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Basement membrane proteins in salivary gland tumours

Distribution of type IV collagen and laminin

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Summary. Immunohistochemical localization of type IV collagen and laminin in normal salivary glands and in salivary gland tumours of various types was studied using rabbit antisera. In normal salivary glands, type IV collagen and laminin were co-localized in basement membranes surrounding acini, ducts, fat cells and peripheral nerves. In salivary gland tumours, three main patterns of co-expression of these basement membrane proteins were distinguished. Linear basement membrane-like staining was detected in duct-cell-derived benign salivary gland tumours and in acinic cell carcinomas. In invasive lesions, however, these basement membrane proteins were distributed in an irregular, interrupted manner, and in many cases they were completely absent. Both benign and malignant salivary gland tumours which have a prominent myoepithelial cell component display a particular deposition of basement membrane molecules adjacent to the modified myoepithelial cells, and at the margins of extracellular matrix deposits within these tumours.

Key words: Basement membrane proteins – Type IV collagen – Laminin – Salivary gland tumours – Immunohistochemistry

Introduction

Basement membranes (BM) are highly organized sheet-like extracellular matrices which separate epithelial and endothelial cells from the adjacent interstitial tissue. BMs also surround adipocytes, Schwann cells and muscle cells. Thus, BMs represent ubiquitous extracellular structures compartmentalizing tissues (Martinez-Hernandez and Amenta 1983; Madri et al. 1984). BMs contain type IV collagen, laminins, entactin and heparan sulphate proteoglycans (Timpl and Dziadek 1986). Laminin-related proteins form a family of tissue-specific components of BM including merosin (Leivo and Engvall 1988; Ehrig et al. 1990) and s-laminin (Hunter et al. 1989). Type IV collagen forms the framework of BM (Timpl et al. 1981), while laminins are the most important non-collagenous proteins of these matrices (Foidart et al. 1980; Martin and Timpl 1987; Engvall et al. 1990). They function as semi-permeable filters and as structural support for tissues, but they also influence cell differentiation, cell migration and neurite outgrowth (Martinez-Hernandez and Amenta 1983). BMs form a barrier for the spread of malignant tumour cells and microorganisms. Thus, the integrity of BMs is significant in the biological behaviour of tumours.

Relatively little attention has been focused on the distribution of BM proteins in salivary gland tumours. Linear BM-like deposits of type IV collagen (Chen et al. 1988) and laminin (Toida et al. 1984) were found lining pseudocysts in adenoid cystic carcinomas, and disruption of BM has been reported in a number of malignant salivary gland tumours (Caselitz et al. 1988). In these tumours, several cell types may contribute to the synthesis of BM proteins, including acinic cells, ductal cells and myoepithelial cells (MECs) (Sobue et al. 1989; Shirasuna et al. 1990). The present study investigates the distribution of BM proteins in various types of salivary gland neoplasms with special interest in the role of MECs in the biosynthesis and deposition of these extracellular matrices. Our results indicate that type IV collagen and laminin are not restricted only to BMs between the epithelial and stromal components of these tumours but, in addition, considerable amounts of both proteins are found in association with proliferating MECs.

Materials and methods

We reviewed 296 salivary gland tumours from the files of the Department of Pathology at University of Helsinki. Seventy-four cases were selected for immunohistochemical studies of BM proteins (Ta-

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Table 1. Material of salivary gland tumours

	Number of cases	
Basal cell adenoma	8	
Canalicular adenoma	1	
Myoepithelioma (spindle-cell)	3	
Myoepithelioma (plasmacytoid and mixed type)	10	
Pleomorphic adenoma	14	
Warthin's tumor	10	
Carcinoma in pleomorphic adenoma	1	
Adenoid cystic carcinoma	11	
Polymorphous low-grade carcinoma	1	
Acinic cell carcinoma	5	
Mucoepidermoid carcinoma	5	
Adenocarcinoma	3	
Squamous cell carcinoma	1	
Undifferentiated carcinoma	1	
Normal salivary gland		20
Total	74	20

ble 1). The criteria for selection included representative morphological characteristics and an adequate tumour mass. The lesions were classified according to diagnostic criteria proposed recently (Seifert et al. 1990). In addition, samples of normal tissue from 20 major and minor salivary glands were examined.

