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Expression of tumor necrosis factor α, TNF-related apoptosis-inducing ligand, and their associated molecules in ameloblastomas

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BACKGROUND: To clarify the roles of the apoptosis signaling pathway mediated by death receptors in oncogenesis and cytodifferentiation of odontogenic tumors, expression of tumor necrosis factor α (TNFα), TNF-related apoptosis-inducing ligand (TRAIL), and their associated molecules was analyzed in ameloblastomas as well as in tooth germs.

METHODS: Tissue specimens of 10 tooth germs, 40 benign ameloblastomas, and five malignant ameloblastomas were examined by reverse transcriptase–polymerase chain reaction (RT-PCR) and immunohistochemistry to determine the expression of TNFα, TNF receptor I (TNFRI), TRAIL, TRAIL receptor 1 (TRAIL-R1), TRAIL-R2, caspase-8, and nuclear factor-κB (NF-κB).

RESULTS: Expression of TNFα, TNFRI, TRAIL, TRAIL-R1, TRAIL-R2, and NF-κB mRNA was detected in most samples of normal and neoplastic odontogenic tissues. Expression of caspase-8 mRNA was identified in six of 33 ameloblastomas, but not in 10 tooth germs or one malignant ameloblastoma. Immunohistochemical reactivity for TNFα, TRAIL, their receptors, and NF-κB was detected in both normal and neoplastic odontogenic tissues. Epithelial expression of TNFα was focal in about 50% of tooth germs and ameloblastomas, and TNFα expression in neoplastic cells was significantly higher in follicular ameloblastomas than in plexiform ameloblastomas. TRAIL reactivity was evident in epithelial cells neighboring the basement membrane. Receptors for TNFα and TRAIL were diffusely expressed in both normal and neoplastic odontogenic epithelium. Expression of caspase-8 was found in some neoplastic cells in three of 37 ameloblastomas, but not in 10 tooth germs or five malignant ameloblastomas. Nuclear NF-κB expression was much lower than cytoplasmic expression in both normal and neoplastic odontogenic epithelium.

CONCLUSION: Expression of TNFα, TRAIL, and their receptors in tooth germs and ameloblastomas suggests that these death factors might be involved in cytodifferentiation of odontogenic epithelium and tissue structuring of ameloblastomas. Expression of caspase-8 and NF-κB suggests that signaling of TNFα and TRAIL minimally affects the biological properties of odontogenic epithelial components.

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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 (1–4). 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, 4). Histologically, ameloblastoma shows considerable variation, including follicular, plexiform, acanthomatous, granular cell, basal cell, and desmoplastic types (1). 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 (1). Recently, malignant ameloblastoma has been subclassified into metastasizing ameloblastoma and ameloblastic carcinoma on the basis of metastatic spread and cytological malignant features (3). Several recent studies have detected genetic and cytogenetic alterations in these epithelial odontogenic tumors (5, 6); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

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There are two alternative pathways that initiate apoptosis: one is mediated by mitochondria, and another is mediated by death receptors on the cell surface (7). Tumor necrosis factor α (TNFα) and TNF-related apoptosis-inducing ligand (TRAIL), members of the TNF family, are natural ligands for the death receptors (death ligands) and can interact with several distinct receptors (7, 8). TNF receptor I (TNFRI), TRAIL receptor 1 (TRAIL-R1), and TRAIL-R2, members of the TNFR family, are death receptors activated by their respective ligands and contain intercellular death domains essential for the transmission of death signals (8, 9). When the ligands bind to their death receptors, the death domains attract adaptor proteins, which recruit the inactive proform of caspase-8, an initiator of apoptosis (9, 10). The death receptors for TNFα and TRAIL also activate nuclear factor-κB (NF-κB), a transcription factor encoding apoptosis inhibitory and cell survival factors, via other adaptor proteins (9, 11). Thus, signaling of TNFα and TRAIL functions as a regulator of both cell death and survival, and aberration of the signaling pathway has been identified in a variety of tumors (12–19).

