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Follicular dendritic cell-secreted protein may enhance osteoclastogenesis in periodontal disease

Jianru Liu[a,*], Huan Bian[b,*], Ruiyu Ding[c,*], Xiaopei Chi[c], and Yixiang Wang[d]

[a]Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China; [b]Department of Stomatology, the First Affiliated Hospital of the Chinese PLA General Hospital, Beijing, China; [c]Department of VIP dental service, Peking University School and Hospital of Stomatology, Beijing, China; [d]Central laboratory, Peking University School and Hospital of Stomatology, Beijing, China

ABSTRACT

Purpose of the study: Follicular dendritic cell-secreted protein (FDC-SP) has been found to be expressed in periodontal ligament (PDL), a layer of soft connective tissue between tooth root and alveolar bone, and involved in immunoreaction. This study was performed to explore the potential role of FDC-SP in periodontal disease. *Materials and Methods*: The human periodontal ligament cells (hPDLCs) were stimulated with *Porphyromonas gingivalis* (*P. gingivalis*) lipopolysaccharide (LPS) and FDC-SP expression was examined by real-time PCR and western blot. Then this molecule was overexpressed or silenced in hPDLCs by transfection of FDC-SP expression plasmids or its small-interfering (si) RNA, respectively, and the effects of FDC-SP on expression of osteogenesis- and osteoclastogenesis-related genes in hPDLCs were analyzed by real-time PCR and western blot. *Results*: Our results showed that *P. gingivalis* LPS upregulated FDC-SP expression in hPDLCs. Overexpression of FDC-SP could decrease the expression of osteogenesis-related genes, increase the expression of osteoclastogenesis-related genes and RANKL/OPG ratio in hPDLCs. Meanwhile, silence of FDC-SP expression in hPDLCs remarkably inversed the above results. *Conclusions*: LPS-induced upregulation of FDC-SP expression in hPDLCs may enhance osteoclastogenesis in periodontal disease.

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FDC-SP; lipopolysaccharide; osteoclastogenesis; osteogenesis; periodontal ligament cells

Introduction

Periodontitis is an inflammatory disease of periodontal connective tissue (1). It is of microbial origin and is characterized by destruction of attachment apparatus of tooth, involving alveolar bone resorption (2,3). In healthy people, alveolar bone is in a remodeling balance, which means that osteoclasts-mediated alveolar bone resorption is in balance with osteoblasts-mediated osteogenesis (4). However, in periodontitis, the integrated cellular systems are interfered by many factors which lead to the dysregulation of remodeling and the consequent loss of bone matrix (4,5).

Alveolar bone and tooth root are separated by periodontal ligament (PDL), a layer of soft connective tissue which is composed of collagen fiber bundles and various cells (6). As the most common cell type in PDL, periodontal ligament cells (PDLCs) act as fibroblasts by secreting extracellular matrix and organizing themselves into collagen fiber bundles. In addition, they also serve as mesenchymal stem cells, which have a potential to differentiate into osteoblasts/cementoblasts (7,8).

Follicular dendritic cell-secreted protein (FDC-SP) is an 85-amino acid peptide secreted from primary follicular dendritic cells in human tonsils (9). Since it was discovered, this protein has been considered to be an immune molecule which regulates follicular dendritic cell–B cell interaction. Recently, FDC-SP was found to be expressed in PDL (10). Several studies show that overexpression of FDC-SP in PDLCs *in vitro* can keep the fibroblastic characteristics and inhibit the osteogenic differentiation as well as the following mineralization of these cells (11,12). However, whether FDC-SP is involved in alveolar bone remodeling in periodontal disease remains unknown.

In this study, we used lipopolysaccharide (LPS), one of the most important virulence factors of periodontal pathogens during the development and progression of periodontitis (13,14), to stimulate human PDLCs (hPDLCs) *in vitro* and evaluated the expression of FDC-SP. Furthermore, we overexpressed and silenced FDC-SP in hPDLCs, respectively, and then examined the influence of FDC-SP on expression of osteogenesis- and osteoclastogenesis-related genes to evaluate the potential role of FDC-SP in periodontitis.

CONTACT Yixiang Wang kqwangyx@sina.com Central Laboratory, Peking University School and Hospital of Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, China.

*These authors contributed equally to this work.