All specimens were processed routinely. Paraffin blocks were available in all cases. For light microscopy, staining with haematoxylin and eosin, van Gieson and periodic acid Schiff-alcian blue (pH 2.5) methods was performed. In some cases, Weigert's resorcin fuchsin and a modification of Gomori's stain for reticulin were also used (Bancroft 1977).

For immunohistochemical analysis, the following antibodies were employed: rabbit antiserum to mouse EHS tumour laminin (chain composition A-B1-B2) (dilution 1:300; Gibco, Grand Island, N.Y.), rabbit antiserum to type IV collagen (1:200; Heyl, Berlin, FRG), and rabbit antibodies to cow S-100 protein (1:100; Dakopatts, Glostrup, Denmark). Mouse monoclonal antibodies to muscle actin HHF 35 (1:7500; Enzo Biochemicals, New York), glial fibrillary acidic protein (GFAP; 1:50; Dako), vimentin (1:5; Dako) and to cytokeratins 8, 18 and 19 (Moll et al. 1982) (dilution 1:5; a generous gift of Dr. Ismo Virtanen, Helsinki, Finland) (Virtanen et al. 1985) were also used.

Sections 4 μm thick were made and deparaffinized. For immunohistochemistry of BM proteins, a proteolytic pre-treatment was found to be essential as formalin fixation abolished the immunoreactions for type IV collagen and laminin completely. Sections stained for type IV collagen and laminin were first treated with 1% pepsin (Merck, Darmstadt, FRG) pH 1.8 for 40 min at 37° C to enhance the availability of antigenic determinants. Sections stained for muscle actin, GFAP and cytokeratins were first digested with 0.4% pepsin in 0.01 N HCl for 30 min at 37° C.

The sections were then exposed to a 0.1% solution of hydrogen peroxide in absolute methanol to inactivate endogenous peroxidases. When staining for S-100 protein, immunolabelling was performed using a PAP method (Dako) as described previously (Sternberger et al. 1970). For all other antigens, an ABC procedure (Vector Laboratories, Burlingame, Calif) was employed (Hsu and Rane 1984). Anti-rabbit IgG and anti-mouse IgG conjugates were diluted 1:300. Following these steps, 3-amino-9-ethyl-carbazole (Sigma, St. Louis, Mo) in 0.03% hydrogen peroxide was used as chromogen.

For controls, normal rabbit serum or phosphate-buffered saline were used instead of the primary antibody. For comparison, normal salivary gland tissue, fat tissue and peripheral nerves were also studied using immunohistochemistry.

Results

In normal major and minor salivary glands, there was a distinct linear staining for type IV collagen and laminin in BMs surrounding the acini and the terminal duct system. Prominent staining for both proteins was seen throughout the entire thickness of the walls of all blood vessels. Fat cells and peripheral nerves were outlined by a thin continuous BM positive for both proteins (Fig. 1).

Four basal cell adenomas and one case of canalicular adenoma were examined. In the canalicular adenoma there was a linear staining for both type IV collagen and laminin along the BM separating the epithelial cords from adjacent loose hypocellular stroma (Fig. 2A). In basal cell adenomas, type IV collagen and laminin were also found adjacent to stromal MECs (Fig. 2B).

In spindle cell myoepitheliomas, there was diffuse strong labelling for type IV collagen and laminin between all tumour cells (Fig. 2C). In plasmacytoid type myoepitheliomas, distinct staining surrounded many MECs and, in addition, prominent linear BM staining was seen around extracellular matrix deposits present within the tumour (Fig. 2D).

