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Basement membranes in fetal, adult normal, hyperplastic and neoplastic human prostate

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Summary. The distribution of the various basement membrane (BM) components (type IV collagen, laminin and heparan sulphate proteoglycan) was studied in fetal, adult normal, hyperplastic and neoplastic prostates in formalin- and ethanol-fixed paraffin-embedded specimens. Stromal, epithelial and neoplastic BMs expressed differential susceptibility to pepsin treatment, suggesting conformational differences in the expression of epitopes on BM proteins in distinct anatomical structures and various lesions of the human prostate. In fetal prostate the acinar BM was regular and continuous in contrast to normal adult prostate and various hyperplastic conditions where the acinar BM was locally thickened or unreactive to the anti-BM antibodies. The localization pattern of BM components in grade I and grade II phases of prostatic cancer did not differ essentially from those found in various hyperplastic lesions. Regardless of the histopathological grade of malignancy, prostatic carcinoma cells were surrounded by distinct pericellular and periacinar membranes which were present even at points of contact with the stroma. This suggests that stroma invasion is invariably associated with neoplastic BM formations. Immunohistochemical evidence of the stromal or epithelial origin of neoplastic BMs could not be found. However, the consistent extracellular distribution of neoplastic BM components in contact with the stroma indicates that the elaboration of BM material requires a stromal influence.

Key words: Prostate – Carcinoma – Laminin – Type IV collagen – Heparan sulphate proteoglycan

Introduction

The basement membrane (BM) in the prostate, as in other parenchymal organs, represents an interphase between the epithelium and the extracellular matrix. Its integrity maintains the structural and functional relationship between the secretory epithelium and the stroma. All BMs are biochemically characterized by a set of proteins that include type IV collagen (Bornstein and Sage 1980), laminin (Timpl et al. 1979) and heparan sulphate proteoglycan (HSPG) (Hassell et al. 1980; Iozzo 1985).

Several immunohistochemical studies have shown that BM constituents, such as laminin and type IV collagen, are lost in many invasive tumours in contrast to their benign counterparts (Albrechtsen et al. 1981; Siegal et al. 1981; Barsky et al. 1983; Liotta et al. 1983; Charpin et al. 1986; Willebrand et al. 1986; Furness and Lam 1987).

Recently, laminin immunoreactivity has been studied in normal, hyperplastic and neoplastic human prostate showing that laminin immunoreactivity is lost with dedifferentiation (Sinha et al. 1989). However, no data have yet been provided about the structural pattern of BMs in invasive prostate cancer compared to their look-alike benign counterparts.

We have examined the distribution of the various BM components (type IV collagen, laminin, HSPG) in fetal, adult normal and in various hyperplastic and neoplastic conditions of human prostate. The effect of pepsin treatment on the immunoreactivity and visualization of these well-defined BM antigens was analysed in formalin- and ethanol-fixed tissue. Special attention was paid to the epithelial-stromal junction in carcinomas. For this purpose the BMs and the prostate epithelium were visualized simultaneously by demonstrating the BM antigen and the prostate-specific epithelial marker PSA in the same section.

Materials and methods

The material comprised 18 prostatectomy specimens resected for prostate cancer, 5 cystoprostatectomy specimens removed for bladder carcinoma and 80 transurethral resection specimens for urinary obstruction. In addition, fetal ($n=7$) and adult normal ($n=5$) prostate glands from autopsy (collected 8–15 h after death) were exam-

