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ORIGINAL ARTICLE

Expression of bone morphogenetic proteins and their associated molecules in ameloblastomas and adenomatoid odontogenic tumors

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OBJECTIVE: To further clarify the roles of regulators of embryonic development, bone morphogenetic protein (BMPs) and their associated molecules, in oncogenesis and cytodifferentiation of odontogenic tumors, the expression of these regulator molecules were analyzed in epithelial odontogenic tumors as well as in tooth germs.
MATERIALS AND METHODS: Tooth germs, ameloblastomas, adenomatoid odontogenic tumors, and malignant ameloblastomas were examined by RT-PCR and immunohistochemistry for detection of BMP-2, -4, -7, BMP receptors I and II (BMPR-I, BMPR-II), core-binding factor α1 (CBFA1), and osterix.
RESULTS: mRNA expression of BMPs, BMPRs, CBFA1, and osterix was detected in all odontogenic tissues. Immunohistochemical reactivity for BMPs, BMPRs, and CBFA1 was detected in both epithelial and mesenchymal cells of tooth germs and epithelial odontogenic tumors. BMPs and BMPRs were evidently expressed in odontogenic epithelial cells in tooth germs and epithelial odontogenic tumors. Acanthomatous ameloblastomas showed increased BMP-7 reactivity in keratinizing cells. Nuclear CBFA1 expression was detected scatteredly in odontogenic epithelial cells in normal and neoplastic odontogenic tissues, as well as in some mesenchymal cells in tooth germs and in some stromal cells in epithelial odontogenic tumors. Ameloblastic carcinomas showed low reactivity for BMPs, BMPRs, and CBFA1.
CONCLUSION: BMPs and their associated molecules might play a role in cytodifferentiation of normal and neoplastic odontogenic epithelium via epithelial–mesenchymal interactions.
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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 (Kramer *et al*, 1992; Eversole, 1999; Melrose, 1999; Sciubba *et al*, 2001). 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 (Kramer *et al*, 1992; Melrose, 1999; Sciubba *et al*, 2001). Histologically, ameloblastoma shows considerable variation, including follicular, plexiform, acanthomatous, granular cell, basal cell, and desmoplastic types (Kramer *et al*, 1992). Adenomatoid odontogenic tumor (AOT) is an uncommon benign and usually cystic tumor of odontogenic epithelial origin with duct-like structures and exerts various degrees of inductive effects on adjacent mesenchyme (Kramer *et al*, 1992; Sciubba *et al*, 2001). Malignant ameloblastoma is defined as a neoplasm in which the pattern of an ameloblastoma and cytological features of malignancy are shown by the primary growth in the jaws and/or by any metastatic growth (Kramer *et al*, 1992). Recently, malignant ameloblastoma has been subclassified into metastasizing ameloblastoma and ameloblastic carcinoma on the basis of metastatic spread and cytological malignant features (Eversole, 1999). Several recent studies have detected genetic and cytogenetic alterations in these epithelial odontogenic tumors (Heikinheimo *et al*, 2002; Jaakelainen *et al*, 2002); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

Bone morphogenetic proteins (BMPs), the largest subgroup within the transforming growth factor-beta (TGF-β) superfamily, were originally isolated as factors

that induce bone and cartilage formation, and recent works have revealed that BMPs play a critical role in cell proliferation, differentiation, chemotaxis, and apoptosis during developmental processes (Urist, 1965; Wozney *et al*, 1988; Hogan, 1996; Yamaguchi *et al*, 2000). BMP signals are transduced by binding to a heteromeric complex of BMP receptors (BMPRs), BMP type I and II receptors (BMPR-I and BMPR-II), which have serine/threonine kinase activity, and signaling from the cell membrane to nucleus is propagated by SMAD proteins (Kawabata *et al*, 1998; Yamaguchi *et al*, 2000). Expression of BMPs and BMPRs has been identified in tooth germ tissues, suggesting that BMPs, especially BMP-2, -4, and -7, regulate tooth development (Vainio *et al*, 1993; Helder *et al*, 1995; Iseki *et al*, 1995; Vaahtokari *et al*, 1996). Several neoplasms express BMPs and/or BMPRs, suggesting that these factors are associated with not only pathological mineralization but also with tumor development and progression (Yoshikawa *et al*, 1994; Katoh and Terada, 1996; Kim *et al*, 2000; Jin *et al*, 2001). Core-binding factor α1 (CBFA1), also referred to as runt-related protein 2 (RUNX2), is a transcription factor in the RUNX family (Ogawa *et al*, 1993; Komori, 2002). CBFA1 is provided by BMP signaling and acts as a master regulator of osteoblast differentiation and chondrocyte maturation (Yamaguchi *et al*, 2000; Komori, 2002). Expression of CBFA1 has been detected during tooth development, suggesting that CBFA1 is required for epithelial–mesenchymal interactions regulating tooth formation (D'Souza *et al*, 1999). Osterix is a recently identified zinc finger-containing transcription factor, which acts downstream of CBFA1 for further osteoblast differentiation and bone formation (Nakashima *et al*, 2002). These transcription factors have been identified in some tumors and are believed to control tumor cell differentiation and progression (Yang *et al*, 2001; Riminucci *et al*, 2003; Huang *et al*, 2004).

