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Downregulation of matrix metalloproteinases in hyperplastic dental follicles results in abnormal tooth eruption

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In this study, we compared the gene expression profiles of non-syndromic hyperplastic dental follicle (HDF) fibroblasts and normal dental follicle (NDF) fibroblasts using cDNA microarrays, quantitative PCR, and immunohistochemical staining. Microarray analysis showed that several collagens genes were upregulated in the HDF's, including collagen types I, IV, VIII, and XI and TIMP-1, -3, and -4 (fold ratio > 2.0). In contrast, the expression of MMP-1, -3, -10, and -16 together with IL-8 was more than two fold downregulated. The differential expression of the genes encoding alkaline phosphatase, MMP-1, -3, -8, and IL-8 was confirmed by quantitative RT-PCR, while that of 24 HDFs and 18 NDFs was confirmed by immunohistochemical analysis. However, HDFs showed stronger expression of MMP-3 than NDFs ($P < 0.001$). Collectively, these results indicate that defective regulation of MMPs mediating connective tissue remodeling may be responsible for abnormal tooth eruption. [BMB reports 2008; 41(4): 322-327]

INTRODUCTION

Tooth eruption is a complex mechanism which requires an eruption force from the erupting tooth and the degradation of overlying tissue. The osteoclastic removal of bone barriers and enzymatic lysis of the connective tissue barrier are very important in creating an eruption pathway. Hence, abnormal tooth eruption is one sign of the dysfunction of osteoclasts in osteopetrotic animals (1). However, there is a lack of evidence that collagen metabolism is required for the eruption of teeth.

Loss of eruptive force is frequently observed, and is related to the prolonged retention of the deciduous tooth, or to tooth trauma (2). Some cases show a thick fibrous tooth follicle which hampers tooth eruption (3). While eruption disorders usually affect single teeth, multiple eruption disorders are usually syndrome-related (4). Non-syndromic multiple eruption disorders are rare (5).

The hyperplastic dental follicle (HDF) is related to tooth eruption disorder (6). Some HDF may be diagnosed as pericoronal myxofibrous hyperplasia (7) and these fibrous hyperplasias obstruct tooth eruption (7). Matrix metalloproteinases (MMPs) have an important role in tissue remodeling. During mouse tooth development, various MMPs are expressed in the branchial arch (8), and interactions between MMPs and tissue inhibitor metalloproteinases (TIMPs) are important for extracellular matrix turnover and tooth eruption (8). For example, MT1-MMP is a membrane-bound matrix metalloproteinase, which has been shown to be involved in pericellular matrix digestion by activating a pro-metalloproteinase, presumably pro-MMP-2 (9). Deficiency of MT1-MMP results in inhibition of both root development and tooth eruption (10). Therefore, proper remodeling of the connective tissue is essential for tooth eruption (10). Another example is the failure to digest collagen fiber, which results in an eruption disorder of the incisors in periostin knockout mice (11). Tooth eruption involves extensive remodeling of the surrounding extracellular matrix (12). In the erupting tooth, some osteoblasts and osteocytes degrade the extracellular matrix with MMPs and modulate the degradation by inhibiting the activity of MMPs via TIMPs (13). Therefore, it is plausible that abnormal connective tissue remodeling due to aberrant expression of MMPs and TIMPs causes the abnormal tooth eruption shown in non-syndromic HDFs.

In this study, we focused on the expression of MMPs and TIMPs in HDFs and NDFs. The differentially expressed genes were confirmed by real-time PCR and immunohistochemistry.