Materials and methods

Cell culture and stimulation

hPDLCs were obtained from the mid-one-third of root surfaces of healthy individuals (age range from 20 to 30 years old) who underwent the third molar extraction at the Department of Oral and Maxillofacial Surgery in Peking University School and Hospital of Stomatology. The study was approved by the Medical Ethical Committee of this hospital (Ethics Approval No. PKUSSIRB-2012017), and written informed consents were obtained from all the donors before tooth extraction. The middle-third-of-root PDL tissues were cut into small fragments (1 mm^3) in Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, Scotland, UK), containing 10% fetal bovine serum (FBS; HyClone, Logan, UT), and were cultured in 35-mm dishes. The cultured PDLCs were identified as vimentin-positive and pan-CK-negative fibroblastic cells using immunocytochemistry, which indicated that the PDLCs were without epithelial original cell contamination (data not shown). To eliminate any potential bias of PDLCs from different donors, the third passage PDLCs from the four donors were mixed together and the cells at fourth to eighth passages were used in all the following experiments. For LPS treatment experiments, 2×10^5 hPDLCs were cultured in 6-well plates with 2 mL complete DMEM medium containing 1 μg/mL or 10 μg/mL *Porphyromonas gingivalis* (*P. gingivalis*) LPS (Catalog number: tltl-pglps, Invivogen, San Diego, CA) for 12 h and 24 h and harvested to subject to real-time PCR and western blot analysis. All cultures were maintained at 37°C with a humidified gas mixture of 5% CO_2/95% air.

Transfection of FDC-SP expression plasmids

hPDLCs were seeded at 2×10^5/well into 6-well plates and cultured in 2 mL DMEM supplemented with 10% FBS for overnight. Then, the cells were transfected with pCDNA3.1-myc vector or pcDNA3.1-hFDC-SP plasmid with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. At 24 h and 48 h post-transfection, the cells were harvested for real-time PCR and western blot analysis, respectively.

RNA interference

hPDLCs were transfected with 100 pM FDC-SP small-interfering (si) RNA or scramble siRNA (control) (Guangzhou RiboBio Co., Ltd., Guangzhou, China). The human FDC-SP-specific siRNA sequence is 5′-TCCATGGTTTAGAC GTAAT-3′. Transfection of siRNA was performed using Lipofectamine 2000, according to the manufacturer's instructions. The cell viability after transfection was also detected using Trypan blue exclusion tests. At 24 h post-transfection, some of the cells were harvested and subjected to real-time PCR and at 48 h post-transfection the others were harvested for western blot.

Real-time polymerase chain reaction

Total RNA was extracted from cells by using the TRIzol reagent (Invitrogen), and then treated with DNase I for 30 min, reversely transcribed into single-stranded cDNA using M-MLV reverse transcriptase (Promega, Madison, WI) according to the manufacturer's protocol. Real-time PCR was performed in an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Life Technologies, Warrington, UK) using SYBR green reagent (Roche, Indianapolis, IN). β-actin was used as the endogenous control. The primers for each gene were listed in Table 1 as follows: FDC-SP, runt-related transcription factor 2 (RUNX2), bone sialoprotein (BSP), alkaline phosphatase (ALP), osteocalcin (OCN), receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), and β-actin. The standard PCR conditions were 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions were performed in triplicate. The expression levels of the target transcripts in each sample were calculated by the comparative $2^{-\Delta\Delta Ct}$ method after normalization to the expression of β-actin.

Table 1. Real-time PCR primers used in this study.

Target gene	Sequence (5′–3′)	Product size (bp)
FDC-SP	AGTGGCTGTTGGTTTCCCAG GGCGAAATGGATATGGGTAAGG	117
RUNX2	TCCTATGACCAGTCTTACCCCT GGCTCTTCTTACTGAGAGTGGAA	193
BSP	GAACCTCGTGGGGACAATTAC CATCATAGCCATCGTAGCCTTG	81
ALP	ATGGGATGGGTGTaCTCCACA CCACGAAGGGGAACTTGTC	108
OCN	CACTCCTCGCCCTATTGGC CCCTCCTGCTTGGACACAAAG	112
OPG	GGAACCCCAGAGCGAAATACA CCTGAAGAATGCCTCCTCACA	226
RANKL	ACATATCGTTGGATCACAGCACAT CAAAAGGCTGAGCTTCAAGCTT	101
β-actin	CATGTACGTTGCTATCCAGGC CTCCTTAATGTCACGCACGAT	280

