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Immunohistochemical detection of insulin-like growth factors, platelet-derived growth factor, and their receptors in ameloblastic tumors

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BACKGROUND: To evaluate the roles of growth factors in oncogenesis and cytodifferentiation of odontogenic tumors, expression of insulin-like growth factors (IGFs), platelet-derived growth factor (PDGF), and their receptors was analyzed in ameloblastic tumors as well as in tooth germs.

METHODS: Tissue specimens of 10 tooth germs, 47 ameloblastomas, and five malignant ameloblastic tumors were examined immunohistochemically with the use of antibodies against IGF-I, IGF-II, IGF-I receptor (IGF-IR), PDGF A-chain, PDGF B-chain, PDGF α-receptor, and PDGF β-receptor.

RESULTS: Immunohistochemical reactivity for IGFs, PDGF chains, and their receptors was detected predominantly in odontogenic epithelial cells near the basement membrane in tooth germs and in benign and malignant ameloblastic tumors. The expression levels of IGF-II and PDGF chains were significantly higher in ameloblastic tumors than in tooth germs. Malignant ameloblastic tumors showed higher reactivity for PDGF chains than benign ameloblastomas and higher reactivity for platelet-derived growth factor receptors than tooth germs. The expression levels of PDGF chains were significantly higher in follicular ameloblastomas than in plexiform ameloblastomas. Desmoplastic ameloblastomas showed higher expression of IGFs and IGF-IR when compared with other ameloblastoma subtypes.

CONCLUSION: Expression of IGFs, PDGF, and their receptors in tooth germs and ameloblastic tumors suggests that these growth factor signals contribute to cell proliferation or survival in both normal and neoplastic odontogenic tissues. Expression of these molecules in odontogenic tissues possibly affects interactions with the bone microenvironment during tooth development and intraosseous progression of ameloblastic tumors. Altered expression of the ligands and receptors in ameloblastic tumors may be involved in oncogenesis, malignant potential, and tumor cell differentiation.

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Introduction

Tumors arising from epithelium of the odontogenic apparatus or from its derivatives or remnants exhibit considerable histological variation and are classified into several benign and malignant entities (1, 2). Ameloblastoma is the most frequently encountered tumor arising from odontogenic epithelium and is characterized by a benign but locally invasive behavior with a high risk of recurrence (1, 2). Histologically, ameloblastoma shows considerable variation, including follicular, plexiform, acanthomatous, granular cell, basal cell, and desmoplastic variants (2). Malignant counterparts of ameloblastoma are classified into metastasizing ameloblastoma and ameloblastic carcinoma on the basis of metastatic spread and cytological malignant features (2). Recent studies have identified genetic and molecular alterations in these epithelial odontogenic tumors (3, 4); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

Growth factors are hormone-like polypeptides that transmit signals by binding specific high-affinity cell surface receptors and involving subsequent signaling molecules (5–7). They usually act in a paracrine or autocrine manner without endocrine secretion (5). Dysfunction of growth factors, growth factor receptors, or signaling components results in pathologic conditions, including neoplasia (5, 7, 8). Insulin-like growth factor (IGF) ligands (IGF-I and -II) were isolated as serum factors with insulin-like activity not suppressed by anti-insulin antibody (9). IGFs are produced in the

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largest amounts by the liver and secreted into the circulation where they mediate the effects of growth hormone. In addition to the endocrine axis, IGFs are produced by most extrahepatic organs, where they are involved in many autocrine/paracrine types of actions. The biological actions of IGFs are predominantly mediated by IGF-I receptor (IGF-IR), a transmembrane receptor with tyrosine kinase activity, and regulate cell growth and metabolism (6, 8). Platelet-derived growth factor (PDGF) was identified in human platelets as a growth promoting factor for fibroblasts, smooth muscle cells, and glial cells (10). PDGF family proteins are homo- and hetero-dimeric molecules of related A- and B-polypeptide chains (PDGF-A and -B), and the B-chain is structurally and functionally very similar to the oncogene product of simian sarcoma virus, v-sis (7, 11). PDGF isoforms bind and activate two different receptors with tyrosine kinase domains, PDGF α- and β-receptors (PDGFR-α and -β), and serve to regulate mitogenic and motogenic signals (7). IGFs and PDGF, as well as transforming growth factor-β (TGF-β) and fibroblast growth factors (FGFs), are stored in bone matrix, and these molecules participate in local regulation of bone metabolism in various bone lesions, including bone metastases of malignancies (12, 13).

