ORAL MICROBIOLOGY AND IMMUNOLOGY

*Oral Microbiology Immunology 2009: 24: 384–389*
*Printed in Singapore. All rights reserved*



ORAL MICROBIOLOGY AND IMMUNOLOGY

# Regulatory roles of β-catenin and AP-1 on osteoprotegerin production in interleukin-1α-stimulated periodontal ligament cells

**T. Suda[1], T. Nagasawa[2], N. Wara-aswapati[1,3], H. Kobayashi[1], K. Iwasaki[4], R. Yashiro[1], D. Hormdee[3], H. Nitta[5], I. Ishikawa[6], Y. Izumi[1,7]**

[1]Section of Periodontology, Department of Hard Tissue Engineering, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Oral Rehabilitation Division of Periodontology and Endodontology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan, [3]Department of Periodontology, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand, [4]Department of Oral and Maxillofacial Surgery, Tokyo Women's Medical University, [5]Behavioral Dentistry, Department of Comprehensive Oral Care, Tokyo Medical and Dental University, Tokyo, Japan, [6]Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan, [7]Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo Medical and Dental University, Tokyo, Japan

*Suda T, Nagasawa T, Wara-aswapati N, Kobayashi H, Iwasaki K, Yashiro R, Hormdee D, Nitta H, Ishikawa I, Izumi Y. Regulatory roles of β-catenin and AP-1 on osteoprotegerin production in interleukin-1α-stimulated periodontal ligament cells.*
*Oral Microbiol Immunol 2009: 24: 384–389.* © 2009 John Wiley & Sons A/S.

**Background:** Periodontitis is a chronic inflammatory disease characterized by the enhanced expression of inflammatory mediators leading to alveolar bone resorption. Osteoprotegerin (OPG) plays a suppressive role in cytokine-induced osteoclastogenesis. In osteoblasts, OPG expression is upregulated by β-catenin but downregulated by the transcription factor activator protein-1 (AP-1; *c-fos*/*c-jun*). The purpose of this study was to examine the roles of β-catenin and AP-1 in interleukin-1α (IL-1α) -induced OPG production in human gingival fibroblasts (hGFs) and periodontal ligament (PDL) cells.
**Methods:** Expression of *c-fos* and c-*jun* messenger RNA was measured by reverse transcription-polymerase chain reaction and OPG production was analysed by enzyme-linked immunosorbent assay. The nuclear AP-1 activity was quantified using an AP-1 microplate assay. The effect of the Wnt canonical pathway on OPG production was evaluated using small interfering (si) RNA for β-catenin and the effect of AP-1 on OPG production was evaluated using the AP-1 inhibitor curcumin.
**Results:** Levels of c-*fos* messenger RNA and nuclear AP-1 activity were higher in PDL cells than in hGFs. When stimulated with IL-1α, PDL cells had significantly higher c-*fos* expression and lower OPG production compared with hGFs. The siRNA for β-catenin suppressed the IL-1α-induced OPG production in both PDL cells and hGFs, whereas the AP-1 inhibitor curcumin augmented the IL-1α-induced OPG production in PDL cells, but not in hGFs.
**Conclusion:** The present study suggests that β-catenin enhances IL-1α-induced OPG production in both PDL cells and hGFs, whereas AP-1 suppresses IL-1α-induced OPG production in PDL cells. Higher expression of c-*fos* in PDL cells than in hGFs may implicate a role of PDL cells in alveolar bone resorption in periodontitis.

Key words: activator protein-1; β-catenin; interleukin-1; osteoprotegerin; periodontitis

Tomonari Suda or Toshiyuki Nagasawa, Section of Periodontology, Department of Hard Tissue Engineering, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
Tel.: + 81 3 5803 5488; fax: + 81 3 5803 0196; e-mail: suda.peri@tmd.ac.jp or nagasawa@hoku-iryo-u.ac.jp

Accepted for publication March 31, 2009

Periodontal disease is an infectious disease characterized by chronic gingival inflammation and alveolar bone resorption that leads to tooth loss. In response to bacterial stimuli, inflammatory cytokines are produced in periodontal tissue and advance bone destruction. The receptor activator of nuclear factor-κB ligand (RANKL) is essential for osteoclast differentiation and is expressed in osteoblasts and activated lymphocytes. RANKL expression is increased in periodontitis tissue compared with healthy periodontal tissue (4, 20, 21). Osteoprotegerin (OPG) is a decoy receptor for RANKL (27). Alveolar bone resorption in periodontitis patients is regulated by the balance between RANKL and OPG in tissues with periodontitis (2, 20).