In pleomorphic adenomas, distinct staining for type IV collagen and laminin was regularly seen around tubular structures in solid epithelial areas as well as at the interface where cellular areas bordered chondromyxoid regions (Fig. 3A). Furthermore, type IV collagen and laminin were co-localized in the form of linear or augmented immunoreactivity in many intercellular slits or pseudoglandular spaces in solid areas, mainly near the border with myxoid areas (Fig. 3A). Expression of type IV collagen was usually linear, whereas laminin was dispersed in more diffuse manner with particular augmentation in areas of focal squamous metaplasia (Fig. 3B).

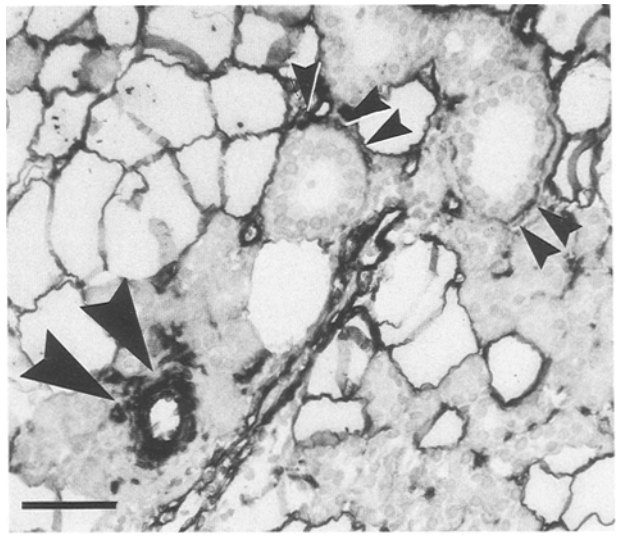

Fig. 1. Normal parotid gland. Staining for type IV collagen shows linear basement membranes around acini (*small arrowheads*), ducts and fat cells. Prominent staining is seen throughout blood vessel walls (*large arrowheads*). Immunoperoxidase stain and haematoxylin counterstain. × 240. *Bar* = 50 μm

