoid tumour" (Chen and Schiff 1983; Young and Clement 1987). It is composed of small, round and irregular glands embedded in a stroma containing mesenchymal cells with plump nuclei resembling reactive fibroblasts. The lesions may be circumscribed or poorly defined and infiltrative. Both examples in this series showed positive staining with 34βE12 in acinar cells, as well as in scattered spindle cells in the stroma (Fig. 5).

Atypical adenomatous hyperplasia was as described by Gleason (1985). This lesion has also been included under the term "adenosis" by other authors (Brawn 1982; Hedrick and Epstein 1989). The cases were characterized by a proliferation of small acini at the periphery of an otherwise typical hyperplastic nodule. Many of the small acini appeared to bud off clearly benign parent glands and to be lined by a single layer of epithelial cells with nuclei having an open chromatin pattern. Rare cells contained small nucleoli. The cells tended to have a cuboidal shape with clear-to-faintly eosinophilic cytoplasm. The appearance has led some authors to speculate that this represents a premalignant lesion (Kastendieck 1980; Helpap 1980). In the present study all 13 cases evaluated showed some staining with 34βE12. In most cases this consisted of scattered weak-to-strongly-positive cells in some but not all of the acini. In glands containing positive cells, a complete layer of basal cells was infrequent (Fig. 6).

All cases of small-acinar adenocarcinoma included in the study were of Gleason patterns 1 and 2 with individual glands lined by a single-layered epithelium with many nuclei having prominent nucleoli. A complete absence of reactivity with 34βE12 was present in all 21 cases (Fig. 7).

The AE1/AE3 cytokeratin was positive in all the benign and malignant lesions examined. Variable staining with PSA and PAP occurred in cases of atrophy, while the remainder of the material was uniformly reactive with these stains.

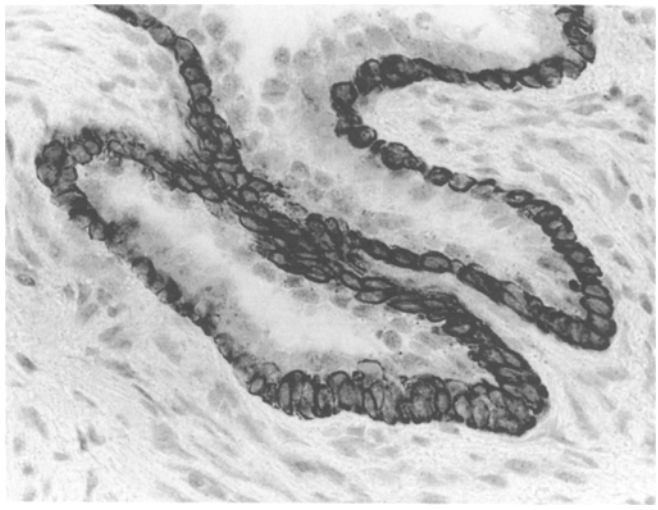

Fig. 1. Normal prostate acinus stained with high-molecular-weight cytokeratin (34βE12), demonstrating intense cytoplasmic positivity confined to basal cells. ×400

Table 1. Summary of immunoreactivity with 34βE12

Glandular pattern	No. cases	Positive staining
Atypical adenomatous hyperplasia	13	13
Atrophy	16	16
Post-sclerotic hyperplasia	5	5
Basal cell hyperplasia	11	11
Fibroepithelial nodule	2	2
Small-acinar adenocarcinoma	21	0

Discussion

The antibody 34βE12, also known by its catalogue number EAB903, was used in this study as a marker of prostatic basal cells. It is a monoclonal antibody raised against human stratum corneum, which reacts with keratins of molecular weights; 49, 51, 57 and 66 K. The cytokeratin cocktail AE1/AE3 recognizes a series of ker-