Our previous studies confirmed apoptotic cells and apoptosis-related factors, such as the bcl-2 family proteins, the inhibitor of apoptosis protein (IAP) family proteins, p53 and its associates, Fas, Fas ligand, and heat shock proteins, in tooth germs and ameloblastomas, suggesting that apoptotic cell death has an important role in oncogenesis or cytodifferentiation of odontogenic epithelium (20–25). In the present study, expression of TNFα, TRAIL, and their associated molecules in benign and malignant ameloblastomas as well as in tooth germs was examined using reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry to clarify the possible role of these death factors and their signaling in epithelial odontogenic tumors.

Materials and methods

Tissue preparation

Specimens were surgically removed from 45 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 World Health Orgaization histological typing of odontogenic tumors (1). The tumors comprised 40 ameloblastomas and five malignant ameloblastomas. Ameloblastomas were divided into 22 follicular and 18 plexiform types, including 13 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 (3). 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, enucleated for orthodontic reasons at the initial stage of crown mineralization, were similarly prepared and compared with the epithelial odontogenic tumors.

RT-PCR

Total RNA was extracted from frozen tissue samples of 10 tooth germs, 33 ameloblastomas, 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). Primers used in this study are listed in Table 1. A housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used as an internal control for the examination of 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 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.

Immunohistochemistry

The tissue sections of 10 tooth germs, 37 ameloblastomas, and five malignant ameloblastomas 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). After treatment with normal serum for 30 min, the sections were incubated with primary antibodies at 4°C overnight. The applied antibodies are listed in Table 1. 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 methylgreen. For control studies of the antibodies, the serial sections were treated with phosphate-buffered saline, mouse anti-OPD4 (CD45RO) monoclonal antibody (subclass IgG1; Dako, Glostrup, Denmark), and normal goat IgG instead of the primary antibodies and were confirmed to be unstained.

Statistical analysis

The statistical significance of differences in the percentages of cases with mRNA/cDNA expression or with immunohistochemical reactivity was analyzed by the Mann–Whitney *U*-test for differences between two

Table 1 Primers and antibodies

	Primer		*Antibody*		
	Sequence 5′-3′	*Product (bp)*	*Clonality*	*Source*	*Dilution*
TNFα	Forward: TAGCCCATGTTGTAGCAAACCCTCAAGCT Reverse: TCACAGGGCAATGATCCCAAAGTAGACCT	428	Monoclonal (Mouse IgG1)	Santa Cruz Biotechnology, Santa Cruz, CA, USA	1:20
TNFRI	Forward: ACCAAGTGCCACAAAGGAAC Reverse: CTGCAATTGAAGCACTGGAA	263	Polyclonal (Goat IgG)	R&D Systems, Minneapolis, MN, USA	1:20
TRIAL	Forward: GGGACCCCAATGACGAAGAG Reverse: CAACTAAAAAGGCCCCGAAAAA	648	Monoclonal (Mouse IgG1)	Novocastra, Newcastle, UK	1:30
TRIAL-R1	Forward: CGATGTGGTCAGAGCTGGTACAGC Reverse: GGACAGGCAGAGCCTGTGCCATC	216	Polyclonal (Goat IgG)	R&D Systems	1:20
TRIAL-R2	Forward: GGGAGCCGCTCATGAGGAAGTTGG Reverse: GGCAAGTCTCTCTCCCAGCGTCTC	151	Polyclonal (Goat IgG)	Santa Cruz Biotechnology	1:30
Caspase-8	Forward: TCTGGAGCATCTGCTGTCTG Reverse: CCTGCCTGGTGTCTGAAGTT	436	Monoclonal (Mouse IgG1)	Novocastra	1:20
NF-κB	Forward: CACTTATGGACAACTATGAGGTCTCTGG Reverse: CTGTCTTGTGGACAACGCAGTGGAATTTTAGG	406	Monoclonal (Mouse IgG1)	Santa Cruz Biotechnology	1:30
GAPDH	Forward: GGAGTCAACGGATTTGGT Reverse: GTGATGGGATTTCCATTGAT	206			

groups or the Kruskal–Wallis test for differences among three or more groups. *P*-values of <0.05 were considered to indicate statistical significance.

