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An Immunohistochemical Study of Odontogenic Mixed Tumours

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Five cases of odontogenic mixed tumour comprising of an ameloblastic fibroma, an adenomatoid odontogenic tumour, an odonto-ameloblastoma and two ameloblastic fibro-odontomas were immunohistochemically investigated. Odontogenic epithelial cells were fully positive for cytokeratin detected by antibody KL-1, although there were some differences in its intensity. In contrast, for tenascin, only immature dental papilla-like mesenchymal tissue, especially around the dental lamina-like odontogenic epithelium, was positive, while the myxomatous area and connective tissue were negative. Positive vimentin staining was observed in some areas of immature dental papilla-like cells as well as the basement membrane of odontogenic epithelium in the ameloblastic fibroma, suggesting that this tumour had developed at the early stage of tooth formation. Proliferating nuclear cell antigen-positive cells were generally rarely seen, but were frequently observed in epithelial cells of the ameloblastic fibroma and odonto-ameloblastoma. These observations suggest that tumour cells in each odontogenic mixed tumour possess characteristic proteins associated with proliferation potential and that ameloblastic fibroma and odonto-ameloblastoma have higher proliferation potential among the tumours examined.

Keywords: odontogenic mixed tumour, immunohistochemistry, keratin, tenascin, vimentin, S-100 protein, proliferating cell nuclear antigen

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INTRODUCTION

IN ODONTOGENIC tumours, which develop at various stages of tooth formation, both ectodermal and mesenchymal odontogenic components show an elaborate proliferation and mutual stimulation [1]. Therefore, odontogenic tumours show a variety of characteristic features dependent on the tumour cell origin and the stage of tumour cell differentiation. Odontogenic tumours range from mixed tumours with the potential for proliferation, such as ameloblastic fibroma, to the completely differentiated complex and compound odontomas [2, 3]. Therefore, exploration of the tumour cell proliferation and differentiation in odontogenic tumours may provide useful information on the understanding of these tumours.

In odontogenic tumours and developing tooth germs, proteins such as keratins [4–7], tenascin [8, 9], vimentin [10, 11], S-100 protein [12], and also proliferating cell nuclear antigen (PCNA) [13], have been investigated immunohistochemically. The local existence of each protein has been clarified and proteins characteristic to the proliferation potential of tumour cells and cells in the tooth germ have been specialised [4, 5, 8–11]. In addition, it has been proved that the appearance and disappearance of these proteins are correlated with the differentiation of cells in the tooth germ [4, 5, 8–11]. However, identification of local existence of these proteins in odontogenic mixed tumours, especially in relatively rare ones, is not sufficient for the understanding of these tumours.

Recently, we encountered five relatively rare odontogenic mixed tumours comprising an ameloblastic fibroma, adenomatoid odontogenic tumour, and odonto-ameloblastoma, and two ameloblastic fibro-odontomas. We investigated the clinical, histological and immunohistochemical patterns of these tumours in order to evaluate their proliferation potential.

MATERIALS AND METHODS

Tumours

The five odontogenic mixed tumours were subjected to the immunohistochemical investigations described below. The clinical presentation in each patient and the X-ray images of the tumours are presented in Table 1 and Fig. 1A–E, respectively. Histological findings and diagnosis are shown in Fig. 2A–E.

Histological and immunohistochemical examinations

Surgically extirpated tumours were processed for histological and immunhistochemical examinations. The tissues were fixed in 10% neutral formalin, and decalcified when necessary. Paraffin-embedded tissues were serially sectioned at a thickness of 5 μm and stained with haematoxylin–eosin and

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Table 1. Clinical findings of odontogenic mixed tumours