Western blot

The cells were harvested and lysed in RIPA buffer (Applygen, Beijing, China) containing proteinase inhibitors and phosphatase inhibitors. After measuring the protein concentration by BCA kit (Thermo, Rockford, IL), equal amounts (30 μg) of protein samples were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to poly-vinylidenedifluoride membranes by wet blotting. The membranes were blocked in 10% non-fat dry milk for 1 h and probed with antibodies against FDC-SP (1:300 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), BSP (1:300 dilution; Santa Cruz Biotechnology), OPG (1:300 dilution; Santa Cruz Biotechnology), RANKL (1:300 dilution; Santa Cruz Biotechnology), RUNX2 (1:300 dilution; Santa Cruz Biotechnology), or β-actin (1:1000 dilution; Santa Cruz Biotechnology) separately at 4°C overnight. After incubation with peroxidase-linked secondary antibodies for 1 h at room temperature, the ECL reagent (Thermo) was used to visualize the immunoreactive proteins. Then, we analyzed the results with densitometric analysis software Quantity One v4.6.2 (Bio-Rad, Hercules, CA). β-actin was used as a loading control and relative optical density of each protein band was calculated.

Statistical analysis

All experiments were performed three times. Data are expressed as the means ± SD. Differences between means were assessed by independent sample *t*-test of SPSS. The level of significance (p) was set as <0.05.

Results

P. gingivalis LPS increased the expression of FDC-SP in hPDLCs

In order to investigate whether the expression of FDC-SP in hPDLCs could be affected by periodontal pathogens during the development and progression of periodontitis, hPDLCs were stimulated with *P. gingivalis* LPS *in vitro* for 12 h and 24 h and then harvested RNA or protein to detect FDC-SP expression through real-time PCR and western blot. As expected, *P. gingivalis* LPS increased FDC-SP expressions in hPDLCs in time- and dose-dependent manners. The expression of FDC-SP at mRNA level increased 1.57 ± 0.55-fold ($p > 0.05$) at 12 h and 2.35 ± 0.41-fold ($p < 0.05$) at 24 h post-stimulation in 1 μg/mL *P. gingivalis* LPS–treated hPDLCs, and 1.86 ± 0.50-fold ($p > 0.05$) at 12 h and 2.84 ± 0.63-fold ($p < 0.05$) at 24 h post-stimulation in 10 μg/mL *P. gingivalis* LPS–treated hPDLCs compared to their corresponding untreated controls (Figure 1A). This result was consistent with western blot analysis, which showed that FDC-SP expression at protein level increased to 1.65 ± 0.04-fold when hPDLCs was stimulated with 1 μg/mL *P. gingivalis* LPS for 24 h ($p < 0.01$, Figure 1B and C). $p < 0.05$ and $p < 0.01$ represent a significant difference and highly significant difference, respectively, in these comparisons.

Overexpression and silence of FDC-SP modulated the expression of osteogenesis- and osteoclastogenesis-related genes in hPDLCs

FDC-SP was overexpressed or silenced in hPDLCs by transfection of FDC-SP expression plasmids or its si RNA, respectively, to explore the effect of FDC-SP on osteogenesis- and osteoclastogenesis-related gene expression in hPDLCs. Real-time PCR results demonstrated that the expression of FDC-SP was significantly upregulated 9.33-fold ($p < 0.01$) at mRNA level and 1.76-fold ($p < 0.01$) at protein level after transfection of FDC-SP expression plasmids (Figure 2A), while it was markedly downregulated 76.3% ($p < 0.01$) at mRNA level and 37.2% ($p < 0.01$) at protein level after transfection of siFDC-SP