Human gingival fibroblasts (hGFs), periodontal ligament (PDL) cells and osteoblasts are major fibroblastic cells in the periodontal tissue. Among inflammatory mediators, interleukin-1 (IL-1) and prostaglandin $E_2$ ($PGE_2$) are actively involved in alveolar bone resorption (1, 11, 22, 25). IL-1 and $PGE_2$ increase RANKL expression in PDL cells (15, 24) and osteoblasts (13, 18), suggesting that alveolar bone resorption by IL-1 and $PGE_2$ might be related to the enhancement of RANKL in the periodontitis tissue. In addition, $PGE_2$ plays a suppressive role in OPG production in osteoblastic cells (28). In contrast to PDL cells and osteoblasts, we have reported that both IL-1 and $PGE_2$ stimulate hGFs to produce OPG (15, 16). The higher OPG production in hGFs than in osteoblastic cells might be a relevant mechanism involved in the role of gingival connective tissue to protect underlying alveolar bone.

Recently, several investigators have reported that the Wnt canonical pathway and activator protein-1 (AP-1) regulate OPG gene expression in osteoblasts (8, 9). *β*-Catenin is the molecular node of the Wnt canonical signaling pathway and plays a variety of important roles in bone development and remodeling (10, 17). This canonical pathway is essential for OPG production (9). AP-1 transcription factor is a dimeric complex composed of *c-fos* family and *c-jun* family proteins and plays a critical role in oncogenesis and normal skeletal growth and homeostasis (7, 30). It is also involved downstream of the protein kinase A (PKA) activation pathway, and suppresses the expression of OPG messenger RNA (mRNA) in osteoblasts (8). We have previously shown that IL-1α-stimulated OPG production in PDL cells and hGFs was stimulated by the protein kinase C signaling pathway and the PKA signaling pathway, respectively (15). Both the Wnt canonical pathway and AP-1 are activated by the PKA signaling pathway (8, 29), suggesting that *β*-catenin and AP-1 may be involved in the regulation of IL-1-stimulated OPG production in PDL cells and hGFs.

So far, there is no previous study reporting the involvement of Wnt signaling and AP-1 in OPG production in PDL cells and hGFs. The purpose of this study was to examine the effects of *β*-catenin and AP-1 on OPG production in PDL cells and hGFs stimulated with IL-1. The possible therapeutic approach of augmenting OPG in periodontal tissues by regulating these molecules is also discussed.

## Materials and methods

### Cell isolation and culture

The hGFs and PDL cells were isolated from four systemically healthy patients aged 20–55 years (one woman and three men; mean age 29.3 ± 17.2 years). Healthy gingival tissues were collected from the collar around orthodontically extracted teeth. PDL cells were explanted from the mid-surface of the root extracted for orthodontic reasons. Following isolation, these cells were cultured in α-minimum essential medium (α-MEM; Kojin Bio, Saitama, Japan) supplemented with 10% fetal bovine serum, penicillin and streptomycin (Wako, Osaka, Japan). The cells were subcultured and used within the range of four to nine passages in this study. The study was approved by the Ethics Committee of Tokyo Medical and Dental University.

### Transfection of small interfering RNA

*β*-Catenin small interfering (si) RNA (Qiagen, Hilden, Germany) and negative control siRNA (Qiagen) were used in this study. One day before transfection, hGFs and PDL cells were seeded on 96-well plates at a cell density of 50%. The siRNA (5 nM) was diluted with opti-MEM (Invitrogen Corp., Carlsbad, CA) and cells were transfected with siRNA (HyperFect transfection reagent; Qiagen) and incubated for 24 h. Efficacy of the transfection was determined using fluorescence-conjugated siRNA. Approximately 80% of PDL cells and hGFs were transfected with siRNA in this system. The efficacy of the transfection was the same for PDL cells and hGFs (data not shown). Following transfection, PDL cells and hGFs were stimulated with 1 ng/ml recombinant human IL-1α (Sigma-Aldrich, St Louis, MO) for 24 h.

### Cell treatment with curcumin

Periodontal ligament cells and hGFs were seeded into 96-well plates at $5 \times 10^3$ cells per well 1 day before treatment with curcumin (Sigma-Aldrich). Following a 1-h or 24-h treatment with curcumin, the cells were stimulated with 1 ng/ml recombinant human IL-1α for 24 h.