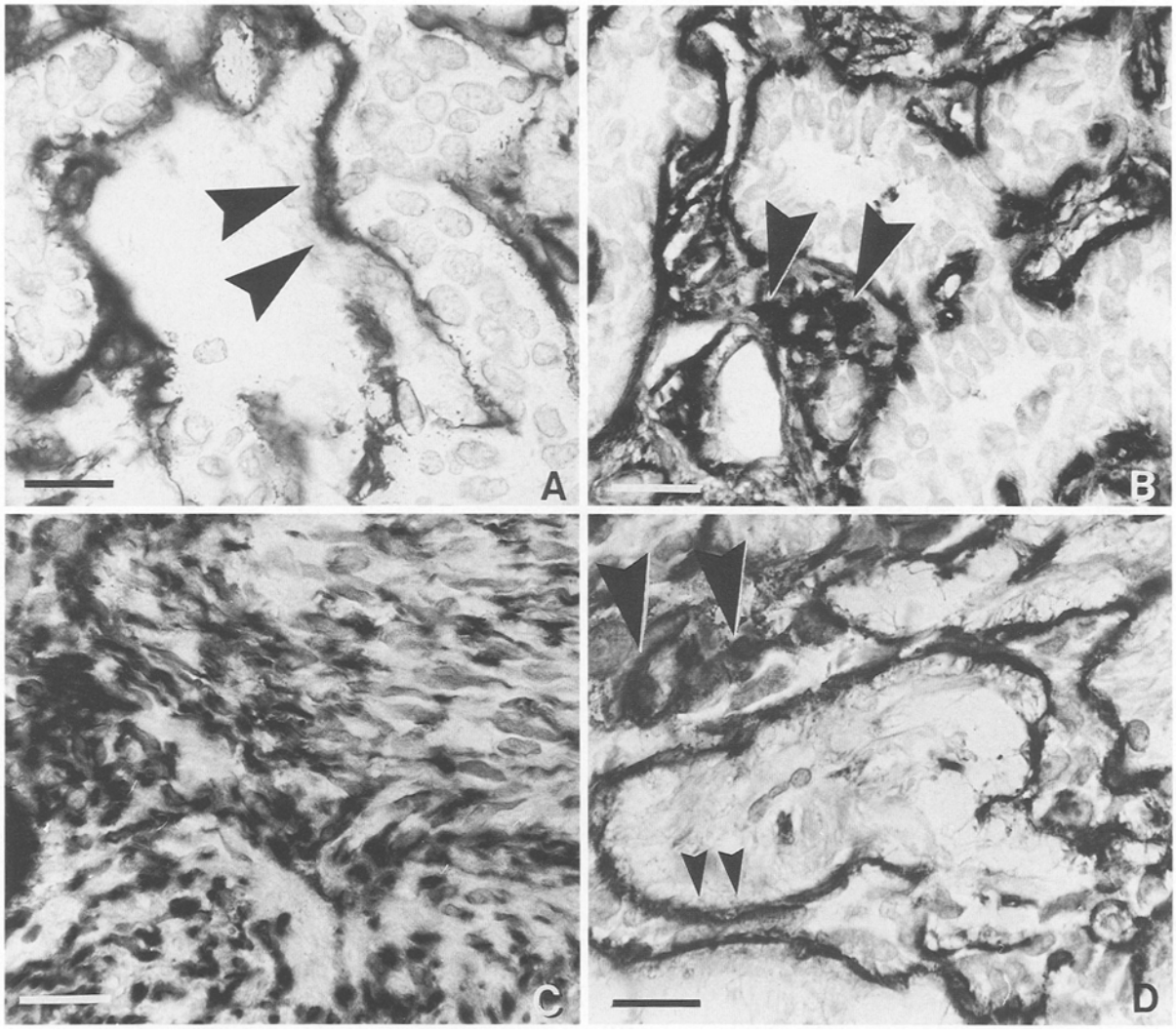

Fig. 2A–D. Benign tumours of parotid gland stained for type IV collagen. **A** Canalicular adenoma. Distinct linear staining separates epithelial cords from loose stroma (*large arrowheads*). **B** Basal cell adenoma shows staining adjacent to stromal myoepithelial cells (*large arrowheads*) in addition to outlines of epithelial basement membranes. **C** In a spindle cell myoepithelioma the staining shows a patchy and interrupted pattern throughout the tumour. **D** Plasmacytoid myoepithelioma shows strong focal staining around some myoepithelial cells (*large arrowheads*) and also linear staining of basement membranes which surround extracellular matrix deposits within the tumour (*small arrowheads*). Immunoperoxidase stain and haematoxylin counterstain. ×600. *Bar* = 20 μm

Solid tumour nests in myxoid areas and even isolated spindle and stellate MECs were stained for type IV collagen and laminin (Fig. 3C).

Adenoid cystic carcinomas (ACC) were classified into three main growth patterns, tubular, cribriform and solid (basaloid) according to criteria described previously (Perzin et al. 1978). In the cribriform type of ACC, there was a strong linear positivity for type IV collagen and laminin along the inner surfaces of the pseudoglandular spaces (Fig. 4A). A thin linear BM was also regularly observed at the edges of the tumour nests (Fig. 4B). The tubular type of ACC, however, displayed strong labelling for both type IV collagen and laminin around the tubular structures (Fig. 4C). The basaloid type of ACC showed little or no expression of either BM protein at the margins of epithelial and stromal compartments, although all blood vessels showed staining for type IV collagen and laminin (Fig. 4D). In all three types of ACC, there was a distinct homogeneous non-linear staining for both type IV collagen and laminin in the interior of the pseudoglandular spaces. In the basaloid type, however, such spaces were few and small.

In a case of polymorphous low-grade carcinoma, staining for both proteins revealed numerous pseudocystic spaces which were outlined by linear BM-like staining for type IV collagen and laminin (Fig. 5A). In mucoepidermoid carcinomas (Fig. 5B), squamous cell carcinomas and mucinous adenocarcinomas staining for type IV collagen showed that the BMs were irregular, interrupted and in many areas even completely absent. In acinic cell carcinomas, however, a continuous BM-like immunoreactivity was observed around tumour acini (Fig. 5C).