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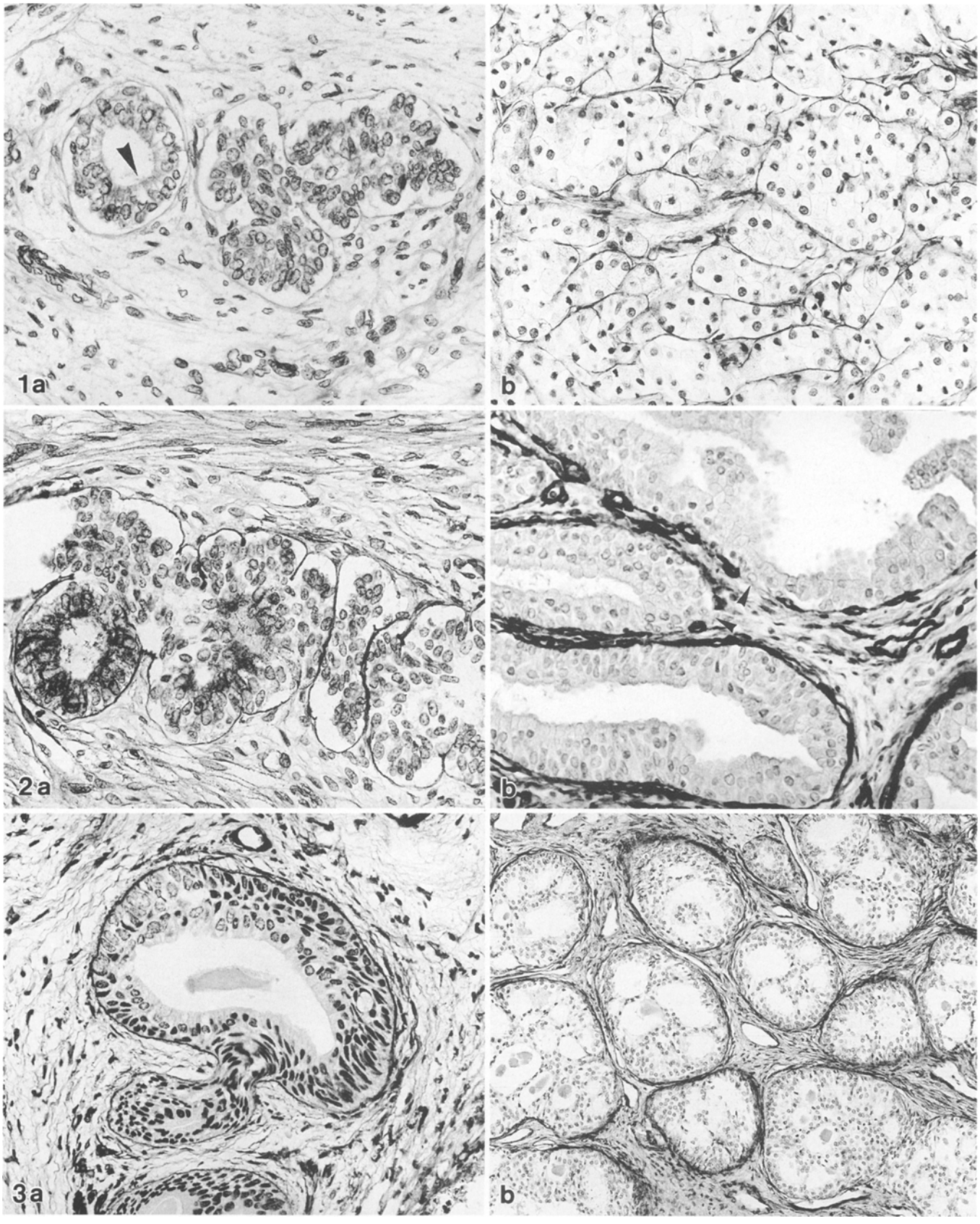


Fig. 1. Immunohistochemical demonstration of *heparan sulphate proteoglycan* (HSPG) in fetal prostate (**a**) and grade II carcinoma (**b**). ×280. A weak HSPG reaction is seen in BMs of fetal glands, around smooth muscle fibres and in the acinar epithelium (*arrow*). Note the distinct periacinar and pericellular BM formation in carcinoma (**b**)

Fig. 2. Fetal (**a**) and normal adult prostate (**b**); laminin, ×280. Intense laminin reactivity is seen in the fetal epithelium. Note the continuous pattern of BMs around fetal glands, while BMs of adult normal glands shows local absence (*arrows*) of laminin reactivity (**b**)

Fig. 3. Atypical basal cell hyperplasia (**a**, ×230) and cribriform hyperplasia (**b**, ×90). Continuous BMs are visualized by laminin immunoreactivity

ined. The material was available as formalin-fixed archival blocks or fresh specimens fixed in 80% ethanol and paraffin embedded.