RESULTS

The microarray results are shown in Table 1. Genes related to connective tissue remodeling or differentially expressed between HDFs and NDFs were selected. Collagen type I, type IV, type VIII, and type XI showed significantly increased expression in HDFs (fold ratio > 2.0, Table 1). Tissue inhibitor metalloproteinase-1 (TIMP-1), TIMP-3, and TIMP-4 also significantly increased their expression (fold ratio > 2.0, Table 1) in HDFs. In contrast, the expression of MMP-1, MMP-3, MMP-10, and MMP-16 decreased (fold ratio < 0.5, Table 1). In particular, MMP-3 was about fold lower in NDFs than HDFs

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Table 1. The results of cDNA microarray

Title	GenBank	Chromosome	Fold ratio
Alkaline phosphatase	NM_000478	1p36.1-p34	6.033
Bone morphogenetic protein 4	NM_001202	14q22-q23	2.221
Collagen, type I, alpha 1	NM_000088	17q21.33	2.036
Collagen, type I, alpha2	NM_000089	7q22.1	2.779
Collagen, type IV, alpha 1	NM_001845	13q34	2.809
Collagen, type VIII, alpha 2	NM_005202	1p34.2	7.690
Collagen, type XI, alpha 1	NM_080629	1p21	2.414
Fibroblast growth factor 7	NM_002009	15q15-q21.1	3.493
Interleukin 26	NM_018402	12q15	3.115
TIMP-1	NM_003254	Xp11.3-p11.23	2.047
TIMP-3	AA837799	22q12.1-q13.2|22q12.3	3.272
TIMP-4	NM_003256	3p25	2.047
Interleukin 8	NM_000584	4q13-q21	0.183
MMP-1	NM_002421	11q22.3	0.225
MMP-3	NM_002422	11q22.3	0.095
MMP-10	NM_002425	11q22.3	0.344
MMP-16	NM_005941	8q21	0.434

(TIMP: tissue inhibitor matrix proteinase, MMP: matrix metalloproteinase)

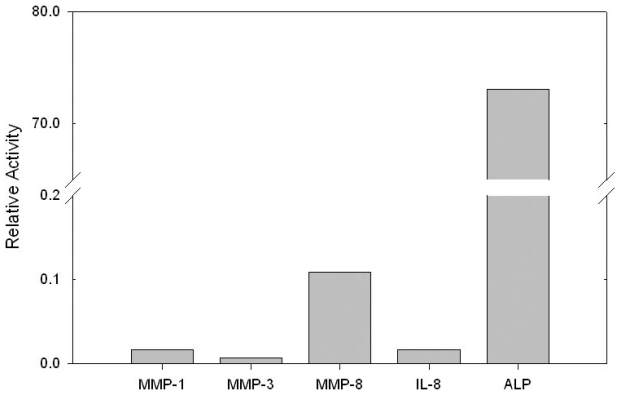

Fig. 1. RNA was extracted from each sample and quantitative RT-PCR performed using the primers shown in Table (Supplementary Materials). Values on the Y-axis indicate the ratios of patient/control (MMP: matrix metalloproteinase, IL: interleukin, ALP: alkaline phosphatase).

(Table 1). Other genes of interest that showed significantly higher expression in HDFs were bone morphogenetic protein-4 (BMP-4), interleukin 26, and fibroblast growth factor-7 (FGF-7) (fold ratio $>$ 2.0, Table 1). Interleukin 8 was down-regulated in HDFs compared to NDFs.

To confirm these differentially expressed genes, quantitative reverse transcriptase-polymerase chain reactions (RT-PCRs) were performed on selected genes including MMP-1, MMP-3, MMP-8, interleukin-8, and alkaline phosphatase. The relative expression of each gene is shown in Fig. 1. The fold ratios for MMP-1, MMP-3, MMP-8, and IL-8 were 0.017, 0.007, 0.108, and 0.017, respectively (Fig. 1). The relative expression of alkaline phosphatase was 73.08 (Fig. 1). These results are in accord

