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Immunohistochemical Demonstration of Tenascin and Fibronectin in Odontogenic Tumours and Human Fetal Tooth Germs

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The distribution of tenascin and fibronectin (plasma fibronectin) was studied immunohistochemically in ameloblastomas, ameloblastic fibromas and ameloblastic carcinomas, as well as in tooth germs using monoclonal antibodies. Tenascin is an extracellular matrix molecule that was shown to be enriched in the embryonic mesenchyme surrounding the budding epithelium in various organs, including the tooth. Tenascin was strongly expressed in the basement membrane zone of the ameloblastomas and in the early tooth germ and the dental lamina, but not in the dental follicle. The expression of tenascin in the ameloblastic fibroma was seen in the basement membrane of the epithelial islands throughout the stromal tissues. There were clear differences in fibronectin expression in the follicular ameloblastoma and ameloblastic carcinoma. The results suggest that tenascin and fibronectin are involved in epithelial mesenchymal interactions of the tooth germ and in odontogenic tumours.
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INTRODUCTION

MOST ODONTOGENIC tumours are benign [1]. Ameloblastoma is usually a locally infiltrative tumour, with a tendency to recur, but is not usually metastatic. It is characterised by a neoplastic epithelium in a mature connective tissue stroma. There are few reports on the immunohistochemical expression of cytokeratin, amelogenin, and enamelin in ameloblastomas and adenomatoid odontogenic tumours [2–5]. In 1983, Shafer *et al.* [6] introduced the term ameloblastic carcinoma to describe ameloblastomas in which there was histological malignant transformation in association with metastatic growth. Ameloblastic carcinomas were classified as an ameloblastoma with anaplastic transformation and metastasis [7]. It would have been useful to have included some malignant odontogenic tumours but they are rare [8].

Tenascin is an extracellular matrix glycoprotein which appears to regulate cell morphology [9–11]. It has a more restricted tissue distribution than fibronectin and can interface with the cell-binding function of fibronectin [12]. Tenascin is most typically expressed in epithelial–mesenchymal interactions during development and in the stroma of carcinoma [11]. Extracellular matrix proteins have been shown to play important roles in cellular growth and differentiation, in complex cell matrix interactions, in normal organ development and tumour progression. There are few reports on the relationship between the extracellular matrix, tenascin in odontogenic tumours and tooth development [13–14]. Here, we compared the distribution of tenascin and fibronectin in some human odontogenic tumours and human tooth germs.

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MATERIALS AND METHODS

Ten ameloblastomas were obtained for this study. Two cases of ameloblastic fibroma and two cases of ameloblastic carcinoma were also examined. Five samples of tooth germs were obtained at autopsy.

Samples were fixed with 10% neutral buffered formalin and embedded in paraffin. For immunohistochemical staining, 3-μm sections were deparaffinised and immersed with 0.3% methanol containing hydrogen peroxide for 30 min. All sections were treated with 0.1% trypsin solution at 37°C for 1 h. Sections were reacted with antibody at 4°C overnight and stained by the PAP method.

Tenascin antibody (mono, Biohit) and fibronectin (plasma, poly, Lipshaw) were diluted to 1:1000. Sections were developed in 0.02% 3,3-diaminobenzidine tetrahydrochloride (Sigma) with 0.01% hydrogen peroxide in Tris-buffered saline solution at room temperature.

RESULTS

In the dental lamina of the bell stage of human tooth germ, tenascin showed a positive reaction on the submucosal

connective tissue side but no reaction on the dental follicle tissue side. There were no morphological differences in the odontogenic epithelial cells on either side (Fig. 1a). In fibronectin staining of the same section, a weak or negative reaction was seen in the condensed mesenchyme surrounding the dental lamina (Fig. 1b). In the cap stage, there were different reactions in tenascin and fibronectin in the human tooth germ (Fig. 2a, b). Intense tenascin accumulation was seen in the dental papilla under the basement membrane, preodontoblastic layer and osteogenic tissue of the alveolar bone. But in the dental follicle, the fibroblastic layer developing to the periodontium was negative for tenascin (Fig. 2a). A marked fibronectin reactivity was evenly present in the dental papilla, dental follicle and osteogenic tissue of the alveolar bone (Fig. 2b). The epithelial components of the tooth germ were negative to both antigens.