The immunohistochemical investigations were performed on fetal ($n=7$), adult normal prostates ($n=5$); benign prostatic ($n=15$), basal-cell ($n=5$), adenomatous ($n=8$), cribriform ($n=7$), postatrophic ($n=5$) and atypical ($n=10$) hyperplasias; prostatic atrophy ($n=4$); granulomatous prostatitis ($n=5$) and adenocarcinomas ($n=25$) graded according to Böcking and Sommerkamp (1980). Grade I formations were found in 5, grade II in 12 and grade III in 16 specimens.

The effect of pepsin on the immunoreactivity of BM antigens was tested in formalin- and ethanol-fixed sections. The sections were incubated at 37° C with pepsin (Sigma, Deisenhofen, FRG) at a constant dilution (2 mg/ml in 0.010 HCl), but with varying incubation times. Endogenous peroxidase was blocked by H_2O_2 (0.3%) prior to the application of antibodies against laminin (monoclonal IgG-rabbit antibody, 1:200; Laboserv, Gießen, FRG), type IV collagen (monoclonal IgG-rabbit antibody, 1:150; Laboserv) and heparan sulphate proteoglycan (polyclonal IgG-rabbit antibody, Schleicher et al. 1989), kindly provided by Dr. Schleicher (Munich, FRG) in a dilution of 1:50. The slides were incubated with these primary antisera for 90 min at 37° C. The ABC rabbit kit raised in goats (Vector Laboratories, Burlingame, USA) was applied for 60 min at 37° C, followed by the avidin-biotin complex.

The location was shown by either conventional diaminobenzidine (DAB) development (Dakopatts, Hamburg, FRG) or by a modified DAB-nickel technique described elsewhere (Bonkhoff and Wernert 1989). Negative controls were performed by replacing the primary antibodies against laminin, type IV collagen and HSPG with a normal rabbit serum which showed no immunostaining.

To assess the epithelial-stromal junction in carcinoma, immunohistochemical double stainings were performed to visualize the BM antigens and the prostate-specific epithelial marker PSA in the same section. In the first staining step laminin, or type IV collagen, was demonstrated, as described above, by the DAB-nickel technique, leaving a black end product. The slides were then dehydrated and moved through an alcohol series to reduce the activity of the PAP complex present in tissue sections. After rinsing in PBS (phosphate-buffered saline, pH 7.2) the slides were incubated for 20 min with a normal porcine serum (Dakopatts) and then with the rabbit monoclonal antibody against the prostate-specific antigen (Dakopatts) for 12 h at 37° C at a dilution of 1:400. Applied next was a porcine anti-rabbit serum (linking antibody) and a PAP complex from pigs for 30 min at room temperature at a dilution of 1:20 and 1:30, respectively. The reaction was visualized by the conventional DAB technique leaving a brownish end product. The nuclei were faintly counterstained by haematoxylin.

Results

In ethanol-fixed untreated sections type IV collagen and laminin immunoreactivity showed distinct BMs in the stroma and around peripheral prostatic ducts, while the acinar BMs of normal and hyperplastic glands were only present focally. Using the same tissue processing, neoplastic BM formations were clearly identified in prostatic adenocarcinoma (PAC). Pretreatment with pepsin for 5 min gave optimal localization of type IV collagen and laminin in stromal, epithelial and neoplastic BMs.

In formalin-fixed, paraffin-embedded tissue, the optimal laminin and type IV collagen reactivity occurred with 10–30 min pepsin treatment to demonstrate stromal and neoplastic BMs and 30–60 min for epithelial membranes. Differences between type IV collagen and laminin immunoreactivity were observed in neither stromal, epithelial nor neoplastic BM material.

Even with optimal tissue processing, the HSPG immunoreactivity was weak and demonstrated complete BM formations only in PAC and in fetal glands (Fig. 1). Epithelial BMs of normal and hyperplastic glands showed a weak and fragmented HSPG staining. Cytoplasmic HSPG localization was only observed in the secretory epithelium of fetal glands (Fig. 1a).