Results

mRNA expression

The RT-PCR analysis identified expression of mRNA transcripts for TNFα, TRAIL, and their associated molecules in normal and neoplastic odontogenic tissue (Fig. 1; Table 1). Expression of TNFα mRNA was detected in all tooth germs and ameloblastomas, except for two benign ameloblastomas. TNFRI mRNA was expressed in seven of 10 tooth germs, 25 of 33 ameloblastomas, and one of one malignant ameloblastoma. TRAIL mRNA was recognized in eight of 10 tooth germs, 29 of 33 ameloblastomas, and one of one malignant ameloblastoma. Expression of TRAIL-R1, TRAIL-R2, and NF-κB mRNA was detected in all 33 ameloblastomas and the one malignant ameloblastoma as well as the 10 tooth germs. Caspase-8 mRNA was identified in six benign ameloblastomas, but not in tooth germs or the malignant ameloblastoma. There was no distinct difference in mRNA expression of TNFα, TRAIL, and their associated molecules among the different types or subtypes of ameloblastomas. Differences in the RT-PCR profiles of these molecules did not reach statistical significance.

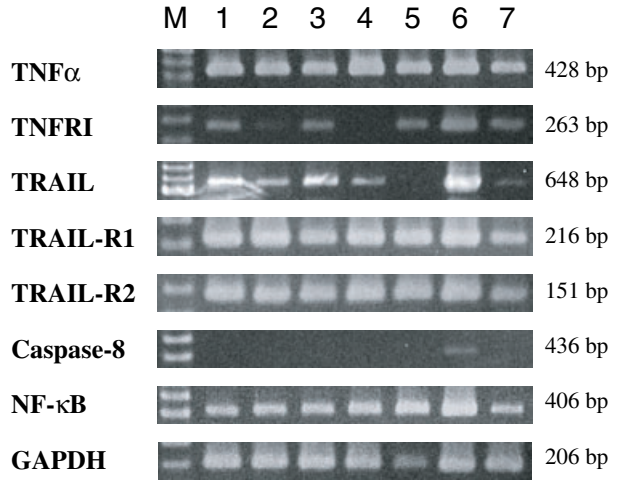

Figure 1 RT-PCR analysis of mRNA transcripts for TNFα, TRAIL, and their associated molecules in tooth germs and ameloblastomas. (M: molecular-weight standard; 1, 2: tooth germs; 3–6: ameloblastomas; 7: malignant ameloblastoma) GAPDH was run as a control to ascertain the integrity of mRNA/cDNA.

Immunohistochemical reactivity

Immunohistochemical reactivity for TNFα and TRAIL was detected in the cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Figs 2a and 3a,b; Table 2). Endothelial cells in tooth germs and benign and malignant ameloblastomas were reactive with anti-TNFα and anti-TRAIL antibodies. In four of 10 tooth germs, faint expression of TNFα was found in inner enamel epithelium. Ameloblastomas showed faint TNFα reactivity in neoplastic cells neighboring the basement membrane in 13 of 21 follicular cases and five of 16 plexiform cases (Fig. 2a). TNFα expression in follicular ameloblastomas was significantly higher than that in plexiform ameloblastomas ($P < 0.05$). Keratinizing cells and granular cells did not show TNFα reactivity in acanthomatous or granular cell ameloblastomas. One basal cell ameloblastoma and two desmoplastic ameloblastomas exhibited faint TNFα reactivity in most neoplastic cells. One metastasizing ameloblastoma showed a TNFα expression pattern similar to that of benign ameloblastomas, and one ameloblastic carcinoma demonstrated faint TNFα reactivity in some neoplastic cells. Weak TRAIL reactivity was found in inner and outer enamel epithelium and dental lamina of all tooth germ tissues. In 20 follicular, 15 plexiform, and two metastasizing ameloblastomas, TRAIL was expressed in most peripheral columnar or cuboidal cells and in fewer central polyhedral cells (Fig. 3a). Keratinizing cells in acanthomatous ameloblastomas showed decreased