Previous studies have confirmed the expression of various growth factors and their receptors, including epidermal growth factor, TGF-α and -β, FGFs, and hepatocyte growth factor, in odontogenic tumors, suggesting that these growth factors contribute to cell proliferation and differentiation of odontogenic tissues (14–18). In the present study, immunohistochemical expression of IGFs, PDGF, and their receptors was examined in benign and malignant ameloblastic tumors as well as in tooth germs to evaluate the roles of these growth factors in oncogenesis and cytodifferentiation of epithelial odontogenic tumors.

Materials and methods

Tissue preparation

Specimens were surgically removed from 52 patients with epithelial odontogenic tumors at the Department of Oral and Maxillofacial Surgery, Tohoku University Dental Hospital, and affiliated hospitals. The specimens were fixed in 10% buffered formalin for one to several days and were embedded in paraffin. The tissue blocks were sliced into 3-μm-thick sections for routine histological and subsequent immunohistochemical examinations. Tissue sections were stained with hematoxylin and eosin for histological diagnosis according to the WHO histological classification of odontogenic tumors (2). The tumors comprised 47 ameloblastomas and five malignant ameloblastic tumors. Ameloblastomas were divided into 24 follicular and 23 plexiform types, including 10 acanthomatous, six granular cell, three basal cell, and four desmoplastic subtypes. Malignant ameloblastic tumors were classified into two metastasizing ameloblastomas and three ameloblastic carcinomas. Specimens of 10 tooth germs of the mandibular third molars, enucleated for orthodontic reasons at the initial stage of crown mineralization, were similarly prepared and compared with the epithelial odontogenic tumors.

Immunohistochemistry

The serial sections were deparaffinized, immersed in methanol with 0.3% hydrogen peroxide, and heated in 0.01 M citrate buffer (pH 6.0) for 10 min by autoclave (121°C, 2 atm). Then, the sections were incubated with primary antibodies at 4°C overnight. The applied antibodies were rabbit anti-IGF-I polyclonal antibody (Lab Vision Corporation, Fremont, CA, USA; diluted at 1:25), goat anti-IGF-II polyclonal antibody (R&D systems, Minneapolis, MN, USA; diluted at 1:25), rabbit anti-IGF-IR polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; diluted at 1:50), rabbit anti-PDGF-A polyclonal antibody (Santa Cruz Biotechnology; diluted at 1:50), rabbit anti-PDGF-B polyclonal antibody (Santa Cruz Biotechnology; diluted at 1:50), rabbit anti-PDGFR-α polyclonal antibody (Lab Vision Corporation; diluted at 1:50), and rabbit anti-PDGFR-β polyclonal antibody (Lab Vision Corporation; diluted at 1:20). The sections were allowed to react with peroxidase-conjugated anti-rabbit IgG (for IGF-I, IGF-IR, PDGF chains, and PDGFRs) or anti-goat IgG (for IGF-II) polyclonal antibody (Histofine Simple Stain MAX-PO; Nichirei, Tokyo, Japan) for 45 min, and reaction products were visualized by immersing the sections in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide for 2–5 min. Nuclei were lightly stained with Mayer's hematoxylin. For control studies of the antibodies, the serial sections were treated with normal rabbit and goat IgG instead of the primary antibodies and were confirmed to be unstained.

Immunohistochemical reactivity for IGFs, PDGF chains, and their receptors was evaluated and classified into four groups: (−) negative in epithelial cells, (+) positive in epithelial cells near the basement membrane, (++) positive in epithelial cells near the basement membrane and some central epithelial cells, and (+++) positive in most epithelial cells. The statistical significance of differences in the percentage of cases with different reactivity levels was analyzed by the Mann–Whitney *U*-test for differences between two groups or the Kruskal–Wallis test for differences among three or more groups. *P*-values < 0.05 were considered to indicate statistical significance.

