Virchows Arch (2000) 436:319–323

ORIGINAL ARTICLE

**Takashi Takata · Mutsumi Miyauchi · Ikuko Ogawa
Yasusei Kudo · Toshitugu Takekoshi · Ming Zhao
Sunao Sato · Hiromasa Nikai · Kazuo Komiyama**

Immunoexpression of transforming growth factor β in desmoplastic ameloblastoma

Received: 22 June 1999 / Accepted: 27 September 1999

Abstract Desmoplastic ameloblastoma (DA) is an unusual subtype of ameloblastoma histologically characterized by the pronounced collagenized stroma. In the present study, the immunolocalization of transforming growth factor beta (TGF-β), one of the most potent local factors for modulating extracellular matrix formation, was observed in DA in order to study its participation in the stromal desmoplasia. Seven cases of DA, including a "hybrid" lesion, were studied together with ten cases of ordinary follicular and plexiform ameloblastomas as the control. In contrast to ordinary ameloblastomas, marked immunoexpression was observed in all DAs but one. In the "hybrid" lesion, TGF-β was not expressed in the area of follicular ameloblastoma but in that of DA. These results show that TGF-β produced by tumor cells of DA plays a part in the desmoplastic matrix formation.

Key words Ameloblastoma · Transforming growth factor-beta · Desmoplasia · Type-IV collagen · Immunohistochemistry

Introduction

Desmoplastic ameloblastoma (DA) is an unusual subtype of ameloblastoma [22, 37]. In contrast to ordinary ameloblastomas, usually involving mandibular molar/ramus areas with multi- or unilocular appearance, DA shows striking tendency to occur in the anterior portions of jaws and radiographic features often resembling fibro-osseous lesions or even malignancies. DA is histologically characterized by the pronounced desmoplastic stroma in which there are compressed tumor islands usually lacking a peripheral layer of ameloblastic cells and a central zone of stellate reticulum.

As for the formation of desmoplastic stroma in DA, Philipsen et al. [22] described preliminary immunohistochemical findings of intense staining for collagen type IV adjacent to tumor islands in DA. They suggested that the finding indicated an active synthesis of extracellular matrix protein and that the desmoplastic stroma in DA was not a scar tissue but rather a newly produced connective tissue. However, the detailed mechanism of the desmoplasia in DA has not been elucidated.

Transforming growth factor beta (TGF-β) is one of the most potent local factors for modulating extracellular matrix formation. In scirrhous gastric [14, 29] and mammary [4] tumors, it is known that TGF-β is produced by tumor cells themselves, resulting in enhanced collagen deposition in stroma. The purpose of this study, therefore, was to localize TGF-β in DA for discussing its participation in the active synthesis of extracellular matrix.

Materials and methods

Eight cases of DA, including a "hybrid" lesion, were retrieved from the pathology files of the Department of Oral Pathology, Hiroshima University School of Dentistry. Immunoexpression of TGF-β was studied in all cases except one in which paraffin-embedded material was not available for further analysis. The clinical findings are summarized in Table 1. Ten cases of ordinary ameloblastoma (five follicular and five plexiform ameloblastomas) were also selected from the file for controls.

Immunohistochemistry

Sections (4.5-μm thick) were cut from 10% neutral formalin-fixed and paraffin-embedded specimens, and mounted on silicon-coated glass slides. The immunostaining was conducted using either an al-

T. Takata (✉) · M. Miyauchi · I. Ogawa · Y. Kudo · T. Takekoshi
M. Zhao · S. Sato · H. Nikai
Department of Oral Pathology,
Hiroshima University School of Dentistry, 1-2-3 Kasumi,
Minami-ku, Hiroshima 734-8553, Japan
e-mail: ttakata@ipc.hiroshima-u.ac.jp
Tel.: +81-82-2575632, Fax: +81-82-2575619

I. Ogawa
Clinical Laboratory, Hiroshima University School of Dentistry,
Hiroshima, Japan

K. Komiyama
Department of Pathology, Nihon University School of Dentistry,
Tokyo, Japan

Table 1 Clinical data of seven desmoplastic ameloblastomas. *I* incisor; *C* canine; *P* premolar; *M* molar regions

Case	Age	Gender	Location	Size (cm)[b]
1	17	Male	mand C-P	3.0×3.0
2	53	Male	max I-C	1.5×2.3
3	54	Male	mand I-P	3.0×7.0
4	52	Male	max I-P	3.5×4.3
5	51	Male	max C-P	1.8×2.1
6	24	Female	mand C-P	2.5×3.0
7[a]	48	Male	mand I-M	5.4×6.6

