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Immunohistochemical detection of platelet-derived endothelial cell growth factor/thymidine phosphorylase and angiopoietins in ameloblastic tumors

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BACKGROUND: To evaluate the roles of angiogenic factors in the development and progression of odontogenic tumors, expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP) and of angiopoietins in ameloblastic tumors as well as in tooth germs.

METHODS: Tissue specimens of 11 tooth germs, 44 ameloblastomas, and five malignant ameloblastic tumors were examined immunohistochemically with the use of antibodies against PD-ECGF/TP and angiopoietin-1 and -2.

RESULTS: Immunohistochemical reactivity for PD-ECGF/TP was detected in mesenchymal cells in tooth germs and stromal cells in ameloblastic tumors, and the level of immunoreactivity for PD-ECGF/TP was significantly higher in ameloblastomas than in tooth germs. Granular cell ameloblastomas showed PD-ECGF/TP reactivity in granular neoplastic cells as well as in stromal cells. Immunoreactivity for angiopoietin-1 and -2 was detected predominantly in odontogenic epithelial cells near the basement membrane in tooth germs and in benign and malignant ameloblastic tumors. Malignant ameloblastic tumors had decreased angiopoietin-1 reactivity and ameloblastic carcinomas had increased angiopoietin-2 reactivity as compared with the respective levels in tooth germs and ameloblastomas. Immunohistochemical reactivity for angiopoietin-2 was slightly higher in follicular ameloblastomas than in plexiform ameloblastomas.

CONCLUSION: Expression of PD-ECGF/TP and angiopoietin-1 and -2 in tooth germs and ameloblastic tumors suggests that these angiogenic factors participate in tooth development and odontogenic tumor progression by regulating angiogenesis. Altered expression of PD-ECGF/TP and angiopoietins in ameloblastic tumors may be involved in oncogenesis, malignant potential, and tumor cell differentiation.

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Keywords: ameloblastoma; angiogenesis; angiopoietin; platelet-derived endothelial cell growth factor/thymidine phosphorylase

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 (3–5); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

Angiogenesis is an essential part of a variety of physiologic and pathologic processes, including embryogenesis, wound healing, inflammation, and tumor progression, and these processes are controlled by numerous different molecules, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), transforming growth factor-β (TGF-β), tumor necrosis factor α (TNFα), and interleukin-8 (IL-8) (6–8). Platelet-derived endothelial cell growth factor (PD-ECGF) was first purified as a novel angiogenic factor present in platelets

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(9, 10) and was thereafter confirmed to be identical to thymidine phosphorylase (TP), an enzyme involved in pyrimidine nucleoside metabolism (11, 12). PD-ECGF/TP stimulates endothelial chemotaxis *in vitro* and promotes angiogenesis *in vivo* enzymatically (13, 14). Angiopoietin-1 and -2 were discovered as ligands for tyrosine kinase with Ig and EGF homology domains (TIE)-2, a receptor tyrosine kinase specifically expressed on endothelial cells (15, 16). Angiopoietin-1 induces phosphorylation and activation of TIE-2 and promotes interactions between endothelial cells and their surrounding supporting cells, resulting in stabilization and maturation of vessels. Angiopoietin-2 competitively binds to TIE-2 and inhibits the stabilizing action of angiopoietin-1, resulting in destabilization of vessels and subsequent angiogenic changes. Angiopoietin-1 is constitutively expressed in adult tissues and is continuously required for stabilization of vessels. In contrast, angiopoietin-2 is highly expressed under conditions required for vascular remodeling or angiogenesis, such as hypoxia and undernutrition, in adult tissues and facilitates angiogenesis (17, 18). Recent studies have revealed that expression of PD-ECGF/TP and angiopoietin-1 and -2 participates in the development, progression, and prognosis of human tumors by modulating angiogenesis (19–21).

Our previous study has confirmed that VEGF expression and microvessel density (MVD) are higher in ameloblastic tumors than in tooth germs, suggesting that the angiogenic factor might promote oncogenesis or malignant transformation of odontogenic epithelium by mediating angiogenesis (22). In the present study, immunohistochemical expression of PD-ECGF/TP and angiopoietin-1 and -2 was examined in benign and malignant ameloblastic tumors as well as in tooth germs to evaluate the role of angiogenic factors in epithelial odontogenic tumors.

