Virchows Archiv A Pathol Anat (1992) 421:217–222

Virchows Archiv A
Pathological Anatomy and Histopathology

Tenascin in salivary gland tumours

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Received November 10, 1991 / Received after revision March 14, 1992 / Accepted March 16, 1992

Summary. The distribution of tenascin immunoreactivity was analysed in salivary gland tissue and in various benign and malignant tumours of the salivary gland. In the non-neoplastic tissue, tenascin was seen in the areas of basement membranes of the ductal epithelium. No immunoreactivity could be observed in the serous or mucous glands. In pleomorphic adenomas, tenascin immunoreactivity could be seen in the stromal compartment. It was more pronounced in the dense stromal areas and chondroid elements than in the myxoid area. In Warthin's tumours, strong tenascin immunoreactivity could be observed in the basement membrane zone of the epithelial component. In the lymphatic component, faint reticular staining could be seen. In adenoid cystic carcinomas, acinic cell tumours and mucoepidermoid carcinomas, tenascin showed a linear stromal distribution. No intracytoplasmic immunoreactivity could be seen in any of the cases. The widespread tenascin positivity in salivary gland tumours suggests that tenascin may play a role in the induction and progression of salivary gland tumours, presumably by interfering with the normal parenchymal-mesenchymal interaction.

Key words: Tenascin – Salivary gland tumours – Immunohistochemistry

Introduction

Tenascin is a six-armed extracellular matrix glycoprotein with a molecular weight of 1,900 kDa (Erickson and Bourdon 1989; Chiquet-Ehrismann 1990). It was discovered independently in several laboratories and, consequently, is known by various names, such as hexabrachion, cytotactin, myotendineous antigen, GP250 and J-1 protein (Erickson and Lightner 1988). The subunits of tenascin show heterogeneity and, in SDS-polyacrylamide gel electrophoresis, three separate tenascin subunits can be found with molecular weights of 240 kDa, 200 kDa and 190 kDa (Chiquet-Ehrismann et al. 1986).

Tenascin is found in embryonic tissues (Chiquet and Famborough 1984; Crossin et al. 1986; Chuong et al. 1987), especially in areas of epithelial-mesenchymal junctions, and in developing brain tissue. It probably has a role in epithelial-mesenchymal induction and cell migration (Chiquet-Ehrismann et al. 1986; Crossin et al. 1986; Chuong et al. 1987; Erickson and Bourdon 1989; Chiquet-Ehrismann 1990). Tenascin is synthesized by glial cells and fibroblasts (Erickson and Bourdon 1989). By in situ hybridization, tenascin synthesis has also been shown in embryonic kidney and lung mesenchymal cells and in lung epithelial cells (Koch et al. 1991; Prieto et al. 1990).

In adult tissues tenascin can be found in healing wounds, in dense connective tissue, such as tendons and ligaments, in cartilaginous tissue and in smooth muscle (Erickson and Bourdon 1989; Chiquet-Ehrismann 1990; Chuong and Chen 1991). Tenascin has also been observed in the basement membranes of different epithelia of ectodermal and endodermal origin (Natali et al. 1991).

In malignant neoplasms, stromal tenascin immunoreactivity has been found in various tumours such as gliomas, sarcomas, melanomas and carcinomas (Bourdon et al. 1983; Erickson and Bourdon 1989; Natali et al. 1990, 1991; Vollmer et al. 1990; Howeedy et al. 1990). It is also been detected in some benign tumours such as naevocellular naevi and benign breast tumours (Howeedy et al. 1990; Natali et al. 1991).

In this study we used immunohistochemical techniques to investigate the distribution of tenascin in salivary gland tissue and neoplasms. We used two monoclonal antibodies, one of which detects tenascin also in formalin-fixed, paraffin-embedded specimens.