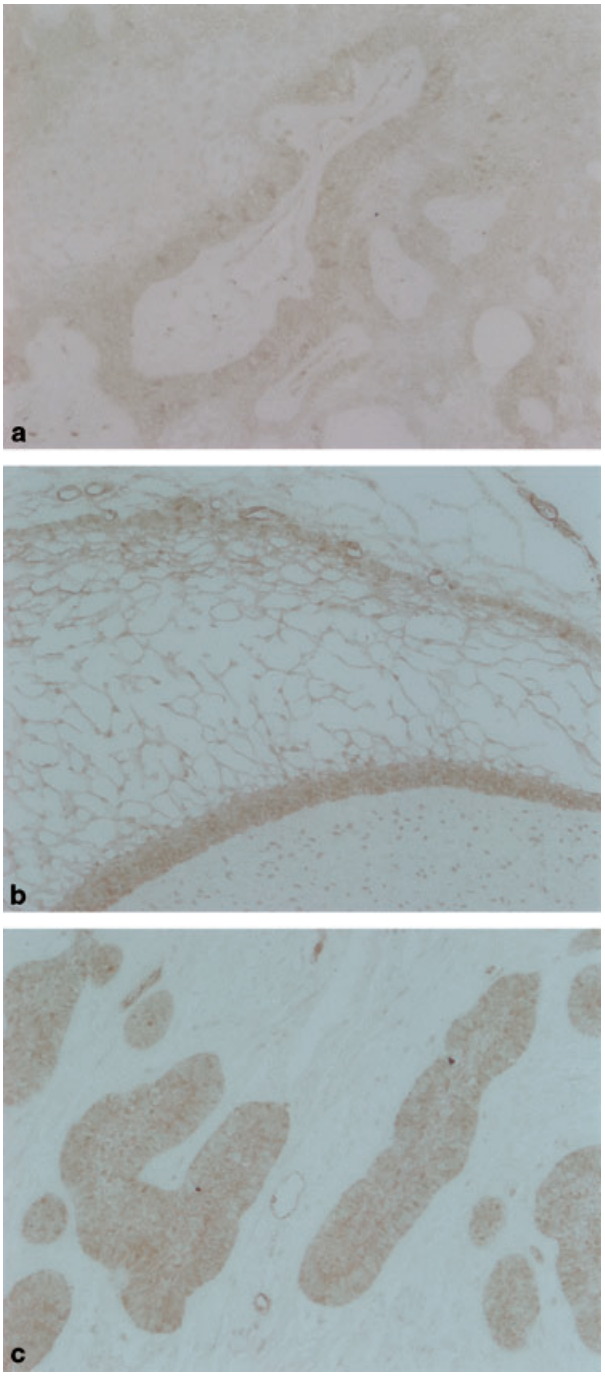

Figure 2 Immunohistochemical reactivity for TNFα (a) and TNFRI (b, c). (a) Plexiform ameloblastoma showing TNFα reactivity in neoplastic cells neighboring the basement membrane. Stromal endothelial cells are also reactive (×105). (b) Tooth germ showing diffuse TNFRI reactivity in enamel organ. Mesenchymal cells are also reactive in dental papilla and dental follicle (×105). (c) Follicular ameloblastoma showing TNFRI reactivity in most neoplastic cells. Stromal fibroblasts and endothelial cells are also reactive (×95).