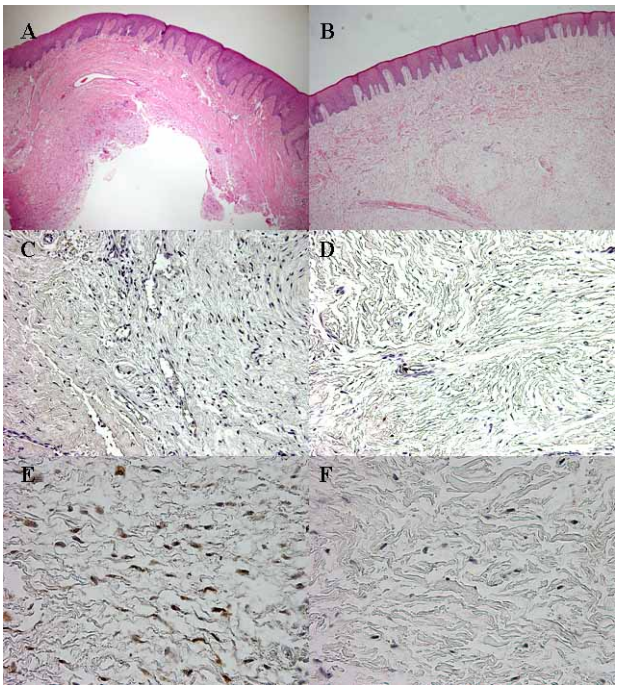

Fig. 2. Histological analysis. the fibrous component was less abundant in the control sample (A, C, E) than in the sample from hyperplastic dental follicles (B, D, F). The dental follicle was thinner in the control (A) than in the hyperplastic dental follicle (B) (H&E, original magnification x40). Though the expression of MMP-1 was higher in the control (C) than in the hyperplastic dental follicle (D) (original magnification x200), this was not evident in general. Expression of MMP-3 was clearly higher in the control (E) than in the hyperplastic dental follicle (F) (original magnification x400).

Table 2. Immunoreactivity in the control and the hyperplastic dental follicle

	Control			Hyperplastic dental follicle		
	0	+	++	0	+	++
MMP-1	7/18 (38.9%)	11/18 (61.1%)	0/18 (0%)	19/24 (79.2%)	5/24 (20.8%)	0/24 (0%)
MMP-3	3/18 (16.7%)	8/18 (44.4%)	7/18 (39.9%)	19/24 (79.2%)	5/24 (20.8%)	0/24 (0%)

($P < 0.001$ in MMP-3)

with the results of the microarray assay (Table 1).

Histological findings are presented in Fig. 2. The thickness of connective tissue was higher in HDFs than NDFs, as expected (Fig. 2A, B). The mean thickness of the dental follicle in the control was 2.08 ± 0.39 mm and 5.34 ± 3.34mm in HDFs (Table 2) ($P < 0.001$). In most control specimens, expression of both MMP-1 (11/18 cases, 61.1%) and MMP-3 (8/18 cases, 44.4%) was visible with a (+) degree of staining (Fig. 2, Table 2). In some control specimens, MMP-3 expression was strong (7/18 cases, 39.9%) with a (++) degree of staining (Table 2). In HDF specimens, however, expression was negligible for both MMP-1 (19/24 cases, 79.2%) and MMP-3 (19/24 cases, 79.2%) (Table 2). When the staining in the two groups was compared using the independent sample t-test, the difference was significant ($P < 0.001$). Some HDFs showed stronger staining for MMP-1 than the control, but the difference was not statistically significant ($P > 0.05$).

DISCUSSION

In this study, we showed abnormal expression of connective tissue remodeling genes in HDFs. The expression of MMPs was greatly decreased (fold ratio < 0.5, Table 1, Fig. 2) while the expression of collagen and TIMPs was significantly increased (fold ratios > 2.0, Table 1). These results indicate that misregulation of connective tissue remodeling in non-syndromic unerupted HDFs may be a cause of defective tooth eruption.

MMPs and TIMPs are important for matrix remodeling of connective tissue. Some studies have suggested that the abnormal expression of MMPs in animal models may be related to the delayed eruption of teeth. Mice deficient in membrane-type 1-MMP show delayed tooth eruption (10, 14). MMP-8 is widely expressed in the periodontium during tooth eruption, and its expression may be coordinated with that of collagens types I and III (15). MMP-2 is also implicated in mouse tooth development (8). Mice without cathepsin K, however, show normal tooth eruption despite increased expression of MMP-9 (16). These results indicate that only some MMPs may be important in tooth eruption. In our experiments, MMP-1, MMP-3, MMP-10, and MMP-16 showed significantly decreased expression (fold ratio < 0.5, Table 1). We think these MMPs may play important roles during tooth eruption.