Materials and methods

Thirty-five cases of benign and malignant salivary gland tumours were selected from the files of the Department of Pathology, Oulu

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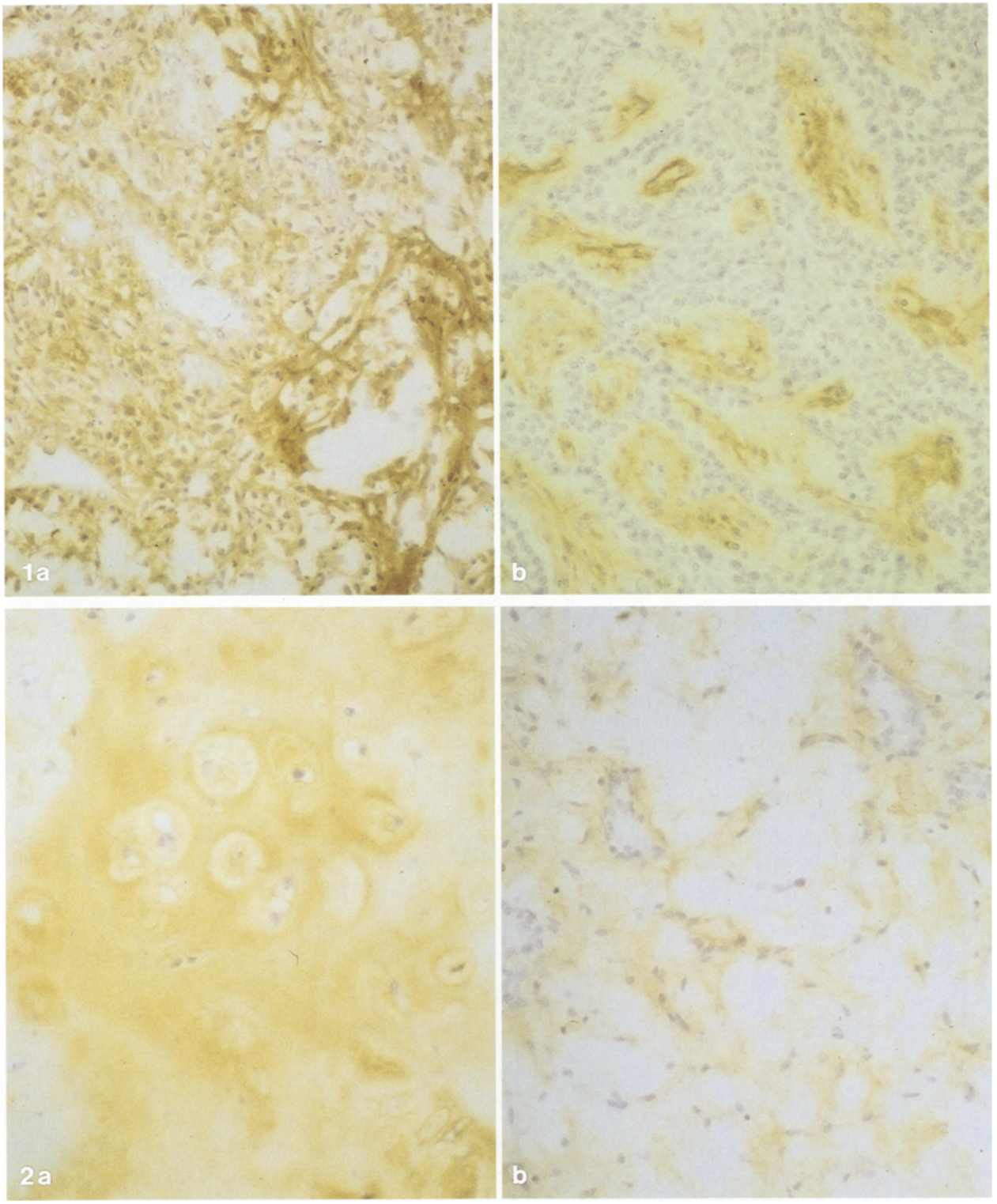
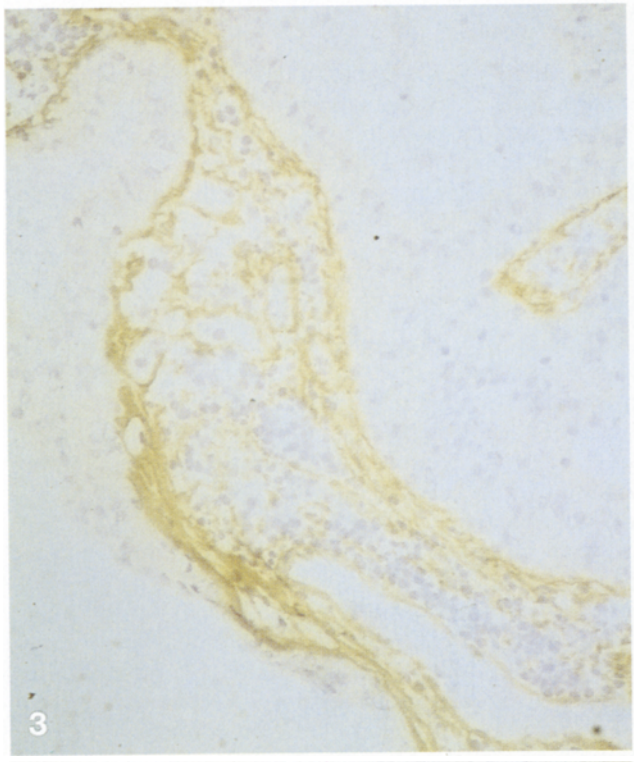
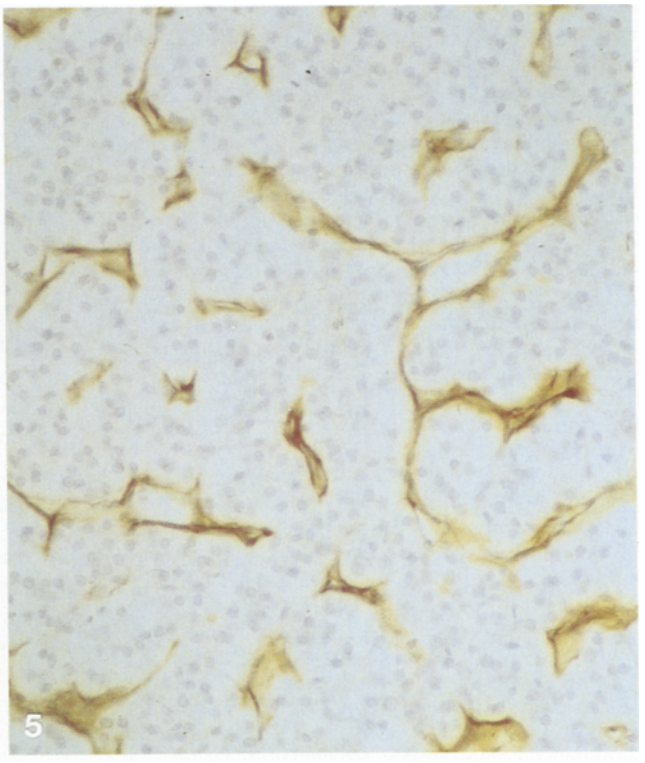


