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Immunoreactive Tenascin in Tumours of Salivary Glands: Evidence for Enhanced Expression in Tumour Stroma and Production by Tumour Cells

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Tenascin, a large molecular weight extracellular glycoprotein expressed at the epithelial–mesenchymal interface during morphogenesis in embryo, wound healing and in the stroma of various benign and malignant tumours was evaluated in a series of primary epithelial tumours of salivary glands using a monoclonal antibody. Normal salivary glands ($n=5$) had linear delicate band-like immunoreactive tenascin in relatively large excretory or intralobular ducts. Pleomorphic adenomas ($n=40$) had heterogeneity of expression in modified myoepithelial cell-associated myxoid, hyaline and chondroid areas. Warthin's tumours ($n=10$) had a linear immunoreactivity profile of tenascin just adjacent to the basal cells of the epithelial tumour component. A heterogeneity of expression with intense to low or negative stromal immunoreactivity was observed in adenoid cystic carcinomas ($n=8$), mucoepidermoid carcinomas ($n=8$), epithelial–myoepithelial carcinomas ($n=4$), polymorphous low-grade carcinomas ($n=3$), papillary cystadenocarcinomas ($n=15$) and undifferentiated carcinomas ($n=3$). In addition, small cystic spaces or lumens of epithelial-lined tubulo-ductal structures in numerous salivary tumours had positive immunoreactivity for tenascin, suggesting its production by the epithelial tumour component. An enhanced expression of tenascin in salivary tumours suggests a role of this protein in the stromal remodelling and tumour growth.

Keywords: salivary gland neoplasms, tenascin, immunohistochemistry

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INTRODUCTION

TENASCIN IS a multifunctional large glycoprotein component of extracellular matrix formed especially at the epithelial–mesenchymal interface and expressed during wound healing and embryonic and tumour development [1–4]. Molecular analysis of cDNA encoding tenascin and the primary structure of amino acid sequences of tenascin have revealed a region homologous to the fibronectin gene, namely a repetitive sequence of type III fibronectin and epidermal growth factor (EGF) and fibrinogen-like sequences with minor species variation [5–8]. The exact function of this glycoprotein, that is expressed spatially and in a site-restricted manner, is not yet fully known but it has been suggested that it plays a role in cellular adhesion, migration and proliferation, demarcation of tissue boundaries, haemagglutination, promotion of cell growth and determination of pathways for neural crest cell migration during foetal development [2, 9–11].

Salivary gland tumours present a most diverse group of tumours in terms of histomorphology. differentiation and clinical course. Various cell types at various levels of differentiation and a complex cell–extracellular matrix interaction have been suggested as one of the contributing factors in producing the diverse histomorphology. Immunoreactive tenascin has been reported in a wide variety of human benign and malignant neoplasms such as cancers of the breast [12, 13], uterine cervix [14], lungs [15], skin [16], colon [17], oral cavity [18], gliomas [19], melanomas [20, 21] and some common tumours of salivary gland origin [22, 23]. We set out the present study to evaluate the expression of tenascin in a large series of salivary gland tumours including rare variants. The pattern of immunoreactivity thus detected is discussed in an

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attempt to elucidate the possible production and role of this glycoprotein in producing the characteristic histopathological features.

MATERIALS AND METHODS

Tissue specimens and histological preparation

Tissue specimens of human salivary gland and tumours surgically excised were evaluated. We observed the following specimens: normal salivary glands ($n=5$), pleomorphic adenoma ($n=40$), Warthin's tumour ($n=10$), adenoid cystic carcinoma ($n=8$), mucoepidermoid carcinoma ($n=8$), epithelial–myoepithelial carcinoma ($n=4$), polymorphous low grade carcinoma ($n=3$), papillary cystadenocarcinoma ($n=15$) and undifferentiated carcinoma ($n=3$). The tumours were diagnosed and classified according to WHO criteria [24]. The tissue specimens were fixed for 6–12 h in 10% formalin and embedded in paraffin. Sections were evaluated by H&E staining and immunostaining of tenascin.