Some genes related to collagen production showed increased expression in HDFs (fold ratio > 2.0, Table 1). These were collagens type I, type IV, type VIII, and type XI. It has been shown that major extracellular components of the periodontal ligament are collagens type I and III (17). The collagenous extracellular matrix is turned over during tooth eruption (18). The connective tissue of the gingiva contains collagen types I, III, IV, V, and VI and the distribution of collagen types varies in periodontitis (19). It seems possible that the upregulated expression of collagens (type I, IV, VIII, XI) in HDFs may result from misregulated connective tissue remodeling. Interestingly, FGF-7 is highly expressed in HDFs, and it may be one of the factors causing the upregulation of collagens, because it is highly expressed in cyclosporine-mediated gingival hyperplasia (20).

Multiple impacted teeth without a definite syndromic disease have been previously reported (5). In osteopetrosis, defective eruption of teeth and the formation of odontoma-like masses have been reported in mice (21). Impairment of bone resorption is closely related to dental abnormalities such as failure of tooth eruption and abnormal tooth morphogenesis (22). BMP-4 was overexpressed in HDFs (Table 1). BMP-4 is important in tooth morphogenesis and it is also highly expressed in odontogenic cysts (23). Though calcification nodules were not found in our samples, ectopic calcification has been frequently reported in tooth follicles (3, 7). The formation of an odontoma-like mass or ectopic calcification may be related to the overexpression of BMP-4. Interestingly, one BMP-4 downstream target gene, alkaline phosphatase, was highly increased in HDFs (Table 1, Fig. 1).

The thickness of connective tissue was increased in HDFs. The mean thickness of the dental follicle in the controls was 2.08 ± 0.39 mm, and it was 5.34 ± 3.34mm in HDFs. If the cases shown HDF are included , the mean thickness of the pericoronal space varies from 0.1 to 5.6 mm (24). When comparing them, the difference was significant ($P < 0.001$). This may be related to the overexpression of collagen (fold ratio > 2.0, Table 1). In the histological sections, the thickness of the epithelium was similar in the two groups but hyperplastic fibrous components were observed only in HDFs (Fig. 2A, B). The expression of MMP-1 in the paraffin sections showed a paint expression in both samples (Table 2). Though the mean expression of MMP-1 was higher in the control than in HDFs, the difference was not statistically significant ($P > 0.05$). However, the expression of MMP-3 was higher in the control

than in HDFs and the difference was statistically significant ($P < 0.001$, Table 2). In the microarray results, the fold ratio of MMP-3 (fold ratio: 0.095) was lower than that of MMP-1 (fold ratio: 0.225, Table 1), and in the quantitative RT-PCR the fold ratio of MMP-1 was 0.017 and that of MMP-3 was 0.007 (Fig. 1). Based on the results of the microarrays and quantitative RT-PCR, it seems clear that expression of MMP-3 in HDFs is more affected than that of MMP-1. BMP-4 was reported to induce the expression of MMP-1 and MMP-3 in oral epithelium (25). However, our results did not confirm this (Table 1). The signaling pathway may be different in oral epithelial cells and in the connective tissue fibroblasts in HDFs. Another explanation might be that the overexpression of BMP-4 in HDFs is due to the impaired expression of MMP-1 and MMP-3. However, the exact mechanism is not clear and further study is required. To the best of our knowledge, this is the first report that lack of MMP activity may be related to tooth eruption disorder in humans.

In conclusion, expression of MMP-1 and MMP-3 was reduced in HDFs. Since the expression of TIMPs and collagens was increased, the thick fibrous component in HDFs may be due to the abnormal expression of MMPs and TIMPs. Hence, misregulation of connective tissue remodeling in non-syndromic unerupted HDF may cause tooth eruption disorder.