Materials and methods

Tissue preparation

Specimens were surgically removed from 49 patients with epithelial odontogenic tumors at the Department of Oral and Maxillofacial Surgery, Tohoku University

Table 1 Immunohistochemical reactivity for PD-ECGF/TP and angiopoietins in tooth germs and ameloblastic tumors

	PD-ECGF/TP			*Angiopoietin-1*			*Angiopoietin-2*		
	−	+	++	−	+	++	−	+	++
Tooth germ ($n = 12$)									
Enamel organ/dental lamina	12 (100)	0 (0)	0 (0)	0 (0)	7 (58)	5 (42)	0 (0)	6 (50)	6 (50)
Dental papilla/dental follicle	0 (0)	12 (100)	0 (0)	0 (0)	12 (100)	0 (0)	0 (0)	11 (92)	1 (8)
Ameloblastoma ($n = 44$)									
neoplastic cells	38 (86)	6 (14)	0 (0)	0 (0)	21 (48)	23 (52)	0 (0)	20 (45)	24 (55)
Stromal cells	0 (0)	18 (41)	26 (59)	1 (2)	42 (96)	1 (2)	0 (0)	39 (89)	5 (11)
Follicular type ($n = 23$)									
Neoplastic cells	18 (78)	5 (22)	0 (0)	0 (0)	11 (48)	12 (52)	0 (0)	8 (35)	15 (65)
Stromal cells	0 (0)	9 (39)	14 (61)	1 (4)	22 (96)	0 (0)	0 (0)	20 (87)	3 (13)
Plexiform type ($n = 21$)									
Neoplastic cells	20 (95)	1 (5)	0 (0)	0 (0)	10 (48)	11 (52)	0 (0)	12 (57)	9 (43)
Stromal cells	0 (0)	9 (43)	12 (57)	0 (0)	20 (95)	1 (5)	0 (0)	19 (90)	2 (10)
Acanthomatous subtype ($n = 10$)									
Neoplastic cells	10 (100)	0 (0)	0 (0)	0 (0)	6 (60)	4 (40)	0 (0)	5 (50)	5 (50)
Stromal cells	0 (0)	4 (40)	6 (60)	1 (10)	9 (90)	0 (0)	0 (0)	10 (100)	0 (0)
Granular subtype ($n = 6$)									
Neoplastic cells	0 (0)	6 (100)	0 (0)	0 (0)	4 (67)	2 (33)	0 (0)	2 (33)	4 (67)
Stromal cells	0 (0)	3 (50)	3 (50)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)
Basal cell subtype ($n = 3$)									
Neoplastic cells	3 (100)	0 (0)	0 (0)	0 (0)	2 (67)	1 (33)	0 (0)	2 (67)	1 (33)
Stromal cells	0 (0)	1 (33)	2 (67)	0 (0)	3 (100)	0 (0)	0 (0)	2 (67)	1 (33)
Desmoplastic subtype ($n = 4$)									
Neoplastic cells	4 (100)	0 (0)	0 (0)	0 (0)	2 (50)	2 (50)	0 (0)	2 (50)	2 (50)
Stromal cells	0 (0)	2 (50)	2 (50)	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)
Non-cellular variation ($n = 21$)									
Neoplastic cells	21 (100)	0 (0)	0 (0)	0 (0)	7 (33)	14 (67)	0 (0)	9 (43)	12 (57)
Stromal cells	0 (0)	8 (38)	13 (62)	0 (0)	20 (95)	1 (5)	0 (0)	17 (81)	4 (19)
Odontogenic carcinoma ($n = 5$)									
Neoplastic cells	5 (100)	0 (0)	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)	2 (40)	3 (60)
Stromal cells	0 (0)	2 (40)	3 (60)	0 (0)	5 (100)	0 (0)	0 (0)	5 (100)	0 (0)
Metastasizing ameloblastoma ($n = 2$)									
Neoplastic cells	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	1 (50)	1 (50)
Stromal cells	0 (0)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)
Ameloblastic carcinoma ($n = 3$)									
Neoplastic cells	3 (100)	0 (0)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	1 (33)	2 (67)
Stromal cells	0 (0)	1 (33)	2 (67)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)

Immunohistochemical reactivity: (−) negative, (+) positive, and (++) strongly positive.
Values in parentheses denote percentage values.
Statistical significance: ***$P < 0.001$.