Fig. 1a, b. Immunoperoxidase staining for the demonstration of tenascin in pleomorphic adenoma. The stromal tissue stains positively for tenascin. The staining is more intense with the EB-100 antibody (**a**) than with 143DB7 (**b**). Immunoperoxidase stain, × 210

Fig. 2a, b. Immunoperoxidase staining for the demonstration of tenascin in pleomorphic adenoma. The cartilaginous component stains strongly for tenascin (**a**) while in the myxoid zones the staining is weak and delicate (**b**). Immunoperoxidase stain, × 210





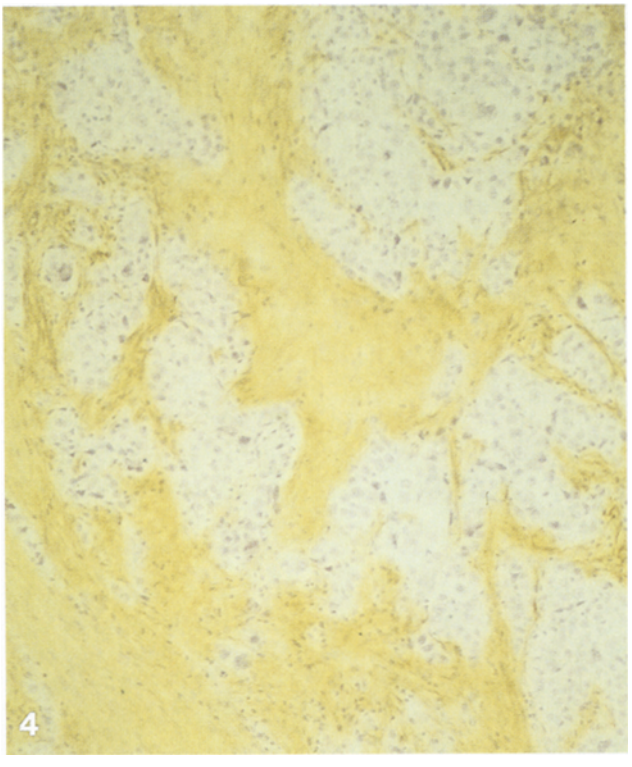


Fig. 3. Immunoperoxidase staining for the demonstration of tenascin in Warthin's tumour. The areas of the basement membranes stain positively. Delicate reticular staining can be observed in the lymphatic areas. Immunoperoxidase stain, ×210

Fig. 4. Immunoperoxidase staining for the demonstration of tenascin in mucoepidermoid carcinoma. The stromal tissue stains strongly for tenascin while the tumour cells are negative. Immunoperoxidase stain, ×155

Fig. 5. Immunoperoxidase staining for the demonstration of tenascin in acinic cell tumour. Delicate positive fibrous strands can be observed in the stroma. Immunoperoxidase stain, ×210

University Central Hospital. The cases were from the years 1985–1990 and the material was fixed in neutral formalin and embedded in paraffin. Some of the material (7 cases) was obtained fresh from the operating theatre and stored in liquid nitrogen. The tumours were classified according to the WHO International classification of salivary gland tumours (Seifert 1991). The diagnosis of all the cases was based on light microscopic examination using conventional haematoxylin and eosin stain.

The material consisted of 16 pleomorphic adenomas, 6 adenolymphomas, 6 mucoepidermoid carcinomas, 3 acinic cell tumours, 3 adenoid cystic carcinomas and 1 pleomorphic sarcoma of the salivary gland. Frequently, the specimens also contained non-neoplastic elements. Eight cases of non-neoplastic salivary gland tissue were also included (4 normal, 4 chronic inflammations of the salivary gland).

We aimed at producing a monoclonal antibody that also detects tenascin efficiently in formalin-fixed material. For that purpose, the mAb 143DB7 was raised as follows. First, tenascin was purified

from the culture medium of human embryonal fibroblasts by using affinity purification with a well-characterized anti-tenascin hybridoma clone 100EB2 (Howeedy et al. 1990). For that purpose, immunoglobulin fraction of 100EB2 was purified from the ascites fluid by using protein G column (Pharmacia, Uppsala, Sweden) in FPLC. It was then coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions. Culture medium of human embryonal fibroblasts was then passed over the column. After extensive washing, the bound tenascin was eluted with 3-[(Cholamidopropyl)-dimethyl ammonio]-1-propanesulfonate (CHAPS) buffer, pH 11, and used for immunization. Three days after the last immunization of BALB/c mice, the hybridoma production was initiated by standard techniques (Köhler and Milstein 1975). The hybridomas were screened, first, by using purified tenascin in enzyme-linked immunoassay, and then by immunohistochemistry on pepsin-treated paraffin sections of selected formalin-fixed tumours. The hybridoma culture 143BD7 was cloned by using standard techniques.

