Pergamon

Oral Oncol, Eur J Cancer, Vol. 31B, No. 4, pp. 275–279, 1995

0964–1955/95 $9.50+0.00

0964-1955(95)00002-X

Expression of Tenascin in Odontogenic Tumours

M. Mori, T. Yamada, T. Doi, H. Ohmura, Y. Takai and P. Shrestha

We investigated the expression of tenascin in a series of odontogenic tumours ($n=63$) of epithelial and epithelial–ectomesenchymal origin by using immunohistochemical methods. A heterogeneity of expression of tenascin was observed in odontogenic tumours. The heterogeneity was most prominent in odontogenic tumours not forming calcified tissues. In these ameloblastomas and adenomatoid odontogenic tumours, tenascin was mainly localised at the epithelial tumour cell–mesenchymal tissue interface. In the calcifying epithelial odontogenic tumour, ameloblastic fibroma and odontoma, a widespread stromal immunoreactivity was observed which was, however, unreactive in the calcified masses. The stellate reticulum-like cells and granular cells of ameloblastoma also showed a positive immunoreactivity for tenascin. The results of the present study suggest that expression of tenascin in the stromal tissue of odontogenic tumours differs according to the potential of forming calcified masses by the tumour cells irrespective of tumour cell morphology.

Keywords: odontogenic tumours, tenascin, immunohistochemistry

Oral Oncol, Eur J Cancer, Vol. 31B, No. 4, pp. 275–279, 1995.

INTRODUCTION

EPITHELIAL TUMOURS, both benign and malignant, are composed of tumour cells and stromal connective tissue. Extracellular matrix glycoproteins are integral components of stromal connective tissue which play an important role in the histomorphological features and behaviour of the tumours. Tumour cell proliferation and invasion are also associated with alterations in the components of extracellular matrix proteins and degradations of the basement membrane.

Tenascin is a multifunctional glycoprotein involved in cell to cell and cell–extracellular matrix interactions and is spatially and temporarily expressed in a site restricted manner at the epithelial–mesenchymal interface during embryonic and fetal development, wound healing and in various solid tumours where it may affect cellular proliferation, differentiation and migration, although the exact function of this large molecular weight protein in neoplastic lesions is largely unknown [1–4].

An enhanced expression of tenascin has been reported in the epithelial–connective tissue interface in oral leukoplakia and a heterogeneity of expression in oral squamous cell carcinoma [5]. Numerous authors have described the expression of tenascin in various tumours [6–14]. In a recent communication from our laboratory, an enhanced and heterogeneous expression of this protein was demonstrated in a variety of salivary gland neoplasms [15]. Although primarily described as a mesenchymal cell product, recent studies have demonstrated its production by epithelial cells in normal as well as various other neoplastic conditions [7, 16–18].

The presence of numerous extracellular matrix proteins has been studied in various odontogenic tumours [19]. The expression of tenascin in a small number of odontogenic tumours and fetal tooth germs and adult teeth have been reported [20, 21]. Therefore, the present study was designed to assess the expression of tenascin in a series of epithelial and epithelial–ectomesemchymal tumours of odontogenic origin with particular attention to their potential to form calcified masses.

MATERIALS AND METHODS

Tissue specimens of odontogenic tumours obtained from surgery, routinely processed by fixation in 10% neutral buffered formalin for 6–12 h and embedded in paraffin, were employed. The odontogenic tumours were ameloblastoma ($n=40$), squamous odontogenic tumour ($n=2$), adenomatoid odontogenic tumour (AOT) ($n=5$), calcifying epithelial odontogenic tumour (CEOT) ($n=3$), calcifying odontogenic cyst (COC) ($n=4$), ameloblastic fibroma ($n=3$), ameloblastic fibro-odontoma ($n=2$) and odontoma ($n=5$). Paraffin sections of 4 μm were used to detect tenascin using a monoclonal antibody to tenascin. A three stage streptavidin–biotin complex immunoperoxidase method was used, the specificity of the antibody and details of the immunohistochemical method have been described previously [5, 15].

RESULTS

A heterogeneous pattern of expression of tenascin was observed in various odontogenic tumours.