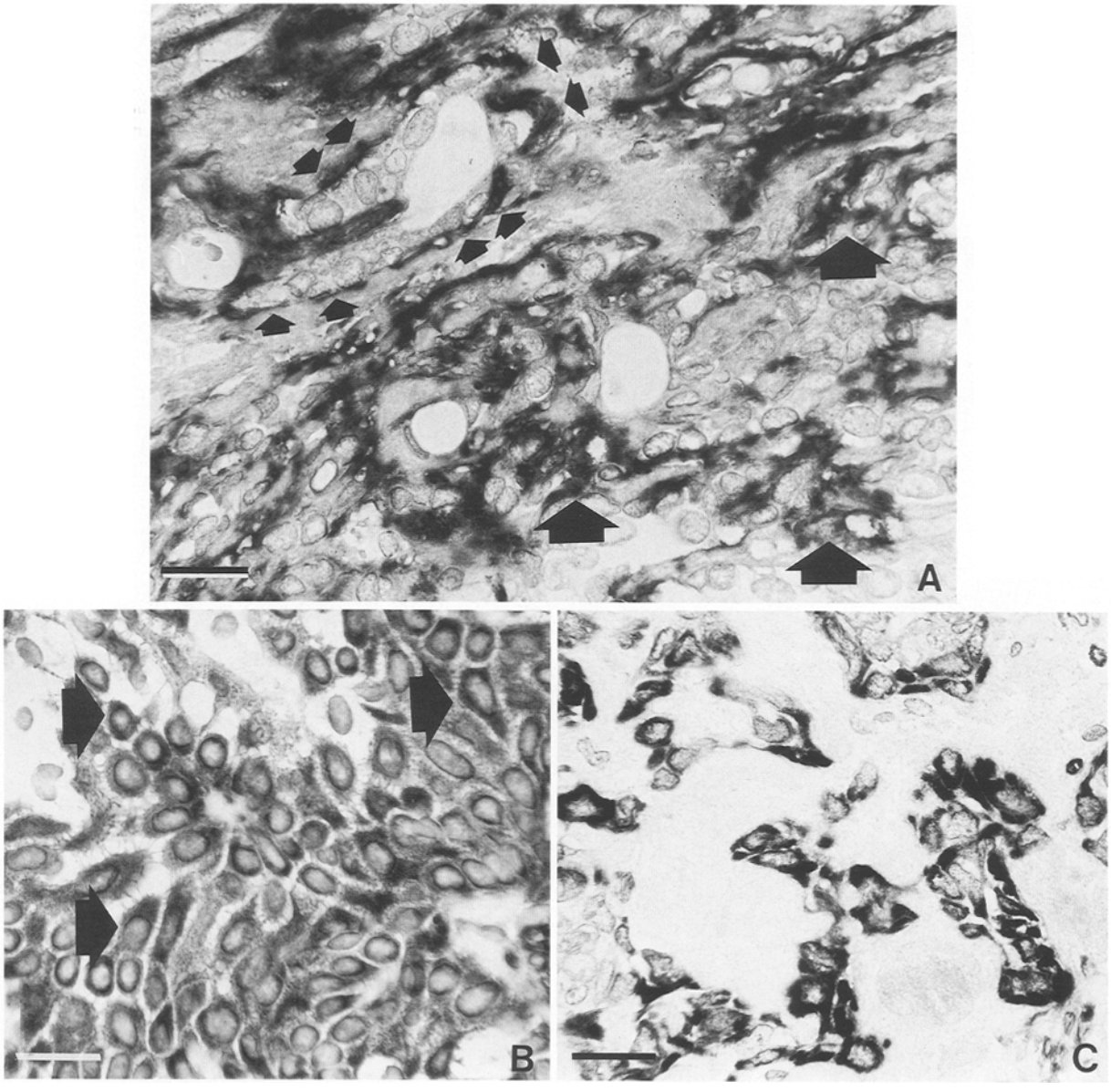

Fig. 3A–C. Pleomorphic adenoma of parotid gland. **A** Tubular structures are outlined by largely linear staining for type IV collagen (*small arrows*) and focal positivity is also seen in clusters of myoepithelial cells (*large arrows*). **B** In foci of squamous metaplasia predominantly intracellular staining for type IV collagen is seen in most cells (*large arrows*). **C** Also in myxoid areas, nests of myoepithelial cells show staining for laminin. Immunoperoxidase stain and haematoxylin counterstain. ×600. *Bar* = 20 μm