### Quantification of AP-1 activity

Nuclear extracts were prepared from unstimulated cells and cells incubated in the presence or absence of 25 μM curcumin for 1 h using a nuclear extraction kit (Marligen Biosciences Inc., Ijamsville, MD). AP-1 activity in the nuclear extracts was quantified using an AP-1 Transcription Factor Microplate Assay (Marligen Biosciences Inc.) based on the specific binding of transcription factors to cognate sequences on the labeled probes. The assays were performed according to the manufacturer's protocol.

### RNA isolation and reverse transcription-polymerase chain reaction

Preparation of RNA was carried out using TriPure isolation reagent (Roche, Indianapolis, IN) according to the manufacturer's instructions. Complementary DNA was synthesized with Ready-to-Go RT-PCR beads (GE Healthcare, Buckinghamshire, UK). Expression of periostin, S100 calcium-binding protein A4 (S100A4), periodontal-ligament-associated protein-1 (PLAP-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were examined using conventional RT-PCR. Primers for periostin, S100A4 and PLAP-1 were designed as follows: periostin forward ACTCTTTGCTCCCACCAATG 810–829, periostin reverse TGATCTCGCGG AATATGTGA 1455–1474; S100A4 forward AGCTTCTTGGGGAAAAGGAC 203–222, S100A4 reverse AACTTGCT CAGCATCAAGCA 475–494; PLAP-1 forward CCAATGTGTCCATTTGGATG 461–480; PLAP-1 reverse GAGGGTT TGCACTCATTTCC 844–863; GAPDH forward GTCAGTGGTGGACCTGAC CT, GAPDH reverse AGGGGTCTACA TGGCAACTG 732–1241.

The nucleotide sequences were derived from the following GenBank accession numbers: periostin, BC106709; S100A4, BC016300; PLAP-1, NM_017680; and GAPDH, NM_002046. Amplification parameters of the four genes were 94/30; 60/30; 72/30 [temperature (°C)/time (s)]. Cycle information is as follows: 25 cycles (periostin), 35 cycles (S100A4), 30 cycles (PLAP-1), and 27 cycles (GAPDH) in a thermal cycler (GeneAmp PCR system 9700; Perkin-Elmer, Norwalk, CT). The PCR products were subjected to electrophoresis on 1% agarose gels, and visualized using ethidium bromide.

The mRNA for c-*fos*, c-*jun* and GAPDH were quantified using a Light Cycler and SYBR green system (Roche). These primers were specifically designed for real-time PCR (c-*fos*, catalog no. 484217; c-*jun*, catalog no. 484231; *β*-catenin, catalog no. 4649966; GAPDH, catalog no. 486884; Search-LC, Heidelberg, Germany) and amplification parameters were set according to the manufacturer's instructions.

#### Measurement of OPG production

To measure the OPG production, enzyme-linked immunosorbent assay was performed according to the manufacturer's protocol using a recombinant human OPG-Fc chimera, monoclonal anti-human OPG/TNFRSF11B antibody and biotinylated anti-human OPG antibody (R&D Systems, Minneapolis, MN).

#### Statistical analysis

Data are expressed as the means ± standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA). When significance was reached with ANOVA, a Fisher post-test for least significant differences (Fisher PLSD) was performed to determine the groups responsible for the significant difference. The level of significance was set at 0.05.

## Results

#### Expression of c-*fos* and c-*jun* mRNA and activity of AP-1 in PDL cells and hGFs

RNA samples from PDL cells and hGFs were checked by RT-PCR using specific primers for periostin, S100A4 and PLAP-1, markers previously reported to be specifically expressed in PDL cells (5, 14, 31). In every hGF and PDL cell line from the same subject, PDL cells consistently expressed higher mRNA levels of periostin, S100A4 and PLAP-1 compared with hGFs (data not shown).

Expression of c-*fos* and c-*jun* mRNA was investigated in PDL cells and hGFs. The c-*fos* expression levels were quite low compared with the c-*jun* expression levels in both cell types. The basal c-*fos* mRNA expression was significantly higher in PDL cells than hGFs (Fig. 1A). The basal c-*jun* mRNA expression was also higher in PDL cells than in hGFs, but the difference was not statistically significant (Fig. 1B). IL-1α stimulation significantly enhanced mRNA expression of c-*fos* and c-*jun* in both PDL cells and hGFs. The IL-1α-stimulated c-*fos* expression in PDL cells was significantly higher than that in hGFs (Fig. 1A). The nuclear AP-1 activity in unstimulated PDL cells was significantly higher than in hGFs (Fig. 1C).