Correspondence to M. Mori.
All authors are at the Department of Oral and Maxillofacial Surgery, Asahi University School of Dentistry, 1851-1 Hozumi, Hozumi-cho, Motosu-gun, Gifu 501-02, Japan.
Received 3 Nov. 1994; provisionally accepted 4 Dec. 1994; revised manuscript received 5 Jan. 1995.

Odontogenic tumours of epithelial origin

Immunoreactivity of tenascin in ameloblastoma was observed in (1) the tumour epithelial island basement membrane–connective tissue interface; (2) the tumour stroma and (3) stellate reticulum-like cells and granular tumour cells of ameloblastoma. In the follicular or plexiform variants of ameloblastoma, the tumour cell island–stromal interface was usually immunoreactive for tenascin. However, there were frequent breaks in the continuity of expression (Fig. 1A, B). Irrespective of the histological variation, the desmoplastic or fibroblastic variants and those with inflammatory infiltrates or hyalinous stroma around the tumour islands also had linear band-like immunoreactivity at the interface (Fig. 1C, D), while, in different areas of the same tumour, the immunoreactivity at the tumour island–stromal interface was also accompanied by reaction products slightly extended into the tumour stromal tissue (Fig. 1E). The cystic ameloblastoma also had immunoreactive tenascin at the mesenchyme adjacent to the basement membrane (Fig. 1F). The stellate reticulum-like cells of follicular ameloblastomas (Fig. 1G) and granular cells of granular ameloblastomas were also immunoreactive for tenascin (Fig. 2H).

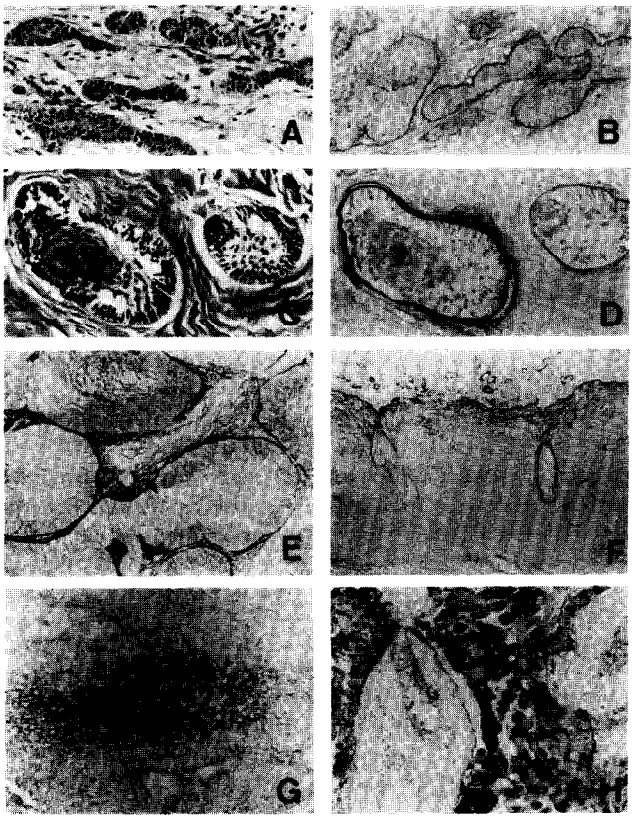

Fig. 1. Ameloblastoma. (A) Follicular ameloblastoma. There are islands of tall columnar cells similar to ameloblasts of the enamel organ surrounding the stellate reticulum-like cells. (B) Tenascin immunostaining in follicular ameloblastoma is seen at the epithelial tumour cell island mesenchymal interface. (C) Follicular ameloblastoma. The stroma show desmoplastic changes. (D) Tenascin is expressed around the tumour cell islands of ameloblastoma while adjacent connective tissue is unreactive. (E) Scanty stromal connective tissue stroma showing abundant tenascin immunostaining in follicular ameloblastoma. (F) Cystic ameloblastoma show immunoreactive tenascin beneath the basement membrane. (G) The stellate reticulum-like cells in tumour nests of follicular ameloblastoma show immunoreactivity of tenascin. (H) Granular cells of ameloblastoma show most intense immunostaining for tenascin.