Our previous study revealed that signal transduction of Sonic hedgehog (SHH), a regulator of embryonic development, might play a role in epithelial–mesenchymal interactions and cell proliferation during the growth of epithelial odontogenic tumors as well as in tooth development (Kumamoto *et al*, 2004). We also showed that the Wnt signaling pathway controlling diverse developmental processes might play a role in oncogenesis and cytodifferentiation of odontogenic epithelium via deregulation of cell proliferation (Kumamoto and Ooya, 2005). In the present study, the expression of BMP-2, -4, -7, and their associated molecules in benign and malignant ameloblastomas and AOTs as well as in tooth germs was examined by reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry to clarify the possible roles of these regulators of developmental processes in epithelial odontogenic tumors.

Materials and methods

Tissue preparation

Specimens were surgically removed from 48 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 typing of odontogenic tumors (Kramer *et al*, 1992). The tumors comprised 37 ameloblastomas, six AOTs, and five malignant ameloblastomas. Ameloblastomas were divided into 21 follicular and 16 plexiform types, including 10 acanthomatous, six granular cell, three basal cell, and four desmoplastic subtypes. Malignant ameloblastomas were classified into two metastasizing ameloblastomas and three ameloblastic carcinomas according to the criteria of Eversole (1999). For RT-PCR analysis, tumor tissues were immediately frozen on dry ice and stored at −80°C. Specimens of 10 tooth germs of the mandibular third molars (age range: 8–13 years old, two males and eight females), enucleated for orthodontic reasons at the initial stage of crown mineralization (bell stage), were similarly prepared and compared with the epithelial odontogenic tumors.

RT-PCR

Total RNA was extracted from frozen tissue samples of seven tooth germs, 23 ameloblastomas, three AOTs, and one malignant ameloblastoma using an RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. First-stranded complementary DNA (cDNA) was synthesized from 1 μg of RNA using an Omniscript RT Kit (Qiagen) with oligo-$(dT)_{15}$ primer (Roche Diagnostics, Mannheim, Germany) as outlined by the manufacturer. The cDNA samples were amplified using a HotstarTaq Master Mix Kit (Qiagen) with specific primers in a DNA thermal cycler (Eppendorf, Hamburg, Germany). The primers used in this study are listed in Table 1 (Gu *et al*, 1996; Ogose *et al*, 1996; Huang *et al*, 2000; Gronthos *et al*, 2003). A housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used as an internal control to examine human gene expression. PCR was performed in a total volume of 50 μl, containing 1 μg of template cDNA and 0.5 μM of each specific primer set. The procedure for amplification included 35 cycles of denaturation at 94 °C for 45 s, annealing at 55°C (for BMP-2, -4, and -7, CBFA1, osterix, and GAPDH) or 52°C (for BMPR-IA and -II) for 45 s, and elongation at 72 °C for 60 s with heat starting at 95 °C for 15 min and final elongation at 72 °C for 10 min. The PCR products were electrophoresed on 2 % agarose gel at 100 V for 30 min and visualized with ethidium bromide. For control studies, RT-PCR using RNase-free water instead of template RNA and cDNA was performed and confirmed not to be amplified. To examine if DNA contamination occurred during our RNA preparation, we amplified GAPDH transcript with a forward primer from exons 2 and 3 and a reverse primer from exon 4. We found that only the 206 bp transcript appeared to be amplified while the DNA expected 284 bp and 1929 bp fragments