Case	Age (years)	Gender	Location	Symptom	Clinical diagnosis	Surgery	Histological diagnosis
1	20	M	ramus ~ $\overline{8-3}$	Cold pain	Ameloblastoma	Mandibulectomy	Ameloblastic fibroma
2	4	F	$\overline{E}$ ~ ramus	Swelling	Odontoma	Extirpation	Ameloblastic fibro-odontoma
3	8	F	$\overline{6E}$	Diasthema	Odontoma	Extirpation	Ameloblastic fibro-odontoma
4	18	F	$\overline{8}$	None existed	Follicular cyst	Extirpation	Adenomatoid odontogenic tumour
5	7	F	$\overline{6}$ ~ ramus	None existed	Odontoma	Extirpation	Odonto-ameloblastoma

Table 2. Immunohistochemical evaluation of odontogenic mixed tumours

Case	Histological diagnosis	Component	Immunohistochemical staining				
			KL-1	Tenascin	Vimentin	S-100	PCNA
1	Ameloblastic fibroma	OE	+ ~ + +	− ~ +	− ~ +	− ~ +	± ~ +
		SR	+	−	−	− ~ +	−
		DP	−	+	+	−	±
2	Ameloblastic fibro-odontoma	OE	+ ~ + +	−	−	−	−
		SR	+	−	−	−	−
		DP	−	+	+	−	−
3	Ameloblastic fibro-odontoma	OE	+	−	−	−	−
		SR	±	−	−	−	−
		DP	−	+	+	−	−
4	Adenomatoid odontogenic tumour	OE I	+	−	−	−	−
		OE II	+	−	−	−	−
		DP	NE	NE	NE	NE	NE
5	Odonto-ameloblastoma	OE	+ ~ + +	−	−	− ~ +	± ~ +
		SR	+	−	−	−	−
		DP	−	+	+	−	−

OE = odontogenic epithelium similar to ameloblasts or dental lamina; SR = stellate reticulum; DP = dental papilla-like mesenchymal tissue; OE I = columnar odontogenic epithelium; OE II = spindle-shaped odontogenic epithelium; NE = not exist.

immunohistochemically. The monoclonal antibodies (Ab) used in this study were those for cytokeratin (KL-1, Immunotech, S.A., France), tenascin (Chemicon International Inc., U.S.A.), vimentin (Dako, U.S.A.), S-100 protein α (Japan Immunoresearch Laboratories Co. Ltd., Japan) and PCNA (PC-10, Dako, Denmark). Deparaffinised sections were immersed in methanol containing 0.3% hydrogen peroxide for 30 min, and stained by the standardised strepto-avidin-biotin method with the aid of Histofine SAB-PO(M) kit (Nichirei, Corp., Japan). The reaction was visualised by 0.05% 3.3'-diaminobenzidine hydrochloride in phosphate-buffered saline containing 0.05% hydrogen peroxide. The sections were counterstained with methyl green.

RESULTS

KL-1

The epithelial cells were generally positive for KL-1. The staining intensity was strong in the dental lamina-like premature odontogenic epithelial islands in ameloblastic fibroma (case 1; Fig. 3A) and odonto-ameloblastoma (case 5), and moderate in the columnar ameloblastic cells in case 5 (Fig. 3B). In the adenomatoid odontogenic tumour (case 4), no difference of the staining intensities in columnar and spindle shaped cells was found. Negative staining for KL-1 was observed in the mesenchymal tissue including the dental papilla-like cells and fibrous connective tissue.

Tenascin

The immature dental papilla-like mesenchymal tissue around the odontogenic epithelium was positive for tenascin in ameloblastic fibroma (Fig. 3C), and ameloblastic fibro-odontoma (cases 2, 3; Fig. 3D) and odonto-amcloblastoma. The mesenchymal tissue showing myxomatous change and connective tissue similar to the dental sac in ameloblastic fibro-odontomas (cases 2, 3) and odonto-ameloblastoma were negative for tenascin. The basement membrane around the dental lamina-like epithelial cells in ameloblastic fibroma (case 1) was also positive for this protein (Fig. 3C).