MATERIALS AND METHODS

Cell cultures and DNA microarrays

Fibroblasts of HDF were taken from a freshly excised dental follicle of a patient who received window opening treatment. Window opening is a procedure that removes the pericoronal tissue of an impacted tooth to promote normal eruption or for orthodontic treatment. Gingival fibroblasts were taken from a second molar of a patient who received crown lengthening treatment. The cells were grown to 80% confluence in Dulbecco's modified Eagle's medium (Gibco, BRL, Gaithersburg, MD) containing 1% penicillin/streptomycin, hFGF (100 μg/ml), and 10% fetal bovine serum (FBS) in a 110mm culture dish in 37℃, 5% CO_2 conditions. After removing the culture medium, total RNA was extracted using Tri-Reagent (Molecular Research Center, Inc. Cincinnati, OH). The subsequent procedure was carried out according to the manufacturer's protocol. The concentration of total RNA was measured with a spectrophotometer.

For DNA microarrays, cDNA was synthesized from the extracted total RNA using a reverse transcriptase kit. The reactions were primed with 3 μl of oligo (dT) and 1 μl of a 10mM dNTP mixture. DEPC-treated tertiary distilled water was added to a total volume of 40 μl. The reaction proceeded at 65°C for 5 minutes, after which the temperature was slowly lowered to room temperature. 2 μl of 10× first-strand buffer, 4 μl of 25 mM $MgCl_2$, 2 μl of 0.1M DTT, 1 μl of RNase inhibitor (40 u/μl), and 1 μl of RTase were then added. The total reaction volume was 50 μl and the reaction proceeded at 42°C for 1 hour.

The extracted mRNA was transferred to chips, as follows (26, 27). 24,200 cDNA fragments from the subtracted library were PCR amplified from individual colonies (Genomictree, Daejon, Korea). They were arrayed as 22(row) × 23(col) × 48(pins) = 24,288 spots in multiple replicas, with each location corresponding to a unique cDNA clone. Genes whose expression is concentration dependent were placed on the microchip to serve as positive internal controls. These genes encoded: β-actin, GAPDH, tubulin-α, hexokinase 1, lactate dehydrogenase A, ribosomal protein L3, ribosomal protein L29, and transcription factor (E2A), and they were spotted at 0.5ng/ul, 2 ng/μl, 8 ng/μl, 32 ng/μl, 128 ng/μl, and 512 ng/μl,. These probes were hybridized with artificial mRNA.

The combined test pool was plotted against the average of the combined control pool. This generated a scatter plot that revealed a subset of genes that were up- or down-regulated by the static compressive force applied group. To improve the reliability, this study was repeated 3-times and the dye swap method was also used (28).

Quantitative RT-PCR

Five of the genes that were significantly up- or down-regulated in the microarrays (MMP-1, MMP-3, MMP-8, interleukin-8, and alkaline phosphatase) were selected for further examination by quantitative RT-PCR using a CYBR green PCR master mix according to the manufacturer's protocol. 3 μg of total RNA was reverse-transcribed and 200 ng of cDNA was used as template in each PCR. 25 μl of CYBR green PCR master mix and 1μl of 10 mM specific primers were combined with the template and water in a total volume of 50 μl. A negative control with no template was included in each assay. The cycle threshold (Ct) values corresponding to the PCR cycle number at which fluorescence emission in real time exceeded the threshold of the base-line emission were determined. The measured values were compared to the corresponding microarray data.

Histology and immunohistochemistry

Twenty-four HDFs were included in this study. The control normal dental follicle (NDF) samples (n=18) were obtained from normally erupting canines during the window opening procedure. The maximum thickness of the dental follicles was measured after window opening.

Goat primary polyclonal antibodies against MMP-1 (sc 6837) and MMP-3 (sc 6839) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and diluted 1:50. The positive control was a squamous cell carcinoma of the oral cavity, and immunostaining without primary antibody was used as a negative control. Sections of 5μm thickness were prepared and immunohistochemical staining was performed with Universal LSAB® + Kits (Dako, Glostrup, Denmark). Subsequent procedures followed the manufacturer's protocol and previous descriptions (23, 29). For antigen retrieval, trypsin was applied to each slide for 5 minutes at room temperature. The sections were counterstained with Mayer's hematoxylin.

