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Immunohistochemical expression of thymosin ß4 in ameloblastomas and odontomas

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Summary. Ameloblastoma is regarded to be a benign odontogenic tumor, but it is destructive, locally invasive and presents a high rate of recurrence. Thymosin ß4 (Tß4) is closely associated with tooth germ development. Tß4 also plays a role in malignant progression and invasion. However, little is known about the function of Tß4 in odontogenic tumors. Thus, we investigated Tß4 expression in ameloblastomas and compared it with odontomas. We immunohistochemically evaluated the expression of Tß4, ameloblastin (AMBN), amelogenin (AMEL) and enamelin (ENAM) in 57 samples of ameloblastomas from 40 patients, and also assessed the expression of these molecules in 11 cases of odontomas, two of ameloblastic fibro-odontomas and one of tooth germ-like structures without the formation of enamel and dentin. Tß4 signals were observed in almost all of the ameloblastomas. The signals were observed in both peripheral columnar cells and central polyhedral/angular cells. Similar findings were observed in tooth germ-like structures, and in the ameloblastomatous nests in the ameloblastic fibro-odontomas. These samples had negative results for AMBN, AMEL and ENAM. Meanwhile, Tß4 signals were not seen in the odontomas, although immunolabeling for AMBN, AMEL and ENAM was observed in the enamel matrix and in some ameloblasts. Ectomesenhymal regions in the odontomas were negative for staining with the antibodies for AMBN, AMEL and ENAM. These results suggest that Tß4 could be associated with morphogenesis and tumor invasion in the ameloblastoma, and that Tß4 may play a role in the behavior of ameloblastoma.

Key words: Thymosin ß4, Ameloblastoma, Odontoma, Tooth germ development, Odontogenic tumor

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Introduction

Diverse odontogenic tumors can arise from the odontogenic remnants, histologically and biologically ranging from hamartomatous proliferations to malignant tumors with metastatic capabilities (Philipsen et al., 2005). Histologically, these tumors are characterized by the presence of soft tissues that morphologically contain elements of enamel organs or dental pulp, the formation of calcified elements resembling enamel, dentin, cementum, or a mixture of these tissues. Although the data of PRC study need to be substantiated, it has been reported that the most common odontogenic tumor is ameloblastoma in Hong Kong, Japan and two African countries, while odontoma is the most common tumor in the US and Canada (Philipsen et al., 2005).

Ameloblastoma is usually a benign odontogenic tumor and progresses slowly, and is characterized by proliferative epithelial nests that resemble the developing enamel organ of the tooth germ. While benign, ameloblastoma is destructive, locally invasive, and presents a high rate of recurrence (Kramer et al., 1992; Gardner et al., 2005). Further, ameloblastoma exhibits invasive growth into the surrounding bone tissue, with small tumor nests developing from the main mass. Therefore, some surgeons prefer wide surgical resection to conservative treatment. Recurrences have been observed more than ten years after the initial

treatment, so long-term follow-up is also necessary (Gardner et al., 2005).

On the contrary, odontomas show slow growth and nonaggressive behavior. They are hamartomas of odontogenic origin rather than tumors, and are composed of multiple small teeth or tooth elements (enamel, dentin, cementum and pulp tissue) in variable proportions and in a haphazard array. The former is regarded as odontoma of the compound type, and the latter as odontoma of the complex type. Recurrence has been reported in cases where there was incomplete removal of a developing odontoma (Prætorius and Piattelli, 2005). Therefore, odontogenic tumors often show features of odontogenesis. It is possible that the mechanism(s) underlying tooth development are active or non-active in many of the odontogenic tumors.

A cDNA subtraction study between the mandibles of embryonic day 10.5 (E10.5) and E12.0 mice was previously conducted to identify genes that might be related to the tooth morphogenesis. Thymosin beta-4 (Tß4) was one of the genes more highly expressed in the E12.0 mandible (Yamaza et al., 2001), and the expression pattern of Tß4 mRNA was closely associated with odontogenesis in the mouse lower first molar (Akhter et al., 2005). In addition, Tß4 over-expressing transgenic mice recently showed enamel hypoplasia-like abnormal tooth development (Cha et al., 2010). We recently reported that *Tβ4* knockdown resulted in a significant growth inhibition of the tooth germ, decreased secretion of matrix metalloproteinase (MMP)-2, reduced cell motility and the upregulation of *E-cadherin* in dental epithelial cells (Ookuma et al., 2013). These data suggest that Tß4 plays an important role in tooth development.

Tß4 is one of the ß-thymosins, which constitute a highly conserved family of actin-binding polypeptides. Tß4 is a 43 amino acid acidic polypeptide (pI 5.1) with a molecular weight of 4.9 kDa (Low et al., 1979, 1981; Low and Goldstein, 1982; Goodall et al, 1983; Yu et al., 1994) that is known to play an important role in cell motility by regulating actin polymerization through actin-sequestering (Safer et al., 1991). The expression of Tß4 has been shown to be related to hepatocyte growth factor (HGF) (Oh et al., 2002), which is also considered to be involved in murine tooth development (Tabata et al., 1996). Philp et al. (2006) have shown that Tß4 increases MMP expression and may play a pivotal role in extracellular matrix remodeling during wound repair. Tß4 can also regulate the migration of gastric and colorectal carcinoma cells (Wang et al., 2004; Ryu et al., 2012). Although Tß4 appears to have different functions in a variety of tissues and cell types, including carcinoma cells, there is little knowledge about the role(s) of Tß4 in ameloblastomas.

In this study, we immunohistochemically investigated the expression pattern of Tß4 in ameloblastomas, as well as the distribution of amelogenesis-related proteins, including ameloblastin (AMBN), amelogenin (AMEL) and enamelin (ENAM), compared with the patterns in odontomas. This report provides evidence of the possible functional roles that this protein may play in the tumorigenesis of ameloblastoma and provides a better understanding of the biology of ameloblastoma.