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 World Health Organization (WHO) histological classification of odontogenic tumors (2). The tumors comprised 44 ameloblastomas and five malignant ameloblastic tumors. Ameloblastomas were divided into 23 follicular and 21 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 11 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 and immersed in methanol with 0.3% hydrogen peroxide. Sections for angiopoietin-1 and -2 immunostaining were heated in 1 mM ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.0) 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 mouse anti-PD-ECGF/TP monoclonal antibody (Lab Vision Corporation, Fremont, CA, USA; subclass IgG1; diluted at 1:40), goat anti-angiopoietin-1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; diluted at 1:30), and goat anti-angiopoietin-2 polyclonal antibody (Santa Cruz Biotechnology; diluted at 1:40). The sections were allowed to react with peroxidase-conjugated anti-mouse IgG (for PD-ECGF/TP) or anti-goat IgG (for angiopoietin-1 and -2) 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 2–3 min. Nuclei were lightly stained with methylgreen. For control studies of the antibodies, the serial sections were treated with phosphate-buffered saline, mouse anti-OPD4 (CD45RO) monoclonal antibody (Dako, Glostrup, Denmark; subclass IgG1), and normal goat IgG instead of the primary antibodies and were confirmed to be unstained.

Immunohistochemical reactivity for PD-ECGF/TP and angiopoietin-1 and -2 was evaluated and classified into three groups: (−) negative, (+) positive, and (++) strongly positive. The statistical significance of differences in the percentages of cases with different reactivity