In the ameloblastic fibroma, tenascin and fibronectin showed different reactions in the stroma. The fibrous stroma and basement membrane immediately adjacent to the epithelial islands had a uniformly intense reaction for tenascin (Fig. 3a), but fibronectin showed a negative or weak reaction in the same area (Fig. 3b). Immuno-reactions for tenascin were variably positive depending upon the site of the mesenchymal tissue component, although in the same area of the fibrous stroma, fibronectin exhibited an evenly negative or weak reaction (Fig. 4a, b).

In the ameloblastomas, the extracellular components of the ameloblastoma stroma exhibited considerable diversity. There were variations in the stromal components: dense and loose connective tissues, a hyalinisation layer and stromal cystic spaces. In the hyalinised stroma, tenascin and fibronectin exhibited both positive and negative responses. In the cystic stroma, tenascin and fibronectin showed negative reactions. In the follicular ameloblastoma, the basement membrane zone showed an irregular linear tenascin positive reaction (Fig. 5a). The dense connective tissue of the stromal area in the follicular ameloblastoma exhibits an even but weak reactivity for fibronectin (Fig. 5b). A partial accumulation of tenascin was found in the basement membrane (Fig. 6). In this case, the stromal round cells were differentiated to dental papilla-like cells. The tenascin-positive basement membrane showed a

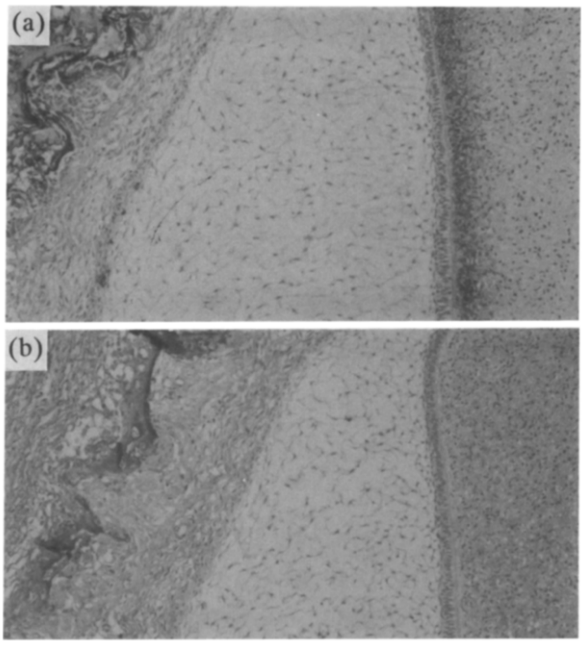

Fig. 2. Developing human tooth germs. Tenascin (a), fibronectin (b). Note the marked reactivity for fibronectin in the area of the dental papilla, dental follicle and osteogenic tissue. Tenascin accumulation is seen in the dental papilla under the enamel epithelium and osteogenic tissue.

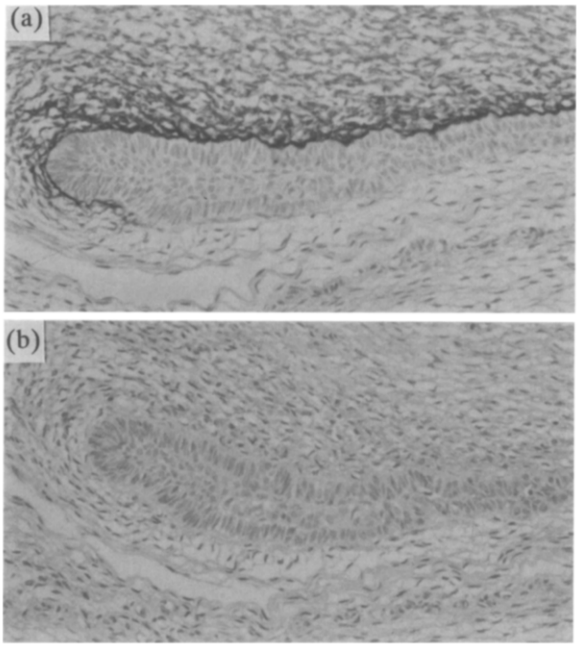

Fig. 1. Dental lamina of human tooth germ. Tenascin (a), fibronectin (b). Tenascin showed a positive reaction on the submucosal connective tissue side, but no reaction on the dental follicle tissue side.