The following observations were made by demonstrating laminin and type IV collagen reactivity in pepsin-treated, formalin or ethanol-fixed tissue. The fetal acinar BM was immunohistochemically characterized by a thin continuous pattern. Some glands showed a strong intracytoplasmic immunoreactivity of type IV collagen and laminin (Fig. 2a). In the adult normal prostate, the

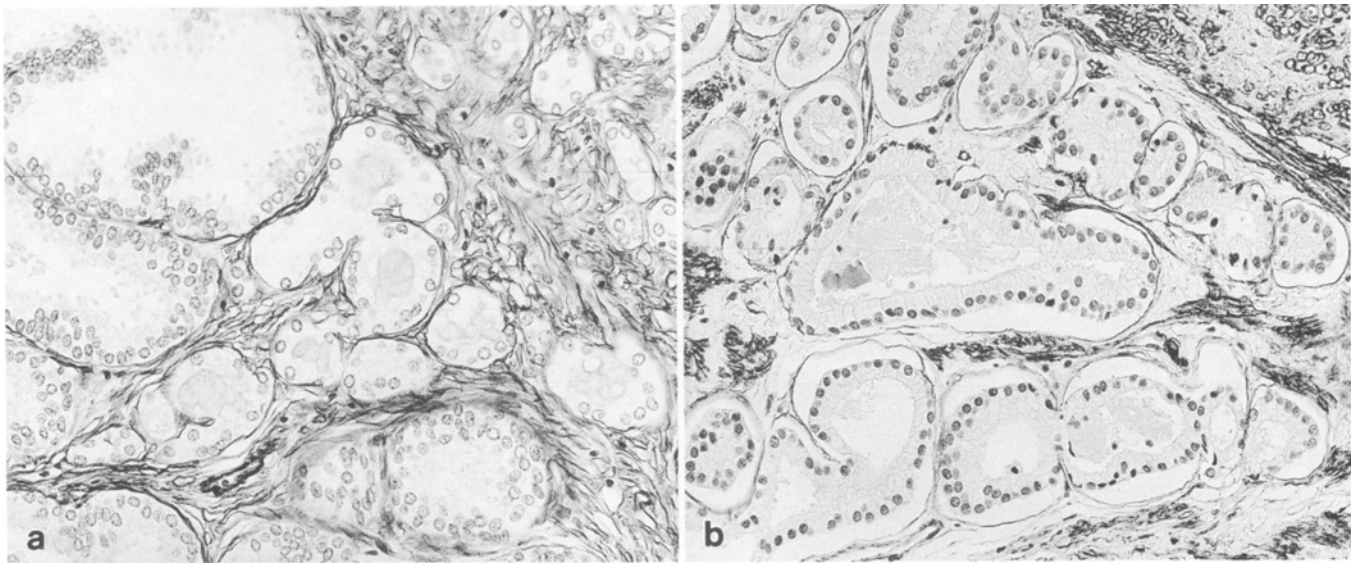
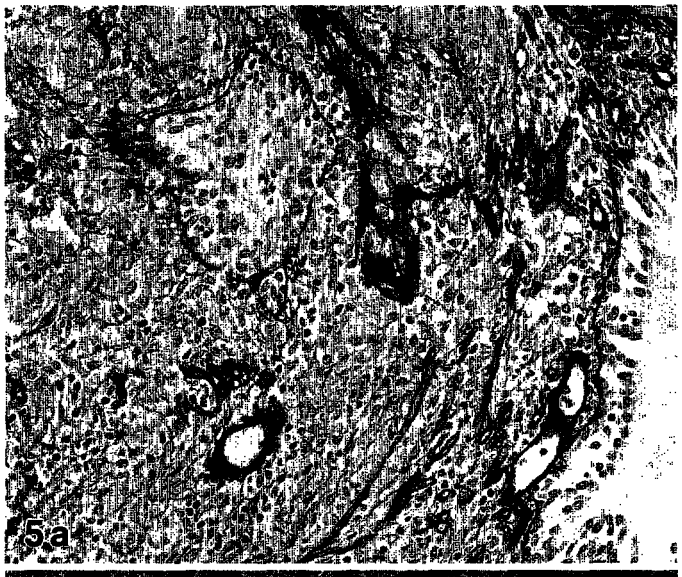