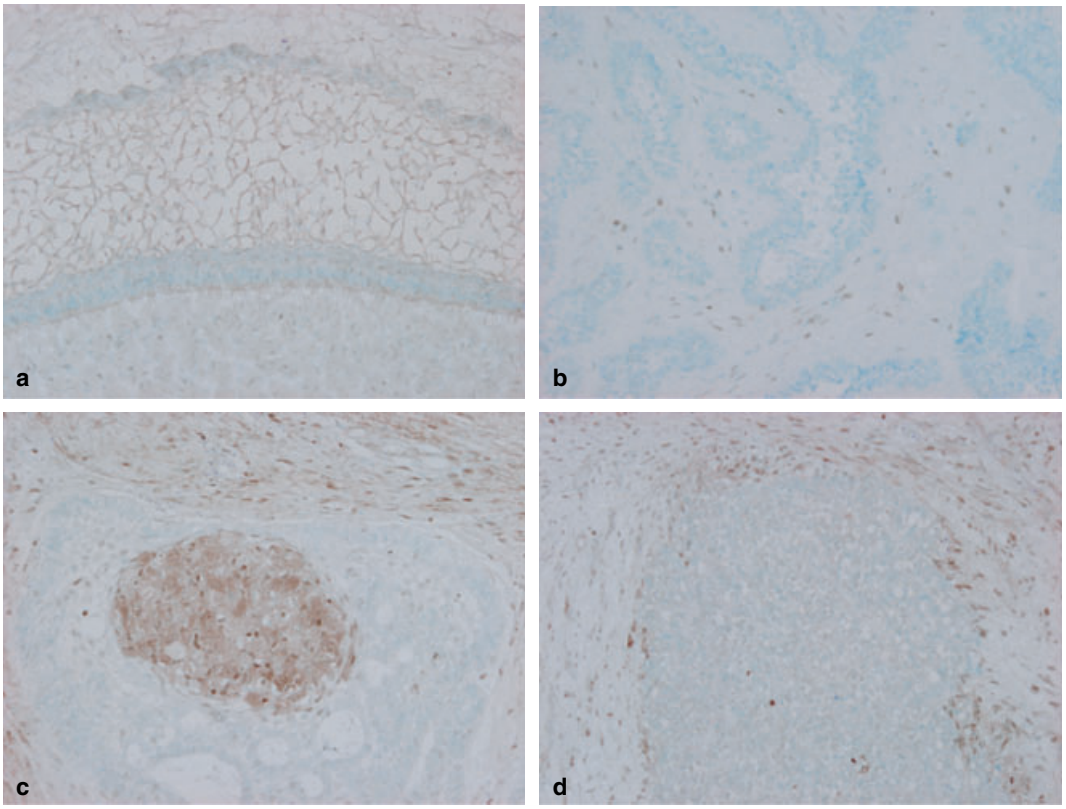

Figure 1 Immunohistochemical reactivity for PD-ECGF/TP. (a) Tooth germ showing weak reactivity in some fibroblastic cells in dental papilla and dental follicle (×110). (b) Follicular ameloblastoma showing reactivity in stromal fibroblasts (×110). (c) Granular ameloblastoma showing reactivity in granular neoplastic cells as well as in stromal fibroblasts (×100). (d) Ameloblastic carcinoma showing reactivity in stromal fibroblasts (×110).

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

Immunohistochemical reactivity for PD-ECGF/TP and angiopoietin-1 and -2 in tooth germs and ameloblastic tumors is summarized in Table 1. Expression of PD-ECGF/TP was detected in the cytoplasm and nuclei of cellular components in normal and neoplastic odontogenic tissues (Fig. 1). In tooth germs, weak reactivity for PD-ECGF/TP was found in some fibroblastic cells in dental papillae and dental follicles (Fig. 1a). Ameloblastomas and malignant ameloblastic tumors showed PD-ECGF/TP reactivity in stromal fibroblasts (Fig. 1b–d). The level of immunohistochemical reactivity for PD-ECGF/TP was significantly higher in the stroma of ameloblastomas than in mesenchymal components of tooth germs ($P < 0.001$; Table 1). Although most epithelial cells in tooth germs and ameloblastic tumors were negative for PD-ECGF/TP, granular neoplastic cells in granular cell ameloblastomas were reactive with PD-ECGF/TP (Fig. 1c). The level of immunohistochemical reactivity for PD-ECGF/TP was significantly higher in the parenchyma of granular cell ameloblastomas than in that of other subtypes of ameloblastomas ($P < 0.001$; Table 1).

Expression of angiopoietin-1 was detected in the cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Fig. 2). In tooth germs, angiopoietin-1 was expressed strongly in inner enamel epithelium and weakly in other epithelial components (Fig. 2a). Some endothelial cells in dental papillae and dental follicles were weakly reactive. Ameloblastomas showed strong angiopoietin-1 reactivity in peripheral columnar or cuboidal cells and weak reactivity in central polyhedral cells (Fig. 2b). Keratinizing cells in acanthomatous ameloblastomas exhibited no expression of angiopoietin-1, and decreased angiopoietin-1 expression was found in granular cells in granular cell ameloblastomas. Most neoplastic cells in basal cell and desmoplastic ameloblastomas demonstrated angiopoietin-1 reactivity. Metastasizing ameloblastomas and ameloblastic carcinomas showed weak reactivity for angiopoietin-1 in neoplastic cells (Fig. 2c,d). In these ameloblastic tumors, some stromal endothelial cells were weakly reactive with angiopoietin-1.

Immunoreactivity for angiopoietin-2 was detected in the cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Fig. 3). In tooth germs, angiopoietin-2 was expressed strongly in inner enamel