Immunohistochemical protocol

The monoclonal anti-tenascin antibody, mouse IgG_1 raised by purified human tenascin without cross-reactivity with fibronectin molecules was obtained from Chemicon International (California, U.S.A.). The sections were processed for immunostaining of tenascin using the streptavidin–biotin complex (ABC) method. After treatment with 0.1% pronase (room temperature, 10 min) and methanol containing 0.03% H_2O_2 to block the endogenous peroxidase and non-immune rabbit serum (1:20, 30 min) for blocking of non-specific reaction, the sections were incubated with anti-tenascin antibody (1:500, room temperature, 1 h). The sections were incubated for 1 h at room temperature in biotinylated anti-mouse rabbit IgG (1:200, Dakopatts, Denmark). Finally, the sections were incubated for 30 min at room temperature in AB complex (1:500, Dakopatts). The sections were visualised for peroxidase activity with 0.025% diaminobenzidine (DAB) including 0.003% H_2O_2 for 10 min. Sections routinely processed by omitting or replacing the primary antibody with an equivalent concentration of untreated mouse serum were used as negative controls and sections of breast carcinoma and oral squamous cell carcinoma known for enhanced expression of tenascin were used as positive controls.

RESULTS

Normal salivary glands

The normal salivary glands showed tenascin immunoreactivity in peripheral connective tissue of large excretory ducts and interlobular duts and no reactivity around acini, intralobular, striated or intercalated ducts (Fig. 1). The immunoreactivity was often seen around the blood vessels and perineural regions.

Warthin's tumour

Warthin's tumour showed a linear immunoreactivity profile of tenascin just adjacent to the basal cells of epithelial tumour component (Fig. 2). The lymphoid stroma, however, was largely negative except for small fibrillar structures (Fig. 3).

Pleomorphic adenoma

Pleomorphic adenomas showed a heterogeneity of expression of tenascin with a widespread immunoreactivity in the so-called stromal tissues. The immunoreactivity was most pronounced around the modified myoepithelial cells surrounding the tubuloductal structure or arranged in sheets or cords in all instances (Fig. 4). The chondroid areas showed a diffuse immunoreactivity with an enhanced reactivity adjacent to the metaplastic chondroid cells (Fig. 5). The myxoid and hyaline areas were also positive (Fig. 6).

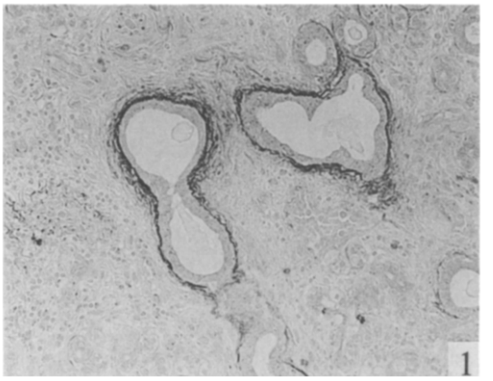


Fig. 1. Normal submandibular gland. Tenascin immunoreactivity is confined to peripheral connective tissue in large excretory or interlobular duct. Original magnification ×100.

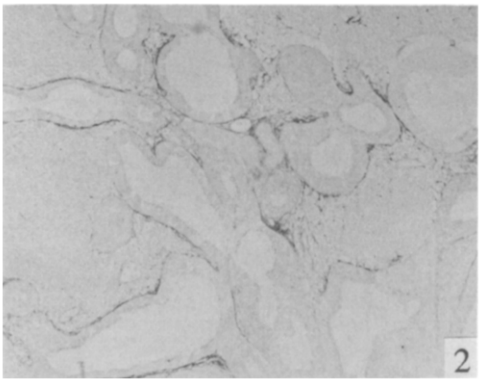


Fig. 2. Warthin's tumour: tenascin immunoreactivity is concentrated at the immediate vicinity of basement membrane beneath the tumour epithelia. Original magnification ×40.