Table 1 List of primers used in this study

	Sequence (5′–3′)	*Anneal (°C)*	*Product (bp)*	*Reference*
BMP-2 (M22489)	Forward: TCATAAAACCTGCAACAGCCAACTCG Reverse: GCTGTACTAGCGACACCCAC	55	671	Ogose *et al* (1996)
BMP-4 (M22490)	Forward: ACTGGTCCACCACAATGTGACACG Reverse: GCTGAAGTCCACATAGAGCGAGTG	55	346	Ogose *et al* (1996)
BMP-7 (X51801)	Forward: TCCGATTCCCTGCCCAAGTG Reverse: AGGCCGTCTTCAGTACCCAGG	55	277	Ogose *et al* (1996)
BMPR-IA (Z22535)	Forward: GAAGAAGATGACCAGGGAGAA Reverse: CCGTCATGAAACCAAGTATGT	52	622	Gu *et al* (1996)
BMPR-II (U25110)	Forward: CTCAGTCCACCTCATTCATTT Reverse: CACCAGTCTATTTCCAGTCAG	52	678	Gu *et al* (1996)
CBFA1 (L40992)	Forward: CAGTTCCCAAGCATTTCATCC Reverse: TCAATATGGTCGCCAAACAG	55	443	Gronthos *et al* (2003)
Osterix (XM62600)	Forward: GCAGCTAGAAGGGAGTGGTG Reverse: GCAGGCAGGTGAACTTCTTC	55	359	Gronthos *et al* (2003)
GAPDH (M33197)	Forward: GGAGTCAACGGATTTGGT Reverse: GTGATGGGATTTCCATTGAT	55	206	Huang *et al* (2000)

were not amplified proving no DNA in our RNA preparation.

Immunohistochemistry

Tissue sections of 10 tooth germs, 37 ameloblastomas, six AOTs, and five malignant ameloblastomas were deparaffinized and immersed in methanol with 0.3% hydrogen peroxide. The sections for BMP-2 and -4 immunostaining were heated in 0.01 M citrate buffer (pH 6.0) for 10 min by autoclave (121°C, 2 atm). After treatment with normal rabbit serum for 30 min, the sections were incubated with primary antibodies at 4°C overnight. The applied antibodies were goat polyclonal antibodies against human BMP-2, -4, -7, BMPR-I, -II, and CBFA1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA; diluted at 1:50). The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibodies with the use of Histofine SAB-PO Kits (Nichirei, Tokyo, Japan). Reaction products were visualized by immersing the sections in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide for 1–3 min. Nuclei were lightly counterstained with methyl green. For control studies of the antibodies, the serial sections were treated with phosphate-buffered saline and normal goat IgG instead of the primary antibodies and were confirmed to be unstained.

Immunohistochemical reactivity for BMPs and their associated molecules was evaluated and classified into four groups: (−) negative, (+) positive in some cells (< 20% of cells positive), (++) positive in many cells (20–80% of cells positive), and (+++) positive in most cells (> 80% of cells positive).

Results

mRNA expression

Reverse transcriptase-PCR analysis identified expression of mRNA transcripts for BMPs and their associated molecules in all 23 ameloblastomas, three AOTs, and one metastasizing ameloblastoma as well as in the seven tooth germ tissues (Figure 1). The PCR products of BMP-2, -4, -7, BMPR-IA, -II, CBFA1, and osterix were 671, 346, 277, 622, 678, 443, and 359 bp, respectively.