The squamous odontogenic tumour showed positive immunoreactivity at the odontogenic epithelial tumour masses—fibrous connective tissue interface, while some areas were unreactive (Fig. 2A, B). The stromal connective tissues were also negative. Calcifying epithelial odontogenic tumours had scanty stromal tissue with the most intense immunoreactivity for tenascin, and squamous or flattened tumour cells with moderate immunoreactivity (Fig. 3A, B).

Precalcified bodies, the eosinophilic areas and well-keratinised bodies were devoid of tenascin. The CEOT cells markedly positive for keratin detected by monoclonal anti-keratin KL1 (Fig. 3C) had slight immunoreactivity for tenascin at the interface of the epithelial cells and connective tissue stroma. In calcifying odontogenic cysts, tenascin was expressed in the mesenchyme beneath the basement membrane of epithelial tumour cells (Fig. 3D) which was, however, widespread in the stroma in some areas. The tumour cells in adenomatoid odontogenic tumours, both pseudoglandular and flattened cells, were negative for tenascin, while narrow stromal clements and blood capillaries in tumour foci expressed conspicuous reaction (Fig. 3E). The large foci of tumour cells with areas of calcification had reaction products localised around the foci of tumour cells and calcified masses.

Tumours of epithelial–ectomesenchymal origin

In composite odontomas, periodontal ligament fibres and mesenchyme adjacent to areas of calcification showed an intense immunoreactivity for tenascin. There were calcified bodies with basophilic and eosinophilic areas accompanied by odontogenic epithelial cells, and tenascin immunoreactivity was strongly positive in the condensed connective tissue fibres (Fig. 3G). Ameloblastic fibro-odontoma had an intense linear band-like immunostaining of tenascin around the basement membrane of the ameloblastic areas. The enamel organ, however, was devoid of immunoreaction while the odontogenic mesenchyme showed a weak diffuse staining (Fig. 4A, B). In ameloblastic fibroma, immunoreactive tenascin was detected in the fibromatous component of the tumour. The peritumour connective tissues showed negative reaction (Fig. 4C, D). The pulp-like tissues adjacent to the odontoblastic layer and dentine-like tissues of odontomas expressed the highest tenascin immunoreactivity (Fig. 4E, F).

DISCUSSION

Tenascin has been found to affect organogenesis during fetal and embryonic development by affecting the epithelial mesen-

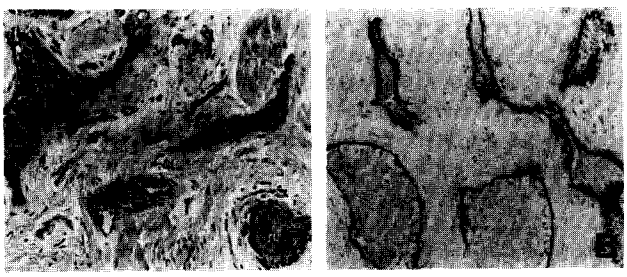

Fig. 2. Squamous odontogenic tumour. (A) The tumour shows islands of squamous epithelial cells with no tall ameloblast like cells. (B) The squamous tumour cell island–connective tissue interface shows immunoreactive tenascin.