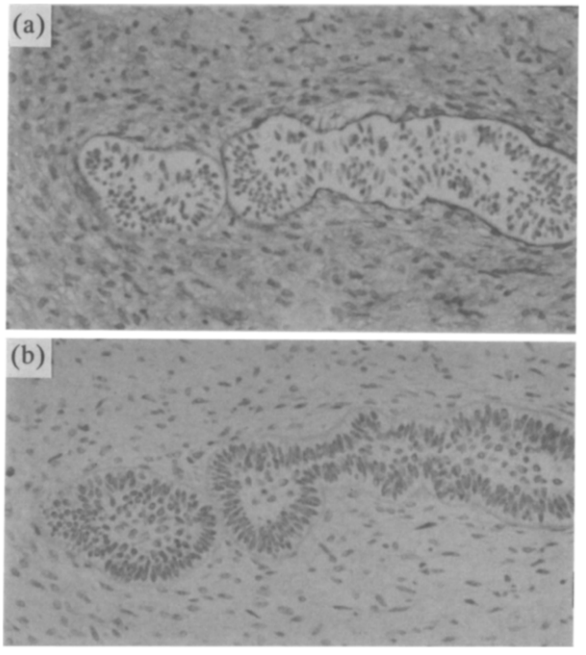

Fig. 3. Tenascin (a) and fibronectin (b) in ameloblastic fibroma. The fibrous stroma of the ameloblastic fibroma shows a uniformly intense reaction for tenascin. Ameloblastic fibroma exhibits a negative or a weak reaction for fibronectin in the fibroblastic stroma.

fuzzy fibrillar material (Fig. 7a), whereas the loose or myxomatous tissues of stromal area of the follicular ameloblastoma exhibited no reaction for fibronectin (Fig. 7b).

The stromal area and basement membrane of the ameloblastic carcinomas showed an irregular strong reaction for tenascin (Fig. 8a). A scattered or granular positive reaction was observed in the epithelial islands of the ameloblastic carcinoma. For fibronectin, the connective tissue stroma of the ameloblastic carcinoma showed an irregular and strong reaction (Fig. 8b).

DISCUSSION

Tenascin expression has been strongly linked to an epithelial–mesenchymal interaction in neoplastic transformation and organogenesis [9–12]. In the embryonic teeth, as well as the

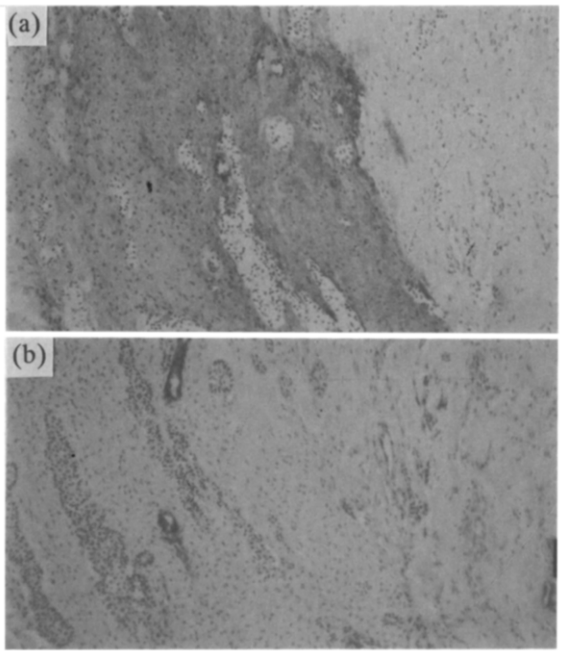

Fig. 4. Tenascin (a) and fibronectin (b) in ameloblastic fibroma. Tenascin showed a strong but patchy reaction compared with the almost negative fibronectin reaction.

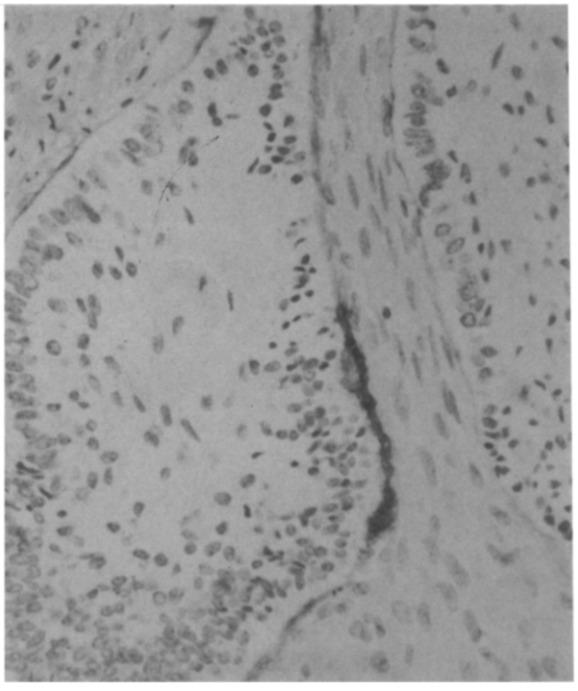

Fig. 6. A partial accumulation of tenascin is evident in the basement membrane of the follicular ameloblastoma.