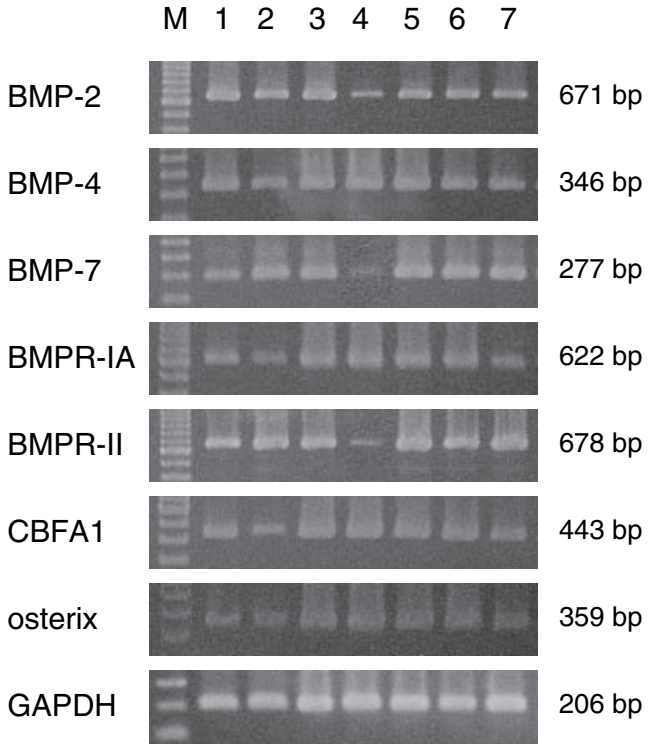

Figure 1 RT-PCR analysis of mRNA transcripts for BMPs and their associated molecules in tooth germs and epithelial odontogenic tumors [M: molecular weight standard (100 bp DNA ladder), 1: tooth germs, 2–4: ameloblastomas (2: follicular, 3: plexiform, 4: granular cell), 5, 6: adenomatoid odontogenic tumors, 7: malignant ameloblastoma (metastasizing ameloblastoma)]. mRNA expression of BMPs and their associated molecules was seen in all samples. The PCR products of BMP-2, -4, -7, BMPR-IA, -II, CBFA1, and osterix were 671, 346, 277, 622, 678, 443, and 359 bp, respectively. GAPDH was run as a control to ascertain the integrity of mRNA/cDNA

Immunohistochemical reactivity

Immunohistochemical reactivity for BMPs and their associated molecules in tooth germs and epithelial

Table 2 Immunohistochemical reactivity for BMPs and their associated molecules in tooth germs and epithelial odontogenic tumors

	BMP-2	*BMP-4*	*BMP-7*	*BMPRs*	*CBFA1*
Tooth germ ($n = 10$)					
Epithelial cells					
Inner enamel epithelium	+	+/++	++/+++	++/+++	++/+++
Outer enamel epithelium	–	–	+/++	+/++	++
Stellate reticulum	–	–	+	+	++
Stratum intermedium	–	–	+	+	+
Dental lamina	–	–	+	+	+
Mesenchymal cells					
Dental papilla	+	+	+	+/++	+/++
Dental follicle	+	+	+	+/++	+/++
Ameloblastoma ($n = 37$)					
Neoplastic cells					
Peripheral cells	+	+/++	++/+++	++/+++	++
Central cells	–	–/+	+	–/+	–/+
Keratinizing cells	–	–	++	–/+	–
Granular cells	–	–	–	–	–
Basal cell variant	++	++	++/+++	++/+++	++/+++
Desmoplastic variant	+	+/++	+/++	++/+++	+
Stromal cells	+	+	+	+/++	+
Adenomatoid odontogenic tumor ($n = 6$)					
Neoplastic cells					
Pseudoglandular cells	++	++/+++	++/+++	++/+++	++/+++
Other cells	++	++/+++	++/+++	++/+++	++/+++
Stromal cells	+	+	+	+/++	+
Metastasizing ameloblastoma ($n = 2$)					
Neoplastic cells					
Peripheral cells	+	+/++	++/+++	++/+++	++
Central cells	–	–	+	–/+	–/+
Stromal cells	+	+	+	+/++	+
Ameloblastic carcinoma ($n = 3$)					
Neoplastic cells	–/+	–/+	–/+	–/+	–/+
Stromal cells	+	+	+	+/++	+

–, Negative; +, positive in some cells (<20% of cells positive); ++, positive in many cells (20–80% of cells positive); +++, positive in most cells (>80% of cells positive).

odontogenic tumors is summarized in Table 2. Immunoreactivity for BMP-2, -4, and -7 was detected in the cytoplasm of cellular components in both normal and neoplastic odontogenic tissues (Figure 2). In tooth germs, BMP-2 and -4 were expressed sporadically in epithelial cells of inner enamel epithelium (Figure 2a), and BMP-4 reactivity was more broadly distributed than BMP-2 reactivity. BMP-7 was recognized in epithelial cells of enamel organs and dental laminae, and reactivity in inner enamel epithelium was stronger than that in other epithelial components. Weak reactivity for BMP-2, -4, and -7 was also found in some fibroblastic cells and endothelial cells of dental papillae and dental follicles. Ameloblastomas showed BMP-2 reactivity in some peripheral columnar or cuboidal neoplastic cells (Figure 2b). BMP-4 and -7 were detected in many peripheral columnar or cuboidal cells and some central polyhedral cells, and BMP-7 reactivity was more broadly distributed than BMP-4 reactivity (Figure 2c,e). Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas were not reactive with BMP-2 or -4, while BMP-7 reactivity was increased in keratinizing cells and lost in granular cells (Figure 2c,e). Basal cell ameloblastomas and desmoplastic ameloblastomas showed BMP-2, -4, and -7 reactivity in neoplastic cells, and the reactivity in desmoplastic ameloblastomas was limited. In AOTs, BMP-2 and -4 were expressed in spindle cells in whorled masses and pseudoglandular cells (Figure 2d), and BMP-4 reactivity was more broadly distributed than BMP-2 reactivity. BMP-7 reactivity was diffusely found in neoplastic cells and was evident in spindle cells in whorled masses and pseudoglandular cells. Metastasizing ameloblastomas showed BMP-2, -4, and -7 expression patterns similar to those of benign ameloblastomas, whereas ameloblastic carcinomas exhibited little or no reactivity for these BMPs (Figure 2f). In these epithelial odontogenic tumors, scattered stromal fibroblasts and endothelial cells showed weak reactivity for these BMPs.

