

Original Article

Asperosaponin VI improves the inflammatory microenvironment through the PERK pathway to promote osteogenic differentiation of periodontal ligament stem cells

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Abstract: Objective: To investigate whether Asperosaponin VI improves the inflammatory microenvironment through the PERK pathway to promote osteogenic differentiation of periodontal ligament stem cells. Methods: Human periodontal ligament stem cells (PDLSCs) were isolated and were divided into control group and TNF- α group. The ER stress induced by TNF- α at a concentration of 10 ng/mL was analyzed. Subsequently, PDLSCs were divided into TNF- α group, TNF- α plus 4-phenylbutyric acid (PBA) group and TNF- α plus Asperosaponin VI group. After expression of PERK siRNA by lentiviral infection, the osteogenic differentiation of PDLSCs after blocking PERK was observed. Effects of saponins VI and 4-PBA on the expression of PDLSC osteogenic genes and PERK pathway molecules in inflammatory microenvironment were analyzed by quantitative PCR and western blotting. Results: After treatment with TNF- α at 10 ng/mL, the mRNA expression of Runx2, ALP and OCN in PDLSCs was decreased, but the mRNA and protein expression of GRP78, PERK, ATF4 and CHOP were significantly increased. Furthermore, after the co-culture with saponin VI at 10 μ mol/L, the abundance of ALP-positive cells and the expression of ALP mRNA were significantly improved, and the mRNA and protein levels of Runx2 and OCN were also increased. Conclusion: Asperosaponin VI may restore the osteogenic differentiation ability of PDLSCs by inhibiting the activation of the PERK pathway in inflammatory conditions.

Keywords: PERK pathway, inflammation, Asperosaponin VI, periodontal ligament stem cells (PDLSCs), osteogenic differentiation capability

Introduction

Human periodontal ligament stem cells (PDLSCs) are adult stem cells with strong self-renewal and self-differentiation ability in periodontal ligament (PDL) tissue [1]. PDLSCs are highly proliferative and differentiated, and can repair and reconstruct periodontal tissue when transplanted into periodontal damage area [2]. However, studies have found that autogenous PDLSCs in patients with periodontitis often fail to promote repairing of bone and PDL, which may be related to significant differences in the function of PDLSCs between healthy individuals and periodontitis patients. Inflammation of periodontal tissue in patients with periodontitis

may impair the differentiation capability of PDLSCs and lead to the damage and reduction of periodontal supporting tissue [3, 4]. Other studies have confirmed that osteogenic differentiation capability of PDLSCs can be reduced by pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α , interleukin-1 β and lipopolysaccharide (LPS) [5, 6]. Therefore, the improvement of periodontal inflammatory microenvironment may affect PDLSC behavior and promote osteogenic differentiation of periodontal ligament stem cells.

Dipsacales, also known as *Dipsacus asperoides* C. Y. Cheng et T. M. Ai, is a common Chinese herbal medicine, which is from the "Shen

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Nong's Herbal Classic", with the effect of nourishing liver and kidney, strengthening bones, and healing fracture. Aspergaponin VI is the main active ingredient extracted from *Dipsacus asperoides* C. Y. Cheng et T. M. Studies have shown that saponins can enhance the osteogenic differentiation ability of rat bone marrow mesenchymal stem cells, and promote bone formation [7]. However, the role of Aspergaponin VI in promoting osteogenic differentiation of periodontal ligament stem cells has not been reported yet.

Endoplasmic reticulum (ER) stress response is a protective mechanism induced by external stimuli (such as ischemia, hypoxia, diabetes and infection) to activate the stacking of unfolded or misfolded proteins in ER. ER chaperone, combined with immunoglobulin (Ig)/glucose regulated protein (GRP) 78, dissociates from the cavity structural domain and induces unfolded protein response (UPR) under ER stress, which can maintain cell homeostasis and reduce protein synthesis by regulating protein translation. Double-stranded RNA activated protein kinase-like ER kinase (PERK) signal transduction is one of the main pathways of UPR activation [8]. Once PERK is dissociated and activated by GRP78 [9], the transcription factor-4 (ATF-4) can be activated and can induce the translation and expression of downstream apoptosis-related factors, leading to cell growth arrest and DNA damage, and finally resulting in apoptosis [10].