Vimentin

The mesenchymal tissue resembling dental papilla in ameloblastic fibroma and ameloblastic fibro-odontomas was strongly stained in some areas (Fig. 3E). The basement

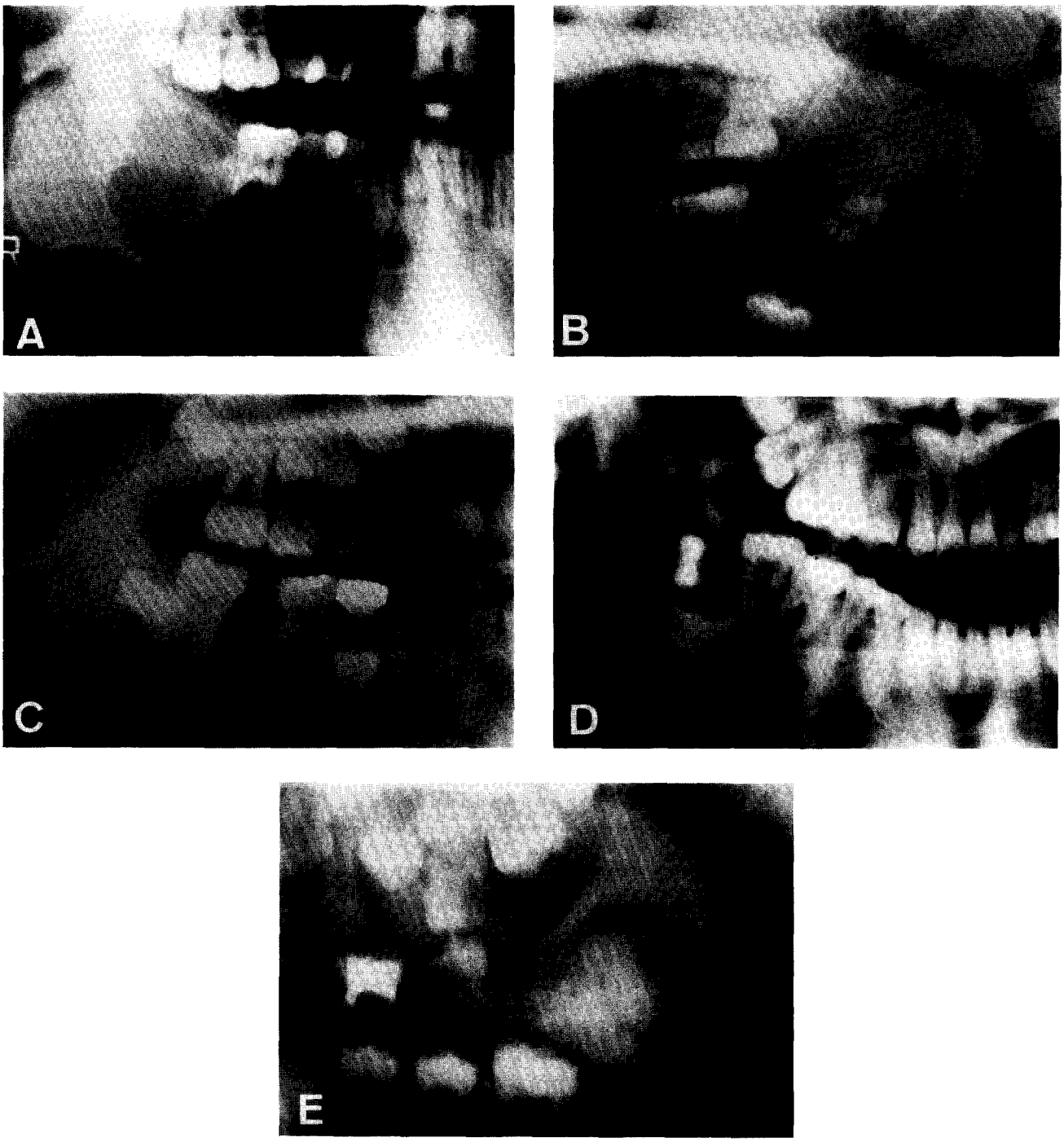

Fig. 1. X-ray pictures of mixed odontogenic tumours. (A) Case 1, multicystic radiolucent shadow in the right mandible. This lesion includes 2nd and 3rd molar teeth and the roots of 654| are resorbed. (B) Case 2, expansive radiolucent lesion including many calcified materials is found at the left mandible. The distal root of |E is resorbed and the 1st molar is dislocated. (C) Case 3, radiolucent lesion is found between the roots of 6| and |E causing the diasthema. The tooth germ of 5| is not visible and the root of |E is slightly resorbed. (D) Case 4, localized cystic lesion including the crown of 8| is seen. The root of 8| is not yet formed. (E) Case 5, cystic lesion including irregularly calcified substance is observed at the left mandible. The tooth germ of |6 is dislocated and those of |78 are not visible.

membrane around the dental lamina-like epithelial cells in ameloblastic fibroma was partially positive for vimentin.

S-100 protein

The mesenchymal tissue and most odontogenic epithelial islands were negative for S-100 protein. However, a few odontogenic epithelial islands with the appearance of dental lamina in ameloblastic fibroma and odonto-ameloblastoma were weakly positive for S-100 protein.

PCNA

PCNA-positive cells were generally very few in all tumours. However, PCNA-positive cells were somewhat more fre-