Two pathologists reviewed all the slides blind to the original

histologic diagnoses, and evaluated them for intensity of staining. The intensity scales were (-) for invisible or trace staining, (+) for visible stain, and (++) for dense, strongly staining slides. Differences between groups were analyzed by the independent samples t-test. The significance level was set at $P < 0.05$.

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REFERENCES

1. Marks, S. C. Jr. (1981) Tooth eruption depends on bone resorption: experimental evidence from osteopetrotic (ia) rats. *Metab. Bone Dis. Relat. Res.* **3**: 107-115.
2. Shey, Z., Leach, P. and Vogel, R. T. (1983) Delayed tooth eruption as a result of trauma. *Pediatr. Dent.* **5**, 150-152.
3. Gomez, R. S., Silva, E. C., Silva-Filho, E. C. and Castro, W. H. (1998) Multiple calcifying hyperplastic dental follicles. *J. Oral Pathol. Med.* **27**, 333-334.
4. De Coster, P. J., Verbeeck, R. M., Holthaus, V., Martens, L. C. and Vral, A. (2006) Seckel syndrome associated with oligodontia, microdontia, enamel hypoplasia, delayed eruption, and dentin dysmineralization: a new variant? *J. Oral Pathol. Med.* **35**, 639-641.
5. Yildirim, D., Yilmaz, H. H. and Aydin, U. (2004) Multiple impacted permanent and deciduous teeth. *Dentomaxillofac. Radiol.* **33**, 133-135.
6. Walker, L. M., Wood, A. J., McDonald, A. and Carpenter, W. (2004) Unerupted mandibular second primary molar with an unusual histopathological finding: a case report. *J. Dent. Child* **71**, 77-79.
7. Yonemochi, H., Noda, T. and Saku, T. (1998) Pericoronal harmartomatous lesions in the opercula of teeth delayed in eruption: an immunohistochemical study of the extracellular matrix. *J. Oral Pathol. Med.* **27**, 441-452.
8. Sahlberg, C., Reponen, P., Tryggvason, K. and Thesleff, I. (1999) Timp-1, -2, and -3 show coexpression with gelatinases A and B during mouse tooth morphogenesis. *Eur. J. Oral Sci.* **107**, 121-130.
9. Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A. and Goldberg, G. I. (1995) Mechanism of cell surface activation of 72 kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. *J. Biol. Chem.* **270**, 5331-5338.
10. Beertsen, W., Holmbeck, K., Niehof, A., Bianco, P., Chrysovergis, K., Birkedal-Hansen, H. and Everts, V. (2002) On the role of MT1-MMP, a matrix metalloproteinase essential to collagen remodeling, in murine molar eruption and root growth. *Eur. J. Oral Sci.* **110**, 445-451.
11. Isao, K., Norio, A., Li, M., Satoshi, K., Yumiko, S. and Akira, K. (2006) Periostin is an extracellular matrix protein required for eruption of incisors in mice. *Biochem. Biophys. Res. Commun.* **342**, 766-772.
12. Marks, S. C. Jr. and Schroeder, H. E. (1996) Tooth eruption: theories and facts. *Anat. Rec.* **245**, 374-393.
13. Maruya, Y., Sasano, Y., Takahashi, I., Kagayama, M. and Mayanagi, H. (2003) Expression of extracellular matrix molecules, MMPs and TIMPs in alveolar bone, cementum and periodontal ligaments during rat tooth eruption. *J. Electron Microsc.* **52**, 593-604.
14. Bartlett, J. D., Zhou, Z., Skobe, Z., Dobeck, J. M. and Tryggvason, K. (2003) Delayed tooth eruption in membrane type-1 matrix metalloproteinase deficient mice. *Connect. Tissue Res.* **44**, 300-304.