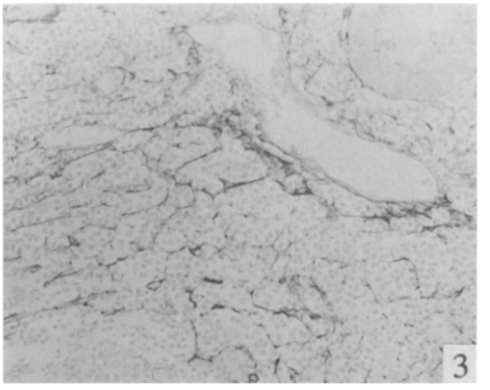


Fig. 3. Warthin's tumour: reticular pattern of immunoreactive tenascin in some instances of lymphoid stroma. Original magnification ×40.

Adenoid cystic carcinoma

The tumour showed immunoreactivity of tenascin around the tumour cell focus arranged in cribriform or tubular pattern

and in basaloid variants, however, not infrequently there was tenascin-negative tumour stroma. The innerside of cyst-like structures particularly in the cribriform type of adenoid cystic carcinoma had intensely positive immunoreactions for tenascin (Fig. 7).

Mucoepidermoid carcinoma (MEC)

A wide variation of tenascin immunoreactivity was observed in MEC with strongly positive focus in or around some tumour cell clusters to weak or negative in the same tumour specimen. There was no correlation between the expression of tenascin and grades of differentiation of MEC.

Polymorphous low-grade adenocarcinoma

Stromal tissues around the tumour cell focus were positive for tenascin (Fig. 8).

Epithelial–myoepithelial carcinoma

Tenascin immunoreactivity was observed around the islands of tumour cells and bands of connective tissue septa (Fig. 9). The lumen of duct-like structures formed in the islands of tumour cells were also positive for tenascin.

Undifferentiated carcinoma

Relatively small areas of tumour stroma had positive immunoreactivity for tenascin suggesting a low expression of tenascin in poorly or undifferentiated salivary tumours.

Papillary cystadenocarcinoma

The stroma around the papillary and papillocystic configuration of tumour cells were strongly positive for tenascin, however, there were some unreactive stromal areas (Figs 10, 11). In addition, small to medium-sized cystic structures with eosinophilic material in their lumen had intense immunoreactivity for tenascin (Figs 12–15).

DISCUSSION

Extracellular matrix protein acts as a substrate for cell attachment and motility in the process of normal development and in tumour proliferation and invasion. The cell origin of salivary gland tumours producing the variation in histomorphology and differentiation is not yet fully substantiated. However, *in vitro* studies employing neoplastic salivary carcinoma cell lines have suggested that tumour cells of neuroectodermal or neural crest in origin have the potential to differentiate into a wide variety of cell types [25]. As tenascin is a multifunctional extracellular glycoprotein with a site-restricted and spatial distribution during embryogenesis and various human neoplasia, and has the potential to regulate the migration of neural crest cells [11], we carried out this immunohistochemical study to elucidate the presence of this glycoprotein in tumours of salivary glands and its possible role. In normal oral mucosa, a delicate thin band of immunoreactive tenascin has been localised. We have recently reported enhanced expression of tenascin in the stromal tissues of oral leukoplakia and squamous cell carcinoma [18].

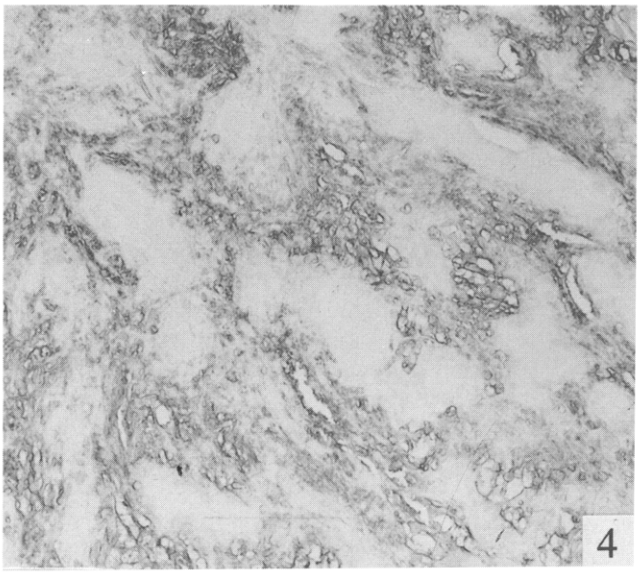
The results of the present study are similar to previously reported studies of tenascin in salivary tumours [22, 23], but they differ in certain aspects. Firstly, the immunoreactivity of tenascin in normal salivary glands in our present study differs from that in previously reported studies. We failed to demonstrate the linear immunoreactivity of tenascin around the acini, intercalated and striated ducts except for relatively large excretory or interlobular ducts of major salivary glands, despite a consistent immunoreactivity around the perivascular and perineural connective tissue, as previously reported. On the other hand, linear tenascin immunostaining in periacinar and ducts of minor oral salivary glands has been noted in patients with graft vs. host disease (unpublished data). The difference between the studies may be attributed to the cross reactivity of the polyclonal and monoclonal antibody to other extracellular matrix glycoprotein.