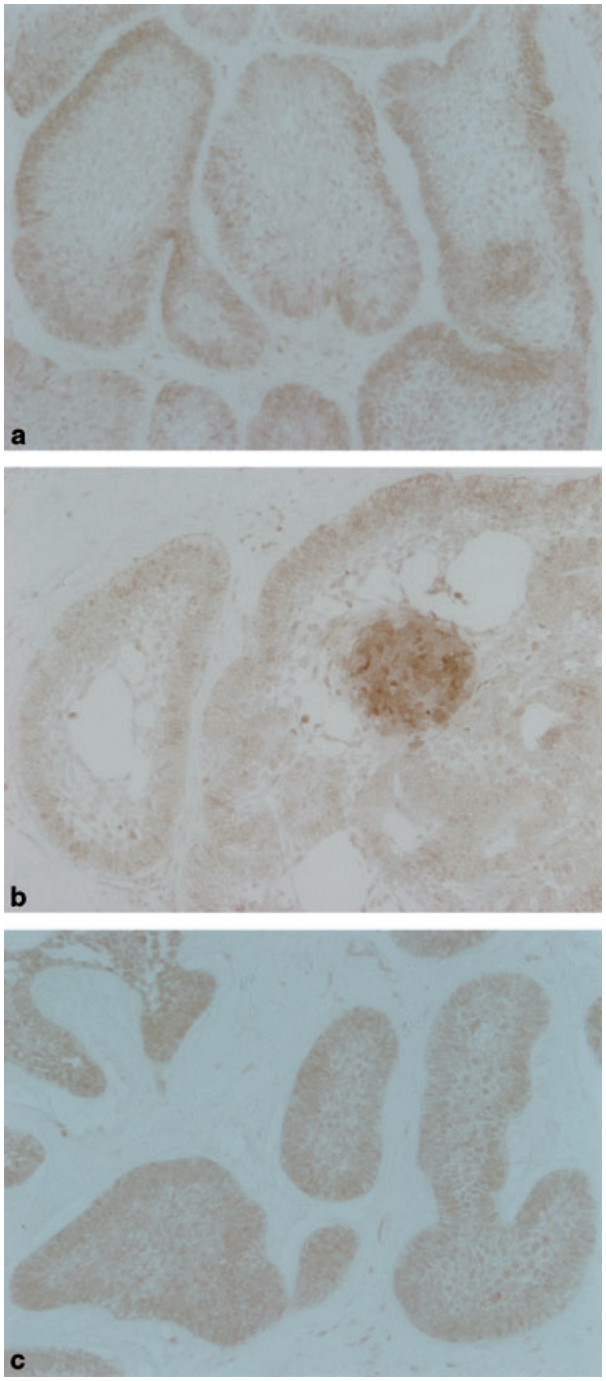

Figure 3 Immunohistochemical reactivity for TRAIL (a, b) and TRAIL-R1 (c). (a) Follicular ameloblastoma showing TRAIL reactivity in most peripheral columnar cells and fewer central polyhedral cells. Stromal endothelial cells are also reactive (×115). (b) Granular cell ameloblastoma showing marked TRAIL reactivity in granular cells (×100). (c) Follicular ameloblastoma showing TRAIL-R1 in most neoplastic cells. Stromal endothelial cells are faintly reactive (×125).

TRAIL reactivity, while granular cells in granular cell ameloblastomas exhibited increased reactivity (Fig. 3b). Basal cell ameloblastomas, desmoplastic ameloblastomas, and ameloblastic carcinomas showed weak TRAIL reactivity in most neoplastic cells in all cases.

Expression of TNFRI, TRAIL-R1, and TRAIL-R2 was recognized in the cell membrane and cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Figs 2b,c and 3c). These death receptors were diffusely expressed in epithelial cells of tooth germs and in neoplastic cells of ameloblastomas in all samples.

Table 2 Expression of TNFα, TRAIL, and their associated molecules in tooth germs and ameloblastomas