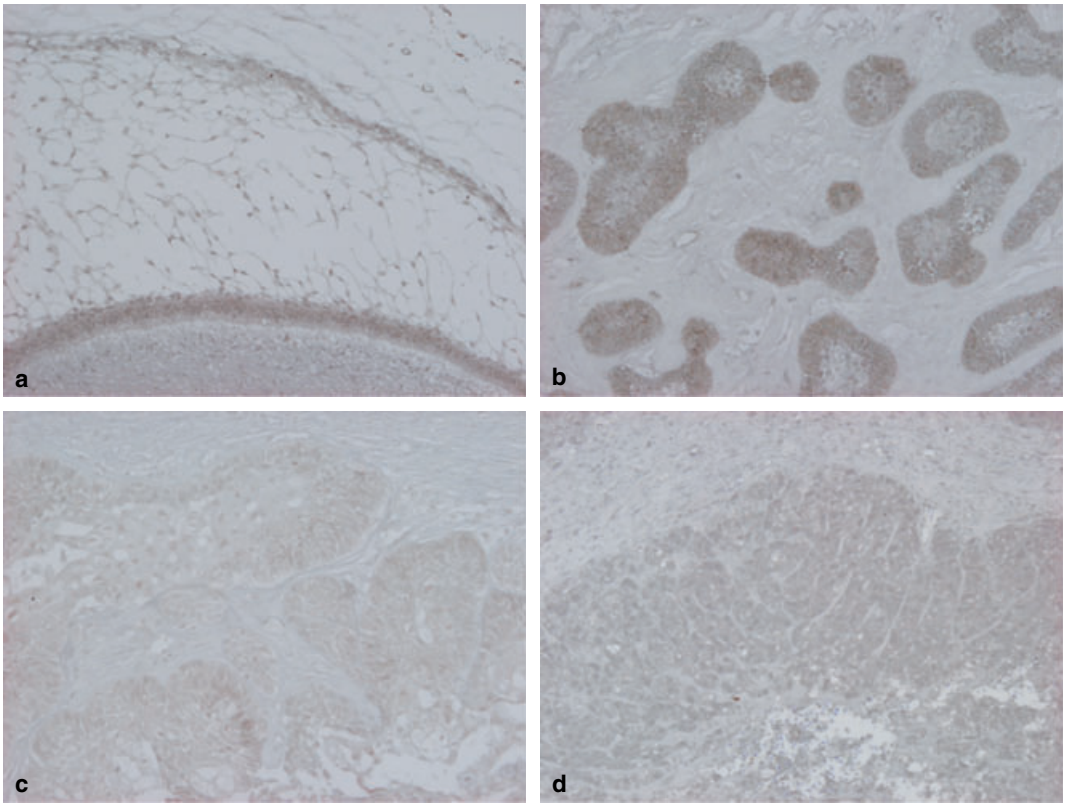

Figure 2 Immunohistochemical reactivity for angiopoietin-1. (a) Tooth germ showing strong reactivity in inner enamel epithelium and weak reactivity in other epithelial components. Some endothelial cells in dental papilla and dental follicle are weakly reactive (×95). (b) Follicular ameloblastoma showing strong reactivity in peripheral columnar cells and weak reactivity in central polyhedral cells. Some stromal endothelial cells are weakly reactive (×115). (c) Metastasizing ameloblastoma showing weak reactivity in neoplastic cells and some stromal endothelial cells (×110). (d) Ameloblastic carcinoma showing weak reactivity in neoplastic cells and some stromal endothelial cells (×115).