BMPR-I and -II were expressed in the cell membrane and cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Figure 3a–d). In tooth germs, BMPR-I and -II were detected in epithelial cells of enamel organs and dental laminae, and reactivity in inner enamel epithelium was stronger than that in other epithelial components (Figure 3a). Fibroblastic cells of dental papillae and dental follicles were also reactive with these BMPRs. Ameloblastomas showed BMPR-I and -II reactivity in many peripheral columnar or cuboidal cells and some central polyhedral cells (Figure 3b,c). Little or no reactivity for these BMPRs was found in keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas. Basal cell ameloblastomas and desmoplastic ameloblastomas exhibited diffuse BMPR-I and -II expression in neoplastic cells, and reactivity in

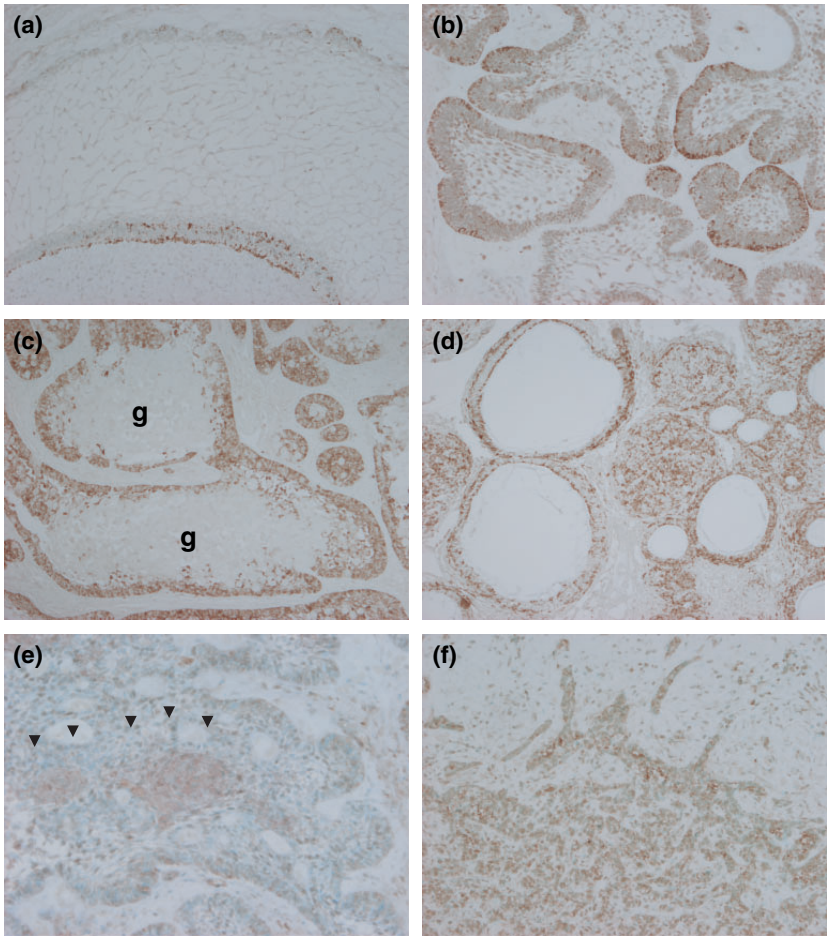

Figure 2 Immunohistochemical reactivity for BMP-2 (**a**, **b**), -4 (**c**, **d**), and -7 (**e**, **f**). (**a**) Tooth germ showing BMP-2 reactivity in some cells of inner enamel epithelium (×80). (**b**) Follicular ameloblastoma showing BMP-2 reactivity in some peripheral columnar neoplastic cells. Some stromal cells are weakly reactive (×105). (**c**) Granular cell ameloblastoma showing BMP-4 reactivity in many peripheral columnar cells and some central polyhedral cells. Granular cells (g) are not reactive. Some stromal cells are weakly reactive (×115). (**d**) Adenomatoid odontogenic tumor showing BMP-4 reactivity in many spindle cells in whorled masses and most pseudoglandular cells (×65). (**e**) Acanthomatous ameloblastoma showing BMP-7 reactivity in many peripheral cuboidal cells and some central polyhedral cells. Keratinizing cells (arrowheads) exhibit increased reactivity. Some stromal cells are weakly reactive (×125). (**f**)Ameloblastic carcinoma showing BMP-7 reactivity in scattered neoplastic cells (×100)