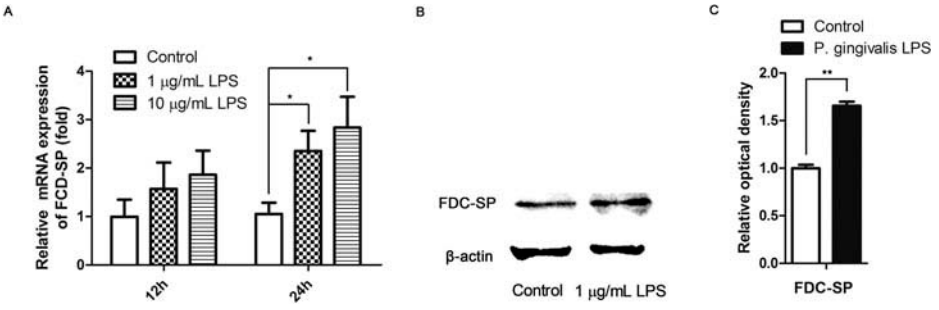

Figure 1. Effect of *P. gingivalis* LPS on the expression of follicular dendritic cell-secreted protein (FDC-SP) in hPDLCs was examined with real-time PCR at 12 h and 24 h after 1 μg/mL and 10 μg/mL *P. gingivalis* LPS treatment and western blot at 24 h after 1 μg/mL *P. gingivalis* LPS stimulation. (A) Relative expression of FDC-SP at mRNA level. (B) Representative western blot bands and (C) the corresponding densitometric analysis of FDC-SP protein expression. *$p < 0.05$; **$p < 0.01$.

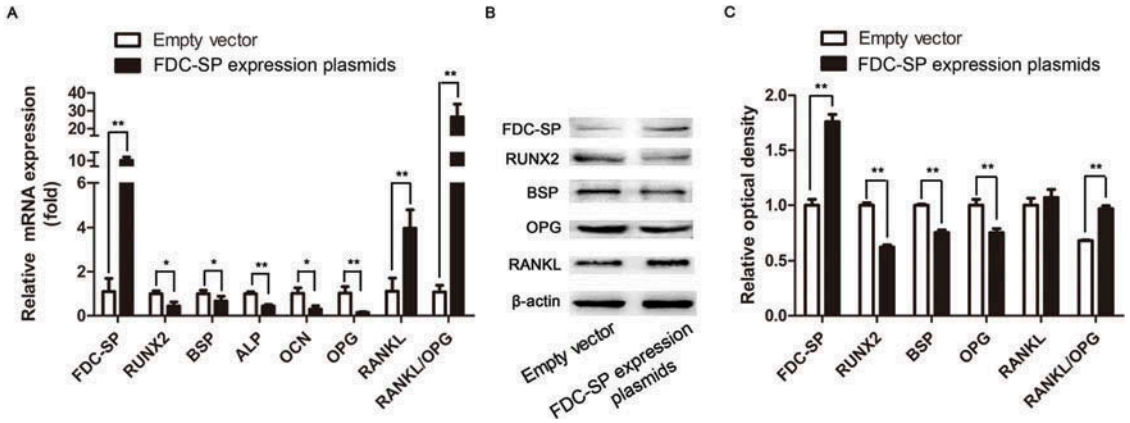

Figure 2. Effect of FDC-SP overexpression on osteogenesis-related biomarkers and osteoclastogenesis-related biomarker RANKL expression in hPDLCs. FDC-SP was overexpressed in hPDLCs by transfection of FDC-SP expression plasmids, and the expression of osteogenic and osteoclastogenic biomarkers was examined by real-time PCR at 24 h after transfection and western blot at 48 h after transfection. (A) Relative mRNA expression of FDC-SP, RUNX2, BSP, ALP, OCN, OPG, and RANKL, and the ratio of RANKL/OPG in hPDLCs transfected with empty vector or FDC-SP expression plasmids. (B) FDC-SP, RUNX2, BSP, OPG, RANKL, and β-actin protein bands were detected by western blot in hPDLCs transfected with empty vector or FDC-SP expression plasmids and (C) densitometric analysis of each band of the western blots. *$p < 0.05$; **$p < 0.01$.