	RT-PCR profile in whole tissue								*Immunohistochemical reactivity in epithelial component*							
	n	*TNFα (%)*	*TNFRI (%)*	*TRAIL (%)*	*TRAIL-R1 (%)*	*TRAIL-R2 (%)*	*Caspase-8 (%)*	*NF-κB (%)*	*n*	*TNFα (%)*	*TNFRI (%)*	*TRAIL (%)*	*TRAIL-R1 (%)*	*TRAIL-R2 (%)*	*Caspase-8 (%)*	*NF-κB (%)*
Tooth germ	10	10 (100)	7 (70)	8 (80)	10 (100)	10 (100)	0 (0)	10 (100)	10	4 (40)	10 (100)	10 (100)	10 (100)	10 (100)	0 (0)	10 (100)
Ameloblastoma	33	31 (94)	25 (76)	29 (88)	33 (100)	33 (100)	6 (18)	33 (100)	37	18 (49)	37 (100)	34 (92)	37 (100)	37 (100)	3 (8)	37 (100)
Follicular type	20	19 (95)	15 (75)	17 (85)	20 (100)	20 (100)	3 (15)	20 (100)	21	13 (62) *	21 (100)	20 (95)	21 (100)	21 (100)	2 (10)	21 (100)
Plexiform type	13	12 (92)	10 (77)	12 (92)	13 (100)	13 (100)	3 (23)	13 (100)	16	5 (31) *	16 (100)	15 (94)	16 (100)	16 (100)	1 (6)	16 (100)
Acanthomatous subtype	13	13 (100)	10 (77)	11 (85)	13 (100)	13 (100)	4 (31)	13 (100)	10	5 (50)	10 (100)	8 (80)	10 (100)	10 (100)	1 (10)	10 (100)
Granular subtype	4	4 (100)	2 (50)	3 (75)	4 (100)	4 (100)	0 (0)	4 (100)	6	2 (33)	6 (100)	6 (100)	6 (100)	6 (100)	0 (0)	6 (100)
Basal cell subtype	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	3	1 (33)	3 (100)	3 (100)	3 (100)	3 (100)	0 (0)	3 (100)
Desmoplastic subtype	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	4	2 (50)	4 (100)	4 (100)	4 (100)	4 (100)	0 (0)	4 (100)
Non-cellular variation	14	12 (86)	11 (79)	13 (93)	14 (100)	14 (100)	2 (14)	14 (100)	14	8 (57)	14 (100)	13 (93)	14 (100)	14 (100)	2 (14)	14 (100)
Malignant ameloblastoma	1	1 (100)	1 (100)	1 (80)	1 (100)	1 (100)	0 (0)	1 (100)	5	2 (40)	5 (100)	5 (100)	5 (100)	5 (100)	0 (0)	5 (100)
Metastasizing ameloblastoma	1	1 (100)	1 (100)	1 (80)	1 (100)	1 (100)	0 (0)	1 (100)	2	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	0 (0)	2 (100)
Ameloblastic Carcinoma	0	–	–	–	–	–	–	–	3	1 (33)	3 (100)	3 (100)	3 (100)	3 (100)	0 (0)	3 (100)

Statistical significance: $^*P < 0.05$.

TNFRI reactivity was also detected in mesenchymal cells of tooth germs and in stromal cells of ameloblastomas, while receptors for TRAIL were weakly expressed in endothelial cells of tooth germs and ameloblastomas.

Immunohistochemical reactivity for caspase-8 was detected in the cytoplasm of neoplastic odontogenic epithelial cells (Fig. 4a). Although tooth germs and malignant ameloblastomas showed no caspase-8 reactivity, benign ameloblastomas showed reactivity in many peripheral columnar or cuboidal cells and in some central polyhedral cells in two follicular cases and one plexiform case. Expression of NF-κB was recognized in the cytoplasm and a few nuclei of cellular components in normal and neoplastic odontogenic tissues (Fig. 4b,c). Fibroblastic cells in tooth germs and ameloblastomas were reactive with anti-NF-κB antibody. In all tooth germ tissues, NF-κB reactivity was strong in inner and outer enamel epithelium and dental lamina, and weak in stratum intermedium and stellate reticulum. Benign and metastasizing ameloblastomas showed NF-κB reactivity in most peripheral columnar or cuboidal cells and some central polyhedral cells in all cases (Fig. 4b). Keratinizing cells and granular cells showed markedly decreased NF-κB reactivity in acanthomatous and granular cell ameloblastomas. All basal cell ameloblastomas, desmoplastic ameloblastomas, and ameloblastic carcinomas showed NF-κB expression in most neoplastic cells (Fig. 4c).

Discussion

TNFα, originally identified as a tumoricidal protein, is a proinflammatory cytokine that functions as a primary mediator of inflammatory and immune responses and can complex two specific receptors, TNFRI and TNFRII (8, 26). TNFRI contains death domains and is responsible for the generation of cytotoxic death signals (9). Expression of TNFα and TNFRI has been identified in some human carcinomas, such as colorectal, mammary, and oral carcinomas, suggesting that these death factors influence the microenvironment of tumors (12, 13, 27). Mice deficient in TNFα are resistant to the development of benign and malignant skin tumors induced by several carcinogens (28). TNFα also induces osteoclast differentiation to activate bone resorption (8, 29). Expression of TNFα in dental follicles regulates processes involved in tooth eruption (30). Several cases of ameloblastoma have been found to express TNFα in neoplastic and endothelial cells (31). In the present study, expression of TNFα and TNFRI was detected in most normal and neoplastic odontogenic tissues, suggesting that these molecules might be associated with local bone resorption. In odontogenic epithelial components, TNFα was expressed focally in about 50% of tooth germs and ameloblastomas, whereas TNFRI expression was diffuse in all odontogenic tissues. Expression of these molecules did not distinctly differ between normal and neoplastic odontogenic tissues; however, expression of TNFα in neoplastic cells was higher in follicular ameloblastomas than in plexiform