[a] "Hybrid" lesion
[b] Described with the greatest diameters in mesio-distal and apico-occlusal directions on a panoramic radiograph

kaline phosphatase (AP) or peroxidase (PO) Histofine streptavidin–biotin complex (SAB) kit (Nichirei, Tokyo, Japan). Briefly, deparaffinized sections were immersed in 0.3% hydrogen peroxide in methanol for 20 min at room temperature to block the endogenous peroxidase activity for the PO method. After rinsing in phosphate-buffered saline (PBS) and incubating with 0.25% casein for 30 min, the sections were incubated with primary antibodies for 24 h at 4°C in a humid atmosphere. The primary antibody used in this study was a monoclonal mouse anti-human TGF-β1 and β2 (Genzyme, Cambridge, Mass.) antibody, which was diluted in 0.01 M PBS to 150 μg/ml following the company instructions. After rinsing in PBS, the sections were incubated with biotinylated rabbit anti-mouse IgG for 30 min. For the AP method, the sections were rinsed in 0.05 M tris-HCl buffer (pH 7.4) and incubated with AP-conjugated streptavidin for 30 min. For the PO method, the sections were rinsed in PBS and incubated in PO-conjugated streptavidin for 30 min. Color was developed with a substrate solution consisting of 100 ml of 0.2 M tris-HCl buffer (pH 8.5), 25 mg naphthol AS-BI phosphate (Sigma, St. Louis, Mo.), 10 mg new fuchsin (Merck, Darmstadt, Germany), 10 mg sodium nitrate (Wako, Osaka, Japan), and 24 mg L-levamisole (Sigma) for AP, or with 3,3'-diaminobentidine tetrahydrochloride (Lipshaw Immunon, Pittsburgh, Pa.) for PO. The sections were counterstained with Mayer's hematoxylin.

We also studied immunohistochemical expression of collagen type IV in DAs, as Philipsen et al. [22] described intense staining for collagen type IV adjacent to tumor islands in DA. For immunohistochemical detection of type-IV collagen, enzymatic retrieval of antigenicity was made in 0.01 N HCl solution containing 0.5% pepsin 1:100 (Wako, Osaka, Japan) for 2 h at 37°C. The antibody against type-IV collagen was purchased from DAKOPATTS A/S (Copenhagen, Denmark) and used with dilution in 0.01 M PBS to 1 in 100. The rest of the procedures were the same as those for immunostaining of TGF-β.

Negative controls were performed by replacing the primary antibodies with PBS.

Results

Histological findings

All cases of DA showed marked stromal collagenization (Fig. 1). Most tumor islands were enclosed by the pronounced highly desmoplastic stroma. There were no inflammatory changes, such as inflammatory cell infiltration and proliferation of inflammatory granulation tissue. Tumor nests were various in size and shape. In some places, the epithelial islands appeared to be compressed by the desmoplastic stroma resulting in narrow strands or cords. Characteristically, the tumor nests were composed of peripheral cuboidal or flattened cells often with nuclear hyperchromatism and central spindle cells often arranged in cellular fascicles and/or whorls (Fig. 1).

A case of "hybrid" lesion showed the characteristics of DA descried above in the incisor to premolar region of the tumor and those of typical follicular ameloblastoma in the molar area of the lesion.

Ordinary ameloblastomas showed nests consisting of peripheral columnar ameloblastic cells and central stellate reticulum-like cells in follicular ameloblastomas and interlacing strands bordered by a single layer of cuboidal cells with an internal area of stellate cells in plexiform ameloblastomas.

Table 2 Immunoexpression of transforming growth factor (TGF)-β in tumor cells and type-IV collagen at the periphery of tumor nests in desmoplastic ameloblastomas (DA) and ordinary ameloblastoma (OA). *OA1–5* follicular ameloblastoma; *OA6–10* plexiform ameloblastoma; – negative; + slightly positive; ++ moderately positive; +++ intensively positive