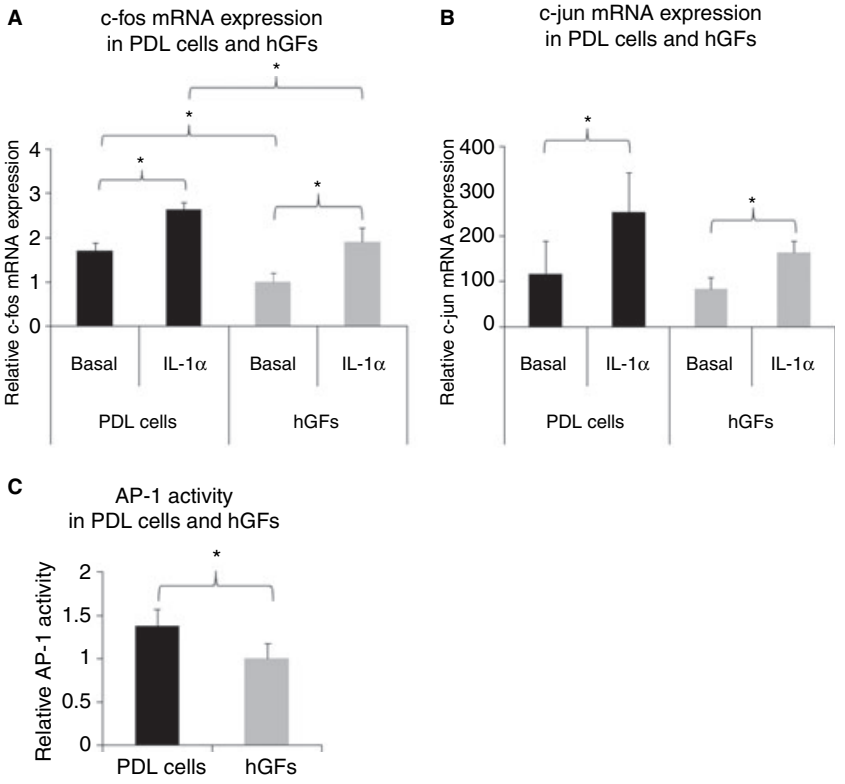


*Fig. 1.* Messenger RNA (mRNA) expression of c-*fos* (A) and c-*jun* (B) in periodontal ligament (PDL) cells and human gingival fibroblasts (hGFs). PDL cells and hGFs were either unstimulated (basal) or stimulated with 1 ng/ml interleukin-1α (IL-1α) for 30 min. Following RNA extraction from the cells, complementary DNA synthesis and real-time polymerase chain reaction analysis for c-*fos*, c-*jun* and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were carried out. The levels of c-*fos* and c-*jun* mRNA expression were expressed as ratios to that of GAPDH mRNA expression and presented as fold change relative to the c-*fos* levels in unstimulated hGFs. (C) Activator protein 1 (AP-1) activity of the nuclear extracts from unstimulated PDL cells and hGFs. Data are expressed as mean ± SD of three separate experiments. An asterisk represents statistical significance at $P < 0.05$.

#### Effect of β-catenin on OPG production in PDL cells and hGFs

The transfected siRNA for β-catenin suppressed about 60% of β-catenin mRNA expression in PDL cells (Fig. 2A). PDL cells produced lower OPG mRNA (Fig. 2B) and OPG protein (Fig. 2C) compared with hGFs (Fig. 2D,E) both at the basal stage and in response to IL-1α stimulation. Silencing β-catenin did not affect basal OPG mRNA and protein in either PDL cells or hGFs. Transfected siRNA for β-catenin significantly suppressed IL-1α-induced OPG production in both PDL cells and hGFs.

#### Effect of curcumin on OPG production in PDL cells and hGFs

Our results showed higher c-*fos* expression (Fig. 1A) and lower OPG production (Fig. 2C) in PDL cells compared with in hGFs. To investigate the role of AP-1 in the regulation of OPG production in PDL cells and hGFs, we pretreated cells with the AP-1 inhibitor curcumin for 1 h before IL-1α stimulation. In PDL cells, curcumin significantly reduced AP-1 activity (Fig. 3C). Curcumin significantly enhanced IL-1α-induced OPG production in PDL cells in a dose-dependent manner (Fig. 3A). In contrast, the effect of curcumin on OPG production in hGFs was not significant (Fig. 3B).