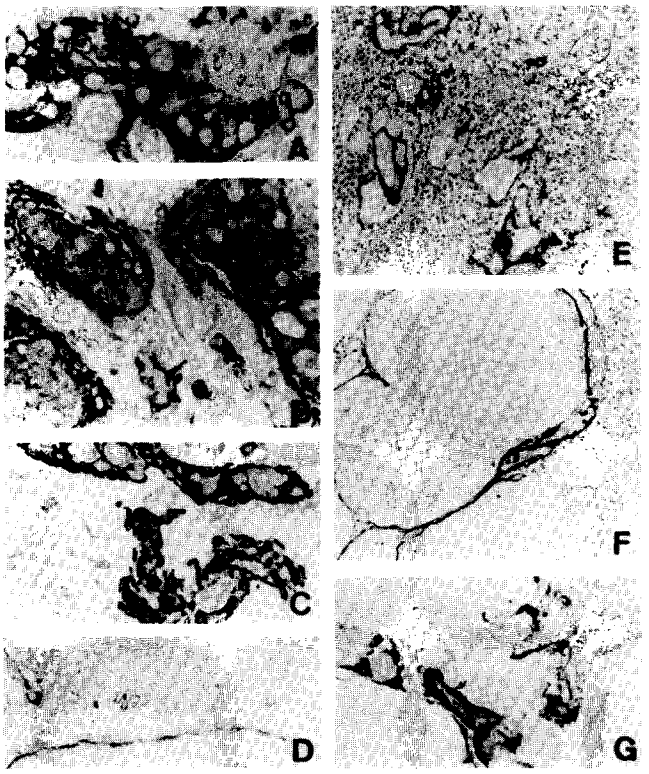

Fig. 3. Tenascin in CEOT, COC and AOT. (A) CEOT show proliferation of polyhedral epithelial cells with areas of calcification. The scanty stromal tissue shows an intense reaction for tenascin. (B) Tenascin in CEOT is markedly concentrated in the peripheral connective tissue stroma and diffusely positive in tumour cells. (C) MoAb KL1 immunostaining of CEOT. The tumour cells are markedly positive for the keratin. (D) Calcifying epithelial cysts show tall basal cells lining the stellate reticulum-like cells where calcified masses are formed in the mesenchyme beneath the epithelium. Tenascin is only positive beneath the basement membrane of the epithelial cells. (E) Adenomatoid odontogenic tumours show multiple duct-like structures which may be accompanied by formation of dysplastic dentine. Tenascin immunostaining in AOT is localised in the scanty stromal tissue around the duct-like structure. (F) Tenascin in AOT is limited at the interface corresponding to the basement membrane of pseudoglandular focus. (G) Complex odontoma show scanty dental epithelium mixed with dental tissues. Periodontal ligament fibres, dentinoid and pulp-like tissues show a marked immunostaining for tenascin.

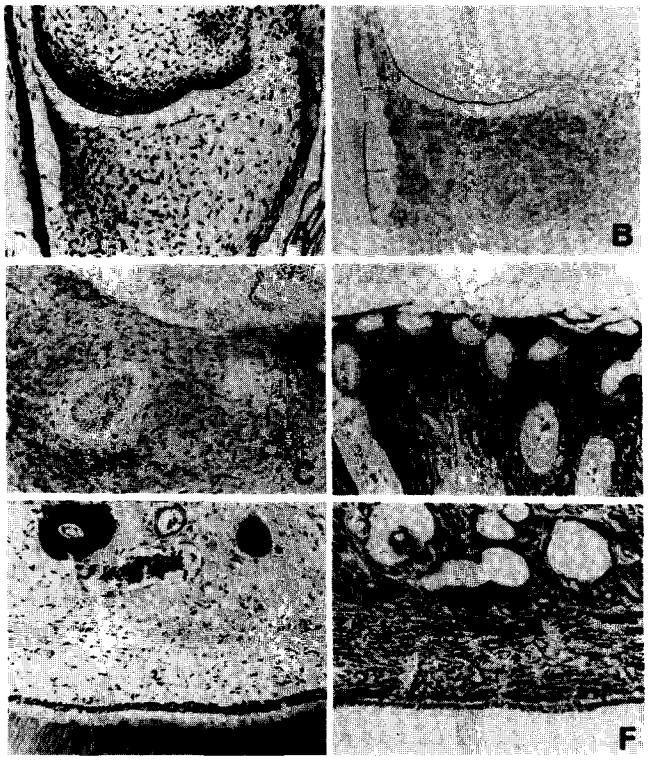

Fig. 4. Tenascin immunoreactivity in odontogenic tumours of ecto-mesenchymal in origin. (A) Ameloblastic fibro-odontoma—histopathologically, there are areas of odontogenic epithelium and odontogenic mesenchyme. (B) Tenascin is confined beneath the basement membrane of the odontogenic epithelium and the mesenchyme is diffusely positive. (C) Ameloblastic fibroma show odontogenic epithelium in a cell rich fibrous mesenchyme. The fibroma component shows abundant immunoreactivity. The epithelial–connective tissue interface is unreactive. (D) Tenascin in ameloblastic fibroma: the tumour stroma (fibroma component) is intensely reactive. However, the normal tissues show no immunoreactive tenascin. (E) Histopathological features of odontoma—the odontoma is composed of pulp-like tissues, odontoblasts, and dentinoid tissues. (F) Tenascin is markedly concentrated in the pulp-like tissues and odontoblasts show a strong immunoreactivity.