(Figure 3A). Moreover, overexpression of FDC-SP significantly downregulated all the examined osteogenesis-related biomarkers including RUNX2 (reduced 56%, $p < 0.05$), BSP (reduced 34%, $p < 0.05$), ALP (reduced 56%, $p < 0.01$), OCN (reduced 71%, $p < 0.05$), and OPG (reduced 86%, $p < 0.01$) and upregulated an osteoclastogenesis-related gene RANKL (increased 2.57-fold, $p < 0.01$) at 24 h after transfection. Moreover, the ratio of RANKL/OPG, an index of osteoclastogenesis, increased 23.6-fold ($p < 0.01$) in FDC-SP transfected hPDLCs (Figure 2A). Western blot results showed the same expression pattern of the examined genes as the real-time PCR results except for RANKL, which was not markedly changed at protein level in FDC-SP overexpressed hPDLCs and empty vector-transfected control cells. However, the ratio of RANKL/OPG was still increased 1.4-fold ($p < 0.01$) in FDC-SP overexpressed hPDLCs (Figure 2B and C). Conversely, downregulation of FDC-SP by siFDC-SP significantly increased RUNX2 (2.13-fold, $p < 0.05$), BSP (2.11-fold, $p < 0.01$), ALP (2.39-fold, $p < 0.01$), OCN (2.07-fold, $p < 0.05$), and OPG (1.68-fold, $p < 0.05$) expression and downregulated RANKL (2.4-fold, $p < 0.01$) expression in hPDLCs at mRNA levels. The ratio of RANKL/OPG decreased 3.89-fold ($p < 0.05$), compared to that in control hPDLCs which were transfected with scramble siRNA (Figure 3A). These

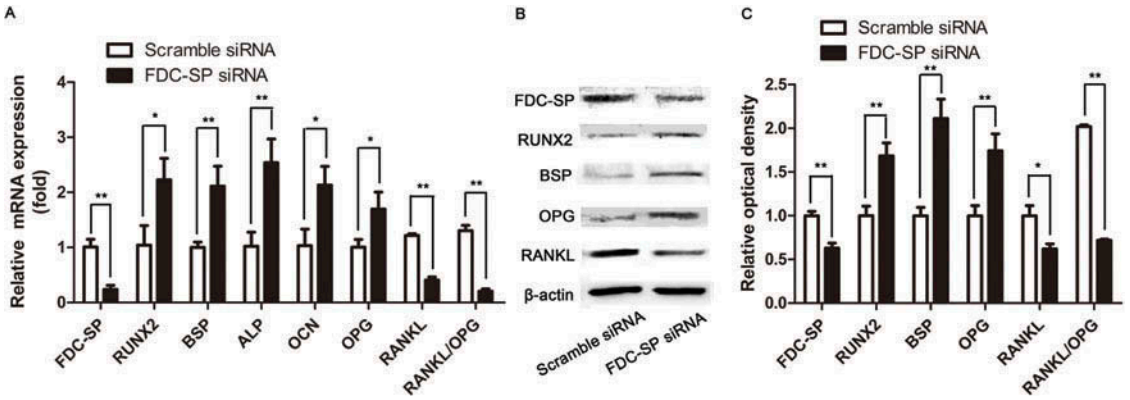

Figure 3. FDC-SP was silenced in hPDLCs by transfecting the FDC-SP siRNA, and the osteogenic and osteoclastogenic biomarkers were examined by real-time PCR at 24 h after transfection and western blot at 48 h after transfection. (A) Relative mRNA expression of FDC-SP, RUNX2, BSP, ALP, OCN, OPG, and RANKL, and ratio of RANKL/OPG in hPDLCs transfected with scramble siRNA or FDC-SP siRNA. (B) FDC-SP, RUNX2, BSP, OPG, RANKL, and β-actin protein bands were detected by western blot in hPDLCs transfected with scramble siRNA or FDC-SP siRNA and (C) densitometric analysis of each band of the western blots. *$p < 0.05$; **$p < 0.01$.

results were further confirmed by western blot analysis (Figure 3B and C).

Discussion

Clinical attachment loss and alveolar bone resorption are characteristics of periodontitis and they are the direct reasons for gomphiasis and tooth loss. Bacterial plaque that contains various periodontal pathogens is the initiating factor for periodontitis (15). LPS has been considered to be one of the most important virulence factors of periodontal pathogens (16). Previous studies reported that LPS derived from Gram-negative bacteria can penetrate into periodontal tissues; recruit and activate immune cells; improve the production of pro-inflammatory mediators such as interleukins-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), and induce the alveolar bone destruction (17–19).

PDL is a unique thin, soft connective tissue between the two different mineralized tissues, alveolar bone and cementum, in the whole body. PDLCs are the basic cellular component of PDL. These heterogeneous cell populations possess the property of multipotential differentiation (20) and may play a pivotal role in alveolar bone remodeling by secreting proteins with osteoblastic or osteoclastic functions (21,22).