The specificity of mAb 143DB7 was shown by immunoblotting of whole human cultured fibroblasts. For immunoblotting, the cells were lysed in electrophoresis sample buffer, boiled and analysed in SDS-polyacrylamide gel electrophoresis, carried out according to Laemmli (1970), by using 6.5% stab gels and reducing conditions. The transfer of the electrophoretically separated polypeptides onto nitrocellulose paper was carried out according to the method of Towbin et al. (1979). To visualize the polypeptide bands, strips of the nitrocellulose sheets were cut and stained with amido black. The immunodetection was done by incubating the nitrocellulose sheets first with mAb 143DB7 and then with peroxidase-coupled rabbit anti-mouse IgG (Dakopatts, Copenhagen, Denmark). 3'-3' Diaminobenzidine was used as a chromogen. In immunoblotting, two distinct bands of 250,000 and 190,000 daltons were revealed. No polypeptide bands were revealed in experiments in which the primary antibody was either omitted or replaced by antibodies to proteins not produced by fibroblasts (not shown).

For paraffin sections, 5-μm-thick sections were deparaffinized in xylene and rehydrated in graded alcohol. The endogenous peroxidase was consumed by treating the sections with 0.3% hydrogen peroxidase in absolute methanol for 30 min. Prior to the immunostaining, the sections were treated with 0.4% pepsin (Merck, Darmstadt, FRG; 10 units/mg) for 30 min at 37° C. Monoclonal mouse antibody (143DB7) to human tenascin was used as the primary antibody. For the immunostaining, the avidin-biotin complex (ABC) method was used (Hsu et al. 1981). The sections were first incubated overnight with the primary antibody at 4° C, followed by biotinylated rabbit anti-mouse secondary antibody (1:200) and the ABC complex (Dakopatts). The colour was developed with diaminobenzidine, whereafter the sections were mounted in an aqueous medium. The sections were counterstained with a light haematoxylin stain. Negative control consisted of substituting PBS (140 ml sodium chloride, 0.01 M phosphate buffer, pH 7.2) for the primary antibody.

For frozen sections, 5-μm-thick sections were cut from the specimens. They were air-dried for 1 h and were then fixed in acetone for 10 min at −22° C. The endogenous peroxidase was consumed by treating the sections with 0.3% hydrogen peroxidase in methanol for 30 min. A monoclonal mouse antibody (EB-100) to human tenascin, with a dilution of 1:5, was used as the primary antibody (Howeedy et al. 1990). The sections were incubated with the primary antibody overnight at 4° C, followed by the biotinylated secondary rabbit anti-mouse antibody (1:200) and the ABC complex (Dakopatts).

The peroxidase reaction was developed with diaminobenzidine, whereafter the sections were mounted in an aqueous medium. The sections were counterstained lightly with haematoxylin. Negative controls were as described previously.

Results

In the normal salivary gland, tenascin immunoreactivity was seen in the basement membranes of the ducts and in the smooth muscle cells in the walls of the small arteries. The mucous and serous glands were negative. The finding was similar in the chronically inflamed salivary gland tissue.

In pleomorphic adenomas, linear immunoreactivity could be observed in the stromal tissue in all cases (Figs. 1, 2). Also the fibrotic capsule stained positive. In the dense, fibrotic areas, the immunoreactivity was stronger than in the myxoid areas (Fig. 2b). Some myxoid islands remained negative for the staining. Also the cartilaginous component stained positive for tenascin (Fig. 2a). Often, a uniform distribution of the staining was seen over the cartilaginous area. Occasionally, however, non-reactive stromal zones could be seen around individual chondrocytes. No intracellular tenascin was seen in tumour cells.

In Warthin's tumours (Fig. 3), tenascin immunoreactivity was seen in the areas of the basement membranes beneath the epithelial cell component. In some areas, positivity also occurred in the fibrous cores of the papillary projections. In the lymphatic component, immunoreactivity was confined to thin reticular fibres. Also the tumour capsule stained positive.

The stroma of all malignant salivary gland tumours was positive for tenascin. The staining was weaker in adenoid cystic carcinomas and in acinic cell tumour than in mucoepidermoid carcinomas. In adenoid cystic carcinomas and mucoepidermoid carcinomas, a linear tenascin immunoreactivity was observed, which sometimes concentrated around the tumour cell islands (Fig. 4). In acinic cell tumours, faintly positive linear strands could be observed (Fig. 5). No intracytoplasmic staining for tenascin was seen in tumour cells.