15. Tsubota, M., Sasano, Y., Takahashi, I., Kagayama, M. and Shimauchi, H. (2002) Expression of MMP-8 and MMP-13 mRNAs in rat periodontium during tooth eruption. *J. Dent. Res.* **81**, 673-678.
16. Okaji, M., Sakai, H., Sakai, E., Shibata, M., Hashimoto, F., Kobayashi, Y., Yoshida, N., Okamoto, K., Yamamoto, K. and Kato, Y. (2003) The regulation of bone resorption in tooth formation and eruption processes in mouse alveolar crest devoid of cathepsin K. *J. Pharmacol. Sci.* **91**, 285-294.
17. Huang, Y. H., Ohsaki, Y. and Kurisu, K. (1991) Distribution of type I and type III collagen in developing periodontal ligament of mice. *Matrix* **11**, 25-35.
18. MacNeil, R. L., Berry, J. E., Strayhorn, C. L., Shigeyama, Y. and Somerman, M. J. (1998) Expression of type I and XII collagen during development of the periodontal ligament in the mouse. *Arch. Oral Biol.* **43**, 779-787.
19. Buduneli, N., Atilla, G., Guner, G. and Oktay, G. (2001) Biochemical analysis of total collagen content and collagen types I, III, IV, V and VI in gingiva of various periodontitis categories. *J. Int. Acad. Periodontol.* **3,** 1-6.
20. Das, S. J., Parkar, M. H. and Olsen, I. (2001) Upregulation of keratinocyte growth factor in cyclosporin A-induced gingival overgrowth. *J. Periodontol.* **72**, 745-752.
21. Symons, A. L., Powell, R. N., Seymour, G. J. and Harbrow, D. J. (1989) Disturbance of tooth and eruption in the microphthalmic (mi) mouse: a light and electron microscopic study. *Arch. Oral Biol.* **34**, 71-76.
22. Felix, R., Hofstetter, W. and Cecchini, M. G. (1996) Recent developments in the understanding of the pathophysiology of osteopetrosis. *Eur. J. Endocrinol.* **134**, 143-156.
23. Kim, S. G., Yang, B. E., Oh, S. H., Min, S. K., Hong, S. P. and Choi, J. Y. (2005) The differential expression pattern of BMP-4 between the dentigerous cyst and the odontogenic keratocyst. *J. Oral Pathol. Med.* **34**: 178-183.
24. Damante, J. H. and Fleury, R. N. (2001) A contribution to the diagnosis of the small dentigerous cyst or the paradental cyst. *Pesqui. Odontol. Bras.* **15**, 238-246.
25. Kim, S. G., Chae, C. H., Cho, B. O., Kim, H. N., Kim, H. J., Kim, I. S. and Choi, J. Y. (2006) Apoptosis of oral epithelial cells in oral lichen planus caused by upregulation of BMP-4. *J. Oral Pathol. Med.* **35**, 37-45.
26. Lee, Y. H., Nahm, D. S., Jung, Y. K., Choi, J. Y., Kim, S. G., Cho, M., Kim, M. H., Chae, C. H. and Kim, S. G. (2007) Differential gene expression of periodontal ligament cells after loading of static compressive force. *J. Periodontol.* **78**, 446-452.
27. Nguyen, L. L., Kriketos, A. D., Hancock, D. P., Caterson, I. D. and Denyer, G. S. (2006) Insulin Resistance Does Not Influence Gene Expression in Skeletal Muscle. *J. Biochem. Mol. Biol.* **39**, 457-463.
28. Yao, B., Rakhade, S. N., Li, Q., Ahmed, S., Krauss, R.,

Draghici, S. and Loeb, J. A. (2004) Accuracy of cDNA microarray methods to detect small gene expression changes induced by neuregulin on breast epithelial cells. *BMC Bioinformatics* **5**, 99.
29. Choi, S. H., Kim, S. Y., An, J. J., Lee, S. H., Kim, D. W., Won, M. H., Kang, T. C., Park, J., Eum, W. S., Kim, J. and Choi, S. Y. (2006) Immunohistochemical Studies of Human Ribosomal Protein S3 (rpS3). *J. Biochem. Mol. Biol.* **39**, 208-215.