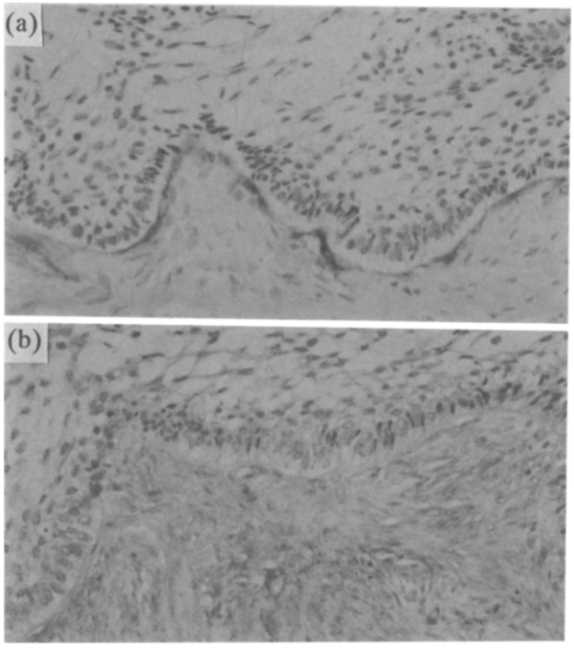

Fig. 5. Note the positively outlined basement membrane in the follicular ameloblastoma for tenascin (a). The dense connective tissue of the stromal area in the follicular ameloblastoma exhibits an even but weak reactivity for fibronectin (b).

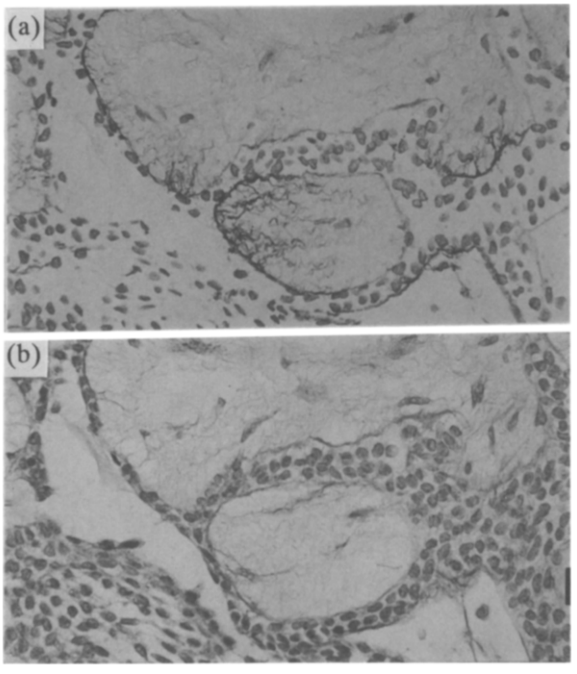

Fig. 7. Note the positively outlined fuzzy basement membrane of the epithelial islands for tenascin (a). The loose or myxomatous connective tissue of the stromal area in the follicular ameloblastoma exhibits no reaction for fibronectin (b).

mammary glands and hair follicles, tenascin is present in the dense, organ-specific mesenchyme surrounding the invaginating epithelial bud, but not in the more distant mesenchyme [9, 10]. It has been proposed that tenascin plays a role in the tissue interactions that govern the early development of these embryonic organs [11]. Previous studies [13, 14] analysed whether changes in the distribution of tenascin can be correlated with certain stages of tooth morphogenesis and cell differentiation. Tenascin was localised in the mouse and rat and in the human teeth at the stage of epithelial bud formation. They showed that tenascin was present in the dental basement membrane at the time of odontoblast differentiation. The dental papilla cells ceased to express tenascin upon differentiation into odontoblasts, and tenascin was completely absent from dentine. It can be speculated that the unusual expression of tenascin in dental mesenchymal cells, as compared with other connective tissues, is associated with its capacity to differentiate into hard tissue forming cells. Heikinheimo *et al.* [13] showed the distribution of tenascin and fibronectin in the ameloblastoma and ameloblastic fibroma, as well as in the developing human tooth. They found that ameloblastic fibromas exhibited an immunoreactivity for tenascin in the basement membrane zone in a similar fashion as during early tooth development. The expression of tenascin in the ameloblastoma was seen throughout the stromal tissues. They think that this difference may be related to the capacity of some ameloblastic fibromas to form dental hard tissues; ameloblastomas lack this capacity. There are some discrepancies between our data and their results in tenascin distribution. Our results show that tenascin was partially present in the basement membrane in the ameloblastoma, as well as in the early tooth germ and in the dental lamina.