Results

IGFs and IGF-IR in tooth germs and ameloblastic tumors

Expression of IGF-I and -II was detected in the cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Table 1) (Fig. 1). Immunohistochemical distribution of these molecules was similar in tooth germs and ameloblastic tumors, and reactivity for IGF-II was slightly weaker than that for IGF-I in these odontogenic tissues, especially in tooth germ tissues. In tooth germs, IGF-I and -II were expressed strongly in inner and outer enamel epithelium and weakly in other epithelial components (Fig. 1a). Some

Table 1 Immunohistochemical reactivity for IGF-I, IGF-II, and IGF-IR in tooth germs and ameloblastic tumors

	n	*IGF-I*				*IGF-II*				*IGF-IR*			
		–	+	+ +	+ + +	–	+	+ +	+ + +	–	+	+ +	+ + +
Tooth germ	10	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	8 (80)	2 (20)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)
Ameloblastoma	47	2 (4)	3 (6)	34 (73)	8 (17)	2 (4)	8 (17)	31 (66)	6 (13)	0 (0)	4 (9)	35 (74)	8 (17)
Follicular type	24	2 (8)	0 (0)	18 (75)	4 (17)	2 (8)	3 (13)	15 (62)	4 (17)	0 (0)	2 (8)	17 (71)	5 (21)
Plexiform type	23	0 (0)	3 (13)	16 (70)	4 (17)	0 (0)	5 (22)	16 (69)	2 (9)	0 (0)	2 (9)	18 (78)	3 (13)
Acanthomatous subtype	10	2 (20)	0 (0)	8 (80)	0 (0)	2 (20)	2 (20)	6 (60)	0 (0)	0 (0)	2 (20)	7 (70)	1 (10)
Granular subtype	6	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	0 (0)	6 (100)	0 (0)
Basal cell subtype	3	0 (0)	0 (0)	1 (33)	2 (67)	0 (0)	1 (33)	0 (0)	2 (67)	0 (0)	0 (0)	1 (33)	2 (67)
Desmoplastic subtype	4	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)	4 (100)
Non-cellular variation	24	0 (0)	3 (13)	19 (79)	2 (8)	0 (0)	5 (21)	19 (79)	0 (0)	0 (0)	2 (8)	21 (88)	1 (4)
Malignant ameloblastic tumor	5	0 (0)	0 (0)	2 (40)	3 (60)	0 (0)	1 (20)	2 (40)	2 (40)	0 (0)	0 (0)	2 (40)	3 (60)
Metastasizing ameloblastoma	2	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)
Ameloblastic carcinoma	3	0 (0)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	1 (33)	2 (67)	0 (0)	0 (0)	0 (0)	3 (100)

Values in parentheses denote percentage values.
–, negative in epithelial cells; +, positive in epithelial cells near the basement membrane; + +, positive in epithelial cells near the basement membrane and some central epithelial cells; and + + +, positive in most epithelial cells.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Figure 1 Immunohistochemical reactivity for insulin-like growth factors (IGFs). (a) Tooth germ showing strong IGF-I reactivity in inner and outer enamel epithelium and weak reactivity in other epithelial components (×70). (b) Plexiform ameloblastoma showing IGF-I reactivity in many peripheral columnar cells and some central polyhedral cells (×110). (c) Ameloblastic carcinoma showing IGF-II reactivity in most neoplastic cells (×110).

fibroblasts and endothelial cells in dental papillae and dental follicles were also weakly reactive with IGFs. Ameloblastomas showed IGF-I and -II reactivity in many peripheral columnar or cuboidal cells and some

central polyhedral cells, except for two cases (Fig. 1b). The level of immunohistochemical reactivity for IGF-II was significantly higher in ameloblastomas than in tooth germs ($P < 0.01$, Table 1). Keratinizing cells in acanthomatous ameloblastomas exhibited no expression of IGFs, while granular cells in granular cell ameloblastomas were reactive with IGFs. Basal cell and desmoplastic ameloblastomas showed IGF-I and -II expression in most neoplastic cells. The levels of immunohistochemical reactivity for IGFs were significantly higher in desmoplastic ameloblastomas than in acanthomatous ameloblastomas ($P < 0.001$), granular cell ameloblastomas ($P < 0.05$), and ameloblastomas without cellular variation ($P < 0.01$, Table 1). Metastasizing ameloblastomas showed IGF-I and -II reactivity in many peripheral columnar or cuboidal cells and some central polyhedral cells, whereas ameloblastic carcinomas showed reactivity for IGFs in most neoplastic cells (Fig. 1c). The level of immunohistochemical reactivity for IGF-II was significantly higher in malignant ameloblastic tumors than in tooth germs ($P < 0.05$, Table 1). In these ameloblastic tumors, some fibroblasts and endothelial cells in the stroma were also weakly reactive with IGFs.