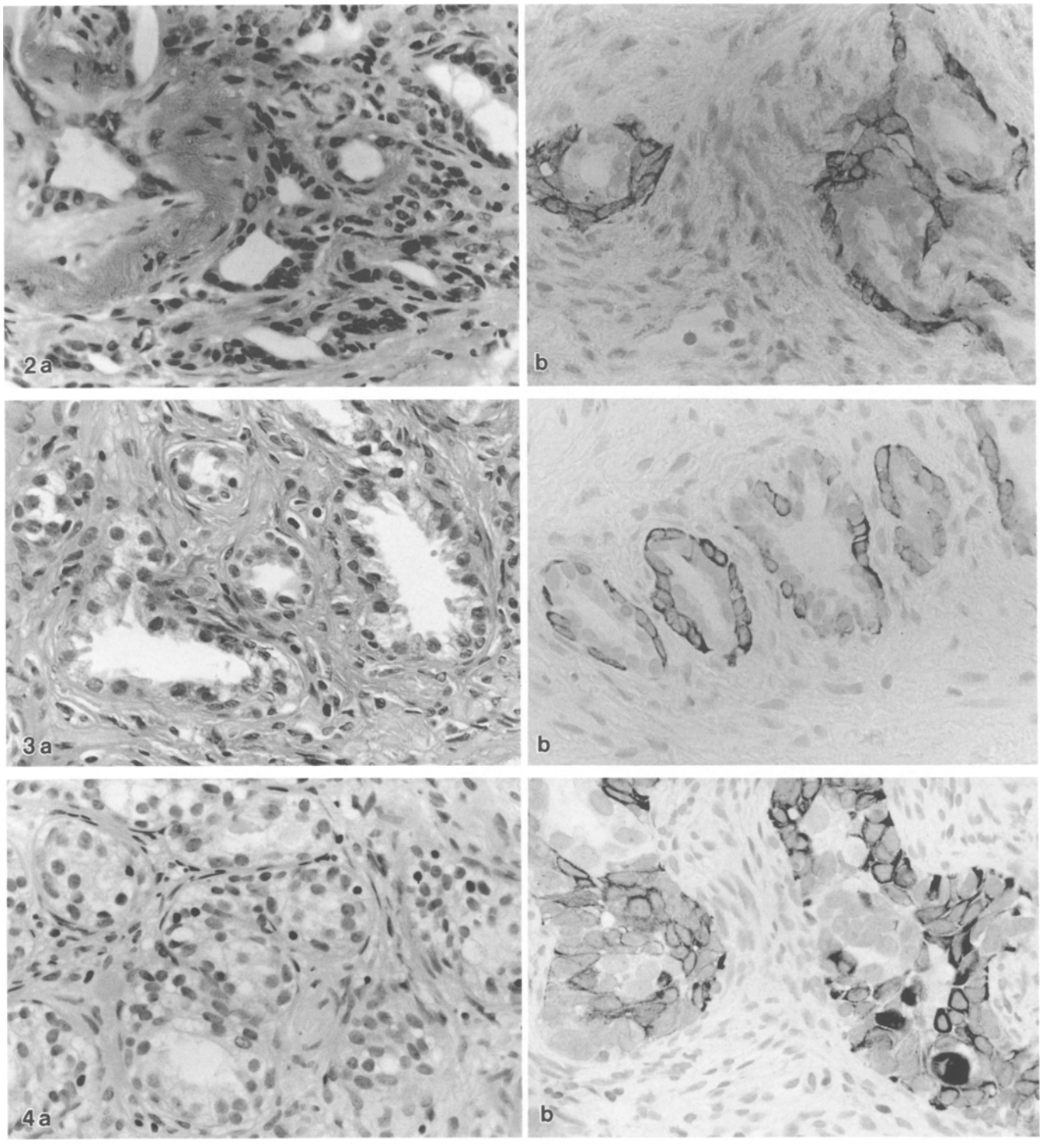

Fig. 2a, b. Prostatic atrophy with basal cells highlighted by immunostaining with antibody 34βE12 **a** Haematoxylin and eosin; **b** CK 34βE12. $\times$400

Fig. 3a, b. Post-sclerotic hyperplasia of prostate with basal cells demonstrated by positive immunostaining with antibody 34βE12 **a** Haematoxylin and eosin; **b** CK 34βE12. $\times$400

Fig. 4a, b. Basal cell hyperplasia of the prostate with diffuse staining of basal cells with antibody CK 34βE12. **a** Haematoxylin and eosin; **b** CK 34βE12. $\times$400