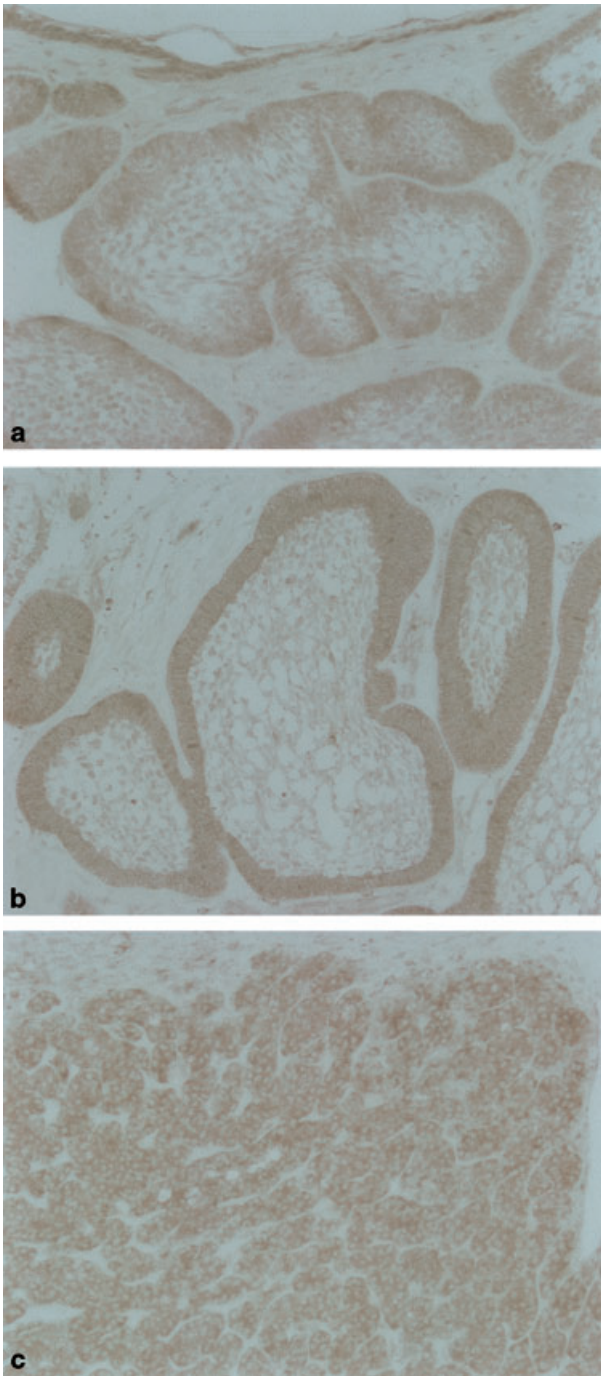

Figure 4 Immunohistochemical reactivity for caspase-8 (a) and NF-κB (b, c). (a) Follicular ameloblastoma showing caspase-8 reactivity in many peripheral columnar cells and some central polyhedral cells (×125). (b) Follicular ameloblastoma showing cytoplasmic NF-κB reactivity in most peripheral columnar cells and some central polyhedral cells. A few nuclei of peripheral neoplastic cells are positive. Stromal fibroblasts are also reactive (×120). (c) Ameloblastic carcinoma showing cytoplasmic NF-κB reactivity in most neoplastic cells. Stromal fibroblasts are also reactive (×90).

ameloblastomas, suggesting that TNFα production by neoplastic cells is involved in tissue structuring of ameloblastomas.