Fig. 4. Adenomatous hyperplasia (**a**) and grade I carcinoma (**b**); ×180, laminin. Both lesions illustrate continuous BM formations. Note the transition from adenomatous hyperplasia to invasive carcinoma without interruption or loss of BMs (**a**)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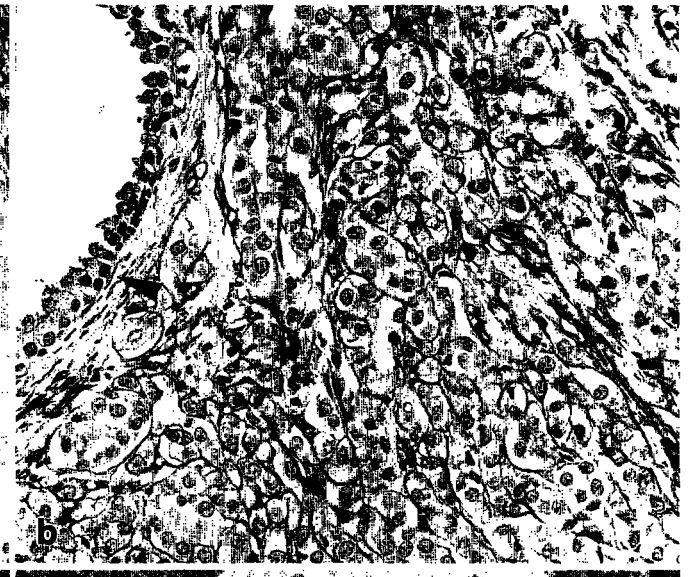
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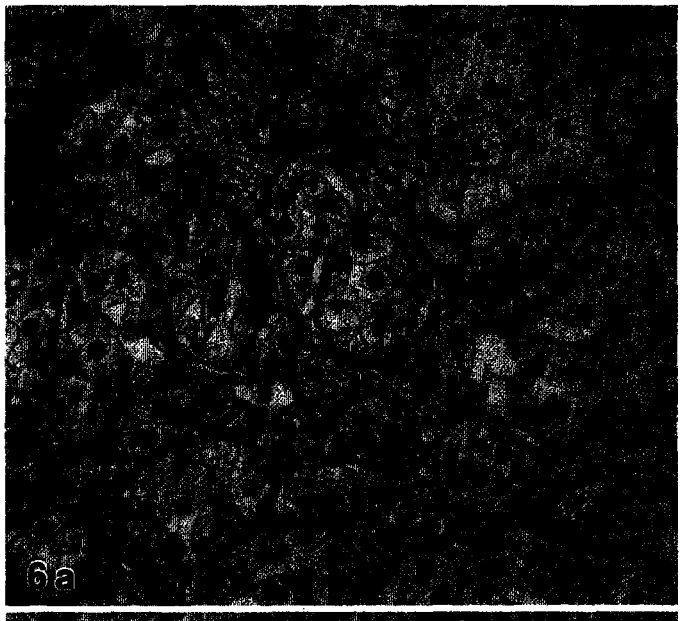
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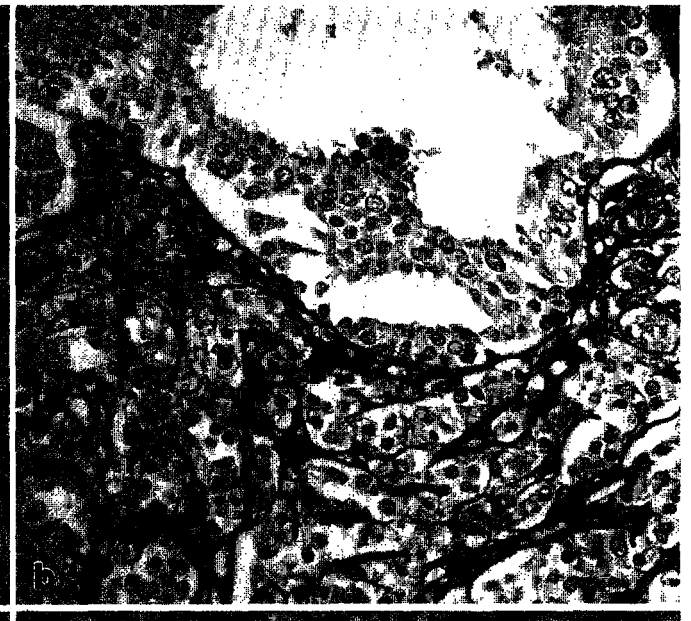
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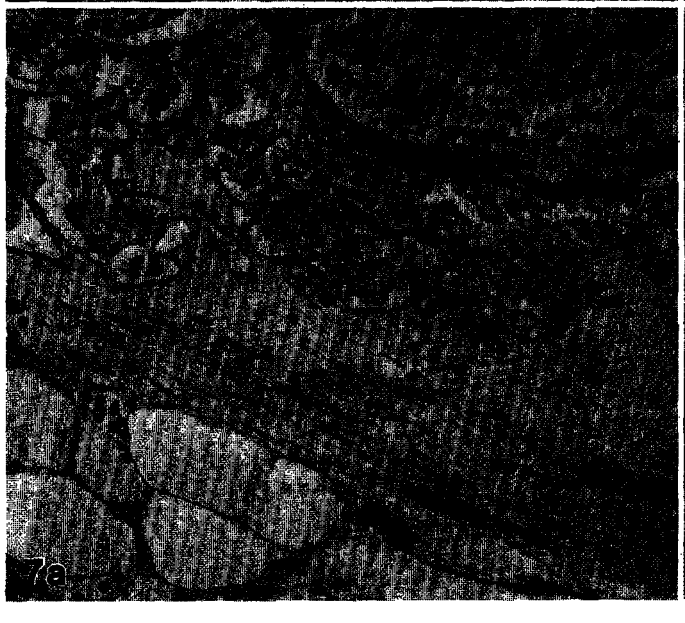
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b