The studies found that PERK signaling may be involved in the development of chronic inflammatory diseases such as rheumatoid arthritis and diabetes [11], and its mediated inflammatory response may be an important participant in MSCs osteoblast differentiation [12, 13]. Therefore, it is possible to promote osteogenic differentiation of periodontal ligament stem cells by regulating the activity of the PERK signaling pathway. Therefore, we hypothesized that saponins may promote the differentiation of periodontal ligament stem cells into osteoblasts, and this effect may be closely related to the activation of PERK signaling pathway.

Based on this, human PDLSCs were isolated and purified, and the inflammatory microenvironment of periodontitis was stimulated by TNF- α , and the Aspergaponin VI was given to intervention. Furthermore, the effects of PERK

signaling on the differentiation potential and ER stress response of periodontal ligament stem cells were evaluated.

Material and methods

Main reagents and instruments

The main reagents and instruments in this study include α -minimum Eagle's medium (α -MEM, Hangzhou Sijiqing Company, Hangzhou, China); fetal bovine serum (Hangzhou Sijiqing Company, Hangzhou, China); 0.25% trypsin (Sigma, USA); Type I collagenase (Sigma company, USA); 4-Benzene Butyric acid (Sigma, USA); alkaline phosphatase assay kit (ALP; Thermo Fisher company, USA); FACS Calibur flow cytometry analysis (BD, USA); reverse transcription kit (Takara company, Japan); anti-GPR78, anti-PERK, anti-ATF4, and anti-CHOP monoclonal antibody (Abcam, USA).

Primary culture and purification of human PDLSCs

From October 2015 to May 2016, six teeth were selected from the wisdom tooth and periodontitis, and the primary culture and purification of human PDLSCs were performed according to the method reported in the literature [14]. The PDL tissue was gently scraped off from the middle third of the root in extraction of tooth. The PDL tissue was cut into 1 mm³ cubes and washed three times in phosphate buffered saline (PBS) containing 100 U/mL penicillin and 100 mg/mL streptomycin (Nantong Biyuntian). The PDL tissue was digested by 3 mg/mL type I collagenase (Sigma Company) at 37°C for 40 minutes, and then transferred to a six-well culture dish. The tissues were cultured in α -minimum essential medium (α -MEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin G and 100 mg/mL streptomycin at 37°C with 5% CO₂. The single-cell cultures were obtained from primary cells by limited dilution technique to separate and purify PDLSCs. The culture medium was renewed every 3 days, and the cells were cultured to about 80% to 90% confluence with trypsin. P3 to P5 generation of subculture cells were used in the experiment. PDLSCs were divided into control group and TNF- α group. It was found that 10 ng/mL TNF- α could effectively induce ER stress [15] and was used in most experiments, then PDLSCs were divided into (1) 10

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ng/mL TNF- α group and (2) 10 ng/mL TNF- α plus 0, 0.5, 1, 5 mM 4-phenylbutyric acid (PBA; Sigma Company, USA) group. The cells were co-cultured with the corresponding drugs for 48 hours. All cell culture reagents were purchased from Thermo Fisher unless otherwise specified.

Detection of cell phenotype by flow cytometry

In order to identify the phenotype of PDLSCs, about 5×10^5 second generation cells were suspended in PBS and mixed with mouse anti-human monoclonal antibodies of FITC-CD31, FITC-CD105, FITC-CD146, PE-CD34, PE-CD44 and PE-CD90. The cells were incubated at 4°C for 30 minutes, washed twice with PBS, and analyzed by FACS Calibur Flow Cytometer (BD Company). All antibodies were purchased from BD Company, USA.