Discussion

This paper describes the presence of regular linear BMs in benign epithelial tumours of salivary glands. In most malignant tumours, however, with the notable exception of ACC and polymorphous low-grade carcinoma, BMs are absent or assembled in a highly irregular fashion. In ACC and polymorphous low-grade carcinoma, where the neoplastic cells presumably are of myoepithelial origin, formation of linear BMs was regularly observed. In the latter tumours and in benign tumours containing actively proliferating MECs, we also found non-linear deposits of BM components.

The linear BM-like deposition of type IV collagen and laminin in salivary gland tumours of pure epithelial origin was reported by Caselitz et al. (1988). The present paper describes a similar pattern of BM proteins in acinic cell carcinomas, in basal cell adenomas, Warthin's tumours, oxyphilic adenomas, and in a case of canalicular adenoma. These neoplasms originate from acinic and ductal cells, and we assume that these cell types are responsible for the synhesis and deposition of the linear BM structures.

Other benign tumours of salivary glands include pleomorphic adenomas and various types of myoepitheliomas where modified MECs form the actively proliferating component of the tumour. These express BM components in both a linear and a patchy non-linear fashion, as already reported by Caselitz et al. (1988). Our results parallel these findings and, furthermore, we point out

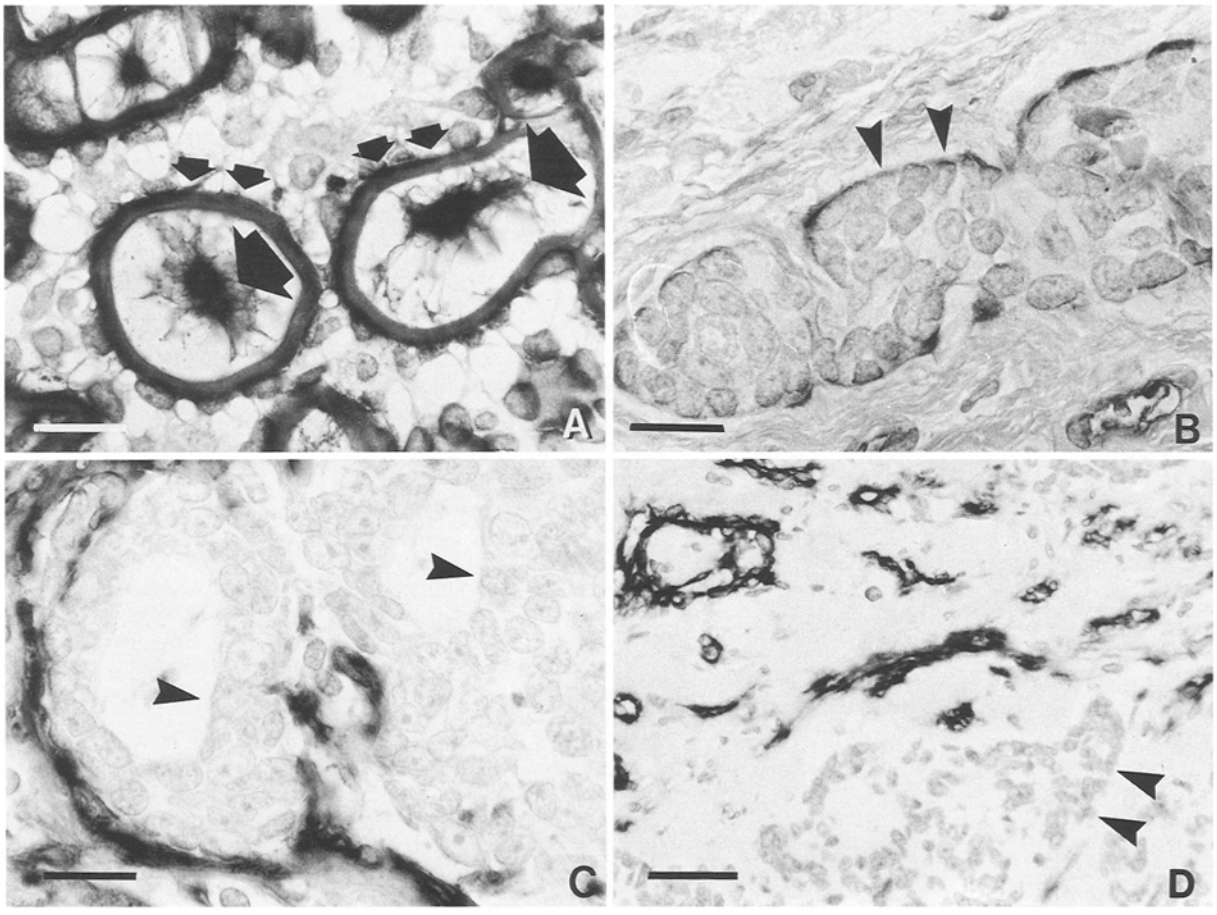

Fig. 4A–D. Adenoid cystic carcinoma of parotid gland stained for type IV collagen. Immunoperoxidase stain and haematoxylin counterstain. **A** Strong linear staining around pseudocysts (*small arrows*) as well as some strongly stained material inside the pseudocysts (*large arrows*) is seen in the cribriform type. ×600. *Bar* = 20 μm. **B** Linear staining is seen at the edges of tumour nests in the cribriform type (*small arrowheads*). ×480. *Bar* = 25 μm. **C** In the tubular type, strong staining is seen around the tubular structures, while the inner surfaces of true lumina are not stained (*small arrowheads*). ×600. *Bar* = 20 μm. **D** In the basaloid type, no staining is seen around tumour nests (*small arrowheads*), while capillary walls of the tumor are strongly stained. ×240. *Bar* = 50 μm