## Discussion

Periodontal ligament cells and hGFs, the major constituents of periodontal tissue, display distinct functional activities in inflammatory periodontal disease, as well as in the remodeling and repairing of periodontal tissue. Periostin (14), S100A4 (5) and PLAP-1 (31) have been identified as PDL-cell-specific markers. In the present study, PDL cells expressed higher levels of periostin, S100A4 and PLAP-1 than did hGFs (data not shown), suggesting that our cultured PDL cells and hGFs retained their different characteristics observed in periodontal tissue.

It has been reported that PDL cells possess osteoblastic characteristics, such as high alkaline phosphatase activity, and the ability to produce bone-like matrix proteins and to form mineralized nodules

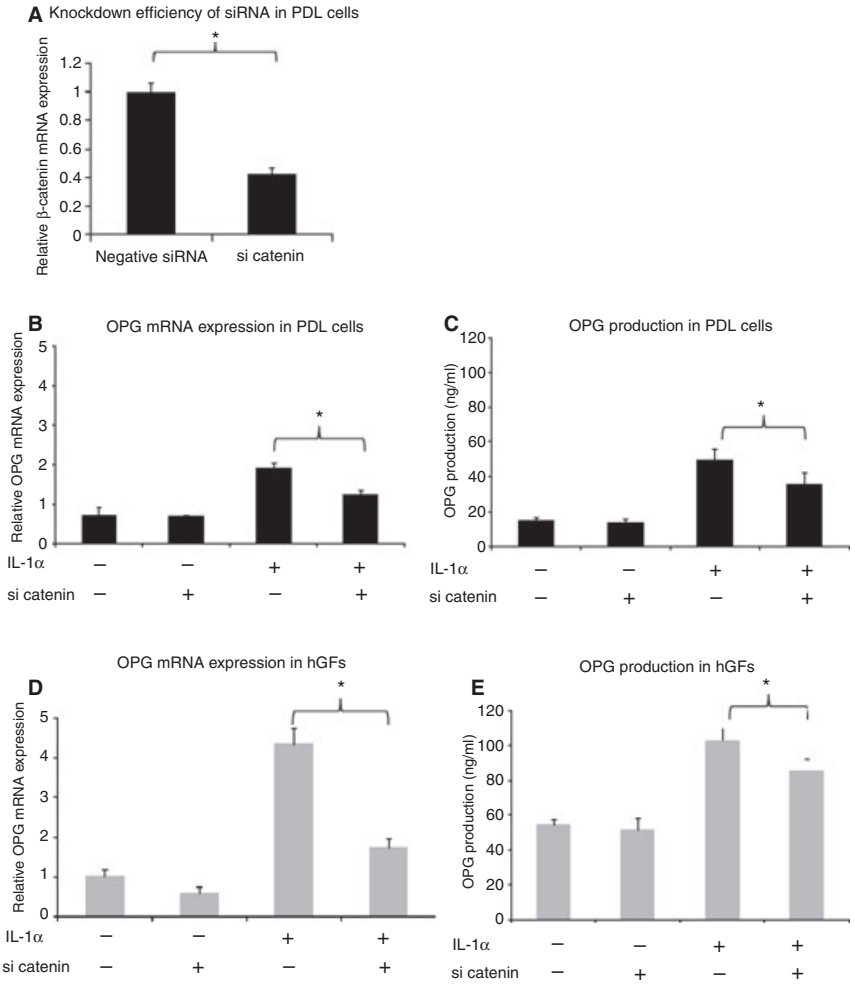


*Fig. 2.* (A) Knockdown efficiency of specific small interfering RNA (siRNA) of *β*-catenin (si catenin) at the messenger RNA (mRNA) level. *β*-Catenin mRNA expression in periodontal ligament (PDL) cells, which were transfected with specific siRNA of *β*-catenin or negative siRNA, was examined using real-time polymerase chain reaction (PCR). (B–E) Osteoprotegerin (OPG) mRNA expression and protein production in PDL cells and human gingival fibroblasts (hGFs) transfected with siRNA for *β*-catenin or negative siRNA. PDL cells and hGFs were transfected with 5 nM siRNA 24 h before stimulation with 1 ng/ml interleukin-1α (IL-1α). The cells were further incubated for 6 h for mRNA assay or for 24 h for protein assay. The mRNA and cultured supernatants were collected, and OPG mRNA and protein were measured by real-time PCR and enzyme-linked immunosorbent assay, respectively. Data are expressed as mean ± SD of three separate experiments. Asterisk represents statistical significance at $P < 0.05$.