Determination of osteogenic and adipogenic differentiation

PDLSCs were vaccinated into six-well culture dishes at the concentration of 1×10^5 cells per hole or 12-well culture dishes with 5×10^4 cells per hole. When the cells reached 80% confluence, they began to differentiate. The original cell culture medium were replaced by osteogenic medium (OM; α -MEM containing 10% FBS, 10 mM β -sodium glycerophosphate, 50 μ g/mL ascorbic acid and 10 nM dexamethasone) or adipogenic medium (α -MEM containing 10% FBS, 0.1 mM indomethacin, 0.5 mM methyl-isobutyl-xanthine, 10 μ g/mL insulin and 1 mM dexamethasone). The culture fluid was changed every 3 days and maintained in the differentiation medium for 1, 2 or 4 weeks. The cells were treated with human TNF- α (1, 10 and 20 ng/mL; R&D, USA) or 4-PBA (0.5, 1, and 5 mM; Sigma, USA) for inducing osteogenic differentiation. A week later, the total RNA was extracted and stained with alkaline phosphatase (ALP; Thermo Fisher) assay kit according to the manufacturer's instructions. ALP staining density was measured by Image J. Two weeks later, the total protein was collected. Four weeks later, the formation of mineralized nodules was assessed by Alizarin red (Sigma, USA) staining, and 100 mM cetylpyridinium chloride (Sigma, USA) was used to extract red dyes at 37°C for 10 minute staining to quantify mineralized nodules. The absorbance of the extracted dyes was measured at 570 nm.

For fat formation and differentiation, lipid vacuoles were stained with oil red O (Sigma, USA) after 4 weeks. All the chemical reagents for inducing differentiation were purchased from Sigma, USA.

Effects of Aspergaponin VI on the expression of PDLSC osteogenic related genes and PERK pathway molecules in inflammatory microenvironment was detected by quantitative PCR

The cells were cultured to second generation cell, and added to a 96-well plate at a density of 1×10^3 cells in 200 μ L medium per well. The control group was cultured in normal cell culture medium, and the TNF- α group was cultured with medium containing 10 ng/mL TNF- α . The Aspergaponin VI group was administered with 10 μ g/L TNF- α and 10 μ mol/L Aspergaponin VI. After 7 days of cultivation, the total RNA was extracted from different groups by TRizol reagent. Reverse transcription was performed using RT kit (Promega). The reaction conditions of PCR were as follows: 94°C, 4 min; 94°C, 40 s; 52°C, 40 s; 72°C, 40 s, 40 cycles. Quantitative real-time PCR was performed on ABI 7500 amplifier using SYBR Premix Ex Taq from Takara. The results of PCR were analyzed by 2- $\Delta\Delta$ Ct method. GAPDH was selected as an internal reference. The primer sequences were as follows: 1) GAPDH: forward, 5'-CTGC-AAGAACAGCATTGCAT-3' and backward, 5'-GACCACCTGGTCCTCAGTGT-3'; 2) PERK: forward, 5'-GTGATAAAGGTTTCGGTTGCTG-3' and backward, 5'-TGTTTTCTGTGGCTCCTCTGG-3'; 3) GRP78: forward, 5'-TCAAGTTCTTGCCGTTCAAGG-3' and backward, 5'-AAATAAGCCTCAGCGGTTCTT-3'; 4) ATF4: forward, 5'-CATGGGTTCTCCAGCGACA-3' and backward, 5'-TCTGGCATGGTTCCAGGTC-3'; 5) CHOP: forward, 5'-CAAGAGGTCCTGTCTCAGATGA-3' and backward, 5'-TCTGTTTCCGTTTCCTGGTTC-3'; 6) ALP: forward, 5'-GGACCATCCACGTCTTCAC-3' and backward, 5'-CCTTGTAGCCAGGCCCATTTG-3'; 7) Runx2: forward, 5'-CCCGTGGCCTTCAAGGT-3' and backward, 5'-CGTTACCCGCCATGACAGTA-3'; 8) Osteocalcin (OCN): forward, 5'-CCCAGGCGCTACCTGTATCAA-3' and backward, 5'-GGTCAGCCAACCTGTCACAGTC-3'.