Materials and methods

Samples

Samples of ameloblastoma, odontoma and other conditions diagnosed in the Department of Oral and Maxillofacial Surgery, Kyushu University Hospital from 2007 to 2012 were examined in this study. Paraffin sections of formalin-fixed samples were used for both the histological and immunohistochemical staining. Hematoxylin and eosin-stained sections were reviewed for every case. The histological type of odontogenic tumors was diagnosed based on the "Head and Neck Tumors" guidelines published in 2005 by the World Health Organization Classification of Tumours (Philipsen et al., 2005). We investigated 71 total cases of odontogenic lesions: 57 samples of ameloblastomas from 40 patients, 11 odontomas (one of which contained multiple small teeth and a tooth germ-like structure without enamel or dentin), two cases of ameloblastic fibro-odontoma (Takeda and Tomich, 2005) and one case consisting of supernumerical tooth germ-like structures without the formation of enamel and dentin. Only odontomas with dental epithelial cell-like and/or ameloblast-like cells were included in the study. This study was approved by the local research ethics committee of Kyushu University.

Antibodies

A rabbit antibody reactive to human Tß4 (A9550) was purchased from Immundiagnostik AG (Bensheim, DEU). The goat antibodies for human AMBN (N-18) and ENAM (C-18) and rabbit antibody for human AMEL (FL-191) were obtained from Santa Cruz (CA, USA). These enamel proteins were used as markers to evaluate the functional differentiation of the neoplastic epithelium in odontogenic tumors (Takata et al., 2000).

Immunohistochemistry

Immunohistochemical staining was performed on 5 μm thick paraffin sections. After deparaffinization and rehydration, endogenous peroxidase activity was eliminated by treatment with 3% hydrogen peroxide in methanol for 20 min. Non-specific protein binding was blocked with 10% goat or rabbit serum for 20 min, and then the sections were reacted with the primary antibodies against Tß4, AMBN and AMEL (diluted 1:1200 each), and against ENAM (diluted 1:800) at 4°C overnight. The sections were incubated with the Fab' fragment of the secondary antibody conjugated with a peroxidase-labeled amino acid polymer (Histofine

Simple Stain MAX PO, Nichirei, Japan) for 30 min at room temperature. After the washing with PBS, their immunoreactivity was visualized with a solution of 3,3'-diaminobenzidine and less than 0.1% hydrogen peroxide (Nichirei). Subsequently, the sections were counter-stained with hematoxylin. For a negative control, PBS was substituted for the primary antibody.

Evaluation of immunohistochemical reactivity

More than one thousand objective cells were examined as a population in at least three different microscopic fields of each area. When the number of objective cells was less than one thousand in a section, all the objective cells were counted. The number of stained cells was divided by the total number of stained and non-stained target cells to calculate the Tß4-positive ratio. Immunohistochemical reactivity was regarded to be negative when all cells were negative for staining or when less than 5% of cells expressed the antigen. Based on the estimated percentage of positively stained tumor cells, the samples were classified into four groups: +++, >75% of cells were positive; ++, 26-75% of cells were positive; +, 5-25% of cells were positive; and - (negative).

The immunoreactivity of activated macrophages in the lymph nodes and/or the striated duct cells in the salivary gland were used as a positive control (Nemolato et al., 2009).

Statistical analysis

Differences among groups were assessed by Mann-Whitney's U-test or Spearman's correction by rank test. A p-value <0.05 was considered to be statistically significant.

Results

Patients' characteristics

Ameloblastomas

Samples of ameloblastoma were obtained from 25 male (62.5%) and 15 female (37.5%) patients, with a mean age of 44.80 years (age range: 5-80 years). The mean age of male patients was 46.84 years and that of female patients was 41.39 years. Thirty-eight cases (95.0%) were from the mandible, and the remaining two were from the maxilla (5.0%). A total of 57 samples of ameloblastomas from 40 patients were assessed and were all found to be tumors of the solid/multicystic type. Histologically, 18 cases dominantly showed a follicular pattern, and 22 cases displayed a dominantly plexiform pattern. The formation of multicysts was observed in three and four of these cases, respectively. The clinicopathological features are summarized in Table 1. No significant correlation was demonstrated between Tß4 immunoreactivity and the patient age, gender, or the location of the ameloblastomas in this study.

Odontomas

The samples with odontoma were obtained from five male (45.5%) and six female (54.5%) patients, with a mean age of 8.08 years (age range: 3-11 years). Seven cases (63.6%) were from the mandible, and the remaining four from the maxilla (36.4%). There were three and eight odontomas of the complex type and compound types, respectively (Prætorius and Piattelli, 2005).

Ameloblastic fibro-odontomas

Two cases of ameloblastic fibro-odontoma were obtained from a 12-year-old male and an 11-year-old female patient. The former was from the molar region of the mandible and the latter from the incisor region of the maxilla.

The other type

The other case occurred in the gingiva of the mandible of a 3-year-old male.

Immunohistochemical findings

Ameloblastomas

Signals for Tß4 were observed in 55 samples of the 57 examined ameloblastoma samples. Many basal polarized cells showed moderate to strong staining in both the follicular and plexiform ameloblastomas (Fig. 1A,B). The central polyhedral/angular cells of all ameloblastomas studied were also positive, varying in intensity. The number and intensity of polyhedral cells stained for Tß4 were higher than those of the angular

Table 1. Clinicopathological features of ameloblastomas in relation to the immunohistochemical reactivity for Tß4 .