Immunoreactivity for IGF-IR was detected on the cell membrane of cellular components of normal and neoplastic odontogenic tissues (Fig. 2). In tooth germs, IGF-IR expression was strong in inner enamel epithelium and weak in other epithelial components. Fibroblasts and endothelial cells in dental papillae and dental follicles were also weakly reactive with IGF-IR. Ameloblastomas and metastasizing ameloblastomas showed strong reactivity for IGF-IR in peripheral columnar or cuboidal cells and weak reactivity in central polyhedral cells (Fig. 2a). Keratinizing cells in acanthomatous ameloblastomas exhibited no or little IGF-IR expression, while granular cells in granular cell ameloblastomas were reactive with IGF-IR (Fig. 2b). Basal cell and desmoplastic ameloblastomas and ameloblastic carcinomas showed reactivity for IGF-IR in most neoplastic cells (Fig. 2c). The level of immunohistochemical reactivity for IGF-IR was significantly higher in desmoplastic ameloblastomas than in acanthomatous ameloblastomas ($P < 0.01$), granular cell ameloblastomas ($P < 0.05$), and ameloblastomas without cellular variation ($P < 0.001$, Table 1). In these ameloblastic tumors, stromal fibroblasts and endothelial cells were also weakly reactive with IGF-IR.

PDGF chains and PDGFRs in tooth germs and ameloblastic tumors

Expression of PDGF-A and -B was detected in the cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Table 2) (Fig. 3). Immunohistochemical reactivity for these molecules was similar in tooth germs and ameloblastic tumors. PDGF chains were not expressed in epithelial components in eight of 10 tooth germs (Fig. 3a), and two tooth germs showed weak reactivity for PDGF chains in inner enamel epithelium. Some endothelial cells in dental papillae and dental follicles were weakly reactive with PDGF chains. Ameloblastomas showed expression of PDGF-A and -B in some peripheral columnar or cuboidal cells in 33 and 32 of 47 cases, respectively (Fig. 3b). The levels