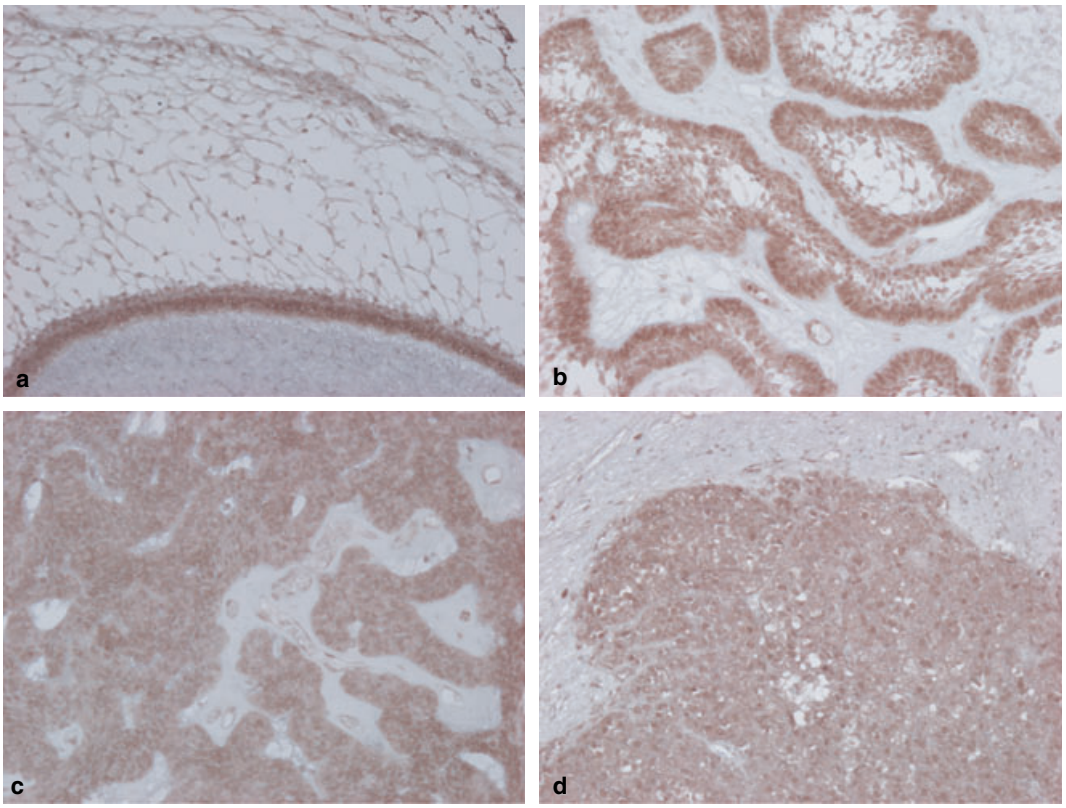

Figure 3 Immunohistochemical reactivity for angiopoietin-2. (a) Tooth germ showing strong reactivity in inner enamel epithelium and weak reactivity in other epithelial components. Many endothelial cells and some fibroblastic cells in dental papilla and dental follicle are also reactive (×95). (b) Follicular ameloblastoma showing strong reactivity in peripheral columnar cells and weak reactivity in central polyhedral cells. Many endothelial cells and some fibroblasts in the stroma are also reactive (×115). (c) Basal cell ameloblastoma showing strong reactivity in most neoplastic cells. Many endothelial cells and some fibroblasts in the stroma are also reactive (×115). (d) Ameloblastic carcinoma showing strong reactivity in most neoplastic cells. Many endothelial cells and some fibroblasts in the stroma are also reactive (×110).

epithelium and weakly in other epithelial components (Fig. 3a). Many endothelial cells and some fibroblastic cells in dental papillae and dental follicles were also reactive. Ameloblastomas and metastasizing ameloblastomas showed strong angiopoietin-2 reactivity in peripheral columnar or cuboidal cells and weak reactivity in central polyhedral cells (Fig. 3b). Keratinizing cells in acanthomatous ameloblastomas exhibited no expression of angiopoietin-2, and decreased angiopoietin-2 expression was found in granular cells in granular cell ameloblastomas. Most neoplastic cells in basal cell and desmoplastic ameloblastomas were reactive with angiopoietin-2 (Fig. 3c). Ameloblastic carcinomas showed moderate to strong reactivity for angiopoietin-2 in most neoplastic cells (Fig. 3d). In the stroma of these ameloblastic tumors, many endothelial cells and some fibroblasts were also reactive with angiopoietin-2.

Discussion

Vessel formation is crucial for the development as well as for the maintenance of normal tissues in higher organisms (7, 17). Homozygous or compound-heterozygous mutations of *PD-ECGF/TP* gene cause mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an autosomal recessive human mitochondrial disease characterized by progressive external ophthalmoplegia, gastrointestinal dysmotility, thin body habitus, peripheral neuropathy, myopathy, leukoencephalopathy, and lactic acidosis (23). Mice deficient in *PD-ECGF/TP* gene show elevated plasma thymidine levels and abnormal myelin structures in the brain (24). Patients with MNGIE and *PD-ECGF/TP*-deficient mice do not have vascular abnormalities, suggesting that the absence of PD-ECGF/TP does not interfere with normal angiogenesis (23, 24). In the present study, immunohistochemical reactivity for PD-ECGF/TP was partial and weak in tooth germs, suggesting that PD-ECGF/TP minimally affects angiogenesis during tooth development. Knockouts of *angiopoietin-1* or *TIE-2* and transgenic overexpression of *angiopoietin-2* result in embryonic death with angiogenic deficits (16, 25, 26). Mice lacking *angiopoietin-2* exhibit major lymphatic vessel defects and die by 2 weeks of age (27). An activating mutation of *TIE-2* gene causes venous malformations (VMs), the most common errors of vascular morphogenesis in humans, characterized by dilated and serpiginous channels (28). Thus, the receptor/ligand system is essential for the assembly of developing vasculature (16, 25–28). In the present study, angiopoietin-1 and -2 were expressed in tooth germs, suggesting

that angiopoietins might control normal angiogenesis during tooth development.