In this study, we found that *P. gingivalis* LPS can upregulate the expression of FDC-SP in hPDLCs *in vitro*, which indicates that FDC-SP can be involved in periodontal pathogen-induced periodontitis. Therefore, we overexpressed and silenced FDC-SP, respectively, in hPDLCs, then examined the osteogenesis-related genes including RUNX2, BSP, ALP, OCN, OPG, and osteoclastogenesis-related gene RANKL to investigate the role of FDC-SP in the regulation of the osteogenesis–osteoclastogenesis balance. ALP is recognized as an early biomarker of osteogenic differentiation (23), and it is associated with mineral formation in calcified tissues (24). BSP is reported to play a pivotal role in the hydroxyapatite deposition and matrix mineralization (25), whereas OCN serves as a biomarker of late-stage osteoblastic differentiation (26). RUNX2 is the master gene of osteodifferentiation (27). This study reported all of these genes were downregulated by FDC-SP overexpression, and these effects could be reversed by silence of FDC-SP expression in hPDLCs, indicating that FDC-SP has a potential to inhibit osteoblastic differentiation of hPDLCs. This result was consistent with previous studies (11,12).

RANKL is a ligand for receptor activator of nuclear factor kappa-B (RANK), which provides osteoclast-specific differentiation signals, while OPG is a soluble decoy RANKL receptor that inhibits osteoclastic differentiation. RANKL/OPG ratio is responsible for determining the bone mass and strength. A high ratio of RANKL/OPG is associated with osteoclast development and bone loss (28,29). In the present study, FDC-SP upregulated RANKL expression and simultaneously attenuated OPG expression in hPDLCs, which indicated that overexpression of FDC-SP promoted osteoclastogenic differentiation. This finding suggests a possible role of FDC-SP in alveolar bone resorption in periodontitis: altering the balance of osteogenesis–osteoclastogenesis through regulating the RANKL/OPG ratio in PDL.

Taken together, in this study, we demonstrated that LPS stimulation could improve the expression of FDC-SP in hPDLCs and the overexpressed FDC-SP could push the osteogenesis–osteoclastogenesis balance toward osteoclastogenesis. It is, for the first time, to provide an alternative explanation for periodontitis in a LPS-FDC-SP-alveolar bone resorption manner. Further researches, especially *in vivo* studies, are needed to explore the precise role of FDC-SP expressed by PDLCs in the progression of periodontitis and alveolar bone loss.

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Declaration of interest

No potential conflicts of interest were noted.

References

1. Lin L, Li C, Liu J, Zhang D, Zhao J, Kou Y, Yu N, Pan Y. Virulence genes of Porphyromonas gingivalis W83 in chronic periodontitis. Acta Odontol Scand 2009;67:258–64.
2. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest 1976;34:235–49.
3. Into T, Inomata M, Kanno Y, Matsuyama T, Machigashira M, Izumi Y, Imamura T, Nakashima M, Noguchi T, Matsushita K. Arginine-specific gingipains from *Porphyromonas gingivalis* deprive protective