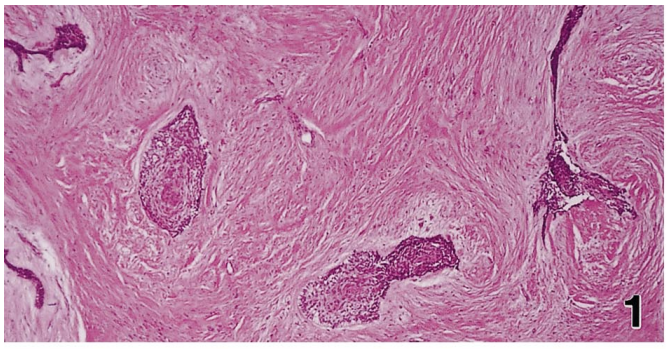
Case		TGF-β	Type-IV collagen
DA1		–	+
DA2		++	++
DA3		+++	+
DA4		++	+
DA5		+++	++
DA6		++	–
DA7 (hybrid)	DA	+++	++
	OA	–	++
OA1		–	++
OA2		–	++
OA3		+	+
OA4		–	–
OA5		–	–
OA6		–	++
OA7		–	++
OA8		–	++
OA9		–	+
OA10		–	–

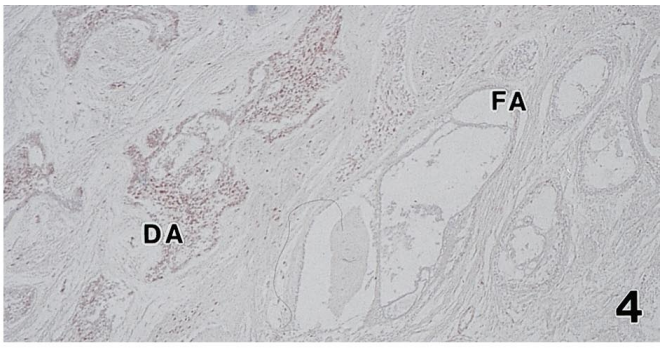
Immunohistochemical findings

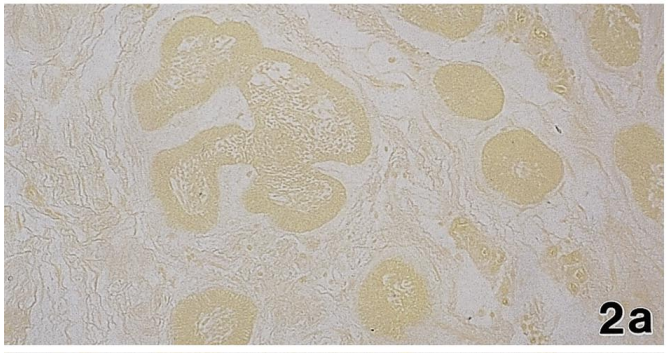
The immunoexpression of TGF-β in tumor cells and collagen type IV at the periphery of tumor nests in seven DAs and ordinary ameloblastomas is summarized in Table 2.

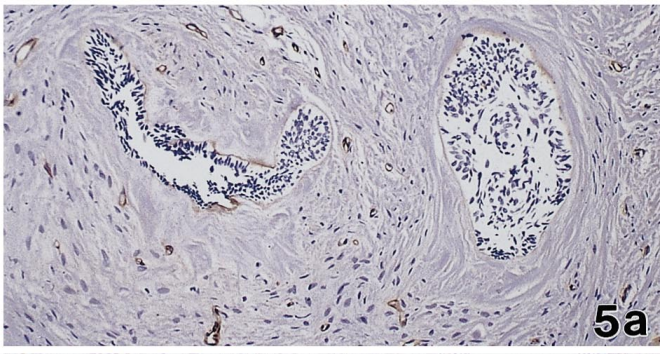
TGF-β

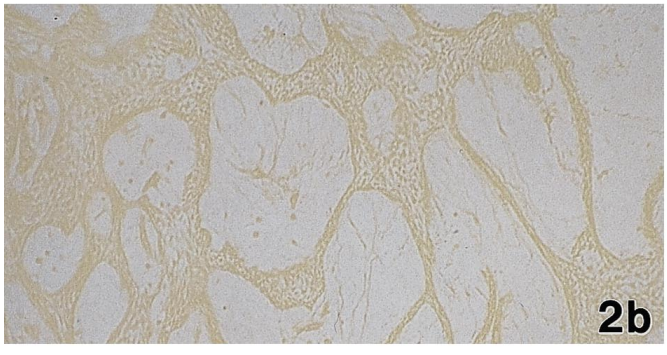
There was no obvious positive staining in ordinary ameloblastomas of the follicular (Fig. 2a) or plexiform type (Fig. 2b). In contrast to the ordinary ameloblastomas, marked immunoexpression was observed in all DAs but one (Fig. 3). Both peripheral and central cells in tumor nests displayed distinct immunopositivity. TGF-β was mainly expressed in the nuclei of the cells. Fibroblasts in highly collagenized stroma often showed slight to moderate positivity, while no staining was seen in extracellular matrices. In the "hybrid" lesion, interestingly, TGF-β was intensively expressed in the area of DA but not in the area of follicular ameloblastoma (Fig. 4).