In normal glands, the immunoreactivity of tenascin in connective tissue surrounding large excretory ducts or interlobular ducts while negative in the tissues surrounding acinar cells, intercalated ducts cells and intralobular ducts suggests that there is a difference in interaction or cell adhesion functions between the ductal basal cells of striated/excretory or intra/interlobular salivary ducts and surrounding connective tissue stroma. In addition, the histological evaluation of ductal cells show that the ductal basal cells in relatively large ducts are continuously arranged as in the normal oral mucosa whereas those on relatively smaller ducts are not continuous [26]. The ductal basal cells of salivary ducts are less well characterised and this difference in ductal basal cell–mesenchymal tissue interactions at various levels needs further study, which may have an implication for the pathogenesis and histogenesis of salivary gland neoplasms.

Remarkable immunoreactivity of tenascin was observed in pleomorphic adenomas. Pleomorphic adenomas show the most diverse histopathological morphology in human neoplasia and usually present with tubulo-duct-like structures containing luminal tumour cells and outer tumour cells termed as modified or neoplastic myoepithelial cells, where the latter are responsible for production of excessive amounts of basal lamina-associated proteins and glycosaminoglycans and, therefore, a variety of so-called stroma ranging from fibrous to hyaline and myxoid to chondroid [27]. As these changes are not the mesenchymal cell product but secondary alterations produced by neoplastic myoepithelial cells [28], a consistent

Figs 4–6. Pleomorphic adenoma of salivary glands. Original magnification ×100. Figure 4. Tenascin immunoreactivity is concentrated at the vicinity of modified myoepithelial cells. Figure 5. Reaction products of tenascin is diffusely present in hyalinous and chondroid areas associated with modified myoepithelial cells. Figure 6. Tenascin immunoreactivity is strongly confined to the myxomatous tissue.