Tß4 immunoreactivity	-	+	++	+++	total
Age (year) (p = 0.15)					
0-20	0	1	1	1	3
20-40	0	0	7	4	11
>40	1	1	8	16	26
Sex (p = 0.06)					
male	1	0	8	16	25
female	0	2	8	5	15
Tumor location (p = 0.19)					
maxilla	0	0	0	2	2
mandible	1	2	16	19	38
Histological subtype (p = 0.07)					
follicular type	0	0	6	12	18
plexiform type	1	2	10	9	22
	1	2	16	21	40

Immunohistochemical reactivity for Tß4 and clinical data were evaluated in the primary specimen when multiple specimens were prepared from the single case at different times.

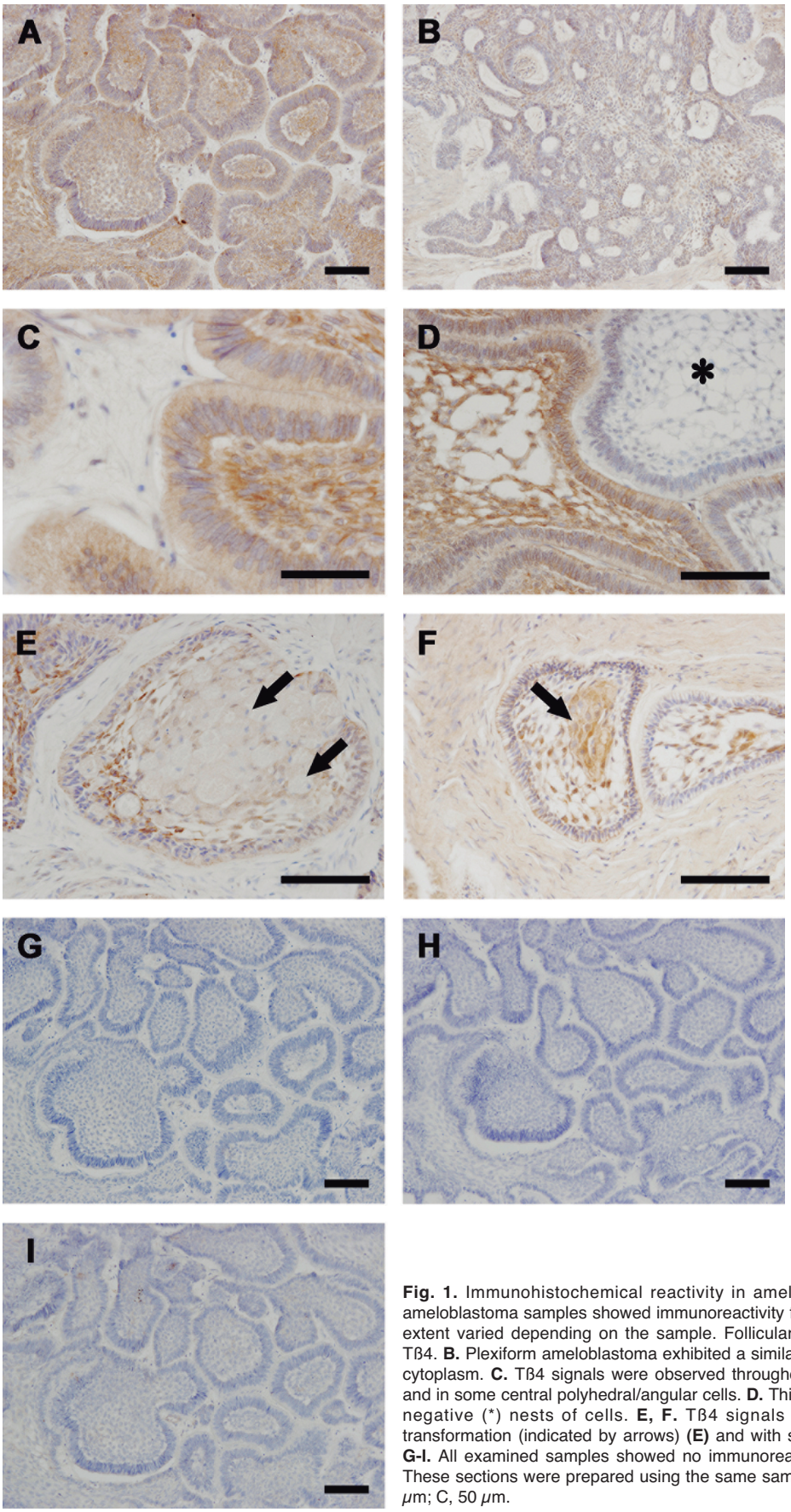

Fig. 1. Immunohistochemical reactivity in ameloblastomas. **A.** Almost all of the examined ameloblastoma samples showed immunoreactivity for Tß4, although their expression intensity and extent varied depending on the sample. Follicular ameloblastoma showed immunoreactivity for Tß4. **B.** Plexiform ameloblastoma exhibited a similar distribution of Tß4 signals throughout the cell cytoplasm. **C.** Tß4 signals were observed throughout the cytoplasm of peripheral columnar cells and in some central polyhedral/angular cells. **D.** This example case showed Tß4-positive and Tß4-negative (*) nests of cells. **E, F.** Tß4 signals were also found in the cells with granular transformation (indicated by arrows) **(E)** and with squamous metaplasia (indicated by arrow) **(F)**. **G-I.** All examined samples showed no immunoreactivity for AMBN **(G)**, AMEL **(H)** or ENAM **(I)**. These sections were prepared using the same sample shown in Fig. 1A. Scale bar: A, B, D-I, 100 μm; C, 50 μm.