chymal interaction, the expression of which, however, is highly restricted or virtually absent in most of the adult tissues. Its expression during epithelial wound healing and in the tumour stroma adjacent to the invading tumour fronts, often with a wide spread of immunoreactivity in the stromal tissues adjacent to invading tumour cells, may be a functional or pathologic alteration in the epithelial mesenchymal interaction at the cellular level under those conditions. The growth factor-like action of tenascin [22–24], and its enhanced expression under the influence of transforming growth factor beta [25], its role in adhesion and migration of various cells [26, 27], may have an implication in tumour cell proliferation, differentiation and not infrequently the histomorphological features which are often the histopathologic diagnostic criteria. Therefore, the study of the extracellular microenvironment has an important role in understanding the neoplastic lesions. The expression and role of various extracellular matrix proteins including tenascin in odontogenic tumours have been carried out [19, 20]. Our findings of expression of tenascin in ameloblastoma do not essentially differ from the findings of those reported earlier [19, 20]. However, in a large series of odontogenic tumours of epithelial and epithelial–ectomesenchymal origin, we were able to demonstrate the difference in the pattern of expression of tenascin in those forming the calcified structures and those without. The present study on the expression of tenascin in benign odontogenic tumours, has particular significance in elucidating and distinguishing the epithelial and epithelial–ectomesenchymal tumours of odontogenic origin in terms of a heterogeneous expression of tenascin.

In the present study, the majority of the benign odontogenic tumours which fail to form calcifying structures had no widespread stromal expression of this glycoprotein. Tenascin in those odontogenic neoplasms were mainly present at the interface of tumour epithelium and the stroma, irrespective of the tumour cell morphology, showing a band-like immunostaining, with frequent breaks and in the fibrous stromal

connective tissue. This pattern of expression is very similar to our previous finding in normal oral mucosa and oral leukoplakia with minimum hyperplasia or dysplasia [5]. Therefore, the state of proliferation and differentiation of tumour cells may determine the extracellular microenvironment or vice versa. On the other hand, those tumours forming calcifying masses had rather widespread stromal immunoreactivity of tenascin, which was, however, not present in calcified masses of CEOT and odontoma.

Histologically, CEOT usually has scanty stromal tissue with a narrow connective tissue surrounding the calcified masses. The epithelial tumour cells are markedly reactive for cytokeratins (Fig. 4C) [28]. Calcified masses in an odontogenic tumour may be enamel or enamel matrix, dentine or dentine-like tissues, cementum or osseous-like tissues. Tenascin has been implicated in the process of chondrogenesis and osteogenesis [29]. In addition, the glycoprotein has been found in normal adult teeth in the dental pulp, odontoblastic layer, cementoblast-pre-cementum zone, and on the periosteal and endosteal surfaces of the alveolar bone [21]. The present study suggests that tenascin may have a role in modulating the extracellular microenvironment so that calcified deposits including the dentine-like materials are formed in the odontogenic tumours.

Classical investigations on stromal tissue in ameloblastoma have shown enzymatic activities for alkaline phosphatase and aminopeptidase in the tumour stromal tissues, particularly in the connective tissue adjacent to the invasive front of ameloblastomas [30, 31]. Extracellular matrix glycoproteins in tumour stromal tissues have been found to be involved in the promotion or inhibition of neoplastic cell proliferation and the presence of alkaline phosphatase and aminopeptidase in the microenvironment of invading fronts of ameloblastoma may be responsible for proteolytic actions during tumour invasion. In addition, the expression of tenascin in the stellate reticulum-like cells in tumour cell islands of ameloblastoma, although not frequently, as seen in the present study, is very similar to observations in the developing tooth germ [19, 20]. It may be suggested that the protein may also have a role in amelogenesis where the cellular alterations within the tooth germ following amelogenesis are still poorly understood. Tenascin, recognised solely as a mesenchymal cell product in the earlier years, has now been found to be produced by various normal and neoplastic epithelial cells [7, 16–18].