Figs 7–9. Heterogeneity of expression of tenascin in malignant tumours of salivary glands. Original magnification ×100. Figure 7. Adenoid cystic carcinoma showing cribriform spaces lined with tumour cells and the positive tenascin immunoreactivity. The tumour stroma in this case is negative. Figure 8. Polymorphous low grade adenocarcinoma of palate shows positive tenascin immunoreactivity in the tumour stroma. Figure 9. Epithelial–myoepithelial carcinoma showing positive tenascin immunoreactivity in the fibrous stroma.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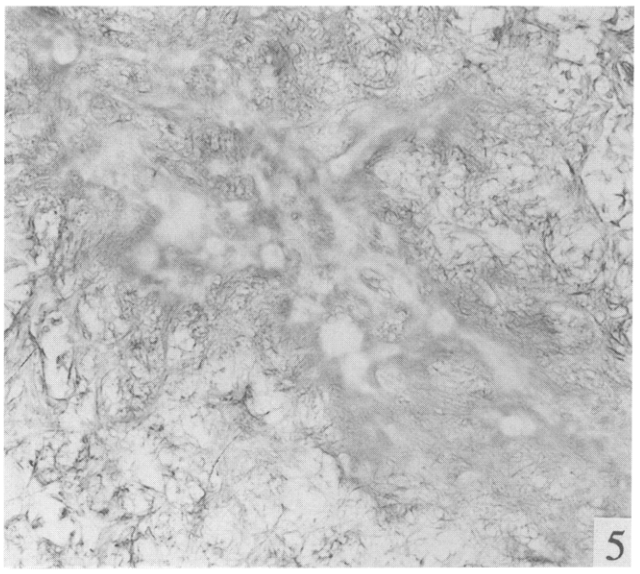