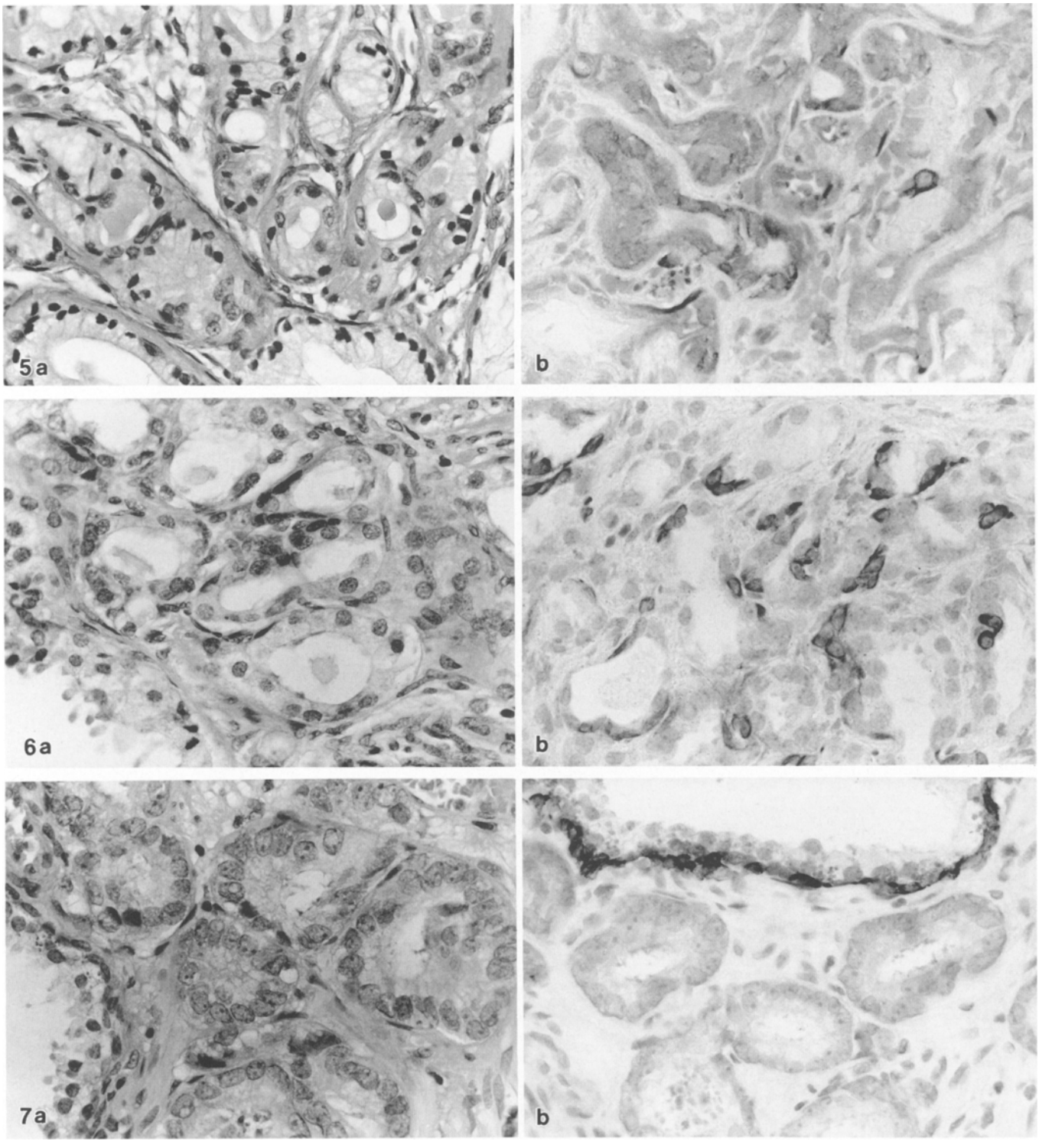

Fig. 5a, b. Fibroepithelial nodule of the prostate with scattered cells demonstrating reactivity for CK 34βE12 **a** Haematoxylin and eosin; **b** CK 34βE12 $\times$400

Fig. 6a, b. Atypical adenomatous hyperplasia of the prostate with incomplete lining of basal cells demonstrated in the acini by immunostaining for high-molecular-weight cytokeratin (**a**) Haematoxylin and eosin (b). CK 34βE12. $\times$400

Fig. 7a, b. Small-acinar adenocarcinoma of the prostate with absence of basal cells indicated by negative staining with antibody 34βE12. Note positive staining of basal cells in benign epithelium at top of **b**. **a** Haematoxylin and eosin; (**b**) CK 34βE12. $\times$400

atin proteins of 50, 56, 58, and 65–67 K. These monoclonal antibodies recognize two different populations of cells in the prostate. High-molecular-weight cytokeratin selectively reacts with the basal cells, whereas the cytokeratin cocktail AE1/AE3 stains the prostatic glandular luminal cells as well as the basal cells. The negative staining of prostatic adenocarcinoma with 34βE12 can be explained on the basis of the loss of basal cells in carcinoma (Totten et al. 1953; Mostofi et al. 1980; Gleason 1985; Kovi 1985; Murphy 1989). It is important to stress that loss of the basal cell layer is not uniform in all prostatic adenocarcinomas; this feature is only a diagnostic criterion in the small-acinar variant. McNeal et al. (1986), in studying cribriform adenocarcinoma of the prostate, found residual basal cells in 9 of 21 cases. Similarly Kovi et al. (1985) found the basal cell layer to be intact in examples of what they considered to be intraductal spread of prostatic adenocarcinoma. It has been suggested by some workers that prostatic adenocarcinoma arises from a malignant clone of either luminal cells or cells located near the luminal surface, with the tumour cells expressing a luminal cell phenotype (Bostwick and Brawer 1987; Nagle et al. 1987). Others have favoured a basal cell or stem cell origin (Kovi 1989). The absence of basal cells in small-acinar adenocarcinoma remains an unexplained phenomenon.