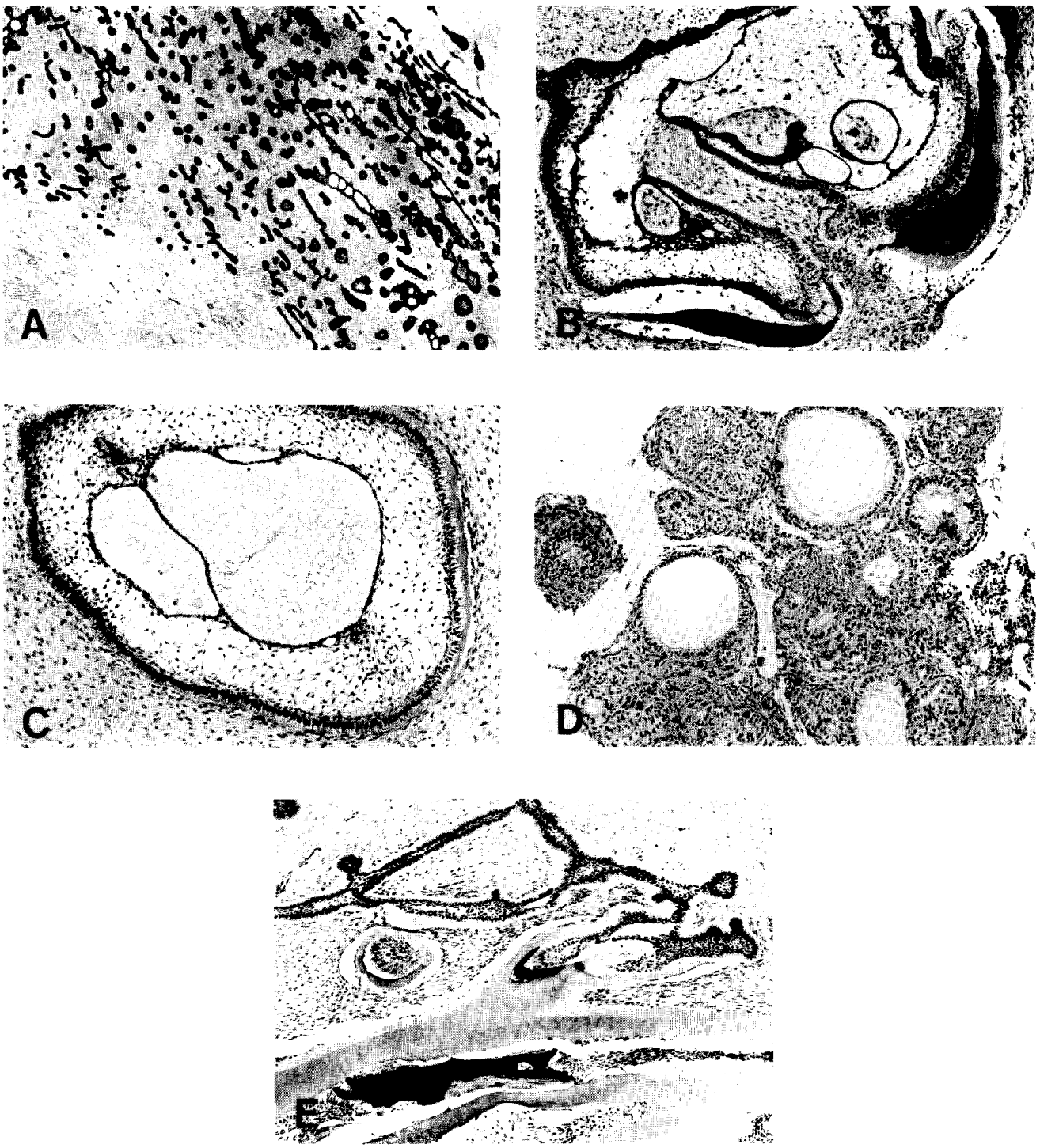

Fig. 2. Histological findings of odontogenic mixed tumours (H&E). (A) Case 1, ameloblastic fibroma, × 40. The odontogenic epithelium proliferated accompanying mesenchymal tissue similar to dental papilla. Most epithelial cells were cuboidal and formed islands similar to dental lamina. Ameloblast-like columnar cells formed nests in which stellate reticulum-like tissue was observed. (B) Case 2, ameloblastic fibro-odontoma, × 100. Nests of odontogenic epithelium were observed in mesenchymal tissue resembling immature dental papilla. In some epithelial islands, high columnar ameloblastic cells had differentiated and showed proliferation accompanying stellate reticulum and stratum intermedium. A hard tissue matrix and calcified dentin and premature enamel were observed. These findings indicated that this tumour was an ameloblastic fibro-odontoma. (C) Case 3, ameloblastic fibro-odontoma, × 100. Immature dental papilla-like mesenchymal tissue was observed with nests of cuboidal and columnar odontogenic epithelial cells. A few calcified materials suspected to be dentin were found around the epithelial cells. (D) Case 4, adenomatoid odontogenic tumour, × 100. The tumour consisted of columnar and spindle-shaped epithelial cells. The columnar cells having polarised nuclei formed a duct-like structure, around which spindle-shaped cells had proliferated. A few small calcified bodies were observed within the epithelial component. (E) Case 5, odonto-ameloblastoma, × 100. The tumour was almost exclusively composed of calcifying odontogenic tissue accompanied by a small amount of soft tissue. Microscopically, formation of dentin with or without dentinal tubules and premature enamel was observed. Around the hard tissue components, proliferated odontogenic epithelium exhibiting an ameloblastic pattern was observed.