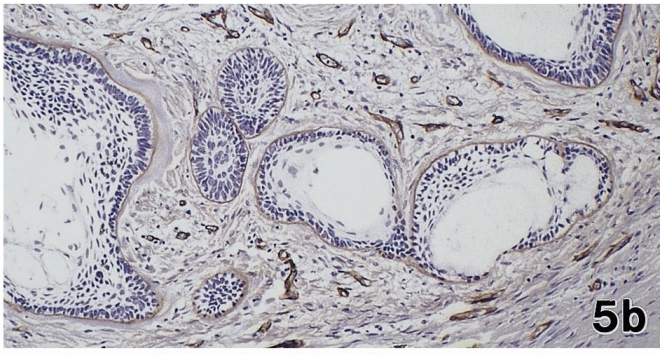


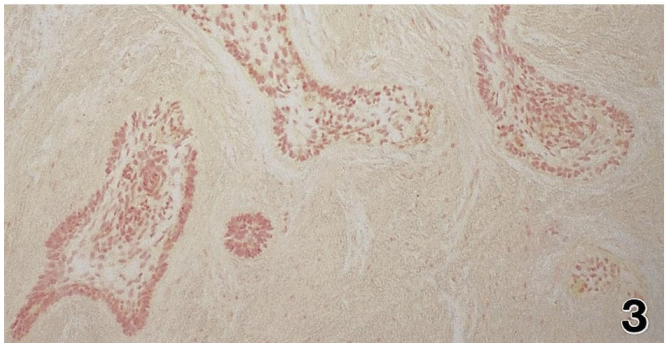












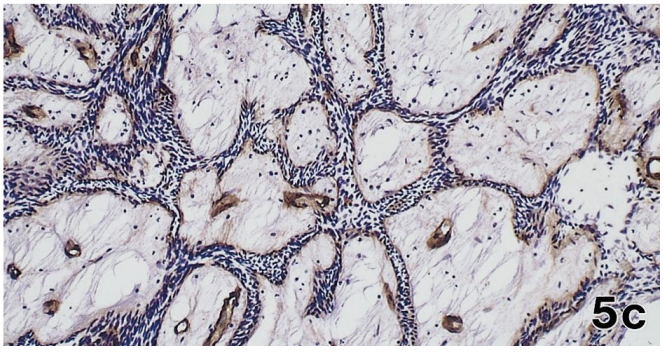


Fig. 1 Histological features of desmoplastic ameloblastoma characterized by the pronounced desmoplastic stroma. Tumor nests, some of which appear to be compressed by the desmoplastic stroma resulting in narrow strands, are usually lacking a peripheral layer of tall ameloblastic cells and a central zone of stellate reticulum. Hemtoxylin and eosin, ×40

Fig. 2 Immunostaining of transforming growth factor (TGF)-β in ordinary ameloblastoma. There is no obvious positive staining in follicular (a) and plexiform (b) ameloblastomas. Immunoalkaline phosphatase method, **a**, **b** ×100

Fig. 3 Immunostaining of transforming growth factor (TGF)-β in desmoplastic ameloblastoma. Marked immunoexpression of TGF-β is observed both in peripheral and central cells in tumor nests. Immunoalkaline phosphatase method, ×100

Fig. 4 Immunostaining of transforming growth factor (TGF)-β in a "hybrid" lesion. TGF-β is intensively expressed in the area of desmoplastic ameloblastoma (*DA*) but not in the area of follicular ameloblastoma (*FA*). Immunoperoxidase method, ×50

Fig. 5 Immunostaining of type-IV collagen in desmoplastic (a), follicular (b) and plexiform (c) ameloblastomas. Distinct expression is seen in the basement membrane of blood vessels. Although both desmoplastic and ordinary ameloblastomas show immunoexpression of type-IV collagen at the periphery of some tumor nests, there is no remarkable difference in the immunoreactivity between the types of ameloblastomas. Immunoperoxidase method, ×100