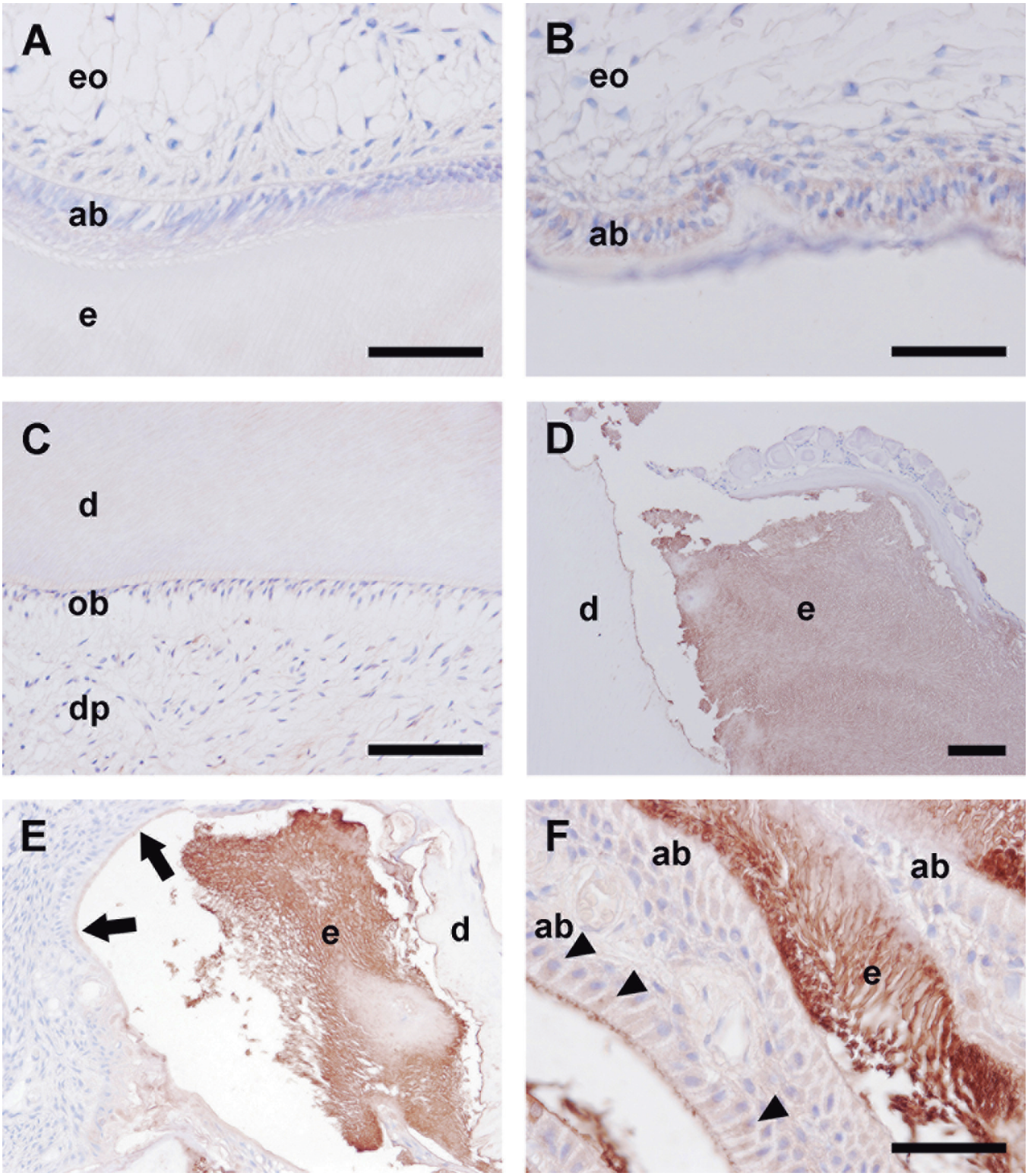

Fig. 2. Immunohistochemical reactivity in odontomas. **A.** The immunostaining for Tß4 was negative in the polarized epithelial cells of the odontomas. **B.** Polarized epithelial cells without enamel matrix were positive for Tß4. **C.** Almost all odontoblasts and dentin were negative for Tß4. Odontoblasts and dental pulp cells occasionally showed faint Tß4 immunoactivity. **D.** The immunoreactivity for AMEL was strong in the enamel matrix, whereas it was negative in the dentin. **E.** AMEL immunoactivity was identified at the interface between the epithelium and enamel matrix (arrows). **F.** Some ameloblastic cells facing the enamel matrix showed faint AMEL immunoactivity (arrowheads). Immunoreactivity for AMBN and ENAM showed similar findings. ab: ameloblasts, d: dentin, e: enamel matrix, dp: dental pulp, eo: enamel organ,ob: odontoblasts, Scale bar: A, B, F, 50 μm; C, D, 100 μm.

cells. Strong signals were occasionally observed in the peripheral polarized cells and central polyhedral cells. The Tß4 protein was distributed throughout cell cytoplasm in both peripheral and central cells (Fig. 1C). Although positive and negative cells were observed in the same tumor nest, Tß4 immunoreactivity tended to be higher in the peripheral polarized cells than in the central cells in the same nest. All cells showed Tß4-positivity in many small nests. In some cases, nests of negative cells or positive cells were mixed in the same tumor (Fig. 1D). The results of the immunohistochemical reactivity evaluation are shown in Table 1. No significant difference was detected in the Tß4 immunoreactivity between ameloblastomas of the follicular type and the plexiform type (p=0.07).