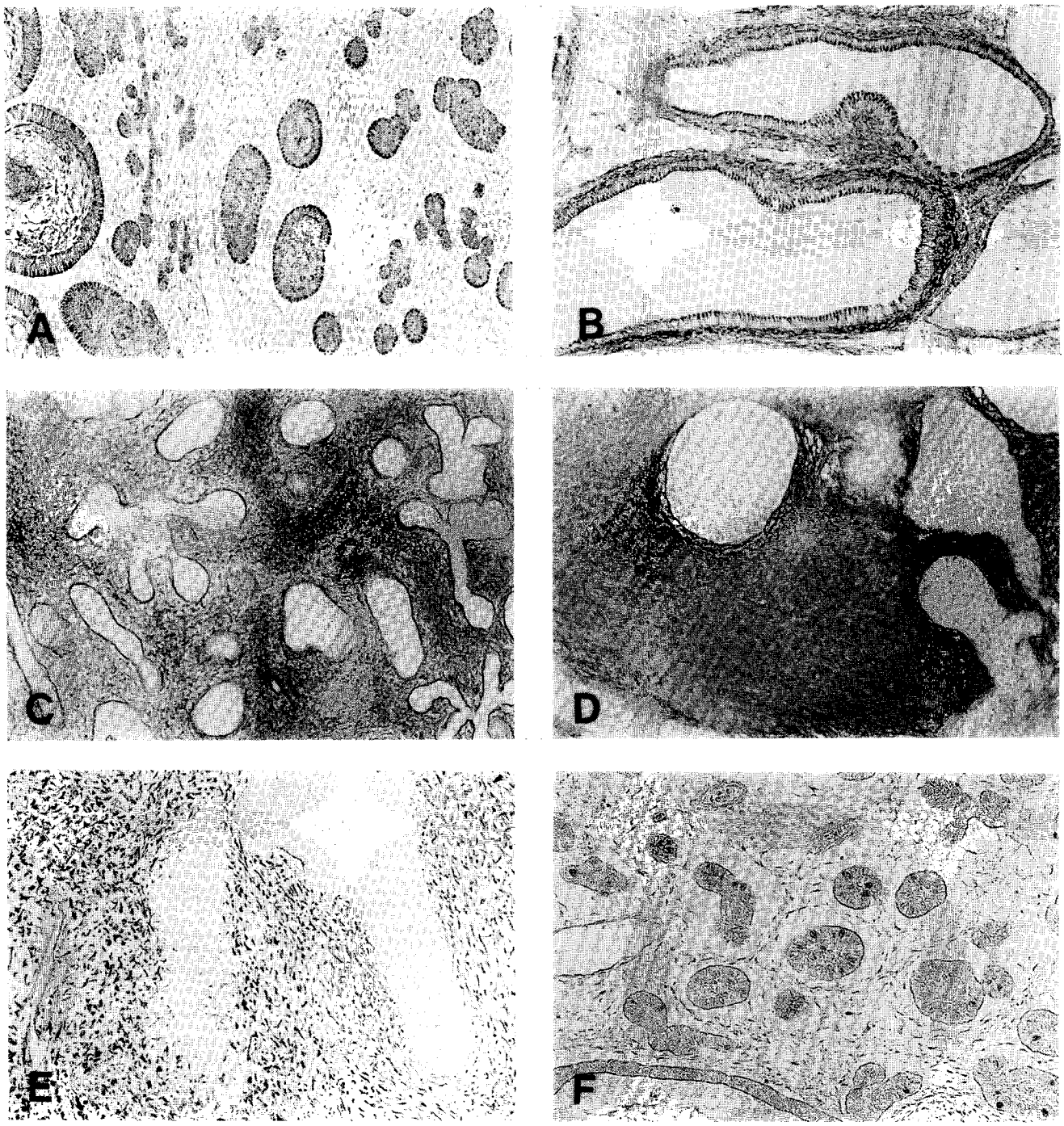

Fig. 3. Immunohistochemical staining of odontogenic mixed tumours. (A) KL-1, case 1, ×200. (B) KL-1, case 5, ×200. (C) Tenascin, case 1, ×200. (D) Tenascin, case 3, ×200. (E) Vimentin, case 3, ×200. (F) PCNA, case 1, ×200.

quently found in the dental lamina-like odontogenic epithelium in ameloblastic fibroma and odonto-ameloblastoma (Fig. 3F). In the former, PCNA-positive cells were also observed in the mesenchymal tissue resembling dental papilla.

DISCUSSION

Odontogenic mixed tumours are relatively rare tumours which consist of components derived from both ectodermal and mesenchymal tissues [1–3]. The biological features of tumours which have developed at the various stages of tooth formation differ depending on the stage of differentiation and the proliferation of the tumour cells [1–3]. In this study, we comparatively investigated five odontogenic mixed tumours immunohistochemically as well as clinically and histologically in an attempt to elucidate the characteristic biological behaviours of these tumours.