In cases of atypical adenomatous hyperplasia, staining with 34βE12 was rather weak and focal. Our results in these cases are similar to those described by Hedrick and Epstein (1989). Bostwick and Brawer (1987) also alluded to this pattern of staining of atypical adenomatous hyperplasia in their paper on prostatic intraepithelial neoplasia. There are two possible explanations for this. One is that the patchy staining is an artefact of formalin fixation. Evidence in support of this theory comes from recent work carried out using 34βE12 in both formalin-fixed and frozen material (Hedrick and Epstein 1989). These authors found that in formalin-fixed normal prostatic tissue a more discontinuous staining pattern of the basal cells was seen with 34βE12, whereas normal prostatic tissue which was frozen showed a continuous staining pattern. They did not, however, study any examples of atypical adenomatous hyperplasia in frozen tissue. Alternatively, atypical adenomatous hyperplasia has been cited as a putative premalignant lesion, and it is believed by some authors that there is a true or real loss of basal cells in this condition, similar to what one finds in cases of severe prostatic intraepithelial neoplasia (Bostwick and Brawer 1987; Gleason 1985; McNeal and Bostwick 1986; Bostwick 1988; Srigley 1988). The evidence in favour of prostatic intraepithelial neoplasia being a preneoplastic lesion, however, is much stronger than that for atypical adenomatous hyperplasia. In contrast to these studies, Chastonay et al. (1986) studied 10 cases of adenosis and reported no reactivity with 34βE12 in any. They used the term "adenosis", as defined by Brawn (1982). Some of the lesions included under this term by Brawn (1982) are identical to atypical adenomatous hyperplasia, as defined in the current report while others, particularly his moderate and severe adenosis, would be considered by some, including us, to represent Gleason pattern 1 or 2 adenocarcinomas. The criteria outlined by Chastonay et al. (1986) appear to describe the latter lesions.

In this study we have evaluated a variety of other small-acinar lesions that may mimic carcinoma (Srigley 1988). Examples of atrophy in Hedrick and Epstein's study (1989) demonstrated positive reactivity for 34βE12 in 92% of acini, a finding confirmed in the current report where all glands showed positivity. None of the prior studies has evaluated examples of post-sclerotic (post-atrophic) hyperplasia, a lesion more easily confused with carcinoma (Srigley 1988). All cases in this study contained basal cells, as demonstrated by staining with 34βE12, confirming their benign nature. Previous reports have shown positive staining in examples of basal cell hyperplasia (Grignon et al. 1988; Hedrick and Epstein 1989) and this was the finding in the current series as well. Finally, two examples of fibroepithelial nodule were evaluated in this study. To our knowledge this lesion has not been previously studied with this antibody. Both showed numerous positive cells in the acini and, interestingly, in some of the what appeared by light microscopy to be stromal cells.

Numerous cases of prostatic adenocarcinoma have been studied with high-molecular-weight cytokeratin (Brawer et al. 1985; Chastonay et al. 1986; Nagle et al. 1987; Bostwick and Brawer 1987; Brawer et al. 1989; Hedrick and Epstein 1989). Combining these reports with ours, a total of 210 cases have been evaluated with only 2 positive results. Both were reported by Chastonay et al. (1986), who suggest that this might be related to their having evaluated only well-differentiated tumours. In our series, all 21 adenocarcinomas were well-differentiated tumours corresponding to Gleason patterns 1 and 2 and we found no reactivity. Hedrick and Epstein's (1989) series also included 19 well-differentiated adenocarcinomas and found no reactivity.

As for the practical usefulness of 34βE12, its main value is the identification of basal cells in small-acinar lesions that otherwise resemble adenocarcinoma. All benign lesions studied showed positive staining to some extent with this antibody. Conversely, it has been our experience and that of most others that adenocarcinomas uniformly demonstrate no reactivity. Thus, it may be concluded that positive staining within a lesion composed of similar glands indicates that this lesion is benign. It must again be stressed that this conclusion is restricted to small-acinar lesions; positive staining may be expected in some other architectural patterns of prostatic adenocarcinoma. In contrast, negative staining within a lesion suggestive of carcinoma should not be interpreted as indicating malignancy, as this may represent a false negative. It goes without saying that careful histological evaluation is essential in the interpretation of this antibody, as is true for all immunohistochemical reagents.

To summarize, complete absence of staining with high-molecular-weight cytokeratin (34βE12) in a suspicious small-acinar lesion should be regarded as very suggestive, but not diagnostic of malignancy. Conversely, positive staining with this antibody in a lesion composed

of similar glands indicates that the lesion is almost certainly benign.

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