Granular transformation and squamous metaplasia were noted in some tumors. The central cells with granular transformation and with squamous metaplasia showed mild and strong Tß4 immunoreactivity, respectively (Fig. 1E,F). Tß4 expression was

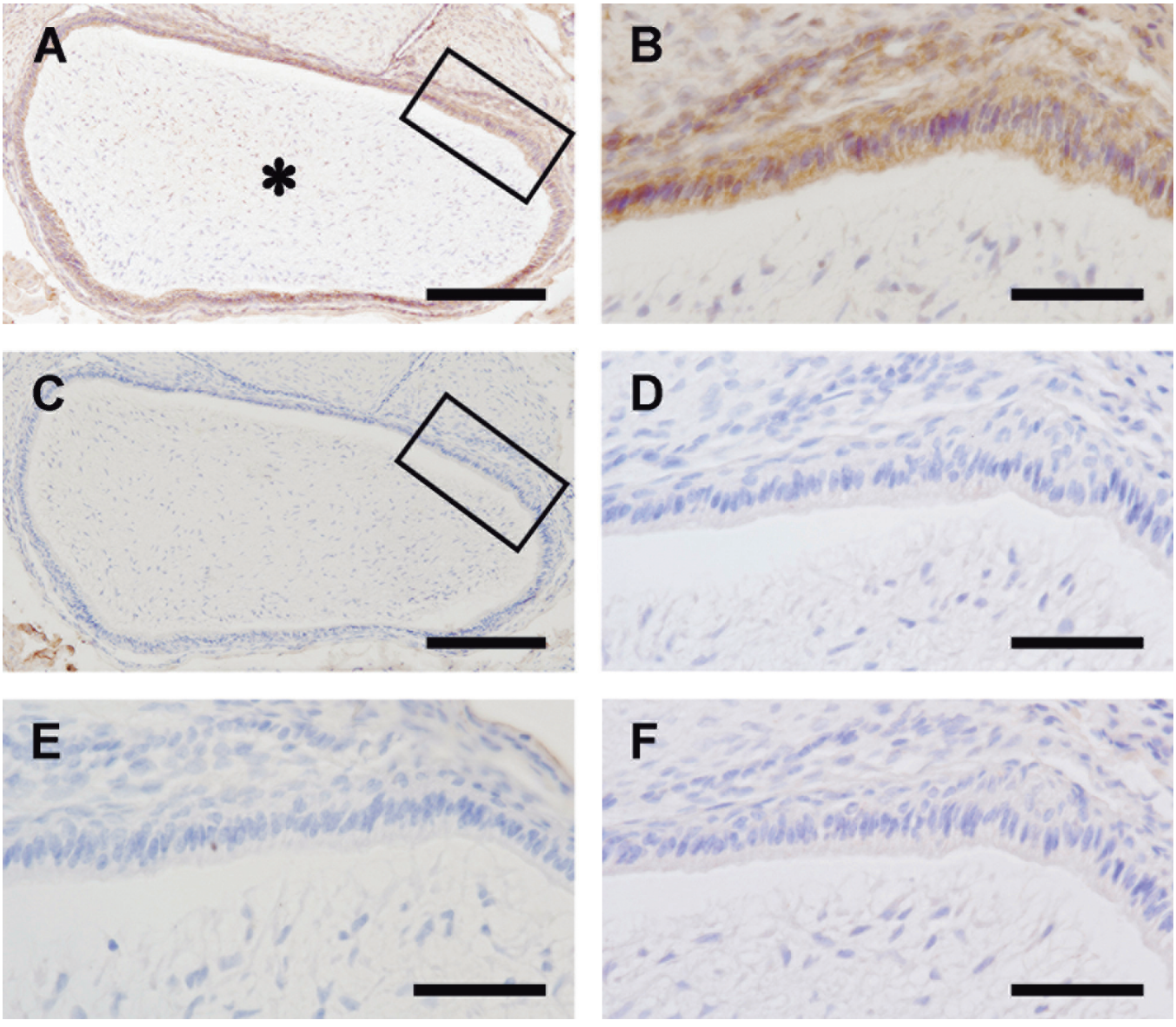

Fig. 3. Protein expression levels in the tooth germ-like structure included in the odontoma. **A.** Immunoreactivity for Tß4 was strong in almost all peripheral columnar cells, while it was scant in the dental pulp-like structure (*). **B.** A higher magnification of the boxed area shown in Fig. 3A. **C.** The tooth germ-like structure in this specimen was negative for AMBN staining. **D.** A higher magnification of the boxed area shown in Fig. 3C. **E, F.** These areas correspond to the area in Fig. 3D, and had negative results for AMEL **(E)** and ENAM **(F)**, respectively. Scale bar: A, C, 400 μm; B, D-F, 50 μm.