Type-IV collagen

Type-IV collagen was distinctly expressed in the basement membrane of blood vessels, peripheral nerves and, if included, covering epithelium (Fig. 5a). Staining intensity was enhanced by retrieval of antigenicity with enzymatic predigestion of the sections. Immunoexpression of type-IV collagen in these structures was used as internal controls. Both the ordinary ameloblastoma and DA showed immunoexpression of type-IV collagen at the periphery of tumor nests (Fig. 5b, c). There was no remarkable difference of type-IV collagen expression between the ordinary ameloblastomas and DA. These findings were true in the "hybrid" lesion where DA and follicular ameloblastoma presented in a histological section.

Control staining

No immunohistochemical localization was seen in the negative controls where the primary antibodies were replaced with PBS.

Discussion

To date, around 70 cases of DA have been published in English literature sporadically [1, 5, 10, 12, 20, 24, 25, 33, 39] and collectively [8, 13, 18, 22, 37] since the first detailed description by Eversole et al. [3]. However, the mechanism of desmoplasia in DA has not been elucidated. Before discussing the mechanism of desmoplasia, we should mind that a fibroplastic reaction can be provoked by an inflammatory reaction in ameloblastomas. Raubenheimer et al. [24] proposed that the diagnosis of a DA should be based on criteria such as the absence of inflammatory changes and the uniform presence of mature, diffuse collagenous stromal tissue compressing the neoplastic epithelial component into strands. The cases of DA examined in the present study fulfilled these criteria.

In comparison with the ordinary ameloblastoma [7, 15, 16, 17, 26, 30, 32, 35], there have been only a few studies on the immunoprofile of various proteins in DA. Siar and Ng [28] described differences in the expression of some antigens such as keratin and vimentin between DA and ordinary ameloblastomas. They supposed that the differences in the expression of the antigens among ameloblastomas of various histological types may be attributed to diverse factors such as dedifferentiation of tumor cells, inherent cellular potentials, and others. Such factors may also relate to extracellular matrix formation. In a report of three cases of DA, Philipsen et al. [22] described intense staining for collagen type IV adjacent to tumor islands in DA and suggested that the tumor stroma in DA was not a scar tissue but rather a newly produced connective tissue. In the present study, however, we could not confirm the excessive deposition of type-IV collagen in DA relative to ordinary ameloblastomas. Technical differences such as the antigen used and retrieval of antigenicity may explain the discrepancy of the results. Unfortunately, we could not compare them, because there was no detailed procedure of immunohistochemistry in their report.

TGFs-β are a family of, at least, three polypeptides in mammals, and the antibody used in the present study recognizes two main polypeptides, TGF-β1 and TGF-β2. TGF-β is synthesized in various cells, including odontogenic epithelium [6], and secreted as a latent complex that requires enzymatic cleavage of carbohydrate groups or acidification to release the active cytokine. The present study demonstrated immunoexpression of TGF-β in tumor cells of DA, but not in those of ordinary ameloblastomas. This distinct difference in TGF-β expression between DA and ordinary ameloblastoma demonstrated first in the present study suggests that TGF-β produced by tumor cells of DA plays a part in the prominent matrix formation. TGF-β is one of the most potent local factors for modulating extracellular matrix formation. It is known that TGF-β stimulates various cells, including fibroblasts, to synthesize extracellular matrix such as collagen, fibronectin, proteoglycans, and others. TGF-β also inhibits degradation of the extracellular matrix both through an inhibitory action that produces matrix metaloproteinase and a stimulatory action that activates inhibitors of the enzymes. In the present study, TGF-β was mainly observed in nuclei of the tumor cells. Similar nuclear expression of TGF-β has been reported in pre-cancerous lesions of the uterine cervix [31] and proliferating chondrocyte [34]. Nuclear translocation has been well known for some cytokines, such as fibroblast growth factor [27, 40] and platelet-derived growth factor [36]. Rakowicz Szulczynska et al. [23] reported nuclear accumulation of TGF-β in a cervical cancer cell line.

In some of previously reported DA cases, active osteoplasia was described in tumors [20, 22, 33]. In vivo studies showed that TGF-β enhanced bone formation, when it was applied to bone site [19, 21], although it is reported that TGF-β shows diverse effects on osteoblastic cell proliferation and differentiation depending on its concentration [2, 9] or the maturation stage of osteoblasts [11, 38]. TGF-β produced by tumor cells may also contribute to some DAs with prominent osteoplasia.