a distinct association between non-linear BM deposits and proliferating MECs in these tumours. Such an association was particularly prominent in spindle-cell myoepitheliomas where modified myoepithelial cells are the only proliferating cell type. Linear BMs, however, seem to be deposited only where MECs are in contact with interstitial matrix deposits such as those seen in plasmacytoid myoepitheliomas and pleomorphic adenomas. Similarly, linear BMs are formed around specialized spherical and crystalloid deposits of interstitial matrix components in salivary gland tumours (Skalova et al. 1992, Skalova and Leivo, 1992). In fact, all salivary gland tumours containing actively proliferating MECs form extracellular matrix deposits and intratumour spaces which are analogous to the pseudocysts of ACCs.

Thus, MECs seem to be a major source of BM material in salivary gland tumours. This conclusion is also supported by cell culture studies (Sobue et al. 1989; Shirasuna et al. 1990). MECs seem to assemble linear BMs only in the presence of interstitial (collagenous) matrix components, resembling many epithelial and endothelial cells in this respect. Such collagenous matrices also influence the shape of MECs, which varies in different locations considerably from stellate and spindle-shaped to angulated (Lam 1985).

The absence of type IV collagen and laminin in malignant salivary gland tumours, such as mucoepidermoid carcinomas, adenocarcinomas, squamous cell carcinomas and acinic cell carcinomas was described by Caselitz et al. (1988). In addition to these tumour types, we also studied the basaloid type ACCs. The absence of BMs around tumour cell clusters in this case suggests a low level of cell differentiation, also evidenced by the absence of muscle actin and S-100 protein (Chen et al. 1988). These tumours are known to have a poorer prognosis than other types of ACC (Perzin et al. 1978).

