Parthenolide Promotes Differentiation of Osteoblasts Through the Wnt/β-Catenin Signaling Pathway in Inflammatory Environments

Xufang Zhang, Qingpiao Chen, Jianwei Liu, Chen Fan, Qi Wei, Zetao Chen, and Xueli Mao

Periodontitis is a progressive inflammatory disease initiated by bacterial biofilm adhering to the tooth surface. If left untreated, periodontitis may lead to tooth loss and destruction of the alveolar bone. Regaining the lost alveolar bone is a clinical challenge because of the limited differentiation ability of osteoblasts in inflammatory environments. We have previously shown the anti-inflammatory and antiosteoclastogenic activities of parthenolide (PTL) in human periodontal ligament-derived cells by inhibiting nuclear factor kappa B (NF-κB) signaling, indicating its potential for periodontitis treatment. In this study, we further examined whether PTL could stimulate differentiation of osteoblasts from human alveolar bone in inflammatory conditions and investigated the involvement of the Wnt/β-catenin signaling pathway during this process. The results showed that PTL significantly stimulated alkaline phosphatase activity, mineralization nodule formation, and osteogenesis-related gene/protein expression of osteoblasts under the stimulation of tumor necrosis factor-α (TNF-α). In addition, PTL inhibited the NF-κB/p50 pathway and resisted the inhibition of Wnt/β-catenin signaling induced by TNF-α. Our results indicate that the stimulatory effect of PTL on the differentiation of osteoblasts in inflammatory environments may involve the activation of the Wnt/β-catenin signaling pathway, and PTL may be a promising component for bone regeneration in periodontitis treatment.

Keywords: parthenolide, osteogenesis, osteoblast, periodontitis, nuclear factor kappa B, Wnt/β-catenin

Introduction

Periodontitis is a destructive and inflammatory disease of the periodontium caused by bacterial biofilm on the tooth surface, leading to loss of the tooth-supporting tissues (Berezow and Darveau 2011). The current clinical therapies for periodontitis yield unsatisfactory outcomes, as the regeneration of the periodontal region is challenged by the complex immune response of the host (Berezow and Darveau 2011; Chang and others 2013; Chen and others 2013). Ideally, strategies should be able to repair and regenerate all damaged periodontal tissue structures, including the cementum, periodontal ligament, and alveolar bone. However, it remains a clinical challenge to regain the lost alveolar bone because of the limited differentiation ability of osteoblasts in inflammatory environments (Chang and others 2013; Chen and others 2013).

Nuclear factor kappa B (NF-κB) is the master regulator of inflammatory response, which can be activated by multiple proinflammatory cytokines, including lipopolysaccharide (LPS) and tumor necrosis factor-α (TNF-α) (Chang and others 2009, 2013; Krum and others 2010). Therefore, the NF-κB signaling pathway might be a promising target to regain bone regeneration in inflammatory environments.

Parthenolide (PTL), an active constituent of the plant Tanacetum parthenium, has been shown to have potential in the treatment of inflammatory diseases by inhibiting the NF-κB signaling pathway (Hehner and others 1999; Yip and others 2004; Ghantous and others 2013; Wang and Li 2015). Our previous study demonstrated the anti-inflammatory and antiosteoclastogenic activities of PTL in human periodontal ligament-derived cells (hPDLCs) in vitro, offering...
fundamental evidence supporting the potential use of PTL in periodontitis treatment (Zhang and others 2014). However, it is unclear whether PTL can be used for regenerating alveolar bone in periodontal tissues. More importantly, the interactions of PTL and osteoblasts in inflammatory environments, including proliferation, osteogenic differentiation (gene expression and protein synthesis), and the possible molecular mechanism, are completely unknown.

The essential roles of the Wnt/β-catenin signaling pathway have been well established in bone formation and tooth morphogenesis (Liu and others 2008; Marie and Hay 2013). The canonical Wnt signaling pathway is one of the signaling cascades transduced by Wnt proteins, involving stabilization and nuclear accumulation of β-catenin and activation of Wnt target genes (Ling and others 2009). However, little is known about the involvement of Wnt/β-catenin signaling in osteoblast differentiation with the interaction of a NF-κB inhibitor (PTL), especially because alveolar bone has limited remodeling potential and is difficult to regenerate. Therefore, the purpose of this study was to investigate the effect of PTL on osteogenic differentiation of cells from alveolar bone under inflammatory conditions, and to further explore the potential molecular mechanism during this process.