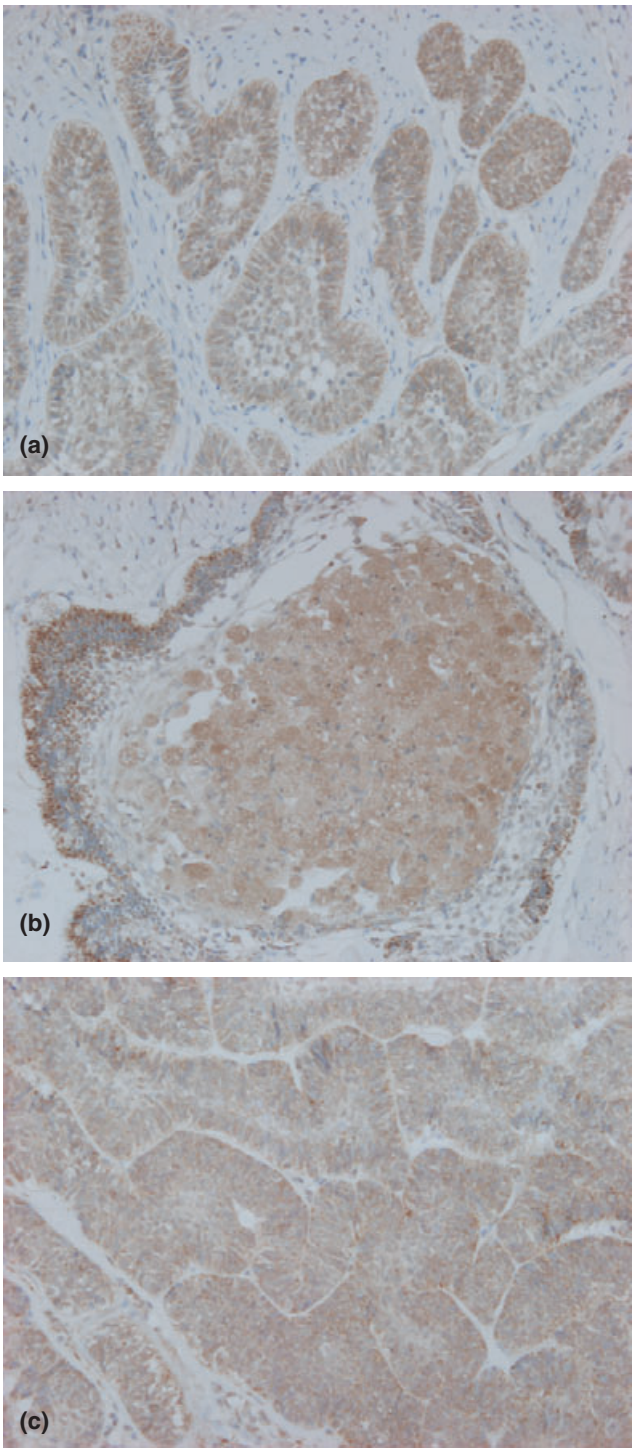

Figure 2 Immunohistochemical reactivity for insulin-like growth factor-I receptor. (a) Follicular ameloblastoma showing strong reactivity in peripheral columnar cells and weak reactivity in central polyhedral cells (×120). (b) Granular cell ameloblastoma showing reactivity in granular neoplastic cells as well as in peripheral neoplastic cells (×100). (c) Metastasizing ameloblastoma showing strong reactivity in peripheral columnar cells and weak reactivity in central polyhedral cells (×120).

Table 2 Immunohistochemical reactivity for PDGF-A, PDGF-B, PDGFR-α, and PDGFR-β in tooth germs and ameloblastic tumors

	n	*PDGF-A*				*PDGF-B*				*PDGF-α*				*PDGF-β*			
		–	+	++	+++	–	+	++	+++	–	+	++	+++	–	+	++	+++
Tooth germ	10	8 (80)	2 (20)	0 (0)	0 (0)	8 (80)	2 (20)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)
Ameloblastoma	47	14 (30)	33 (70)	0 (0)	0 (0)	15 (32)	32 (68)	0 (0)	0 (0)	11 (24)	32 (68)	2 (4)	2 (4)	13 (28)	32 (68)	0 (0)	2 (4)
Follicular type	24	4 (17)	20 (83)	0 (0)	0 (0)	4 (17)	20 (83)	0 (0)	0 (0)	3 (13)	19 (79)	2 (8)	0 (0)	4 (17)	20 (83)	0 (0)	0 (0)
Plexiform type	23	10 (43)	13 (57)	0 (0)	0 (0)	11 (48)	12 (52)	0 (0)	0 (0)	8 (35)	13 (56)	0 (0)	2 (9)	9 (39)	12 (52)	0 (0)	2 (9)
Acanthomatous subtype	10	4 (40)	6 (60)	0 (0)	0 (0)	4 (40)	6 (60)	0 (0)	0 (0)	3 (30)	6 (60)	1 (10)	0 (0)	3 (30)	7 (70)	0 (0)	0 (0)
Granular subtype	6	1 (17)	5 (83)	0 (0)	0 (0)	1 (17)	5 (83)	0 (0)	0 (0)	1 (17)	5 (83)	0 (0)	0 (0)	2 (33)	4 (67)	0 (0)	0 (0)
Basal cell subtype	3	1 (33)	2 (67)	0 (0)	0 (0)	1 (33)	2 (67)	0 (0)	0 (0)	0 (0)	1 (33)	0 (0)	2 (67)	0 (0)	1 (33)	0 (0)	2 (67)
Desmoplastic subtype	4	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)
Non-cellular variation	24	8 (33)	16 (67)	0 (0)	0 (0)	9 (38)	15 (62)	0 (0)	0 (0)	7 (29)	16 (67)	1 (4)	0 (0)	8 (33)	16 (67)	0 (0)	0 (0)
Malignant ameloblastic tumor	5	0 (0)	2 (40)	1 (20)	2 (40)	0 (0)	0 (0)	2 (40)	3 (60)	0 (0)	2 (40)	1 (20)	2 (40)	0 (0)	2 (40)	1 (20)	2 (40)
Metastasizing ameloblastoma	2	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
Ameloblastic carcinoma	3	0 (0)	0 (0)	1 (33)	2 (67)	0 (0)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	1 (33)	2 (67)	0 (0)	0 (0)	1 (33)	2 (67)