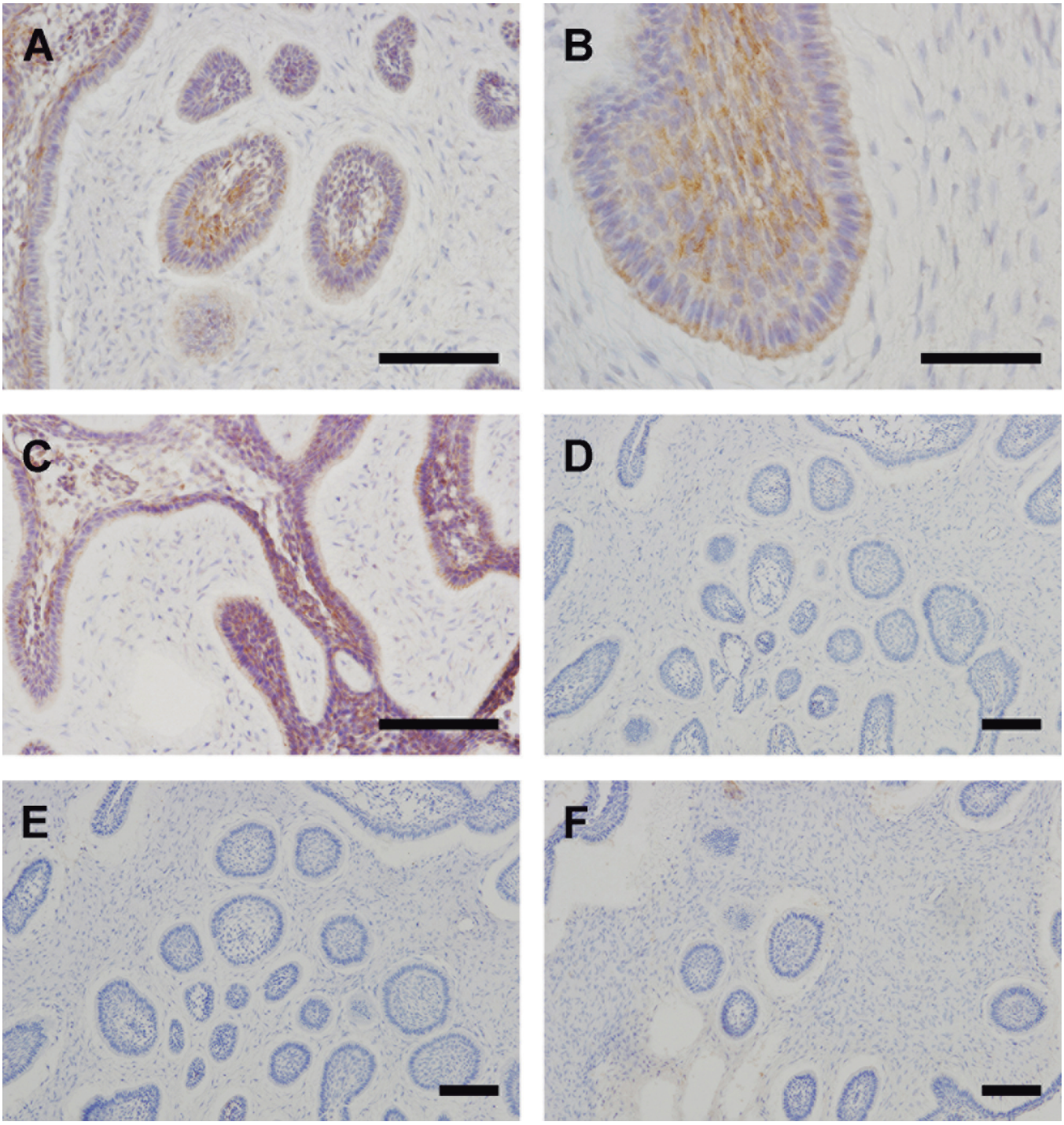

Fig. 4. Protein expression levels in the soft tissue region of the ameloblastic fibro-odontoma. **A.** The immunoreactivity for Tß4 was positive in the epithelial nests, resembling that in ameloblastoma. **B.** Tß4 signals were observed in peripheral columnar cells and in central polyhedral/angular cells. **C.** Tß4 signals were also identified in the basal polarized cells and central cells of the epithelial nests resembling plexiform ameloblastoma. **D-F.** The epithelial nests and mesenchymal components were negative for AMBN **(D)**, AMEL **(E)** and ENAM **(F)**. Scale bar: A, C-F, 100 μm; B, 50 μm.