Materials and Methods

Isolation and culture of osteoblasts

Human osteoblasts were isolated according to previously published methods (Helley and others 1981; Dillon and others 2012). In brief, normal human alveolar bones were obtained from consenting patients with ethical approval from the Queensland University of Technology and were digested with collagenase type II. The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies) containing 10% (v/v) fetal bovine serum (FBS; In Vitro, Australia), 1% (v/v) penicillin/streptomycin (P/S; Life Technologies), and 1% nonessential amino acids (Life Technologies) at 37°C, 5% CO2/95% air, with fresh medium provided every 3 days. Cells of passages 3 through 5 were used for all experiments.

Cell proliferation analysis

The effect of PTL (Sigma-Aldrich) on cell proliferation was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT; Sigma-Aldrich). In brief, osteoblasts were seeded in 96-well plates at a density of 3 × 10^3 cells/well and allowed to adhere and spread for 12 h. The cells were then treated with various concentrations of PTL (0.1, 1, 2.5, 5, and 10 μM) for 1, 3, and 7 days. The combined effect of PTL with TNF-α on cell proliferation was also tested. Next, the MTT solution (0.5 mg/mL) was added to each well at 37°C for 4 h. Then, the mixture was discarded and dissolved in dimethyl sulfoxide (Sigma-Aldrich). After shaking, the absorbance was measured at 495 nm using a SpectraMax Microplate Reader (Molecular Devices).

Mineralization assay

Mineralization in osteoblast cultures was analyzed by Alizarin Red S staining (Sigma-Aldrich) as previously reported (Zhou and others 2013). Osteoblasts were maintained either in 10% FBS/DMEM (as control) or osteogenic medium (OM). OM was composed of high-glucose DMEM supplemented with 10% FBS, 1% P/S, 10 mM β-glycerophosphate (Sigma-Aldrich), 50 μM ascorbic acid (Sigma-Aldrich), and 100 nM dexamethasone (Sigma-Aldrich). Osteoblasts were pretreated with PTL (0, 1, and 2.5 μM) for 1 h and then stimulated with TNF-α (10 ng/mL; Life Technologies) for 14 days. After treatments, the samples were washed twice with phosphate-buffered saline (PBS) and fixed in 100% methanol for 10 min. Then, the samples were washed with distilled water and stained with 1% Alizarin Red S for 20 min at room temperature. Finally, the stained cells were rinsed with distilled water and recorded under an Eclipse TS100 microscope (Nikon, Australia).

Alkaline phosphatase activity assay

Alkaline phosphatase (ALP) activity of osteoblasts after treatment with PTL and TNF-α was measured using a QuantiChrom™ Alkaline Phosphatase Assay Kit (BioAssay Systems) according to the manufacturer’s instructions. Osteoblasts were treated with the mineralization assay. After stimulation with TNF-α for 7 and 14 days, the cells were lysed in 0.2% Triton X-100 and centrifuged at 978 g for 10 min. The supernatant of the medium was harvested for subsequent assay. The optical density (OD) was detected at 405 nm with a SpectraMax Microplate Reader (Molecular Devices). The ALP activity is represented as the OD values divided by the reaction time, and the total protein amount was measured using bicinchoninic acid (BCA; Thermo Fisher Scientific, Australia) Assay.

Quantitative reverse transcriptase polymerase chain reaction

PTL-induced changes in osteoblast gene expression were determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Osteoblasts were pretreated with PTL (0, 1, and 2.5 μM) for 1 h and then stimulated with TNF-α for the indicated time points. Total RNA was extracted using TRIzol® Reagent (Life Technologies). Complementary DNA strands were generated by reverse transcription of 1 μg total RNA using the DyNAamo™ cDNA Synthesis Kit (Finzymes; Genesearch Pty Ltd., Australia) according to the manufacturer’s protocol. qRT-PCR was performed on the ABI 7,500 Thermal Cycler (Applied Biosystems, Australia) using the SYBR® Green detection reagent (Applied Biosystems). Glycereraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as an internal control. The relative amount of RNA was calculated by the 2^−ΔΔCt method. Genes and related specific primers are listed in Table 1.