The expression of tenascin in the ameloblastic fibroma was seen throughout the periepithelial stromal tissues and the basement membrane of the odontogenic epithelial island. In some tissue parts of the stroma of the ameloblastic fibroma, as well as in the dental follicle, tenascin was absent. Our preliminary data show that columnar cells of the follicular ameloblastoma lack a secretory function [2]. The pattern of distribution of tenascin in the ameloblastoma indicates that the peripheral columnar cells of follicular ameloblastoma are compatible with inner enamel epithelial cells of early bell stage of tooth germ.

Our ameloblastic carcinoma cases were characterised by areas of typical ameloblastoma features and areas with an anaplastic appearance [8]. Tenascin is most typically expressed in the stroma and the basement membrane of the ameloblastic carcinoma. There are clear differences of expression in the extracellular matrix of the follicular ameloblastoma and ameloblastic carcinoma.

As a similar pattern of co-expression of vimentin and keratin in ameloblastoma and adenomatoid odontogenic tumour, the epithelial islands of the ameloblastic carcinoma revealed intracellular distribution of tenascin. The finding is also observed in the cells of developing tooth germ. In odontogenic tumours, thus, tenascin expression may be a marker for histological diagnosis and biological stage.

The dental follicle is a mesenchymal structure that surrounds the enamel organ and the dental papilla as a fibrocellular layer, where the cells are oriented in a radial pattern. This structure gives rise to the supporting tissue periodontium of the tooth. Interestingly, the dental follicle appeared as a tenascin-free zone between the dental epithelium and the osteogenic mesenchyme [13, 14]. The dental follicle stains positively with fibronectin and type I and type III procollagen antibodies [15].

In this study, the dental follicle of the tooth germ lacked tenascin but had fibronectin. The osteogenic tissue contained both tenascin and fibronectin. The ameloblastic fibromas showed a positive or negative distribution in the stroma which suggests a differentiation to the papilla of the tooth germ. It is also suggested that in tenascin-negative areas, the stroma cells of the ameloblastic fibroma differentiate to dental follicle tissues. The relative distribution of tenascin and fibronectin can be a marker in histological diagnosis of periodontal and osteogenic fibrous tissues.

Our results also suggest that fibronectin and tenascin may be used as markers in the epithelial-mesenchymal interaction during tooth development and in odontogenic tumours.

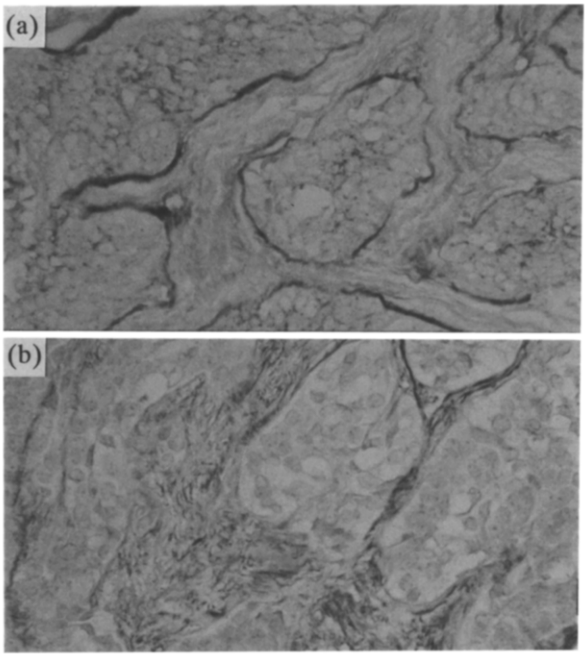

Fig. 8. A basement membrane with a strong positive reaction for tenascin is shown in the ameloblastic carcinoma. Note the scattered and granular positive reaction in the epithelial islands of the ameloblastic carcinoma (a). The stromal area of the ameloblastic carcinoma exhibits a strong irregular reactivity for fibronectin (b).

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