Loss of BMs may be due to decreased synthesis or decreased assembly of secreted components (Albrechtsen et al. 1981; Birembat et al. 1985; Bosman et al. 1985). Alternatively, the loss may be due to increased degradation. Interestingly, a proteolytic enzyme specific for type IV collagen and capable of degrading BMs has been detected in various types of metastatic tumour cells (Liotta et al. 1980, 1981). The breakdown of BMs

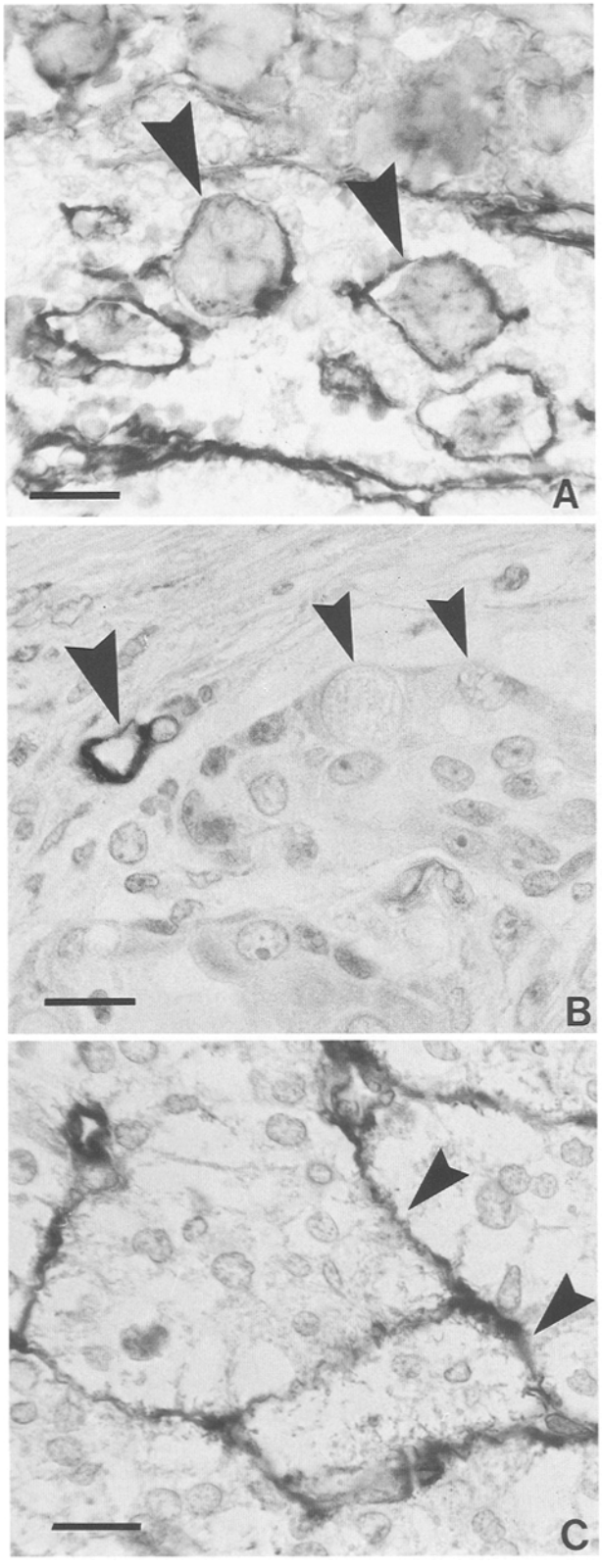

Fig. 5A–C. Carcinomas of parotid gland stained for type IV collagen. **A** Polymorphous low-grade carcinoma shows numerous pseudocystic spaces outlined by linear staining (*large arrowheads*). **B** In mucoepidermoid carcinoma no staining is seen in association with tumour cells (*small arrowheads*), while capillary walls are stained (*large arrowhead*). **C** An acinic cell carcinoma shows linear basement-membrane-like staining around the acini (*small arrowheads*). Immunoperoxidase stain and haematoxylin counterstain, ×600. *Bar* = 20 μm

around tumour cells presumably facilitates their invasiveness and metastatic potential (Liotta et al. 1983, 1984; Bosman et al. 1985).

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