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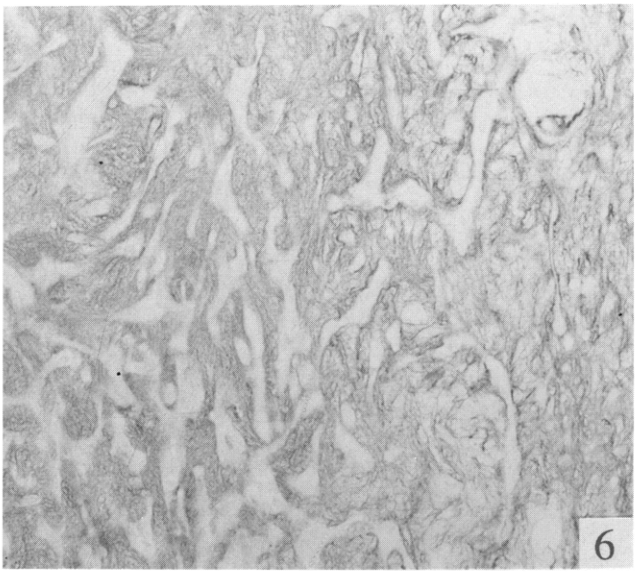
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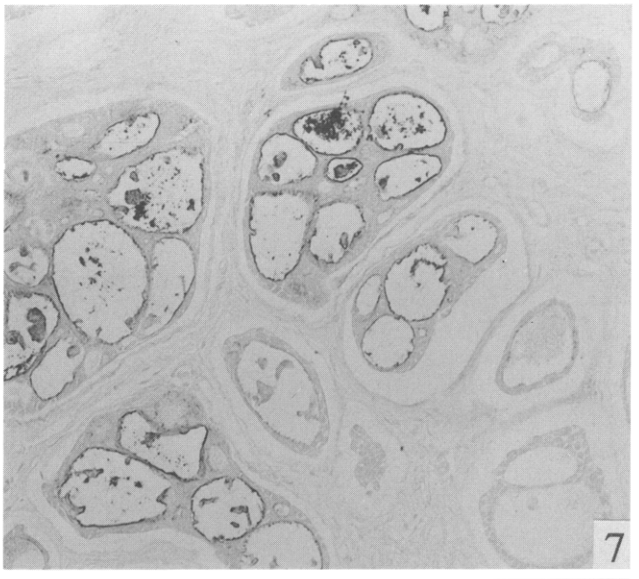
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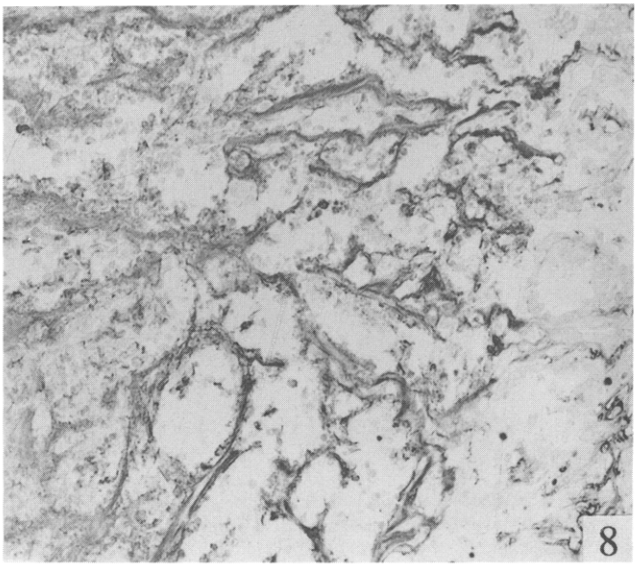
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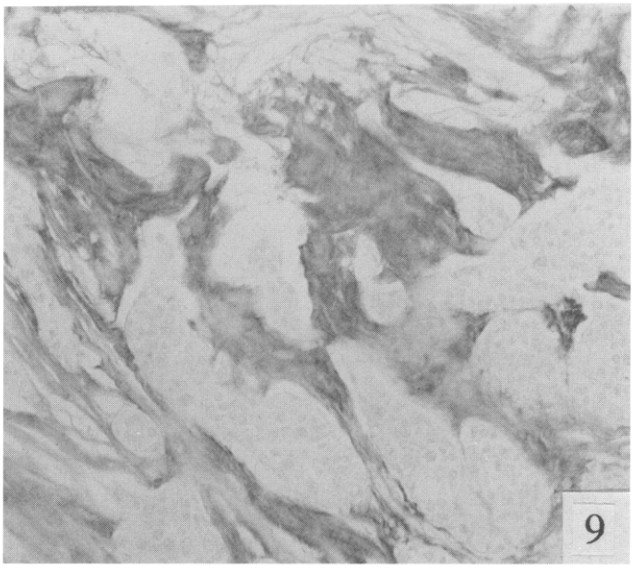
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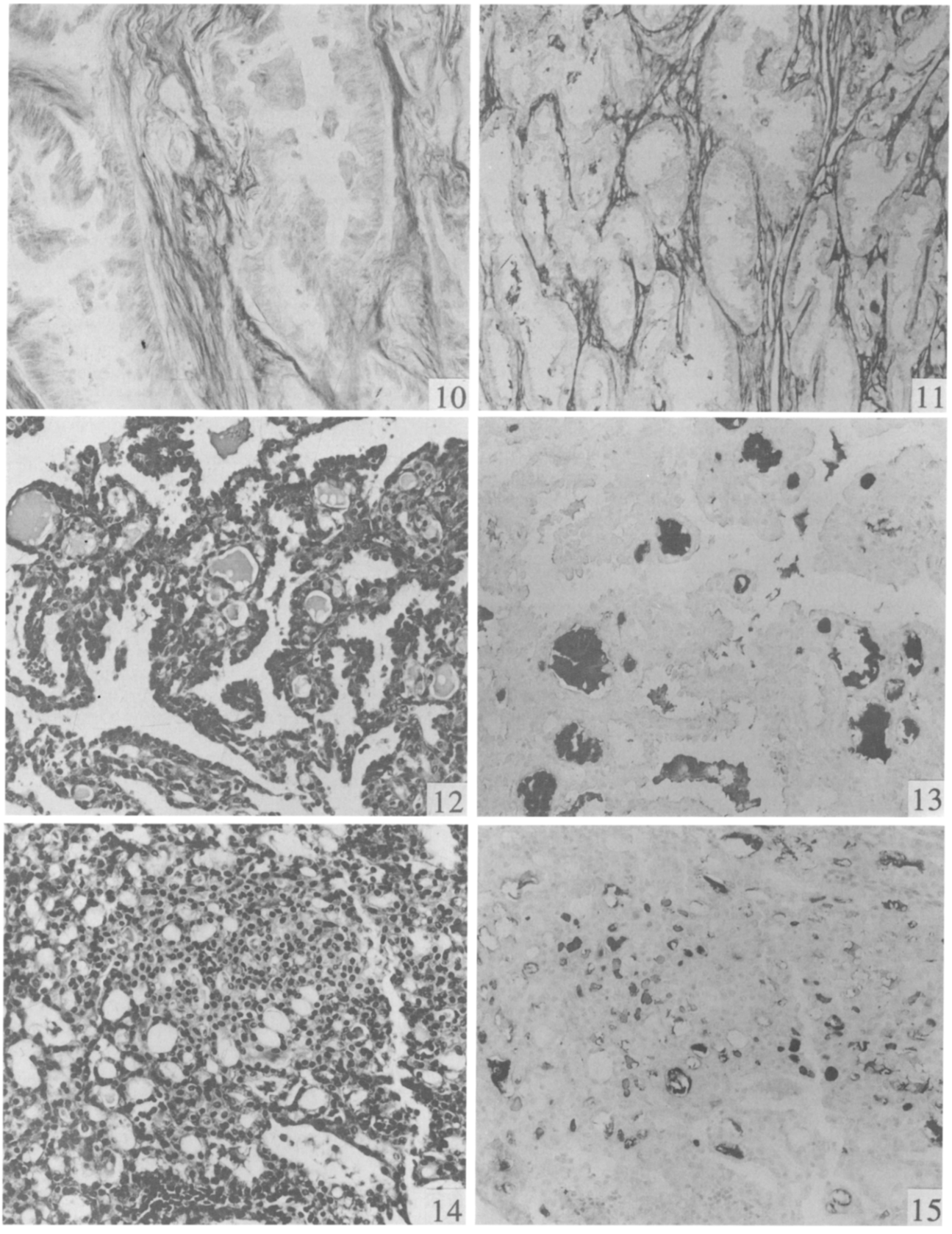