desmoplastic ameloblastomas was low. In AOTs, diffuse immunoreactivity for BMPR-I and -II was identified in neoplastic cells (Figure 3d). Metastasizing ameloblastomas showed BMPR-I and -II expression patterns similar to those of benign ameloblastomas, whereas ameloblastic carcinomas exhibited little or no reactivity for these BMPRs. In these epithelial tumors, stromal fibroblasts were also reactive with BMPR-I and -II.

Immunohistochemical reactivity for CBFA1 was detected in the cytoplasm and some nuclei of cellular components of normal and neoplastic odontogenic tissues (Figure 3e,f). In tooth germs, CBFA1 was expressed in the cytoplasm of many epithelial cells in inner enamel epithelium, stratum intermedium, outer enamel epithelium, and dental laminae and in some nuclei of epithelial cells in stellate reticulum. Scattered nuclei of fibroblastic cells in dental papillae and dental follicles were also reactive with CBFA1. Ameloblastomas showed CBFA1 reactivity in the cytoplasm of many peripheral columnar or cuboidal cells and in some nuclei of central polyhedral cells (Figure 3e). Keratinizing cells and granular cells in ameloblastoma subtypes were not reactive with CBFA1. Basal cell ameloblastomas showed CBFA1 reactivity in the cytoplasm of many or most neoplastic cells, and desmoplastic ameloblastomas were sporadically reactive with CBFA1 in some neoplastic cells. In AOTs, diffuse immunoreactivity for CBFA1 was identified in neoplastic cells, and some nuclei of spindle cells in whorled masses were also positive for CBFA1 (Figure 3f). Metastasizing ameloblastomas showed a CBFA1 expression pattern similar to that of benign ameloblastomas, whereas ameloblastic carcinomas exhibited little or no reactivity for CBFA1. In these epithelial odontogenic tumors, scattered nuclei of stromal fibroblasts were also reactive with CBFA1.

Discussion

Bone morphogenetic proteins function not only as bone-forming factors but also as regulators of early embryogenesis and the development of diverse organs and tissues, including the nervous system, eye, heart, lung, kidney, gonads, and skeletal system (Hogan, 1996; Yamaguchi *et al*, 2000). In mice, deficiency of *BMP-2*, *-4*, or *BMPR-IA* causes embryonic lethality, whereas deficiency of *BMP-7* or *BMPR-IB* leads to developmental abnormalities in the kidney, eye, and skeleton (Luo *et al*, 1995; Mishina *et al*, 1995; Winnier *et al*, 1995; Zhang and Bradley, 1996; Yi *et al*, 2000). Expression of BMP-2, -4, and -7 has been detected within the enamel knot as a signaling center in developing teeth, suggesting that these molecules participate in tooth formation (Vainio *et al*, 1993; Helder *et al*,