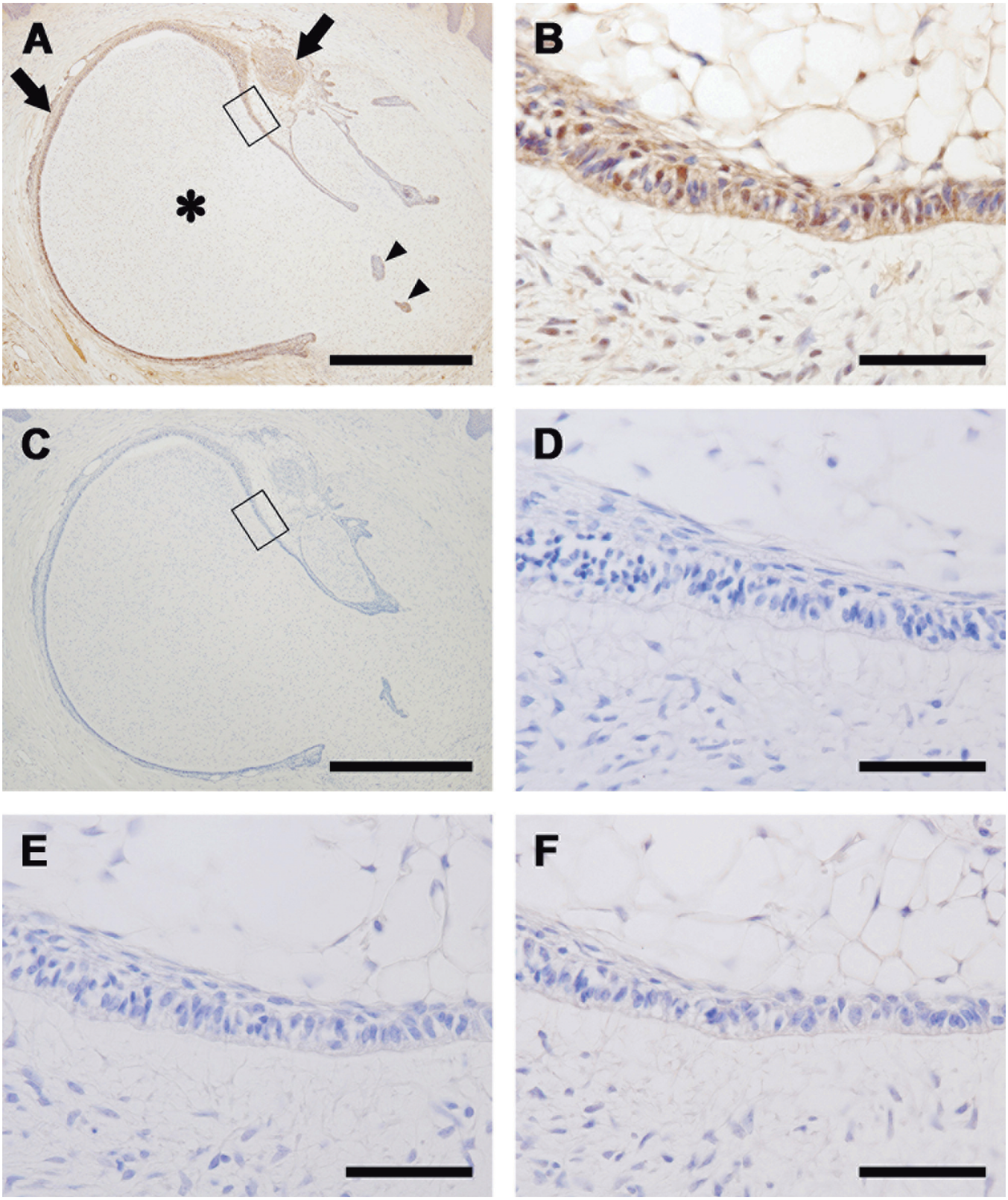

Fig. 5. Immunohistochemical reactivity in the specimen mimicking multiple developing tooth germs without the formation of enamel and dentin. **A.** Strong signals for Tß4 were observed in the enamel organ-like structure (arrows), whereas the protein was absent in the dental pulp-like structure (*). The odontogenic epithelial nests showed positive signals for Tß4 (arrowheads). **B.** A higher magnification of the upper boxed area in Fig. 5A. Immunoreactivity for Tß4 is evident in almost all peripheral columnar cells and in some central angular cells. **C.** Immunolabeling for AMBN was absent in the enamel organ-like structure as well as in the dental pulp-like structure. **D.** A higher magnification of the boxed area in Fig. 5C. **E, F.** These areas corresponded to that shown in Fig. 5D. Signals for AMEL **(E)** and ENAM **(F)** were absent in this specimen. Scale bar: A, C, 500 μm; B, D-F, 50 μm.

consistently detected in the samples removed from the same 14 patients at different times. The immunoreactivity was slightly reduced, but the expression patterns were similar findings.

All of the assessed ameloblastomas were negative for AMBN (Fig. 1G), AMEL (Fig. 1H) and ENAM (Fig. 1I).

Odontomas

The odontogenic epithelium showing postsecretory transition ameloblasts and reduced enamel epithelium was negative for staining with all of the antibodies used in this study (Fig. 2A). The calcificated materials associated with epithelial cells were negative for Tß4, AMBN, AMEL and ENAM (Fig. 2D). Odontogenic polarized epithelial cells showing presecretory ameloblasts were positive for Tß4 (Fig. 2B). These cells also showed faint positivity for AMEL and ENAM, but was not detectable for AMBN. Immunoreactivity for AMBN, AMEL and ENAM was noted at the epithelium-enamel interface (Fig. 2E). Some columnar cells showed intracytoplasmic signals for AMBN, AMEL and ENAM, suggesting that they were secretory ameloblasts (Fig. 2F). Their expression intensity varied among the cells. Strong signals for AMBN, AMEL and ENAM were observed within the enamel matrix in all the odontomas (Fig. 2D-F). Their intensity in the enamel matrix was much higher than that in ameloblast-like cells. These findings were observed in both subtypes of odontoma.

An odontoblastic layer was observed in the pulpal tissue faced with predentin matrix. Most of the cells were negative for Tß4 (Fig. 2C) as well as AMBN, AMEL and ENAM in all of the examined specimens. The dentin matrix in all of the odontomas was negative for staining with all of the antibodies used in this study.

There was one case in which the odontomas contained a tooth germ-like structure without enamel or dentin. The columnar cells at the periphery showed strong immunolabeling for Tß4 (Fig. 3A,B), but were negative for AMBN (Fig. 3C), AMEL (Fig. 3D) or ENAM (Fig. 3E). Meanwhile, the dental pulp-like cells surrounded by these columnar cells were negative for staining with all of the antibodies used in this study (Fig. 3).

Ameloblastic fibro-odontomas

A moderate positive reaction for Tß4 was observed within the peripheral columnar cells in the epithelial nests/strands in the soft tissue. The immunoreactivity in the stellate reticulum-like cells varied in comparison with that in the peripheral columnar cells (Fig. 4A-C). The epithelial elements of this region were negative for staining with all of the other antibodies (Fig. 4D-F). The immunoreactive pattern in the epithelial nests/strands was similar to that observed in the ameloblastomas as mentioned above. On the other hand, the epithelial and mesenchymal cells, and the enamel and dentin in the hard tissue, showed equal immunoreactivity in the odontomas. These findings were similar in both cases.

The other type

The lesion was composed of multiple enamel organ-like structures with a peripheral layer of columnar cells enclosing stellate reticulum-like cells, and a dental papilla-like structure and dental follicle-like structure, mimicking multiple developing tooth germs, but without the formation of enamel and dentin. We believe that the lesion corresponded to the initiation phase of odontoma formation (Silva et al., 2009) because the morphological findings seem to depend on the developmental stage of the tooth germ at discovery (Tabata et al., 1996; Akhter et al., 2005; Philipsen et al., 2005). The specimen had no apparent odontoblastic layer in the pulpal tissue facing the ameloblast-like cells.

Strong signals for Tß4 were noted in the columnar cells at the periphery of the enamel organ-like structure (Fig. 5A,B). Some stellate reticulum-like cells showed weak signals for Tß4. A few cells in the dental pulp-like structure were positive for Tß4. There were no cells that stained positive for AMBN, AMEL or ENAM in this specimen (Fig. 5C-F). The odontogenic epithelial nests were positive for Tß4 (Fig. 5A), but negative for AMBN, AMEL or ENAM.

Discussion

Our results revealed that almost all of the ameloblastoma samples showed expression of Tß4 in the epithelial components, and its expression was higher compared to samples of odontoma. To our knowledge, this study represents the first comprehensive analysis of Tß4 immunoreactivity in odontogenic neoplasms.