Values in parentheses denote percentage values.
–, negative in epithelial cells; +, positive in epithelial cells near the basement membrane; ++, positive in epithelial cells near the basement membrane and some central epithelial cells; and +++, positive in most epithelial cells.
$^{*}P < 0.05$, $^{***}P < 0.001$.

Figure 3 Immunohistochemical reactivity for platelet-derived growth factor (PDGF). (a) Tooth germ showing no reactivity for PDGF-A in epithelial components (×75). (b) Follicular ameloblastoma showing PDGF-B reactivity in peripheral columnar cells (×115). (c) Ameloblastic carcinoma showing PDGF-A reactivity in most neoplastic cells (×110).

of immunohistochemical reactivity for PDGF chains were significantly higher in ameloblastomas than in tooth germs ($P < 0.05$), and follicular ameloblastomas showed significantly higher expression of PDGF chains than plexiform ameloblastomas ($P < 0.05$, Table 2).

Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas exhibited no expression of PDGF chains. Basal cell and desmoplastic ameloblastomas showed PDGF-A and -B reactivity in some neoplastic cells. Metastasizing ameloblastomas were reactive with PDGF chains in some peripheral columnar or cuboidal cells, whereas ameloblastic carcinomas were reactive with PDGF chains in most neoplastic cells (Fig. 3c). The levels of immunohistochemical reactivity for PDGF chains were significantly higher in malignant ameloblastic tumors than in tooth germs ($P < 0.001$) and ameloblastomas ($P < 0.05$, Table 2). In these ameloblastic tumors, some endothelial cells were weakly reactive with PDGF chains.

Immunoreactivity for PDGFR-α and -β was detected on the cell membrane of cellular components in normal and neoplastic odontogenic tissues (Fig. 4). Immunohistochemically, these molecules were similarly distributed in tooth germs and ameloblastic tumors, and reactivity for PDGF-β was slightly weaker than that for PDGF-α in these odontogenic tissues. In tooth germs, PDGFRs were expressed weakly in inner enamel epithelium, as well as in endothelial cells in dental papillae and dental follicles. Ameloblastomas showed expression of PDGFR-α and -β in peripheral columnar or cuboidal cells in 36 and 34 of 47 cases, respectively (Fig. 4a). Some central polyhedral cells in a few ameloblastomas were reactive with PDGFRs. Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas exhibited no expression of PDGFRs. Basal cell ameloblastomas showed PDGFR-α and -β expression in most neoplastic cells (Fig. 4b), while reactivity for PDGFRs in desmoplastic ameloblastomas was found in neoplastic cells neighboring the basement membrane. Metastasizing ameloblastomas showed PDGFR-α and -β reactivity in peripheral columnar or cuboidal cells, whereas ameloblastic carcinomas showed reactivity for PDGFRs in most neoplastic cells (Fig. 4c). The levels of immunohistochemical reactivity for PDGFRs were significantly higher in malignant ameloblastic tumors than in tooth germs ($P < 0.05$, Table 2). In these ameloblastic tumors, stromal endothelial cells were weakly reactive with PDGFRs.