Angiogenesis is prerequisite for the growth, survival, and expansion of tumors and is induced by various angiogenic factors produced in neoplastic cells and/or stromal cells (6, 8, 18, 29). Expression of PD-ECGF/TP has been detected in neoplastic cells as well as in stromal macrophages, lymphocytes, and fibroblasts, and expression levels are elevated in a variety of human tumors (19, 30, 31). PD-ECGF/TP expression has also shown to correlate with MVD and outcomes of neoplastic lesions (14, 30, 31). In the present study, immunohistochemical reactivity for PD-ECGF/TP in ameloblastic tumors was detected predominantly in stromal cells and was significantly higher than that in tooth germs. Our previous study showed that MVD was higher in ameloblastic tumors than in tooth germs (22). These features suggest that PD-ECGF/TP functions as an inducer of angiogenesis in ameloblastic tumors, and up-regulation of PD-ECGF/TP might promote oncogenesis of odontogenic epithelium. Our previous study also revealed that MVD is slightly higher in granular cell ameloblastomas than in other ameloblastoma subtypes (22). In the present study, neoplastic cells with granular cytoplasm were positive for PD-ECGF/TP in ameloblastomas, suggesting that PD-ECGF/TP expression in neoplastic cells might be involved in increased angiogenesis as well as in tumor cell differentiation in ameloblastomas.

Angiopoietins have been implicated in the development of vasculature in various types of tumors, and their expression patterns are complex, but in general the up-regulation of angiopoietin-1 in tumors is less common than elevated expression of angiopoietin-2 (17, 18, 32, 33). In the present study, immunohistochemical reactivity for angiopoietin-1 and -2 was detected in neoplastic and stromal cells in ameloblastic tumors, suggesting that these molecules affect angiogenesis in these epithelial odontogenic tumors. Although expression levels of angiopoietins in ameloblastomas did not markedly differ from those in tooth germs, decreased angiopoietin-1 reactivity in malignant ameloblastic tumors and increased angiopoietin-2 reactivity in ameloblastic carcinomas were found, as compared with those in tooth germs and benign ameloblastomas in this study. Our previous study demonstrated an apparent increase in MVD in malignant ameloblastic tumors (22). These features suggest that the balance between angiopoietin-1 and -2 expression might be associated with the malignant potential of epithelial odontogenic tumors. In the present study, immunohistochemical reactivity for angiopoietin-2 was slightly higher in neoplastic cells of follicular ameloblastomas than in plexiform ameloblastomas. Our previous study demonstrated that microvessels in follicular ameloblastomas were numerous and small, whereas those in plexiform ameloblastomas were scattered and dilated (22). These features suggest that angiopoietin-2 might influence the development and morphology of vasculature in ameloblastomas. Angiopoietin-1 stimulates blood vessel invasion synergistically with VEGF, and VEGF selectively up-regulates angiopoietin-2 (34, 35). Our previous study demonstrated VEGF expression in tooth germs and benign and malignant ameloblastic tumors (22). These features suggest that the expression and function of angiopoietins are affected by VEGF in normal and neoplastic odontogenic tissues.