There was no difference between the results obtained with the two tenascin antibodies. The intensity of the immunostaining was stronger, however, in the EB-100 stained fresh frozen material. The control staining was negative in all cases.

Discussion

In this investigation we analysed the distribution of tenascin in benign and malignant salivary gland tumours and in non-neoplastic salivary gland. The immunostaining was carried out by using two different antibodies, EB-100 and 143DB7, both of which work well on fresh, frozen sections and the latter also on formalin-fixed, paraffin-embedded material. The staining results with the two antibodies were identical. However, the immunostaining of fresh frozen material with EB-100 yielded the most intense staining reaction. This probably depends not only on the difference in the reactivity between the antibodies, but also on the better preservation of the tenascin antigen in fresh frozen material and/or the fact that the antigenic determinants are partly hidden in formalin-fixed material.

In benign salivary gland tumours, stromal positivity was observed both in pleomorphic adenomas and in adenolymphomas. In pleomorphic adenomas, there was a strong stromal immunoreactivity in the dense, fibrotic stroma as well as in the cartilaginous component of the tumour. In the myxoid zones the staining was less intense, suggesting that there is a lower concentration of tenascin in the myxoid zones. Interestingly, pleomorphic adenomas contain fibronectin (Caselitz et al. 1988) and chondroitin sulphate proteoglycans (Harrison and Auger 1991), which both bind tenascin (Chiquet Ehrismann et al. 1991; Erickson and Lightner 1988). Thus the lower degree of staining for tenascin in the myxoid zone could also be a reflection of a lower concentration of these substances in this region.

A diffuse distribution of tenascin could also be seen in the chondroid compartment in pleomorphic adenomas. Tenascin is present in embryonic chrondroid tissue, where it is distributed throughout the tissue, while in adult tissue it is concentrated mainly in the perichondrium (Erickson and Lightner 1988). It may even by possible that the chondrogenic differentiation in pleomorphic adenomas is induced by tenascin. This is supported by the observation that tenascin is associated with chondrogenic differentiation in vivo (Chiquet-Ehrismann 1990).

In Warthin's tumours, as in normal salivary gland, a strong tenascin immunoreactivity was seen in the basement membrane areas beneath the epithelial component. This is in line with previous studies which have shown tenascin in the basement membranes of different epithelia of ectodermal and endodermal origin (Natali et al. 1991). Synthesis of tenascin has also been reported in the epithelial cells of developing lung buds (Koch et al. 1991). A receptor for tenascin has been described that belongs to a family of integrin molecules (Bourdon and Ruoslahti 1989). The epithelial cells of salivary gland ducts, as well as other epithelia, might harbour such surface receptors and tenascin might thus function as a structural protein in the ductal units to retain the integrity and orientation of the epithelial cells.

In malignant salivary gland tumours, stromal tenascin immunoreactivity could be seen in all cases. This is in accordance with previous results which indicate stromal tenascin immunoreactivity in various malignant neoplasms (Bourdon et al. 1983; Erickson and Bourdon 1989; Natali et al. 1990; Vollmer et al. 1990; Howeedy et al. 1990). In the present study, however, there were differences in the intensity of tenascin staining between different types of tumours.

The tissue distribution of tenascin is reminiscent of that of fibronectin, which is also found in the stromal areas, while laminin and type IV collagen are mainly found in the basement membrane areas of these tumours (Caselitz et al. 1988). This similarity in tissue distribution may be due to the fact that the 190 kDa isoform of tenascin is able to attach to fibronectin (Chiquet-Ehrismann et al. 1991).

In tumour tissue tenascin is considered to be synthesized by stromal fibroblasts which are stimulated by cytokines such as transforming growth factor-beta, which is produced by the tumour cells (Pearson et al. 1988; Chiquet-Ehrismann 1990). Tenascin contains epidermal growth factor-like repeats (Jones et al. 1988) and it has been suggested that tenascin might induce tumour growth by an autocrine mechanism (Engel 1989). The growth promotion induced by tenascin may also play a part in the maintenance and progression of both benign and malignant salivary gland tumours.

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