The PCR primers were designed and synthesized by Shanghai Sangon Biotech Company.

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Effects of Aspergaponin VI on the expression of PDLSC osteogenic proteins and PERK pathway molecules in inflammatory microenvironment were detected by western blotting

Western-blotting was used to detect the expression of osteogenic proteins and PERK pathway molecules after protein separation from different cell types. The method is briefly described as follows: after separation of proteins in different groups, Western-blot was used to detect the expression of osteogenic related proteins and PERK pathway molecules. Western-blot method was described as follows: After separation of proteins in different groups, the cell proteins were analyzed by SDS-PAGE gel electrophoresis and transferred to PVDF membrane. The PVDF membrane was blocked with 5% skim milk and incubated with osteogenic related proteins (anti-Runx2 and anti-OCN) and PERK pathway molecules (anti-GRP78, anti-PERK, anti-ATF4 and anti-CHOP monoclonal antibodies) (all antibodies were purchased from Abcam, USA), using GAPDH monoclonal antibody as internal reference. The protein was examined by chemiluminescence. The protein bands were quantified by Image J software, and the protein level was analyzed.

Statistical analysis

GraphPad Prism 6.0 and SPSS 18.0 statistical software were used to analyze the data obtained from the study. For two-group comparison, independent t test was used. For multi-group comparison, one-way ANOVA with post hoc Bonferroni test were used. The data were expressed as mean \pm standard deviation (mean \pm SD). $P < 0.05$ was considered to have statistical significance.

Results

Separation and identification of PDLSCs

PDLSCs purified by limited dilution technique showed fibroblast-like morphology (**Figure 1A**). PDLSC also showed multi-differentiation potential. After 4 weeks of induction, PDLSCs could form red dye-positive mineralized nodules, indicating osteoblasts (**Figure 1B**), and lipid-stained lipid droplets, indicating fat cells (**Figure 1C**). The immunophenotype of PDLSCs was further determined by flow cytometry. PDLSCs are characterized by typical patterns of mesenchy-

mal surface markers, which are positive for CD146, CD105, CD90 and CD44, but negative for CD31 and CD34 (**Figure 1D**).

Effect of Asperosaponin on osteogenic differentiation of PDLSCs

After 1, 2 or 4 weeks of osteogenic induction, the mRNA expression of osteogenic transcription factor Runx2, bone matrix factor ALP and OCN in PDLSCs treated with 10 ng/mL TNF- α were decreased compared with untreated cells, but the mRNA expression of the bone transcription factor Runx2 and the bone matrix factors ALP and OCN in the saponins VI were significantly higher than those in the TNF- α group (**Figure 2A, 2B**). The protein levels of Runx2 and OCN has emerged the same trend (**Figure 2C**). In addition, TNF- α treatment was associated with decreased ALP staining (**Figure 2D**) and the formation of mineralized nodule (**Figure 2E, 2F**) ($P < 0.05$). The ALP staining and mineralized nodule formation in the Asperosaponin VI group was significantly increased compared with the TNF- α group.

Regulatory effect of Asperosaponin VI on PERK pathway

Compared with the control cells cultured in OM without TNF- α , TNF- α treatment (10 ng/mL) resulted in a significant increase in the mRNA and protein expression of GRP78, PERK, ATF4 and CHOP, but the mRNA and protein expression levels of GRP78, PERK, ATF4 and CHOP in saponins VI were significantly lower than those in TNF- α group ($P < 0.05$, **Figure 3A-F**), which suggested that Asperosaponin VI reduced the activation level of PER-pathway-related factors by TNF- α (10 ng/mL).