TRAIL is a recently discovered anticancer agent that selectively induces apoptosis in cancer cells and has minimal or no toxicity against normal tissues (32, 33). Five receptors for TRAIL, TRAIL-R1-5, have been identified, two of which, TRAIL-R1 and TRAIL-R2, carry death domains and transmit apoptotic signals (8, 34). Expression of TRAIL and its receptors has been detected in various human tumors, including lung, ovarian, colorectal, pancreatic, and oral carcinomas, suggesting that TRAIL signaling is involved in endogenous tumor surveillance (18, 19, 35–37). In the present study, TRAIL and its death receptors were expressed in most normal and neoplastic odontogenic tissues. TRAIL expression was rather frequent in odontogenic epithelial components, as compared with TNFα expression, and reactivity was obvious in epithelial cells neighboring the basement membrane in tooth germs and ameloblastomas. In ameloblastoma variants, keratinizing cells in acanthomatous ameloblastomas showed low TRAIL expression, while granular cells in granular cell ameloblastomas demonstrated increased TRAIL expression. These features suggest that expression of TRAIL might influence cytodifferentiation of odontogenic epithelium. Diffuse immunoreactivity for TRAIL-R1 and TRAIL-R2 was found in both normal and neoplastic odontogenic epithelium in addition to TNFRI reactivity. Our previous studies detected apoptotic cells detached from the basement membrane in tooth germs and ameloblastomas and confirmed marked increased apoptotic reactions in keratinizing cells and granular cells in ameloblastoma variants (20, 22). These features indicated no clear correlation between the localization of apoptotic cells and the expression of TNFα, TRAIL, and their death-inducing receptors in these odontogenic tissues.

Caspases are cysteine proteases required for programmed cell death, and 14 mammalian homologues, caspase-1–14, have been identified to date (38). Caspase-8 plays an important role as an apical caspase in death receptor signaling (7, 9, 10). Expression of *caspase-8* has been detected in some tumors, including lung carcinoma, malignant lymphoma, neuroblastoma, and rhabdomyosarcoma, and aberrations of caspase-8 gene, such as mutations and hypermethylation, have been identified in lung carcinoma and neuroblastoma (16, 17, 39–41). In the present study, expression of caspase-8 was found in some neoplastic cells in a few ameloblastomas, but not in tooth germs or malignant ameloblastomas by RT-PCR or immunohistochemistry, suggesting that the apoptosis pathway mediated by death receptors was not strongly activated in odontogenic epithelial components. The transduction pathway of death signals is known to be inhibited by the bcl-2 and IAP family proteins, as well as by cellular Fas-associated death domain protein (FADD)-like interleukin-1β converting enzyme (ICE) inhibitory protein (c-FLIP), an inhibitor of caspase-8 (42–44). Our previous studies detected some apoptosis inhibitory molecules belonging to the bcl-2 and IAP families in normal and neoplastic odontogenic epithelium (21, 25). The apoptosis pathway mediated by death receptors may be suppressed by these apoptosis inhibitors, and programmed cell death might be induced by other apoptosis pathways in odontogenic

epithelial components. NF-κB is an inducible transcription factor well known for its involvement in inflammatory and immune responses (11, 45). Unstimulated NF-κB remains sequestered in the cytoplasm by its inhibitor, inhibitor of NF-κB (IκB), whereas stimulated and liberated NF-κB translocates to the nucleus, where it regulates a variety of genes, including cell proliferation, survival, and anti-apoptotic proteins (11, 46). Constitutive activation of NF-κB has been detected in various malignancies, including mammary, prostatic, pancreatic, and colorectal carcinomas, suggesting that it is associated with oncogenesis and tumor progression (14, 15, 47, 48). Investigation of mice with suppressed NF-κB has revealed that NF-κB is essential for morphogenesis of bone, hair follicles, exocrine glands, and teeth (49). In the present study, nuclear NF-κB expression was much lower than cytoplasmic expression in both normal and neoplastic odontogenic epithelium, suggesting that NF-κB activation by TNFα or TRAIL was low. Thus, apoptotic cell death or cell survival in odontogenic epithelial components is considered to be minimally affected by signaling of TNFα and TRAIL; however, the specific expression of death receptors for TNFα and TRAIL suggests that these proteins are potential therapeutic targets for epithelial odontogenic tumors.

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