epithelial BM was locally thickened or unreactive to anti-BM antibodies (Fig. 2b). Laminin- and type IV collagen immunoreactivity was not detected in secretory epithelia, basal cells, macrophages or fibroblasts (Fig. 2b). In hyperplastic glands laminin- and type IV collagen reactions showed similar staining patterns to those described for normal glands. However, the stroma was generally denser so that the acinar BM was not always sharply delineated from the condensed stromal BM material. Similar results were observed in atrophic prostate glands.

Different forms of prostatic hyperplasia such as basal cells (Fig. 3a), cribriforme (Fig. 3b), postatrophic and adenomatous hyperplasia (Fig. 4a), with or without nuclear atypia, displayed similar localization patterns of BM to those described in normal adult and hyperplastic prostate, and in the G I and G II phases of prostate cancer with focal absence or thickening of the BM. Laminin and type IV collagen immunoreactivity was not detected in the secretory epithelium or basal cells. At the transition points from atypical glands to invasive carcinoma the epithelial BM showed a regular and continuous pattern of staining (Fig. 4a).

Local absence of BMs was observed in normal and hyperplastic glands involved in inflammation. The tumour-like infiltration by histiocytes and macrophages in granulomatous inflammation did not reveal any BM formations (Fig. 5a).

In prostatic adenocarcinoma immunoreactivity of neoplastic BMs was generally more intensive than in adult normal and hyperplastic prostate (Fig. 5b). The structural pattern of BM formation depended on the histological growth pattern. In glandular grade I (Fig. 4b) and grade II (Figs. 1b, 6a) formations the lesions showed a regular continuous BM. Cribriform and papillary proliferations in grade II and grade III phases did not elaborate BM material unless they were in direct contact with the stroma (Fig. 6b). In solid growth patterns of grade III formations, tumour cells were only surrounded by pericellular or periacinar BM material at the epithelial-stromal junction (Figs. 5b, 6). Intraductal tumour components did not contain any BM material. Tumour infiltration of extraprostatic soft tissue was also associated with BM formation (Fig. 7). Intracytoplasmic immunoreactivity of type IV collagen and laminin was not found in tumour cells, neutrophils, macrophages or fibroblasts.