Cytokeratin, one of the intermediate filaments of epithelial cells, is a polypeptide in the molecular weight range from 40 to 68 kDa [14]. Cytokeratins are divided into 19 types according to differences in the constituent peptides [14]. The distribu-

tions of these polypeptides differ with the type of epithelium and the degree of differentiation [15] as well as the pathological condition [16]. There have been several reports regarding the distribution of cytokeratin in the human tooth germ [4–6]. For example, it has been reported that cytokeratins 18 and 19 are present in all cells constituting the dental lamina and enamel organ, and are also found in ameloblastic cells [4]. In contrast, cytokeratins 13 and 16 have been observed in the dental lamina but not in the enamel organ [4]. These findings suggest that the cytokeratin composition of differentiated odontogenic epithelial cells in the enamel organ differs from that in the dental lamina [17]. The histochemical distribution of the cytokeratins reacting with the KL-1 antibody has not been reported in the human tooth germ. However, the positivity of Malassez's epithelial rest and the spinosal and its above layers of epithelium separated from the intact oral mucosa has been reported [7, 18]. In the present study, all odontogenic epithelial cells were found to be positive for KL-1, although there were some differences in intensity. Keratins are a group of epithelial cell-specific cellular matrix, and a variety of different molecular weight keratins are distributed in epithelial cells depending on the cell type and differentiation. Therefore, more detailed detection of the distribution of many cytokeratins is needed for characterisation of the odontogenic tumour cells.

Tenascin was first detected as an extracellular matrix protein associated with the formation of the muscle–tendon junction in chicken embryos [19, 20]. The biological role of tenascin is not yet clear. However, multiple functions of tenascin in cell attachment, migration, proliferation and haemagglutination, have been reported [21]. In the tooth germ, histochemical examinations have revealed that tenascin is localised in the mesenchymal tissue around the dental lamina, and also dental papilla especially in the portion near the basement membrane of odontogenic epithelia [8, 9, 11]. In the present study, tenascin was observed in the immature dental papilla-like mesenchymal tissue, especially around the odontogenic epithelia. It is well known that tenascin is released from mesenchymal cells upon stimulation by epithelial cells, consistent with strong linking in the epithelia–mesenchymal interaction [22]. Therefore, our results appear to indicate that the proliferation and the differentiation of the odontogenic mesenchymal tissue are influenced by the epithelium even in tumours.

Vimentin is considered to be a major protein which constitutes intermediate filaments in the cytoplasm of mesenchymal cells, and it has been frequently examined as a means of identification of mesenchymal tumour components [23]. In the tumours presented, vimentin was observed in the immature dental papilla-like cells. However, the areas with myxomatous change and the connective tissue resembling a dental sac were negative for vimentin. In ameloblastic fibroma (case 1), the basement membrane of the dental lamina-like odontogenic epithelium was positive for vimentin. Epithelial cells [24], and cells in the early stage of tooth formation [25] and in ameloblastic fibroma [26] are occasionally positive for vimentin. These findings appear to indicate that vimentin disappears along with differentiation of odontogenic epithelial cells and that ameloblastic fibroma develop at the early stage of tooth formation.

PCNA is a nuclear protein which is associated with the S-phase of DNA synthesis in association with cell proliferation [27]. Among odontogenic tumours, ameloblastomas have been examined for PCNA [13], and actually few PCNA-positive cells have been observed. However, the percentage of PCNA-positive cells in recurrent ameloblastomas is reported to be significantly higher than that in primary tumours [13]. With the exception of some of the dental lamina-like odontogenic epithelial cells in ameloblastic fibroma (case 1) and odonto-ameloblastoma (case 5), PCNA-positive cells were rarely encountered. These results may reflect the slowly growing nature of odontogenic tumours.

When considering that the basement membrane of odontogenic epithelium in ameloblastic fibroma expressed vimentin and tenascin in parallel with the results in PCNA staining, ameloblastic fibroma and odonto-ameloblastoma appear to have a higher proliferation protential than other odontogenic mixed tumours. Further studies using antibodies reacting with various types of tumour cell components are necessary to elucidate the biological nature of odontogenic tumours.

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