This study demonstrated interesting differences in Tß4 immunoreactivity between ameloblastoma and odontoma. In the epithelial cells of the ameloblastomas, immunoreactivity for Tß4 was significantly higher than those in the odontomas. The former are odontogenic neoplasms derived from odontogenic epithelium comprising immature cells with a lack of enamel matrix, while ameloblastomas exhibit higher local aggressiveness and more frequent recurrence (Gardner et al., 2005). The epithelial cells in the ameloblastomas were negative for AMBN, AMEL and ENAM in this study, as well as in the report of Crivelini et al. (2012). In contrast, odontomas can be regarded as hamartomas rather than odontogenic neoplasms, and are composed of both odontogenic epithelium and mesenchymal dental hard tissues (Prætorius and Piattelli, 2005). The odontogenic epithelial cells show a mature differentiated phenotype with the formation of enamel matrix. Additionally, Tß4 expression was noted in the epithelial nests/strands in the soft tissue of the ameloblastic fibro-odontoma. Tß4 expression was similar to that in the

ameloblastomas. Interestingly, this Tß4 expression pattern was also observed in the tooth germ-like structures examined in this study. However, their expression appears to be temporally limited, based on the results of the odontoma and developing tooth germ (Akhter et al., 2005). In contrast, the ameloblastomas seem to continuously express Tß4, because Tß4 was detected with a similar expression pattern in the samples removed from the same 14 patients at different times. The epithelial cells in ameloblastomas were positive for Tß4 but negative for AMBN, AMEL and ENAM in this study. These enamel proteins are regarded to be useful markers that can be used to evaluate the functional differentiation of the neoplastic epithelium in odontogenic tumors in comparison to the developing tooth germ (Takata et al., 2000). Based on these results, the expression of Tß4 (Akhter et al., 2005), HGF and c-MET (Tabata et al., 1996), and morphology, the ameloblastoma cells may correspond to epithelial cells of the tooth germ at the early bell stage. Some of the ameloblastoma cells may have similar properties to those of the epithelial cells of the tooth germ at the cap stage. Furthermore, Tß4-overexpressing transgenic mice were recently shown to have enamel hypoplasia-like abnormal tooth development (Cha et al., 2010), similar to that observed in the ameloblastin or amelogenin knockout mice (Fukumoto et al., 2004). Tß4 may therefore play an important role in organ development during embryogenesis, since a strong reactivity for Tß4 was also seen in the developing gut (Nemolato et al., 2010), as well as in developing salivary glands (Nemolato et al., 2009). Together with observations during normal tooth development (Akhter et al., 2005; Ookuma et al., 2013) and in malignant cells (Wang et al., 2004; Ryu et al., 2012), these data suggest that Tß4 may contribute to cellular differentiation and the maintenance of morphology in ameloblastoma.

Ameloblastomas may exhibit destructive and locally invasive growth through disruption of the extracellular matrix by MMPs expressed in the ameloblastomas themselves (Pinheiro et al., 2004; Shen et al., 2010; Siqueira et al., 2010). In the hair follicle, Tß4 promoted MMP expression and promoted the migration of stem cells and their immediate progeny to the base of the follicle, inducing differentiation and extracellular matrix remodeling (Philp et al., 2004). Tß4 is also involved in migration of gastric carcinoma cells (Ryu et al., 2012). In comparison to primary colorectal tumors, the matched metastatic liver lesions had relatively upregulated expression of Tß4 and MMP-7, but significantly downregulated levels of E-cadherin and Fas (Wang et al., 2004). The overexpression of Tß4 in colorectal carcinoma cells induced a loss of E-cadherin and a cytosolic accumulation of ß-catenin, leading to a reduction in cell-cell interactions (Huang et al., 2007). Tß4 also regulates actin polymerization, thereby participating in cell motility (Safer et al., 1991). Together, the results of the present and previous studies suggest that Tß4 may regulate the expression of MMPs and E-cadherin, as well as actin polymerization, and therefore may be involved in the migration of ameloblastoma cells as well as that of cells during the tooth germ development (Ookuma et al. 2013).

Tß4 has also been implicated in antiapoptotic activities in various cells, such as corneal epithelial cells (Sosne et al., 2004; Ho et al., 2007), conjunctival cells (Sosne et al., 2006) and nerve cells (Choi et al., 2006, 2007). The continuous expression of Tß4 was observed in the neoplastic epithelial cells in ameloblastomas, but was negative in the epithelial cells of odontomas. Tß4 appeared to be temporally expressed in the tooth-like structures in this study. In another study, it was revealed that, in colon carcinoma cells, Tß4 overexpression induced resistance to apoptosis triggered by FasL and two topoisomerase II inhibitors via the downregulation of Fas and upregulation of Survivin, respectively (Hsiao et al., 2006). In addition, Tß4 overexpression inhibited paclitaxel-mediated apoptosis and induced paclitaxel resistance in the malignant cells (Oh et al., 2006). The continuous expression of Tß4 in the ameloblastomas could contribute to tumor progression, at least in part, by providing antiapoptotic activity.

Interestingly, the expression of Tß4 in the ameloblastomas in our present study closely coincided with the expression of HGF and c-Met, the HGF receptor, in the epithelial tumor cells of ameloblastomas (Kumamoto et al., 2002; Poomsawat et al., 2012). Since HGF upregulates Tß4 expression in HUVECs (Oh et al., 2002), continuous Tß4 expression may take part in the HGF/c-Met signaling pathway that promotes tumor proliferation to influence the biological behavior of the ameloblastoma.

This study demonstrated that Tß4 was expressed in ameloblastoma, as well as in the tooth germ-like structures associated with odontoma. Together with its functions in malignant cells and tooth germ development, Tß4 may also be associated with important proteases and growth factors/molecules that regulate morphogenesis, cell differentiation, invasive tumor growth and apoptosis susceptibility in the ameloblastoma. The results of this study help to provide a better understanding of the cell biology of ameloblastoma. However, further studies of the effectors and cell targets are required to define the detailed Tß4-associated mechanism(s) involved in the development and progression of ameloblastoma.

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