Western blot analysis

Expression of osteogenic markers (ALP, OPN, OCN, and RUNX2) and signaling pathway-related proteins (p50, I-κB, p-p65, p65, ERK, pERK, AXIN2, DKK1, and β-catenin) in osteoblasts after PTL and TNF-α treatment was detected by Western blotting. After cell treatment for indicated time points, the whole cells were lysed in radioimmunoprecipitation assay lysis buffer (Sigma) with a protease inhibitor cocktail and phosphatase inhibitor (Roche, Swiss). The supernatants were collected, and the protein concentration was
TABLE 1. PRIMERS USED IN QUANTITATIVE REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer: forward (F) and reverse (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>F: 5’-TCAGAAGCTAACACCAACGG-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’TGATTCGATCCTGGAGAGGC-3’</td>
</tr>
<tr>
<td>OPN</td>
<td>F: 5’TACGTGCGATCCAGGTAAA-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’TGAGATGGTCGACGGTTAGC-3’</td>
</tr>
<tr>
<td>OCN</td>
<td>F: 5’-GCAGAGGTCGACGGTTAGC-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’-GGCTCCGAGCTTGGATAGAC-3’</td>
</tr>
<tr>
<td>RUNX2</td>
<td>F: 5’-CAGAAGGAAGGGCAGGTGC-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’-GGCTTTCCTTACCTGGTGT-3’</td>
</tr>
<tr>
<td>AXIN2</td>
<td>F: 5’-GCTAGCAGAGCAAGCATGT-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’-GGCTTTCCTTACCTGGTGT-3’</td>
</tr>
<tr>
<td>DKK1</td>
<td>F: 5’TCCGAGGAGAAATTGAGGGA-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’-CAGAAGGAAGGGCAGGTGC-3’</td>
</tr>
<tr>
<td>CTNNB</td>
<td>F: 5’-GCTACTGTTGGATTGCAGAACATC-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’-CCTCCTGCAGCATGCCAGGAGA-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5’TCTGGTTCCTGGCATGATGC-3’</td>
</tr>
<tr>
<td></td>
<td>R: 5’-GCCTTTCCCATTGGTGT-3’</td>
</tr>
</tbody>
</table>

ALP, alkaline phosphatase; AXIN2, axis inhibition protein 2; CTNNB, ß-catenin; DKK1, dickkopf-related protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OCN, osteocalcin; OPN, bone osteopontin.

determined using the BCA assay. Equal amounts of protein (10 μg) were prepared and separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes (Pall Corporation). The membranes were blocked and incubated with primary antibodies overnight at 4°C. The membranes were then washed 3 times and probed with corresponding fluorescent secondary antibodies (Cell Signaling Technology), and GAPDH (1:2,000; Abcam). The membranes were then washed 3 times and probed with corresponding fluorescent secondary antibodies (Cell Signaling) for 1 h at room temperature. Images were then captured and analyzed using the Odyssey Imaging system and software (LI-COR Biosciences).

Statistical analysis

All assays were performed in triplicate, with each treatment tested individually 3 times in cells from 3 different patients. One-way analysis of variance with the Student–Newman–Keuls (S-N-K) test was used to analyze the statistical difference. A P value <0.05 was regarded as statistically significant.

Results

Effect of PTL on proliferation of osteoblasts

To identify the effects of PTL on cell proliferation, osteoblasts were treated with different concentrations of PTL (0.1, 1, 2.5, 5, and 10 μM) for 1, 3, and 7 days. As shown in Fig. 1A, no effect of PTL on osteoblast proliferation was observed at 0.1, 1, and 2.5 μM compared with the control (P > 0.05). However, 5 μM of PTL reduced osteoblast proliferation by 23.2% ± 6.1% and 26.7% ± 2.4% at days 3 and 7 compared with the control (P < 0.05), respectively. In addition, osteoblast proliferation was 32.6% ± 6.7% and 73.3% ± 0.4% below the control (P < 0.05), when exposed to 10 μM PTL for 3 and 7 days, respectively. Taken together, these data indicate that a high concentration has a suppressive effect on the cell proliferation of osteoblasts.