The present study shows that tenascin positive cells are present in the granular cells of granular ameloblastoma, central stellate reticulum-like cells and central tumour cells of cystic changes in follicular ameloblastoma with a heterogeneity of expression of this extracellular matrix glycoprotein. If these findings suggest the production of tenascin by epithelial tumour cells in odontogenic tumours this has to be further investigated at the level of mRNA or cDNA for tenascin. Although the histochemical properties of granular cells and stellate reticulum-like cells are different, they are probably of the same histologic origin. Granular cells have a higher content of lysozomal enzymes such as acid phosphatase and beta-glucurodinase [32] and high affinity for lectin binding [33]. The granular cells may, therefore, have the potential to accumulate and secrete various proteins in the extracellular microenvironment.

1. Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T. Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. *Cell* 1986, **47**, 131–139.
2. Erickson HP, Bourdon MA. Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. *Annu Rev Cell Biol* 1989, **5**, 71–92.
3. Mackie EJ, Halfter W, Liverani D. Induction of tenascin in healing wounds. *J Cell Biol* 1988, **107**, 2757–2767.
4. Natali PE, Nicrotra MR, Bigotii A, *et al.* Comparative analysis of the expression of the extracellular matrix protein tenascin in normal human fetal, adult and tumor tissues. *Int J Cancer* 1991, **47**, 811–816.
5. Shrestha P, Sakamoto F, Takagi H, Yamada T, Mori M. Enhanced tenascin immunoreactivity in leukoplakia and squamous cell carcinoma of the oral cavity: an immunohistochemical study. *Oral Oncol, Eur J Cancer* 1994, **30B**, 132–137.
6. Sakai T, Kawakatsu H, Hirota N, Yokoyama T, Sakakura T, Saito M. Specific expression of tenascin in human colonic neoplasms. *Br J Cancer* 1993, **67**, 1058–1064.
7. Howeedy AA, Virtanen I, Laitinen L, Gould NS, Koukoulis GK, Gould VE. Differential distribution of tenascin in the normal hyperplastic and neoplastic breast. *Lab Invest* 1990, **63**, 798–806.
8. Mackie EJ, Chiquet-Ehrismann R, Adams Pearson C, *et al.* Tenascin is a stromal marker for epithelial malignancy in the mammary gland. *Proc Natl Acad Sci USA* 1987, **84**, 4621–4625.
9. Tiitta O, Wahlström T, Paavonen J, *et al.* Enhanced tenascin expression in cervical and vulvar koilocytotic lesions. *Am J Pathol* 1992, **141**, 907–913.
10. Lightner VA, Gumkowski F, Bigner DD, Erickson HP. Tenascin/hexabrachion in human skin: biochemical identification and localization by light and electron microscopy. *J Cell Biol* 1989, **108**, 2483–2493.
11. Bourdon MA, Wikstrand CJ, Furthmayr H, Mathews TJ, Bigner DD. Human glioma-mesenchymal matrix antigen defined by monoclonal antibody. *Cancer Res* 1983, **43**, 2796–2805.
12. Herlyn M, Graeven U, Speicher D, *et al.* Characteristics of tenascin secreted by human melanoma cells. *Cancer Res* 1991, **51**, 4853–4858.
13. Natali PG, Nicotra MR, Bartolazzi A, *et al.* Expression and production of tenascin in benign and malignant lesion of melanocyte lineage. *Int J Cancer* 1990, **46**, 586–590.
14. Soini Y, Pääkko P, Virtanen I, Lehto VP. Tenascin in salivary gland tumours. *Virchows Arch A Pathol Anat* 1992, **421**, 217–222.
15. Shrestha P, Sumitomo S, Ogata K, *et al.* Immunoreactive tenascin in tumours of salivary glands: evidence for enhanced expression in tumour stroma and production by tumour cells. *Oral Oncol, Eur J Cancer* 1994, **30B**, 393–399.
16. Gould VE, Martinez-Lacabe V, Virtanen I, Sahlin KM, Schwartz NM. Differential distribution of tenascin and cellular fibronectin in acute and chronic renal allograft rejection. *Lab Invest* 1992, **67**, 71–79.
17. Kawakatsu H, Shiurba R, Obara M, Hiraiwa H, Kusakabe M, Sakakura T. Human carcinoma cells synthesize and secrete tenascin *in vitro*. *Jpn J Cancer Res* 1992, **83**, 1073–1080.
18. Mackie EJ, Scott-Burden T, Hahn EW, *et al.* Expression of tenascin by vascular smooth muscle cells. Alternations in hypertensive rats and stimulation by angiotensin II. *Am J Pathol* 1992, **141**, 377–388.
19. Heikinheimo K, Morgan PR, Happonen R-P, Stenman G, Virtanen I. Distribution of extracellular matrix proteins in odontogenic tumors and developing teeth. *Virchows Arch B Cell Pathol* 1991, **61**, 101–109.
20. Nagai N, Yamachika E, Nishijima K, *et al.* Immunohistochemical demonstration of tenascin and fibronectin in odontogenic tumours and human fetal tooth germs. *Oral Oncol, Eur J Cancer* 1994, **30B**, 191–195.
21. Lukinmaa PL, Mackie EJ, Thesleff I. Immunohistochemical localization of the matrix glycoproteins—tenascin and the ED-sequence-containing form of cellular fibronectin—in human permanent teeth and periodontal ligament. *J Dent Res* 1991, **70**, 19–26.
22. Jones F, Hoffman S, Cunningham B, Edelman G. A detailed structure model of cytotactin: protein homologies, alternative RNA splicing, and binding regions. *Proc Natl Acad Sci USA* 1989, **86**, 1905–1909.
23. Siri A, Carnemolla B, Saginati M, *et al.* Human tenascin: primary structure, pre-mRNA splicing patterns and localization of the