References

1. Ashman SG, Corio RL, Eisele DW, Murphy MT (1993) Desmoplastic ameloblastoma. A case report and literature review. Oral Surg Oral Med Oral Pathol 75:479–482
2. Centrella M, McCarthy TL, Canalis E (1987) Transforming growth factor beta is a bifunctional regulator of replication and collagen synthesis in osteoblast-enriched cell cultures from fetal rat bone. J Biol Chem 262:2869–2874
3. Eversole LR, Leider AS, Hansen LS (1984) Ameloblastomas with pronounced desmoplasia. J Oral Maxillofac Surg 42:735–740
4. Frazier KS, Grotendorst GR (1997) Expression of connective tissue growth factor mRNA in the fibrous stroma of mammary tumors. Int J Biochem Cell Biol 29:153–161

5. Fukushima D, Kobayashi H, Takeda I, Yamane G, Tanaka Y (1997) A case of desmoplastic ameloblastoma of the maxilla. Bull Tokyo Dent Coll 38:223–227
6. Gao J, Symons AL, Bartold PM (1998) Expression of transforming growth factor- beta 1 (TGF-beta 1) in the developing periodontium of rats. J Dent Res 77:1708–1716
7. Gao YH, Yang LJ, Yamaguchi A (1997) Immunohistochemical demonstration of bone morphogenetic protein in odontogenic tumors. J Oral Pathol Med 26:273–277
8. Higuchi Y, Nakamura N, Ohishi M, Tashiro H (1991) Unusual ameloblastoma with extensive stromal desmoplasia. J Craniomaxillofac Surg 19:323–327
9. Ibbotson KJ, Orcutt CM, Anglin AM, D'Souza SM (1989) Effects of transforming growth factors beta 1 and beta 2 on a mouse clonal, osteoblast-like cell line MC3T3-E1. J Bone Miner Res 4:37–45
10. Kaffe I, Buchner A, Taicher S (1993) Radiologic features of desmoplastic variant of ameloblastoma. Oral Surg Oral Med Oral Pathol 76:525–529
11. Katagiri T, Lee T, Takeshima H, Suda T, Tanaka H, Omura S (1990) Transforming growth factor-beta modulates proliferation and differentiation of mouse clonal osteoblastic MC3T3-E1 cells depending on their maturation stages. Bone Miner 11:285–293
12. Kawai T, Kishino M, Hiranuma H, Sasai T, Ishida T (1999) A unique case of desmoplastic ameloblastoma of the mandible. Report of a case and brief review of the English language literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 87:258–263
13. Keszler A, Paparella ML, Dominguez FV (1996) Desmoplastic and non-desmoplastic ameloblastoma: a comparative clinicopathological analysis. Oral Dis 2:228–231
14. Mahara K, Kato J, Terui T, Takimoto R, Horimoto M, Murakami T, Mogi Y, Watanabe N, Kohgo Y, Niitsu Y (1994) Transforming growth factor beta 1 secreted from scirrhous gastric cancer cells is associated with excess collagen deposition in the tissue. Br J Cancer 69:777–783
15. Mitsuyasu T, Harada H, Higuchi Y, Kimura K, Nakamura N, Katsuki T, Kubota E, Toyoshima K, Ohishi M (1997) Immunohistochemical demonstration of bcl-2 protein in ameloblastoma. J Oral Pathol Med 26:345–348
16. Mori M, Yamada K, Kasai T, Yamada T, Shimokawa H, Sasaki S (1991) Immunohistochemical expression of amelogenins in odontogenic epithelial tumours and cysts. Virchows Arch 418:319–325
17. Myoken Y, Myoken Y, Okamoto T, Sato JD, Takada K (1995) Immunohistochemical localization of fibroblast growth factor-1 (FGF-1) and FGF-2 in cultured human ameloblastoma epithelial cells and ameloblastoma tissues. J Oral Pathol Med 24:387–392
18. Ng KH, Siar CH (1993) Desmoplastic variant of ameloblastoma in Malaysians. Br J Oral Maxillofac Surg 31:299–303
19. Noda M, Camilliere JJ (1989) In vivo stimulation of bone formation by transforming growth factor-beta. Endocrinology 124:2991–2994
20. Okada Y, Sugimura M, Ishida T (1986) Ameloblastoma accompanied by prominent bone formation. J Oral Maxillofac Surg 44:555–557