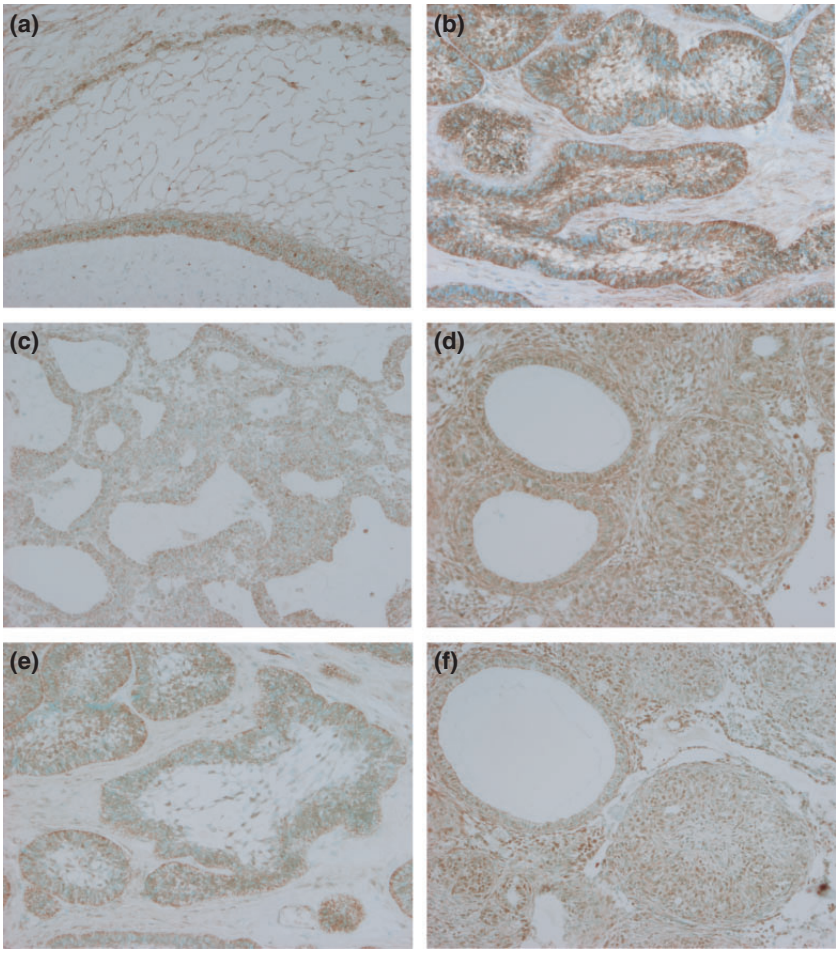

Figure 3 Immunohistochemical reactivity for BMPR-I (**a**, **b**), -II (**c**, **d**), and CBFA1 (**e**, **f**). (**a**) Tooth germ showing strong BMPR-I reactivity in inner and outer enamel epithelium and weak reactivity in stratum intermedium and stellate reticulum. Some mesenchymal cells in dental papilla and dental follicle are also reactive (×95). (**b**) Follicular ameloblastoma showing BMPR-I reactivity in most peripheral columnar cells and some central polyhedral cells. Stromal cells are also reactive (×110). (**c**) Plexiform ameloblastoma showing BMPR-II reactivity in many peripheral cuboidal cells and a few central polyhedral cells. Stromal cells are also reactive (×105). (**d**) Adenomatoid odontogenic tumor showing diffuse BMPR-II reactivity in neoplastic cells (×105). (**e**) Follicular ameloblastoma showing CBFA1 reactivity in the cytoplasm of peripheral columnar cells and in some nuclei of central polyhedral cells. Stromal cells are weakly reactive (×115). (**f**) Adenomatoid odontogenic tumor showing diffuse CBFA1 reactivity in the cytoplasm of neoplastic cells. Some nuclei of spindle cells in whorled masses are also reactive (×80)

1995; Vaahtokari *et al*, 1996). BMP-4 signaling is involved in the establishment of tooth identity (Tucker *et al*, 1998). BMPRs are expressed in various stages of tooth development (Iseki *et al*, 1995; Ikeda *et al*, 1996). In the present study, BMP-2, -4, -7, BMPR-I, and -II were identified in tooth germ tissues at the initial stage of crown mineralization. BMP-2 and -4 are targets of SHH signaling in developmental processes (Bitgood and McMahon, 1995). Our previous study confirmed the expression of SHH signaling molecules in tooth germs (Kumamoto *et al*, 2004). These features support roles of SHH and these BMPs in epithelial–mesenchymal interactions during tooth development (Bitgood and McMahon, 1995).

Overexpression of BMPs and BMPRs is involved in pathological ossification or ectopic osteogenesis in several bone lesions, such as fibrodysplasia ossificans progressiva and ossification of the posterior longitudinal ligament (Shafritz *et al*, 1996; Yonemori *et al*, 1997). Expression of BMPs and/or BMPRs has also been detected in neoplasms, including osteosarcoma, prostate carcinoma, mammary carcinoma, gastric carcinoma, colorectal carcinoma, oral carcinoma, and salivary gland tumor, suggesting that BMP signals affect not only osteoplastic potential but also tumor development and progression (Yoshikawa *et al*, 1994; Katoh and Terada, 1996; Kusafuka *et al*, 1998; Arnold *et al*, 1999; Kim *et al*, 2000; Imai *et al*, 2001; Jin *et al*, 2001). Immunohistochemical expression of BMP has been examined in various odontogenic tumors, and BMP reactivity has been recognized in mesenchymal and mixed odontogenic tumors but not in epithelial odontogenic tumors, including ameloblastoma, AOT, and calcifying epithelial odontogenic tumor (Gao *et al*, 1997). In the present study, mRNA expression of BMP-2, -4, -7, BMPR-IA, and -II was detected in all epithelial odontogenic tumors by RT-PCR. Immunohistochemical reactivity for these BMPs and BMPRs was identified in both neoplastic and stromal cells of epithelial odontogenic tumors, suggesting that these BMPs act via paracrine and autocrine mechanisms and are involved in epithelial–mesenchymal interactions. Expression of BMPs and BMPRs was evident in neoplastic cells neighboring the basement membrane in benign and metastasizing ameloblastomas and in spindle neoplastic cells forming whorled masses and pseudoglandular cells in AOTs. These features suggest that these BMP signals influence the cytodifferentiation of neoplastic odontogenic epithelium. Our previous study revealed increased immunoreactivity for TGF-β receptors in keratinizing cells in acanthomatous ameloblastomas (Kumamoto *et al*, 2002). In the present study, acanthomatous ameloblastomas showed strong BMP-7 reactivity in keratinizing cells. These features suggest that signaling of these TGF-β superfamily