(3, 23, 33). An *in vitro* study of osteoblast maturation demonstrated that levels of all fos and jun proteins were highest in proliferating osteoblasts and declined during the extracellular matrix maturation stage (19). Furthermore, AP-1 suppressed promoter activity of osteocalcin (19), a protein associated with the late stage of osteoblast differentiation and mineralization. S100A4 and PLAP-1 have also been shown to negatively regulate mineralization (6, 32). Hence, our results showing high expression levels of S100A4, PLAP-1 (data not shown) and *c-fos* (Fig. 1A) in PDL cells suggest that PDL cells are cognate with osteoblasts in the immature stage and preferentially express these markers to maintain the PDL characteristics, including inhibition of mineralization.

In osteoblasts, the dominant negative form of c-*fos* augments the basal expression of OPG mRNA, indicating that OPG production is suppressed by AP-1 transcription factor (8). In this study, we found that the expression level of c-*fos* in PDL cells and AP-1 activity of the nuclear extract from PDL cells were higher than that in hGFs, suggesting that the amount of AP-1 might be higher in PDL cells. OPG production was lower in PDL cells than in hGFs (Fig. 2). The AP-1 inhibitor curcumin, which interferes with AP-1/DNA binding and suppresses AP-1 transcription activity (26), was able to enhance the IL-1-induced OPG production in PDL cells (Fig. 3A). These results are consistent with previous report in osteoblasts indicating that AP-1 also inhibits OPG production in PDL cells. Our findings implicate some similarities between osteoblasts and PDL cells in the regulation of osteoclastogenesis. We speculate that the higher level of AP-1 caused by high expression of c-*fos* in osteoblastic cells, including osteoblasts and PDL cells, leads to lower OPG production in these cells compared with hGFs. Therefore, reduced OPG production in osteoblasts may result in the enhancement of osteoclastogenesis and bone resorption. The results of our present study circumstantially suggest that AP-1 may be involved in the suppression of OPG production in PDL cells. However, the effect of curcumin on the cell may not be restricted to only AP-1, and other molecules may also be involved in the regulation of OPG production in PDL cells.

In contrast to the inhibitory effect of AP-1, the activation of the Wnt canonical pathway augments OPG production in osteoblasts (9). In this study, we found that IL-1-induced OPG production in both PDL cells and hGFs is suppressed by the siRNA for *β*-catenin (Fig. 2), suggesting that the Wnt signaling pathway is involved in the augmentation of OPG production in these cells. We postulate that the net production of OPG in these cells depends on the interplay between the activation by the Wnt canonical pathway and the suppression by AP-1.

In osteoblasts, the activation of the PKA signaling pathway is important for osteoclastogenesis and bone resorption. OPG promoter activity in osteoblasts is temporarily augmented, and thereafter suppressed, by PKA signal activation, indicating that the regulation of OPG production by the PKA signaling pathway is biphasic (12). As AP-1 and the Wnt canonical pathway are mediated by the PKA signaling pathway (8, 29), regulation of OPG production by AP-1 and *β*-catenin in PDL cells and hGFs may be influenced by PKA signaling and as a consequence it is biphasic.

In conclusion, our study suggests that *β*-catenin and AP-1 differentially regulated OPG production in PDL cells and hGFs. The IL-1-induced OPG production is activated by the Wnt canonical pathway in both PDL cells and hGFs. Furthermore, our results circumstantially suggest that AP-1 may be involved in the suppression of OPG