21. Oue Y, Kanatani H, Kiyoki M, Eto Y, Ogata E, Matsumoto T (1994) Effect of local injection of activin A on bone formation in newborn rats. Bone 15:361–366
22. Philipsen HP, Ormiston IW, Reichart PA (1992) The desmo- and osteoplastic ameloblastoma. Histologic variant or clinicopathologic entity? Case reports. Int J Oral Maxillofac Surg 21:352–357
23. Rakowicz Szulczynska EM, McIntosh DG, Smith M (1994) Growth factor- mediated mechanisms of nicotine-dependent carcinogenesis. Carcinogenesis 15:1839–1846
24. Raubenheimer EJ, Heerden WF van, Noffke CE (1995) Infrequent clinicopathological findings in 108 ameloblastomas. J Oral Pathol Med 24:227–232
25. Sakashita H, Miyata M, Okabe K, Kurumaya H (1998) Desmoplastic ameloblastoma in the maxilla: a case report. J Oral Maxillofac Surg 56:783–786
26. Saku T, Okabe H, Shimokawa H (1992) Immunohistochemical demonstration of enamel proteins in odontogenic tumors. J Oral Pathol Med 21:113–119
27. Shiurba RA, Jing N, Sakakura T, Godsave SF (1991) Nuclear translocation of fibroblast growth factor during Xenopus mesoderm induction. Development 113:487–493
28. Siar CH, Ng KH (1993) Patterns of expression of intermediate filaments and S-100 protein in desmoplastic ameloblastoma. J Nihon Univ Sch Dent 35:104–108
29. Tahara E (1990) Growth factors and oncogenes in human gastrointestinal carcinomas. J Cancer Res Clin Oncol 116:121–131
30. Takahashi H, Tsuda N, Yamabe S, Fujita S, Okabe H (1995) Immunohistochemical detection of alpha 1-antitrypsin, alpha 1-antichymotrypsin, transferrin and ferritin in ameloblastoma. Anal Cell Pathol 9:135–150
31. Tervahauta A, Syrjanen S, Yliskoski M, Gold LI, Syrjanen K (1994) Expression of transforming growth factor-beta 1 and -beta 2 in human papillomavirus (HPV)-associated lesions of the uterine cervix. Gynecol Oncol 54:349–356
32. Thesleff I, Ekblom P (1984) Distribution of keratin and laminin in ameloblastoma. Comparison with developing tooth and epidermoid carcinoma. J Oral Pathol 13:85–96
33. Thompson IO, Rensburg LJ van, Phillips VM (1996) Desmoplastic ameloblastoma: correlative histopathology, radiology and CT-MR imaging. J Oral Pathol Med 25:405–410
34. Thorp BH, Anderson I, Jakowlew SB (1992) Transforming growth factor-beta 1, -beta 2 and -beta 3 in cartilage and bone cells during endochondral ossification in the chick. Development 114:907–911
35. Ueno S, Miyagawa T, Kaji R, Mushimoto K, Shirasu R (1994) Immunohistochemical investigation of epidermal growth factor receptor expression in ameloblastomas. J Pathol 173:33–38
36. Van den Eijnden van Raaij AJ, van Maurik P, Boonstra J, van Zoelen EJ, de Laat SW (1988) Ultrastructural localization of platelet-derived growth factor and related factors in normal and transformed cells. Exp Cell Res 178:479–492
37. Waldron CA, el Mofty SK (1987) A histopathologic study of 116 ameloblastomas with special reference to the desmoplastic variant. Oral Surg Oral Med Oral Pathol 63:441–451
38. Wrana JL, Maeno M, Hawrylyshyn B, Yao KL, Domenicucci C, Sodek J (1988) Differential effects of transforming growth factor-beta on the synthesis of extracellular matrix proteins by normal fetal rat calvarial bone cell populations. J Cell Biol 106:915–924
39. Yoshimura Y, Saito H (1990) Desmoplastic variant of ameloblastoma: report of a case and review of the literature. J Oral Maxillofac Surg 48:1231–1235
40. Zhan X, Hu X, Friedman S, Maciag T (1992) Analysis of endogenous and exogenous nuclear translocation of fibroblast growth factor-1 in NIH 3T3 cells. Biochem Biophys Res Commun 188:982–991