Figs 10–15. Papillary cystadenocarcinoma. Figures 10, 11. Papillary cystadenocarcinoma showing positive tenascin reaction in the stromal fibrous tissue. A heterogeneity in intensity of reaction is seen in the fibrous tissue adjacent to the tumour cells (original magnification Figure 10: ×100, Figure 11: ×40). Figures 12, 14. H&E staining. Papillary cystadenocarcinoma with poorly developed stroma but with numerous small (Figure 12) and smaller (Figure 14) cyst-like spaces containing eosinophilic deposits. Tenascin immunoreactivity is strong in the intracystic eosinophilic deposits suggesting tumour cells produce tenascin into lumen (Figures 13, 15). Original magnification ×100.

pattern of immunoreactivity of tenascin in these areas suggests a cardinal role of this glycoprotein in the production of modified myoepithelial cells in pleomorphic adenoma. The chondroid changes in pleomorphic adenoma do not differ from normal hyaline cartilage at the immunohistochemical and ultrastructural level [29]. A recent study from our laboratory has demonstrated bone morphogenetic protein responsible for chondrogenesis and osteogenesis in modified myoepithelial cells of pleomorphic adenoma [30]. As tenascin has been found to play a role in chondrogenesis and osteogenesis during embryonic development [31], a similar function of this extracellular matrix protein in pleomorphic adenoma may be anticipated, responsible for chondroid changes.

The linear immunoreactivity of tenascin adjacent to the basal cells of epithelial components in Warthin's tumour suggests a role of tenascin in delineating epithelial and stromal tissues in tumours as seen in the normal epithelial–mesenchymal interface in embryogenesis. Although we failed to demonstrate the source of tenascin (epithelial or mesenchymal) in our previous study, the characteristic pattern of immunoreactivity in the lumens of duct-like structures formed by tumour cells in adenoid cystic carcinoma, mucoepidermoid carcinoma, epithelial–myoepithelial carcinoma, polymorphous low-grade adenocarcinoma and papillary cystadenocarcinoma without stromal participation clearly demonstrates the production of tenascin by tumour cells. Tenascin was previously thought to be solely a mesenchymal cell product, and tumour cells may induce mesynchymal cells to produce it. In addition, a variety of cells such as arterial smooth muscle cells and epithelial tumour cells have been found to produce tenascin at various levels of differentiation [12, 32–34].

On the basis of the present study, tenascin distribution in salivary tumours may be divided into the following types. Firstly, its distribution as in pleomorphic adenoma where the tumour areas with modified myoepithelial cell containing myxoid, hyaline and chondroid changes are positive. Secondly, the presence of tenascin at the interface of epithelial/mesenchymal tumour tissues, was unreliable in benign or malignant histomorphology. Lastly, tenascin was produced by tumour cells at sites such as in lumens formed by epithelial tumour cells, which are not in direct communication with the supporting stroma. In conclusion, the presence of tenascin around the large excretory ducts suggests a different basal cell–mesenchymal interaction in the ductal system of normal glands and its enhanced expression suggests that it may have an important role in the growth and differentiation of salivary gland tumours by altering epithelial–mesenchymal interactions.

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