References

1. Sciubba JJ, Fantasia JE, Kahn LB. *Tumors and cysts of the jaw*. Washington, D.C.: Armed Forces institute of Pathology, 2001; 71–99.
2. Philipsen HP, Reichart PA, Slootweg PJ, et al. Odontogenic tumours. In: Barnes L, Eveson JW, Reichart PA, Sidransky D, eds. *WHO classification head and neck tumours*. Lyon: IARC Press, 2005; 283–327.
3. Heikinheimo K, Jee KJ, Niini T, et al. Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. *J Dent Res* 2002; **81**: 525–30.
4. Jaakelainen K, Jee KJ, Leivo I, Saloniemi I, Knuutila S, Heikinheimo K. Cell proliferation and chromosomal changes in human ameloblastoma. *Cancer Genet Cytogenet* 2002; **136**: 31–7.
5. Kumamoto H. Molecular pathology of odontogenic tumors. *J Oral Pathol Med* 2006; **35**: 65–74.
6. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**: 1182–6.
7. Risau W. Mechanisms of angiogenesis. *Nature* 1997; **386**: 671–4.
8. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000; **407**: 249–57.
9. Miyazono K, Okabe T, Urabe A, Takaku F, Heldin CH. Purification and properties of an endothelial cell growth factor from human platelets. *J Biol Chem* 1987; **262**: 4098–103.
10. Ishikawa F, Miyazono K, Hellman U, et al. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. *Nature* 1989; **338**: 557–62.
11. Furukawa T, Yoshimura A, Sumizawa T, et al. Angiogenic factor. *Nature* 1992; **356**: 668.
12. Moghaddam A, Bicknell R. Expression of platelet-derived endothelial cell growth factor in *Escherichia coli* and confirmation of its thymidine phosphorylase activity. *Biochemistry* 1992; **31**: 12141–6.
13. Miyadera K, Sumizawa T, Haraguchi M, et al. Role of thymidine phosphorylase activity in the angiogenic effect of platelet derived endothelial cell growth factor/thymidine phosphorylase. *Cancer Res* 1995; **55**: 1687–90.
14. Akiyama S, Furukawa T, Sumizawa T, et al. The role of thymidine phosphorylase, an angiogenic enzyme, in tumor progression. *Cancer Sci* 2004; **95**: 851–7.
15. Davis S, Aldrich TH, Jones PF, et al. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* 1996; **87**: 1161–9.
16. Maisonpierre PC, Suri C, Jones PF, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 1997; **277**: 55–60.
17. Gale NW, Yancopoulos GD. Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. *Genes Dev* 1999; **13**: 1055–66.
18. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000; **407**: 242–8.

19. Takebayashi Y, Yamada K, Miyadera K, et al. The activity and expression of thymidine phosphorylase in human solid tumours. *Eur J Cancer* 1996; **32A**: 1227–32.
20. Jones PF. Not just angiogenesis–wider roles for the angiopoietins. *J Pathol* 2003; **201**: 515–27.
21. Tait CR, Jones PF. Angiopoietins in tumours: the angiogenic switch. *J Pathol* 2004; **204**: 1–10.
22. Kumamoto H, Ohki K, Ooya K. Association between vascular endothelial growth factor (VEGF) expression and tumor angiogenesis in ameloblastomas. *J Oral Pathol Med* 2002; **31**: 28–34.
23. Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science* 1999; **283**: 689–92.
24. Haraguchi M, Tsujimoto H, Fukushima M, et al. Targeted deletion of both thymidine phosphorylase and uridine phosphorylase and consequent disorders in mice. *Mol Cell Biol* 2002; **22**: 5212–21.
25. Dumont DJ, Gradwohl G, Fong GH, et al. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. *Genes Dev* 1994; **8**: 1897–909.
26. Suri C, Jones PF, Patan S, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* 1996; **87**: 1171–80.
27. Gale NW, Thurston G, Hackett SF, et al. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. *Dev Cell* 2002; **3**: 411–23.
28. Vikkula M, Boon LM, Carraway KL III, et al. Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2. *Cell* 1996; **87**: 1181–90.
29. Folkman J. The vascularization of tumors. *Sci Am* 1976; **234**: 58–64, 70–3.
30. Toi M, Hoshina S, Taniguchi T, Yamamoto Y, Ishitsuka H, Tominaga T. Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast cancer. *Int J Cancer* 1995; **64**: 79–82.
31. Takebayashi Y, Akiyama S, Akiba S, et al. Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. *J Natl Cancer Inst* 1996; **88**: 1110–7.
32. Tanaka S, Mori M, Sakamoto Y, Makuuchi M, Sugimachi K, Wands JR. Biologic significance of angiopoietin-2 expression in human hepatocellular carcinoma. *J Clin Invest* 1999; **103**: 341–5.
33. Ahmad SA, Liu W, Jung YD, et al. Differential expression of angiopoietin-1 and angiopoietin-2 in colon carcinoma. A possible mechanism for the initiation of angiogenesis. *Cancer* 2001; **92**: 1138–43.
34. Koblizek TI, Weiss C, Yancopoulos GD, Deutsch U, Risau W. Angiopoietin-1 induces sprouting angiogenesis in vitro. *Curr Biol* 1998; **8**: 529–32.
35. Oh H, Takagi H, Suzuma K, Otani A, Matsumura M, Honda Y. Hypoxia and vascular endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial cells. *J Biol Chem* 1999; **274**: 15732–9.