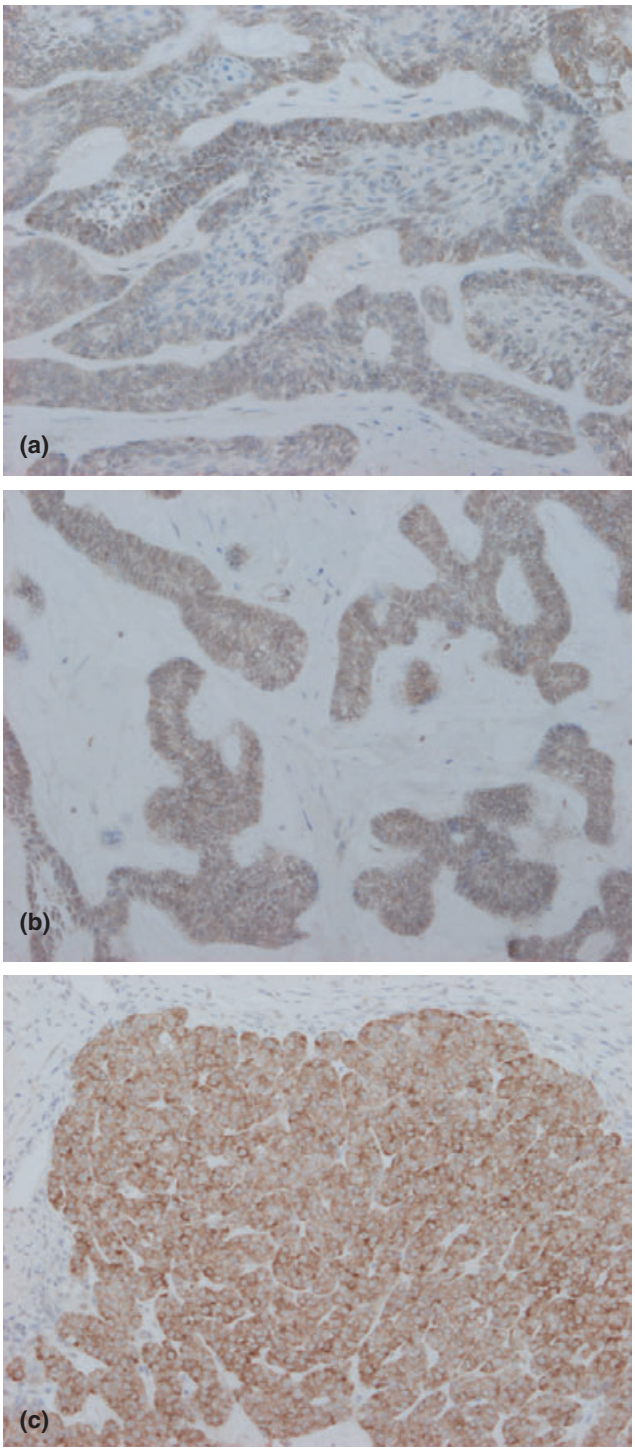

Figure 4 Immunohistochemical reactivity for platelet-derived growth factor receptors (PDGFRs). (a) Follicular ameloblastoma showing PDGF-α reactivity in peripheral columnar cells (×105). (b) Basal cell ameloblastoma showing PDGF-β reactivity in most neoplastic cells (×110). (c) Ameloblastic carcinoma showing PDGF-α reactivity in most neoplastic cells (×115).

Discussion

Expression of IGFs is widely distributed in most organs and tissues of the human fetus (19). Mice lacking *IGF-I*, *IGF-II*, or *IGF-IR* genes show severe growth retardation and die at or shortly after birth (20, 21). In humans, IGF-I deficiency causes Laron syndrome, characterized by dwarfism with obesity and osteopenia/osteoporosis (22). Transactivation of *IGF-II* in mice results in most of the symptoms of Beckwith–Widemann syndrome, a fetal overgrowth syndrome with skeletal abnormalities, and is associated with fetal and neonatal lethality (23). PDGF and PDGFRs are expressed in various tissues during development (11). Mice deficient for *PDGF-B* or *PDGFR*-β die during embryogenesis or perinatally in association with defective development of the kidney and blood vessels (24, 25). Knockout of *PDGF-A* causes defects in lung development and leads to death at about 3 weeks of age (26). *PDGFR*-α knockout mice show cranial malformations and deficient myotome formation

(27). Thus, these growth factors and their receptors are essential for major proliferative and differentiation pathways in the developmental processes of mammals. Expression of IGFs, PDGF, and their receptors has been detected temporally and spatially in rodent tooth germs, suggesting that these molecules play a role in tooth development (28–31). In the present study, immunoreactivity for IGFs, PDGF, and their receptors was found in epithelial and mesenchymal components of human tooth germs at the initial stage of crown mineralization, suggesting that these molecules might regulate cell proliferation and differentiation during tooth development via autocrine and paracrine mechanisms.