PTL enhanced differentiation of osteoblasts in inflammatory microenvironments

It is well known that proinflammatory factors, especially interleukin-1β (IL-1β), IL-6, and TNF-α, are major mediators that inhibit the differentiation potential of osteoblasts. In this study, TNF-α (10 ng/mL) was used to mimic the inflammatory microenvironment of periodontitis. Cell proliferation assay showed that 10 ng/mL TNF-α combined with or without PTL (at 1 or 2.5 μM) had no obvious effect...
FIG. 2. Effects of PTL on osteogenic differentiation. (A) Mineralization in osteoblasts after treatment with TNF-α and PTL (1.0 and 2.5 μM) was observed using Alizarin Red S staining at 14 days. (B) ALP activity in osteoblasts at 7 and 14 days. The ALP activity is represented as the OD values divided by the reaction time and the total amount of protein (OD 405/min/μg). Error bars indicate mean ± SEM (n = 3). *P < 0.05 versus the OM group, whereas #P < 0.05 versus the TNF group. ALP, alkaline phosphatase; OM, osteogenic medium.

FIG. 3. Effects of PTL on the protein expression of osteogenic markers. Osteoblasts were treated with TNF-α and PTL (1 and 2.5 μM) for 3 and 7 days and the expression of proteins was detected by Western blotting. GAPDH was included as loading control. The intensities of the bands were measured with densitometry and first normalized to GAPDH and then further converted to the percentage of the control (containing 10% FBS/DMEM only). Error bars indicate mean ± SEM (n = 3). *P < 0.05 versus the OM group, whereas #P < 0.05 versus the TNF-α group. DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
on osteoblast proliferation (Fig. 1B). The effect of PTL on calcium deposition of osteoblasts under TNF-α stimulation was observed by Alizarin Red S staining. As shown in Fig. 2A, mineralized nodules could be detected in osteoblasts cultured in OM; however, the addition of TNF-α remarkably reduced calcium deposition. Pretreatment of osteoblasts with 1 and 2.5 μM PTL enhanced calcium deposition compared with that of the TNF-α only group. Furthermore, osteoblasts pretreated with PTL showed a significantly (P < 0.05) higher ALP activity at 7 and 14 days than those treated with TNF-α only (Fig. 2B). These data indicated that PTL resisted the mineralization-inhibiting ability of TNF-α.

**Effects of PTL on the expression of osteogenic markers in osteoblasts**

To further identify the effects of PTL on osteoblast differentiation, the expression of osteogenic markers (ALP, OPN, OCN, and RUNX2) was investigated. In both protein and gene levels (Figs. 3 and 4), ALP, OPN, OCN, and RUNX2 expression was all upregulated in osteoblasts pretreated with PTL 2.5 μM than cells treated with TNF-α (10 ng/mL) only at days 3 and 7 (P < 0.05). In addition, 1 μM of PTL increased ALP expression at day 3, whereas increasing OCN and RUNX2 expression at day 7 at both protein and gene levels. ALP genes were also increased in osteoblasts when exposed to TNF-α and 1 μM of PTL for 7 days than the TNF-α only group (P < 0.05) (Fig. 4).

**Effect of PTL on the NF-κB signaling pathway in osteoblasts**

The effects of PTL on the expression of NF-κB, I-κB, and ERK in osteoblasts were evaluated as they play vital roles in mediating the inflammation process in periodontitis. As shown in Fig. 5, the expression of NF-κB/p50, p-p65, and p-ERK was remarkably increased when exposed to TNF-α (10 ng/mL) for 30 and 60 min, whereas I-κB expression was decreased in response to TNF-α at 60 min. Pretreatment with 1 and 2.5 μM of PTL significantly downregulated p-ERK and p50 expression as well as enhanced I-κB expression in osteoblasts at 60 min compared with that of the TNF-α only group. PTL of 2.5μM also downregulated p-ERK and p50

**FIG. 4.** Effects of PTL on the gene expression of osteogenic markers. After treatment with TNF-α with/without PTL, total RNA was collected at days 3 and 7. The expression of the target gene was first normalized to GAPDH and then further converted to the percentage of the control. Error bars indicate mean ± SEM (n = 3). *P < 0.05 versus the OM group, whereas †P < 0.05 versus the TNF-α group.
expression in osteoblasts at 30 min compared with that of the TNF-α only group. However, no obvious effect of PTL on p-p65 expression was observed at either time point.

**Activation of the Wnt/β-catenin signaling pathway in osteoblasts stimulated by PTL**

The effects of PTL on the expression of the Wnt/β-catenin signaling pathway components in osteoblasts were also evaluated as they have been demonstrated to participate in the osteogenic differentiation process. As shown in Fig. 6A, PTL obviously upregulated AXIN2 and β-catenin and downregulated DKK1 protein expression compared with the TNF-α (10 ng/mL) only group at day 3, although it had no significant effect on this signaling pathway on day 1. Gene expression of AXIN2, β-catenin, and DKK1 showed similar expression patterns on days 1 and 3 (Fig. 6B–D).