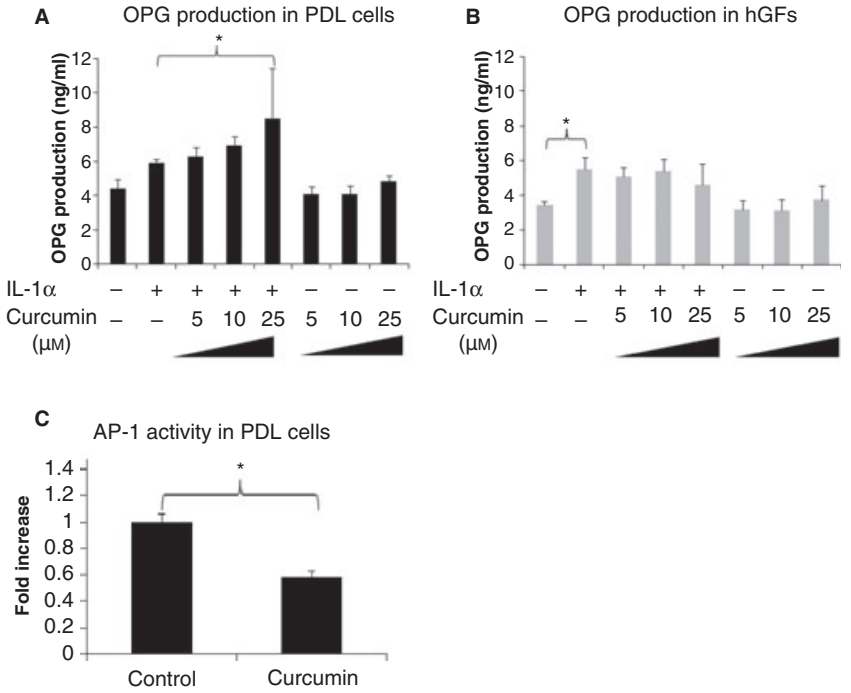


*Fig. 3.* Osteoprotegerin (OPG) production in periodontal ligament (PDL) cells (A) and human gingival fibroblasts (hGFs) (B) treated with the activator protein-1 (AP-1) inhibitor curcumin. PDL cells and hGFs were pretreated with various concentrations of curcumin for 1 h and stimulated with 1 ng/ml interleukin-1α (IL-1α) for 24 h. Cultured supernatants were collected and OPG production was measured by enzyme-linked immunosorbent assay. (C) AP-1 activity of the nuclear extract from PDL cells. Before nuclear extraction, PDL cells were treated with 25 μM curcumin for 1 h. Data are expressed as mean ± SD of three separate experiments. An asterisk represents statistical significance at $P < 0.05$.

production in PDL cells. The insignificant suppression by AP-1 in hGFs may involve a mechanism contributing to the higher OPG production in hGFs. These results implicate the potential of hGFs to protect the underlying alveolar bone and the role of PDL cells in bone resorption in periodontal disease. Therefore, inhibition of AP-1 and activation of β-catenin might be applicable therapeutic targets for preventing alveolar bone resorption in periodontitis.

## Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research (c)(2) (nos. 18592258 and 20592425), Ministry of Education, Culture, Sports, Science and Technology of Japan, JSPS Invitation Fellowship for Research in Japan (N.W.) and the grant from the Japanese Ministry of Education, Global Center of Excellence (GCOE) Program, "International Research Center for Molecular Science in Tooth and Bone Disease".

## References

1. Boch JA, Wara-aswapati N, Auron PE. Interleukin 1 signal transduction – current concepts and relevance to periodontitis. J Dent Res 2001: **80**: 400–407.
2. Bostanci N, Ilgenli T, Emingil G et al. Differential expression of receptor activator of nuclear factor-κB ligand and osteoprotegerin mRNA in periodontal diseases. J Periodontal Res 2007: **42**: 287–293.
3. Cho MI, Matsuda N, Lin WL, Moshier A, Ramakrishnan PR. *In vitro* formation of mineralized nodules by periodontal ligament cells from the rat. Calcif Tissue Int 1992: **50**: 459–467.
4. Crotti T, Smith MD, Hirsch R et al. Receptor activator NF κB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. J Periodontal Res 2003: **38**: 380–387.
5. Duarte WR, Kasugai S, Iimura T, et al. cDNA cloning of S100 calcium-binding proteins from bovine periodontal ligament and their expression in oral tissues. J Dent Res 1998: **77**: 1694–1699.
6. Duarte WR, Shibata T, Takenaga K et al. S100A4: a novel negative regulator of mineralization and osteoblast differentiation. J Bone Miner Res 2003: **18**: 493–501.
7. Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 2003: **3**: 859–868.
8. Fu Q, Jilka RL, Manolagas SC, O'Brien CA. Parathyroid hormone stimulates receptor activator of NFκB ligand and inhibits osteoprotegerin expression via protein kinase A activation of cAMP-response element-binding protein. J Biol Chem 2002: **277**: 48868–48875.
9. Glass DA 2nd, Bialek P, Ahn JD et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 2005: **8**: 751–764.
10. Glass DA 2nd, Karsenty G. *In vivo* analysis of Wnt signaling in bone. Endocrinology 2007: **148**: 2630–2634.
11. Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol 2003: **74**: 391–401.
12. Halladay DL, Miles RR, Thirunavukkarasu K, Chandrasekhar S, Martin TJ, Onyia JE. Identification of signal transduction pathways and promoter sequences that mediate parathyroid hormone 1–38 inhibition of osteoprotegerin gene expression. J Cell Biochem 2001: **84**: 1–11.
13. Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Interleukin-1β and tumor necrosis factor-α, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Bone 1999: **25**: 255–259.
14. Horiuchi K, Amizuka N, Takeshita S et al. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor β. J Bone Miner Res 1999: **14**: 1239–1249.
15. Hormdee D, Nagasawa T, Kiji M et al. Protein kinase-A-dependent osteoprotegerin production on interleukin-1 stimulation in human gingival fibroblasts is distinct from periodontal ligament fibroblasts. Clin Exp Immunol 2005: **142**: 490–497.
16. Kiji M, Nagasawa T, Hormdee D et al. Internal prostaglandin synthesis augments osteoprotegerin production in human gingival fibroblasts stimulated by lipopolysaccharide. Clin Exp Immunol 2007: **149**: 327–334.
17. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest 2006: **116**: 1202–1209.
18. Liu XH, Kirschenbaum A, Yao S, Levine AC. Cross-talk between the interleukin-6 and prostaglandin $E_2$ signaling systems results in enhancement of osteoclastogenesis through effects on the osteoprotegerin/receptor activator of nuclear factor-κB (RANK) ligand/RANK system. Endocrinology 2005: **146**: 1991–1998.
19. McCabe LR, Banerjee C, Kundu R et al. Developmental expression and activities of specific fos and jun proteins are functionally related to osteoblast maturation: role of Fra-2 and Jun D during differentiation. Endocrinology 1996: **137**: 4398–4408.
20. Nagasawa T, Kiji M, Yashiro R et al. Roles of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin in periodontal health and disease. Periodontol 2000 2007: **43**: 65–84.
21. Nagasawa T, Kobayashi H, Kiji M et al. LPS-stimulated human gingival fibroblasts inhibit the differentiation of monocytes into osteoclasts through the production of osteoprotegerin. Clin Exp Immunol 2002: **130**: 338–344.