members might be associated with cell death of neoplastic odontogenic epithelium. Previous studies have demonstrated up-regulation of TGF-β and its receptors in desmoplastic ameloblastomas (Takata *et al*, 2000; Kumamoto *et al*, 2002), whereas our study showed no obvious expression of BMPs or BMPRs in these tumors. Our study also showed pretty low reactivity for these BMPs and their receptors in ameloblastic carcinomas, indicating a departure from odontogenic differentiation because of malignant transformation of odontogenic epithelium.

Core-binding factor α1, first identified as a T lymphocyte-specific factor, has been shown to be essential for osteoblast differentiation by the laboratory works of knockout mice (Ogawa *et al*, 1993; Komori *et al*, 1997; Otto *et al*, 1997). Expression of Cbfa1 has been recognized in tooth germ tissues, and *Cbfa1*-deficient mice show severely abnormal tooth formation (D'Souza *et al*, 1999). Mutations in *CBFA1* cause cleidocranial dysplasia, an autosomal-dominant disorder in humans characterized by defective bone formation as well as dental defects, including supernumerary teeth, prolonged retention of deciduous teeth, and delayed eruption of permanent dentition (Mundlos *et al*, 1997). In the present study, expression of CBFA1 was identified in human tooth germ tissues by RT-PCR and immunohistochemistry. No bone formation occurs in osterix-null mice, suggesting that osterix is required for osteoblast differentiation and bone formation (Nakashima *et al*, 2002). To date, the roles of osterix in tooth development remain unclear. In this study, osterix mRNA was identified in all tooth germ samples, suggesting that this transcription factor might also play a certain role in tooth formation.

Cbfa1 cooperates strongly to induce T-cell lymphoma in transgenic mice ectopically expressing *c-myc* in T lymphocytes (Stewart *et al*, 1997). Expression of CBFA1 has been recognized in some human tumors, including renal cell carcinoma, prostate carcinoma, mammary carcinoma, malignant melanoma, and giant cell tumor of bone, and transcriptional controls by CBFA1 are apparently involved in the progression of these neoplastic cells (Yang *et al*, 2001; Barnes *et al*, 2003; Riminucci *et al*, 2003; Huang *et al*, 2004; Van Wijngaarden *et al*, 2004). In the present study, CBFA1 mRNA was detected in all epithelial odontogenic tumors by RT-PCR. Immunohistochemically, nuclear CBFA1 expression was recognized scatteredly in central neoplastic cells in benign and metastasizing ameloblastomas and in spindle cells forming whorled masses in AOTs, as well as in some stromal fibroblasts; moreover, ameloblastic carcinomas showed low CBFA1 reactivity in neoplastic cells. These features suggest that CBFA1 affects the differentiation of neoplastic odontogenic epithelial cells through transcriptional controls. Osterix transcripts have been detected in human bone lesions, such as giant cell tumor of bone and periapical inflammatory lesions (Huang *et al*, 2004; Maeda *et al*, 2004), while the roles of osterix in neoplastic development are unclear. In the present study, epithelial odontogenic tumors expressed osterix mRNA, as did tooth germ tissues. These features suggest that this transcriptional regulator of osteogenesis potentially functions as a differentiation factor of odontogenic tissues, similar to BMPs and CBFA1.

In conclusion, expression of BMPs, their receptors, CBFA1, and osterix in tooth germs and epithelial odontogenic tumors suggests that these regulators of developmental processes might play a role in cytodifferentiation of normal and neoplastic odontogenic epithelium via epithelial–mesenchymal interactions.

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