**Discussion**

This study showed that under inflammatory conditions, PTL significantly stimulated the ALP activity, mineralization nodule formation, and osteogenesis-related gene/protein expression of osteoblasts from alveolar bone. In addition, PTL inhibited the NF-κB and ERK signaling pathways and resisted the inhibition of Wnt/β-catenin signaling induced by TNF-α. Our results indicate that the stimulatory effect of PTL on the differentiation of osteoblasts in inflammatory environments may involve the activation of the Wnt/β-catenin signaling pathway, and PTL may be a promising component for bone regeneration in periodontitis treatment.

In clinical cases of bone defect, such as periodontitis, bone regeneration is often hampered by inflammatory environments. Abundant evidence suggests that inflammatory cytokines such as TNF-α inhibit osteoblast differentiation by suppression of RUNX2, reduce bone formation by mature osteoblasts, inhibit differentiation of osteoblasts from precursor cells, and increase osteoclastic resorption (Hashimoto and others 1989; Gilbert and others 2000, 2002). Our previous study showed the anti-inflammatory effect of PTL in periodontitis, as it could suppress the expression of inflammatory cytokines in LPS-stimulated hPDLCs (Zhang and others 2014). However, whether the anti-inflammatory effect of PTL could rescue the impaired osteogenic differentiation of osteoblasts remains unknown. In this study, we first examined the cytotoxicity of PTL on osteoblasts, and
the result showed that PTL at concentrations of 1 and 2.5 μM is nontoxic to cells; these concentrations also fall within the nontoxic pharmacological concentrations reported previously (1–10 μM) (Ghantous and others 2013). Then, TNF-α was used to mimic the inflammatory environment in periodontitis and the effect of PTL on osteogenesis in vitro was examined. ALP is known as a marker for preosteoblast differentiation and osteoblast mineralization (Komori 2006). RUNX2 is regarded as a key transcription factor of osteoblast differentiation (Liu and Lee 2013). OPN and OCN are also well-defined genes that play a role in the process of mineral deposition (Komori 2006). Pretreatment of osteoblasts with PTL overcame, at least in part, the inhibitory effect of calcium deposition caused by TNF-α. Furthermore, osteoblasts pretreated with PTL showed a significantly higher ALP activity and osteogenic gene (ALP, OPN, OCN, and RUNX2) expression of osteoblasts than those under TNF-α stimulation group. The result indicates that PTL possesses the potential to rescue the impaired osteogenic differentiation caused by TNF-α in vitro.

The transcriptional factor NF-κB has been found to play a central role in the inflammation cascade (Gasparini and Feldmann 2012). Previous studies have shown that the anti-inflammatory effect of PTL is obtained by targeting different components of the NF-κB pathway (Hehner and others 1999; Yip and others 2004; Zhang and others 2014). During inflammatory response, the classical pathway of NF-κB activation is achieved through a heterodimer of p50 and p65 (Gasparini and Feldmann 2012; Napetschnig and Wu 2013).

In the inactive state, NF-κB dimers are bound to its inhibitor IκB and reside in the cytoplasm. Phosphorylation of IκB by IκB kinase leads to IκB degradation by the ubiquitin

**FIG. 6.** Effects of PTL on Wnt/β-catenin signaling pathways in osteoblasts. (A) Protein expression of Axin2, Dkk1, and β-catenin in cells treated with TNF-α with/without PTL. Gene expression of (B) AXIN2, (C) DKK1, and (D) CTNNB in osteoblast treated with TNF-α with/without PTL. Error bars indicate mean ± SEM (n = 3). *P < 0.05 versus the TNF-α group.
pathway, so that the NF-κB p50/p65 dimer translocates to the nucleus and initiates transcription (Gasparini and Feldmann 2012; Napetschnig and Wu 2013). Our results demonstrated that in TNF-α-stimulated osteoblasts, PTL inhibits the NF-κB pathway by decreasing NF-κB p50 expression and promotes IκB degradation, which is consistent with our previous study in an ex vivo LPS-stimulated hPDLCs model (Zhang and others 2014). However, unlike other reports that showed that PTL inhibits p65 translocation in macrophages and osteoclasts, we did not observe that PTL has an effect on p65 phosphorylation of osteoblasts in this study. The discrepancy might be because of diverse cell types and different experimental conditions.