Fig. 5. Granulomatous prostatis (**a**, ×180) and grade III carcinoma (**b**, ×230); type IV collagen/PSA. Observe the total absence of BM formations within the tumour-like histiocytic infiltrates (**a**) while distinct BM material is formed around tumour cells in direct contact with the stroma (**b**). Note the fragmentary acinar BM (*arrow*) in ethanol-fixed tissue not pretreated with pepsin

Fig. 6. Grade II (**a**, ×280) and grade III (**b**, ×230) carcinoma; type IV collagen/PSA. Note the distinct periacinar and pericellular BMs in both lesions. The cribriform proliferations show BM formations at the epithelial-stromal junction only (**b**)

Fig. 7. Infiltration of extraprostatic soft tissue (**a**) and skeletal muscle bundles (**b**) by prostate cancer with distinct pericellular and periacinar periacinar BM formations, type IV collagen/PSA. ×230. The neoplastic epithelium is retracted from BMs (fixation artefact)

Discussion

Analysis of the effects of pepsin treatment on the demonstration of BMs in ethanol- and formalin-fixed tissue provides strong evidence that laminin, type IV collagen and HSPG immunoreactivity differ in normal acinar and stromal as well as in neoplastic BM formations. The selective immuno-enhancement effect of pepsin on BM antigens is well known, but its exact mechanism remains unclear (Barsky et al. 1984). The action of pepsin may be related to its ability to solubilize portions of the molecule masking immunoreactive BM epitopes (Leu et al. 1986). Therefore, the differential susceptibility to the duration of pepsin exposure most likely reflects conformational differences in the expression of epitopes on BM proteins in acinar, stromal and neoplastic BMs.

No differences between type IV collagen and laminin immunoreactivity are observed in stromal, epithelial or neoplastic BMs. Compared with laminin and type IV collagen, the HSPG reactivity is weak and only demonstrates distinct BMs in fetal prostate and prostatic adenocarcinoma (PAC). Whether or not the weak HSPG staining detected in the current study reflects a low content of HSPG in prostatic BMs needs further investigation.

The acinar BM in the fetal prostate is characterized immunohistochemically by a thin, regular and continuous pattern of staining. In adult, normal prostate and various non-neoplastic conditions (different forms of hyperplasia, atrophy, prostatitis) the acinar BM is locally thickened and shows focal absence of laminin or type IV collagen immunoreactivity. The local absence of BM antigens may reflect a physiological process of repair and remodulation of the acinar BM.

In contrast with a recent study (Sinha et al. 1989), we do not see intracytoplasmic laminin or type IV collagen immunoreactivity in either normal adult, or in hyperplastic or neoplastic prostate epithelium. This discrepancy is probably due to differences in antibody specificities. Interestingly, we observe intracytoplasmic laminin, type IV collagen and HSPG immunoreactivity in fetal glands, suggesting that the BM components of acinar membranes are produced in the secretory epithelium.

The demonstration of BM components has been reported to be a useful diagnostic tool in distinguishing various carcinomas from their in situ equivalents or their look-alike benign counterparts (Albrechtsen et al. 1981; Siegal et al. 1981; Barsky et al. 1983; Liotta et al. 1983; Charpin et al. 1986; Willebrand et al. 1986; Furness and Lam 1987). In small biopsy specimens, various hyperplasias (post-atrophic, adenomatous, cribriform) can be confused with PAC, especially if the hyperplastic glands display cellular atypia. According to our results, the immunohistochemical pattern of BM formations in these hyperplastic lesions does not differ significantly from glandular formations in grade I and grade II phases of

PAC. This indicates that the qualitative assessment of basement membranes does not provide a diagnostic aid in distinguishing invasive PACs from their look-alike benign counterparts in the human prostate. Only granulomatous prostatitis, which may mimic solid growth patterns of grade III carcinomas, can easily be differentiated from the latter by the total absence of BM formations.