functions of secretory leucocyte protease inhibitor in periodontal tissue. Clin Exp Immunol 2006;145:545–54.
4. Nair SP, Meghji S, Wilson M, Reddi K, White P, Henderson B. Bacterially induced bone destruction: mechanisms and misconceptions. Infect Immun 1996;64:2371–80.
5. Kikuchi T, Mitani A, Noguchi T. The mechanism of bone resorption in chronic inflammation of periodontal disease. Clin Calcium 2006;16:241–7.
6. Kaku M, Yamauchi M. Mechano-regulation of collagen biosynthesis in periodontal ligament. J Prosthodont Res 2014;58:193–207.
7. Ohta S, Yamada S, Matuzaka K, Inoue T. The behavior of stem cells and progenitor cells in the periodontal ligament during wound healing as observed using immunohistochemical methods. J Periodontal Res 2008;43:595–603.
8. Bloemen V, Schoenmaker T, de Vries TJ, Everts V. Direct cell-cell contact between periodontal ligament fibroblasts and osteoclast precursors synergistically increases the expression of genes related to osteoclastogenesis. J Cell Physiol 2010;222:565–73.
9. Marshall AJ, Du Q, Draves KE, Shikishima Y, HayGlass KT, Clark EA. FDC-SP, a novel secreted protein expressed by follicular dendritic cells. J Immunol 2002;169:2381–9.
10. Nakamura S, Terashima T, Yoshida T, Iseki S, Takano Y, Ishikawa I, Shinomura T. Identification of genes preferentially expressed in periodontal ligament: specific expression of a novel secreted protein, FDC-SP. Biochem Biophys Res Commun 2005;338:1197–203.
11. Wei N, Yu H, Yang S, Yang X, Yuan Q, Man Y, Gong P. Effect of FDC-SP on the phenotype expression of cultured periodontal ligament cells. Arch Med Sci 2011;7:235–41.
12. Xiang L, Ma L, He Y, Wei N, Gong P. Osteogenic differentiation of human periodontal ligament cells after transfection with recombinant lentiviral vector containing follicular dendritic cell secreted protein. J Periodontal Res 2014;49:554–62.
13. Zhang Y, Li X. Lipopolysaccharide-regulated production of bone sialoprotein and interleukin-8 in human periodontal ligament fibroblasts: the role of toll-like receptors 2 and 4 and the MAPK pathway. J Periodontal Res 2014.
14. Moradi J, Abbasipour F, Zaringhalam J, Maleki B, Ziaee N, Khodadoustan A, Janahmadi M. Anethole, a medicinal plant compound, decreases the production of pro-inflammatory TNF-alpha and IL-1beta in a rat model of LPS-induced periodontitis. Iran J Pharm Res 2014;13:1319–25.
15. Kornman KS. Mapping the pathogenesis of periodontitis: a new look. J Periodontol 2008;79:1560–8.
16. Wang PL, Ohura K. Porphyromonas gingivalis lipopolysaccharide signaling in gingival fibroblasts-CD14 and Toll-like receptors. Crit Rev Oral Biol Med 2002;13:132–42.
17. Page RC, Offenbacher S, Schroeder HE, Seymour G, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000 1997;14:216–48.
18. Agarwal S, Piesco NP, Johns LP, Riccelli AE. Differential expression of IL-1 beta, TNF-alpha, IL-6, and IL-8 in human monocytes in response to lipopolysaccharides from different microbes. J Dent Res 1995;74:1057–65.
19. Bae WJ, Shin MR, Kang SK, Jun Z, Kim JY, Lee SC, Kim EC. HIF-2 inhibition suppresses inflammatory responses and osteoclastic differentiation in human periodontal ligament cells. J Cell Biochem 2015;116:1241–55.
20. McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. J Periodontal Res 1991;26:144–54.
21. Beertsen W, McCulloch CA, Sodek J. The periodontal ligament: a unique, multifunctional connective tissue. Periodontol 2000 1997;13:20–40.
22. Oshiro T, Shiotani A, Shibasaki Y, Sasaki T. Osteoclast induction in periodontal tissue during experimental movement of incisors in osteoprotegerin-deficient mice. Anat Rec 2002;266:218–25.
23. Kuru L, Griffiths GS, Petrie A, Olsen I. Alkaline phosphatase activity is upregulated in regenerating human periodontal cells. J Periodontal Res 1999;34:123–7.
24. Beertsen W, Van den Bos T. Alkaline phosphatase induces the deposition of calcified layers in relation to dentin: an in vitro study to mimic the formation of afibrillar acellular cementum. J Dent Res 1991;70:176–81.
25. Gordon JA, Tye CE, Sampaio AV, Underhill TM, Hunter GK, Goldberg HA. Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization in vitro. Bone 2007;41:462–73.
26. Kim K, Dean D, Mikos AG, Fisher JP. Effect of initial cell seeding density on early osteogenic signal expression of rat bone marrow stromal cells cultured on cross-linked poly (propylene fumarate) disks. Biomacromolecules 2009;10:1810–7.
27. Wang H, Mao Y, Zhang B, Wang T, Li F, Fu S, Xue Y, Yang T, Wen X, Ding Y, Duan X. Chloride channel ClC-3 promotion of osteogenic differentiation through Runx2. J Cell Biochem 2010;111:49–58.
28. Vega D, Maalouf NM, Sakhaee K. CLINICAL Review #: the role of receptor activator of nuclear factor-kappaB (RANK)/RANK ligand/osteoprotegerin: clinical implications. J Clin Endocrinol Metab 2007;92:4514–21.
29. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys 2008;473:139–46.