Expression of IGFs and IGF-IR has been detected in various human malignancies, such as lung, breast, prostate, renal, and colorectal carcinomas, suggesting that these molecules induce cell proliferation and inhibition of apoptosis in an autocrine or paracrine manner (8, 32, 33). Most primary tumors and established tumor cell lines express high levels of IGF-II and IGF-IR, while some tumors overexpress IGF-I (8). Loss of imprinting of *IGF-II* gene has been reported to play an important role in oncogenesis in some tumors, including Wilms' tumor, rhabdomyosarcoma, and lung and colorectal carcinomas (8, 34). In the present study, immunoreactivity for IGFs and IGF-IR was found in ameloblastic tumors, suggesting that IGF signals contribute to cell proliferation or survival in these epithelial odontogenic tumors. The expression levels of IGF-II in ameloblastomas and malignant ameloblastic tumors were significantly higher than that in tooth germs, suggesting that an autocrine effect of IGF-II might be involved in oncogenesis of odontogenic epithelium.

Insulin-like growth factors, the most abundant growth factors in bone, are released from bone in response to bone resorption, and released IGFs have important roles in stimulating cell proliferation and upregulating cellular metabolism in metastatic cancer cells invading bone, such as breast and prostate cancer cells (13, 35). In the present study, IGF-IR was expressed in neoplastic cells of all ameloblastic tumors, suggesting that ameloblastic tumors with osteolytic progression are influenced by IGFs released from resorbed bone. The biological activities of IGFs include stimulation of collagen production and downregulation of collagenase synthesis in fibroblasts (9). Previous studies have shown that desmoplastic ameloblastoma expresses high levels of TGF-β and its receptors (16, 18). In the present study, immunoreactivity for IGFs and IGF-IR was high in desmoplastic ameloblastomas when compared with other ameloblastoma subtypes. These features suggest that IGFs, as well as TGF-β, might participate in stromal desmoplasia in the ameloblastoma variant.

Gene amplification and/or overexpression of PDGF and its receptors have been implicated in autocrine as well as paracrine mechanisms in various types of human malignancies, including glioblastoma, osteosarcoma, and thyroid, breast, and colorectal carcinomas (7, 36, 37). Chronic myelomonocytic leukemia is associated with chromosomal translocation of *PDGFR*-β gene (38), and gastrointestinal stromal tumor shows activating mutations of *PDGFR*-α gene as well as *c-kit* gene (39). In the present study, PDGF-A, -B, PDGFR-α, and -β were expressed in neoplastic cells of many ameloblastic tumors. The expression levels of PDGF chains in ameloblastic tumors were significantly higher than those in tooth germs, and malignant ameloblastic tumors showed higher expression of PDGF chains than ameloblastomas. PDGFR-α and -β reactivity in malignant ameloblastic tumors was significantly higher than that in tooth germs. These features suggest that PDGF and its receptor system might participate in tumorigenesis and malignant transformation of odontogenic epithelium. In addition, the expression levels of PDGF-A and -B in follicular ameloblastomas were significantly higher than those in plexiform ameloblastomas, suggesting that PDGF signaling might be involved in tissue structuring in ameloblastomas.

Platelet-derived growth factor-BB produced by breast cancer cells has been reported to have a causative role in the development of osteosclerotic bone metastasis of malignancies (40). Activation of PDGFRs has been correlated with the bone-metastatic potential of prostate and breast carcinomas (41, 42). Expression of PDGF chains and PDGFRs in ameloblastic tumors, as shown in the present study, might affect interactions with the bone microenvironment during intraosseous progression of these ameloblastic tumors. PDGF has an important role in the later stages of blood vessel formation by stimulating the development of pericytes and vascular smooth muscle cells (7), and PDGF produced by neoplastic cells facilitates tumor angiogenesis (43, 44). In our previous study, microvessel density in ameloblastic tumors was significantly higher than that in tooth germs, and follicular ameloblastomas possessed many small blood vessels when compared with plexiform ameloblastomas (45). In the present study, these characteristics of blood vessel distribution were consistent with the immunoreactivity for PDGF chains. These features suggest that PDGF might also function as an angiogenic factor in these epithelial odontogenic tumors.

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