No immunohistochemical evidence of interruption or loss of BMs was found during the transition from intraepithelial to invasive neoplasms. Based on the structural integrity of BMs, the entities carcinoma in situ and (micro)-invasive carcinoma cannot be clearly defined in prostate malignancy.

Many tumours may produce defective BMs which are structurally and biochemically different from those found in the equivalent normal and nonneoplastic tissue (Liotta et al. 1986; Martinez Hernandez 1988; Damjanov 1990). It has been reported that in epithelial tumours such as those from breast (Albrechtsen et al. 1981; Siegal et al. 1981; Barsky et al. 1983, 1984; Liotta et al. 1983; Liotta 1984; Nielson et al. 1983; Willebrand et al. 1986), colon/rectum (Forster et al. 1984; Havenith et al. 1988), pancreas (Barsky et al. 1983; Haberern-Blood et al. 1987) and transitional cell carcinoma of bladder (Conn et al. 1987; Daher et al. 1987; Zuk et al. 1989) the structural disintegration and loss of BMs correlate with tumour dedifferentiation, invasiveness and prognosis. Similar results have recently been reported in prostate cancer showing that loss of laminin reactivity is proportional to the degree of tumour dedifferentiation (Sinha et al. 1989).

Our results suggest that the immunoreactivity of the major BM components – laminin, type IV collagen and HSPG – does not correlate with the histological grade of PAC. Regardless of the histological grade, prostatic carcinoma cells are surrounded by distinct BM formations whose immunoreactivity is generally stronger than in normal and non-neoplastic conditions. In contrast to breast and endometrial disorders (Charpin et al. 1989), PAC contains more BM material than the equivalent normal and non-neoplastic tissue.

According to our results, neoplastic BM formations are detected at the epithelial-stromal junction only. In contrast to their invasive counterparts, intraductal tumour components do not contain any BM material since the neoplastic tissue grows free in luminal spaces. In the same way, neoplasms with papillary and cribriform patterns of growth do not elaborate BM material unless these lesions contact stroma directly. These findings provide strong morphological evidence that stroma invasion is invariably associated with the formation of neoplastic membranes. However, it remains unclear whether neoplastic BM material derives from tumour or stromal cells, since type IV collagen, laminin or HSPG reactivity are not detected in either stromal or neoplastic cells. In situ hybridization techniques demonstrating specific mRNA sequences of BM components may further elucidate the origin of neoplastic BMs. Nevertheless, the consistent extracellular distribution of neoplastic BMs in contact with the stroma indicates that the elaboration of BM material requires a stromal influence. The ability to induce BM formation is obviously not limited to the fibromuscular stroma. Invasion of extraprostatic soft tissue, including fat tissue and muscle bundles, by PAC is also associated with formation of BM material.

A general feature of PAC (unlike most carcinomas) is the absence of tumour desmoplasia and inflammation. This may be explained by the presence of BMs between tumour cells and the stroma, thus preventing immunological contact between these two compartments which would induce desmoplasia and inflammation.

We draw the following conclusions from the present morphological study:

1. The immunoreactivity of the major BM components laminin, type IV collagen and HSPG differs in normal acinar and stromal BMs as well as in neoplastic BM formations. There is differential susceptibility to pepsin treatment, suggesting conformational differences of BMs in distinct anatomical structures and various lesions of human prostate.
2. The qualitative assessment of BMs in the prostate does not provide any diagnostic aid to distinguish invasive PACs from their look-alike benign counterparts or to differentiate in situ neoplasia from invasive cancer. Only granulomatous prostatitis can be distinguished easily from the latter by the total absence of BMs.
3. In PACs, regardless of the histological grade of malignancy, distinct periacinar and pericellular BMs are produced at the epithelial-stromal junction, suggesting that stromal invasion is invariably associated with neoplastic BM formations.
4. Although the origin of neoplastic BM material remains unclear, the consistent extracellular distribution of BM components in contact with the stroma indicates a stromal influence on elaboration of BM material. This possible inductional mechanism does not appear to be specific for the fibromuscular prostatic stroma.

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