22. Noguchi K, Ishikawa I. The roles of cyclooxygenase-2 and prostaglandin $E_2$ in periodontal disease. Periodontol 2000 2007: **43**: 85–101.
23. Nohutcu RM, McCauley LK, Koh AJ, Somerman MJ. Expression of extracellular matrix proteins in human periodontal ligament cells during mineralization *in vitro*. J Periodontol 1997: **68**: 320–327.
24. Nukaga J, Kobayashi M, Shinki T et al. Regulatory effects of interleukin-1beta and prostaglandin $E_2$ on expression of receptor activator of nuclear factor-κB ligand in human periodontal ligament cells. J Periodontol 2004: **75**: 249–259.
25. Offenbacher S, Heasman PA, Collins JG. Modulation of host $PGE_2$ secretion as a determinant of periodontal disease expression. J Periodontol 1993: **64**: 432–444.
26. Park CH, Lee JH, Yang CH. Curcumin derivatives inhibit the formation of Jun-Fos-DNA complex independently of their conserved cysteine residues. J Biochem Mol Biol 2005: **38**: 474–480.
27. Simonet WS, Lacey DL, Dunstan CR et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997: **89**: 309–319.
28. Suda K, Udagawa N, Sato N et al. Suppression of osteoprotegerin expression by prostaglandin $E_2$ is crucially involved in lipopolysaccharide-induced osteoclast formation. J Immunol 2004: **172**: 2504–2510.
29. Suzuki A, Ozono K, Kubota T, Kondou H, Tachikawa K, Michigami T. PTH/cAMP/PKA signaling facilitates canonical Wnt signaling via inactivation of glycogen synthase kinase-3beta in osteoblastic Saos-2 cells. J Cell Biochem 2008: **104**: 304–317.
30. Wagner EF. Functions of AP1 (Fos/Jun) in bone development. Ann Rheum Dis 2002: **61** (suppl 2): ii40–ii42.
31. Yamada S, Murakami S, Matoba R et al. Expression profile of active genes in human periodontal ligament and isolation of PLAP-1, a novel SLRP family gene. Gene 2001: **275**: 279–286.
32. Yamada S, Tomoeda M, Ozawa Y et al. PLAP-1/asporin, a novel negative regulator of periodontal ligament mineralization. J Biol Chem 2007: **282**: 23070–23080.
33. Yamashita Y, Sato M, Noguchi T. Alkaline phosphatase in the periodontal ligament of the rabbit and macaque monkey. Arch Oral Biol 1987: **32**: 677–678.