epitopes recognized by two monoclonal antibodies. *Nucleic Acids Res* 1991, **19**, 525–531.
24. Sriramarao P, Bourdon MA. A novel tenascin type III repeat is a part of a complex of tenascin mRNA alternative splices. *Nucleic Acid Res* 1993, **21**, 163–168.
25. Pearson CA, Pearson D, Shibahara S, Hofsteenge J, Chiquet-Ehrismann R. Tenascin: cDNA cloning and induction by TGF-β. *EMBO J* 1988, 7, 2977–2982.
26. Erickson HP, Lightner VA. Hexabrachion protein (tenascin, cytotactin, brachionectin) in connective tissues, embryonic brain and tumors. *Adv Cell Biol* 1988, **2**, 55–90.
27. Mackie EJ, Tucker RP, Halfter W, Chiquet-Ehrismann R. The distribution of tenascin coincides with pathways of neural crest cell migration. *Development* 1988, **102**, 237–250.
28. Mori M, Tatemoto Y, Yamamoto N, Okada Y. Immunohistochemical localization of intermediate filament proteins in calcifying epithelial odontogenic tumors. *J Oral Pathol* 1988, **17**, 236–240.
29. Mackie EJ, Thesleff I, Chiquet-Ehrismann R. Tenascin is associated with chondrogenic and osteogenic differentiation *in vivo* and promotes chondrogenesis *in vitro*. *J Cell Biol* 1987, **105**, 2569–2579.
30. Mori M, Okamoto Y, Oka R, Mizushima T. Enzyme histochemical demonstration of ameloblastoma. *Oral Surgery* 1964, **17**, 235–250.
31. Okamoto Y, Oka R, Mori M. Histochemical study of aminopeptidase in tumors of the oral regions. *Oral Surg* 1963, **16**, 733–737.
32. Mori M. Histochemical evaluation of enzymes in ameloblastic tumors-acanthomatous and granular-cell ameloblastoma. *J Oral Surg* 1970, **28**, 825–831.
33. Mori M, Nakai M, Tsukitani K, Kobayashi K. Biological significance in granular cells of ameloblastoma—histochemical identification of sugar residues and filamentous proteins, morphometry and DNA cytometry of granular cells. *Cell Mol Biol* 1985, **31**, 265–279.