The Wnt/β-catenin signaling pathway is an important regulator in bone development and regeneration. For instance, the Wnt/β-catenin pathway stimulates preosteoblast replication, induces osteoblastogenesis, promotes osteogenesis by directly stimulating RUNX2 gene, and inhibits apoptosis of osteoblasts and osteocytes (Kato and others 2002; Bodine and others 2004; Gaur and others 2005; Holmen and others 2005). Wnt/β-catenin signaling is activated when Wnt ligands (Wnt3a) bind to their receptors and lead to the accumulation of β-catenin in cytoplasm (Reya and Clevers 2005). Consequently, β-catenin initiates target gene transcription together with the transcriptional factor LEF/TCF (Reya and Clevers 2005). Axin2 is regarded as the reporter gene for intensity and duration of the Wnt/β-catenin pathway (Chia and Costantini 2005). DKK1 is a secreted protein, which is an antagonistic inhibitor of the Wnt signaling pathway that acts by isolating the Wnt coreceptor LRP6 (Lewis and others 2008). In this study, TNF-α was found to downregulate Wnt/β-catenin signals in osteoblasts, as shown by decreased expression of AXIN2 and β-catenin and increased DKK1, whereas PTL treatment recovered the activation of AXIN2 and β-catenin and downregulated DKK1. Therefore, the results suggest that PTL resists the inhibition of the Wnt/β-catenin signaling pathway caused by TNF-α in osteoblasts.

This study demonstrated that NF-κB inhibition by PTL downregulated DKK1 and recovered the activation of AXIN2 and β-catenin, which also implied the cross-talk between the NF-κB and β-catenin signaling pathways. DKK1 is a well-known Wnt signaling inhibitor, which suppresses nuclear β-catenin accumulation and correlates with inflammatory cytokine levels in chondrocytes of osteoarthritis (Weng and others 2009). Therefore, it is reasonable to speculate that in inflammatory environments, the activated NF-κB upregulated DKK1 through an unknown mechanism and then suppressed the accumulation of β-catenin. A recent study reported that NF-κB signaling promoted β-catenin degradation through induction of Smurf1 and Smurf2 (Chang and others 2013); in contrast, Wnt/β-catenin signaling activation led to the inhibition of NF-κB/p65 nucleus translocation through GSK-3β (Chen and others 2013). These studies also implied the interaction between the NF-κB and Wnt/β-catenin signaling pathways. However, the underlying molecular mechanisms of the cross-regulation between these 2 pathways require verification in future studies.

Periodontitis is a chronic and preventable dental disease, which can be prohibited before its occurrence or intervened in its early stage. In our previous and current studies, we found that PTL inhibits the inflammatory response and promotes osteoblast differentiation impaired by TNF-α in vitro. This evidence supports further investigation into the effect of PTL in periodontitis using animal models. In future studies, we will optimize the formulation of PTL for in vivo experiment and clinical use. Clinically, PTL could be utilized as an important ingredient to control inflammation and promote bone regeneration in periodontitis. For instance, PTL can be added into toothpaste for daily care or incorporated into periodontal dressing paste, which can be applied to periodontal lesions after scaling treatment or periodontal flap operation.

Conclusion

In summary, PTL showed the ability to promote osteoblast differentiation impaired by TNF-α. The possible mechanism may be related to the inhibitory effect of PTL on the NF-κB/p50 and ERK signaling pathways and, furthermore, the activation of the Wnt/β-catenin pathway in osteoblasts. Therefore, PTL has the potential to be used as a bone regeneration supplement in periodontitis.

Acknowledgments

Funding for this study was provided by Natural Science Foundation of Guangdong, China (2016A030313262), and Science and Technology Program of Guangzhou, China (201510010223).

Author Disclosure Statement

No competing financial interests exist.

References


PARTHENOLIDE PROMOTES DIFFERENTIATION OF OSTEOBLASTS


Address correspondence to:
Dr. Xueli Mao
Guangdong Province Key Laboratory of Stomatology
Department of Operative Dentistry and Endodontics
Guanghua School of Stomatology
Sun Yat-sen University
Guangzhou 510055
People’s Republic of China

E-mail: maoxuel@mail.sysu.edu.cn

Received 16 March 2017/Accepted 22 June 2017