Effect of ER stress inhibitor 4-PBA on osteogenic differentiation of PDLSCs involving TNF- α

During osteogenic induction, the cells were pre-treated in OM containing 4-PBA for 12 hours, and then cultured with 10 ng/mL TNF- α without 4-PBA every 3 days for 4 weeks. Compared with 0 mM 4-PBA, 0.5, 1 and 5 mM 4-PBA showed significantly positive correlation to the mRNA level of ALP and Runx2 (**Figure 4A, 4B**) and the protein level of Runx2 and OCN (**Figure 4C**) ($P < 0.05$). In addition, ALP staining (**Figure 4D**) showed significantly positive correlation to mineralized nodule formation (**Figure 4E**) and

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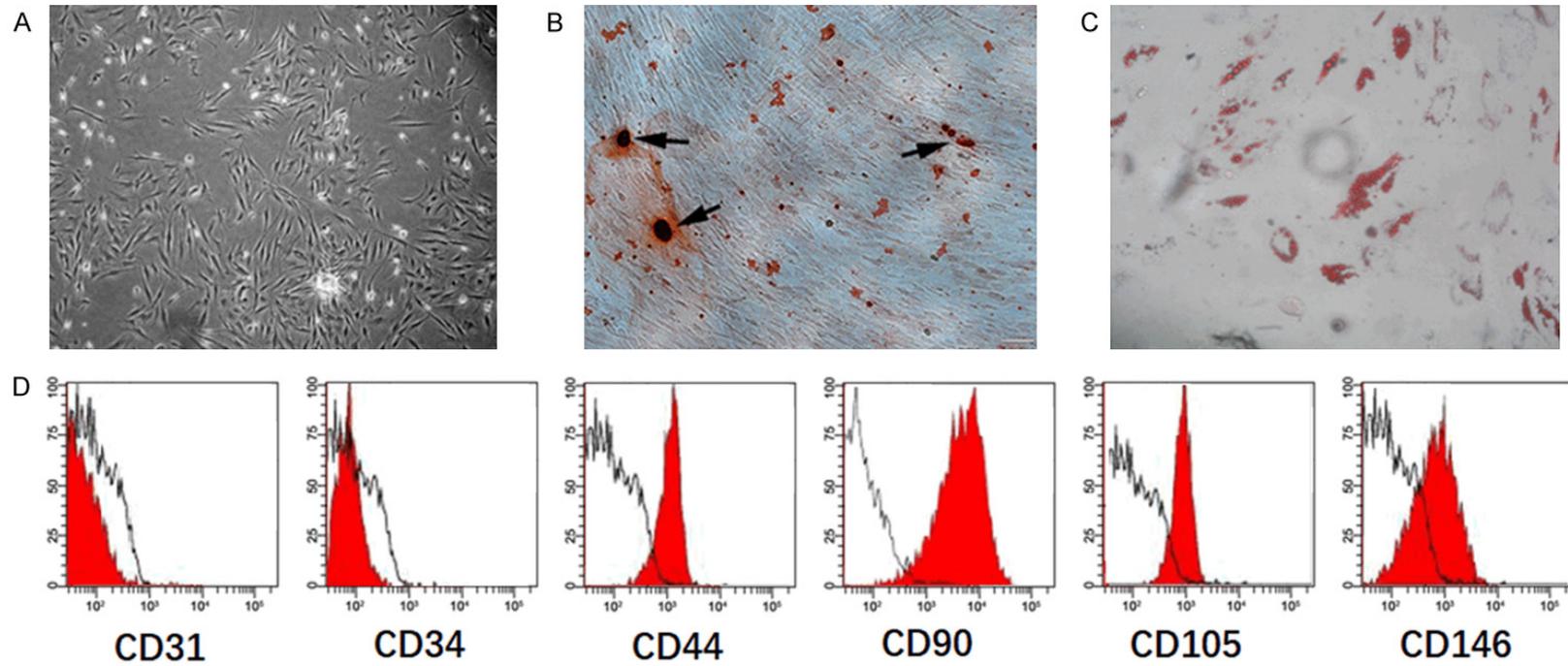


Figure 1. Separation and identification of PDLSCs. A. Morphology; B. Alizarin red staining; C. Oil red staining; D. Flow cytometry.

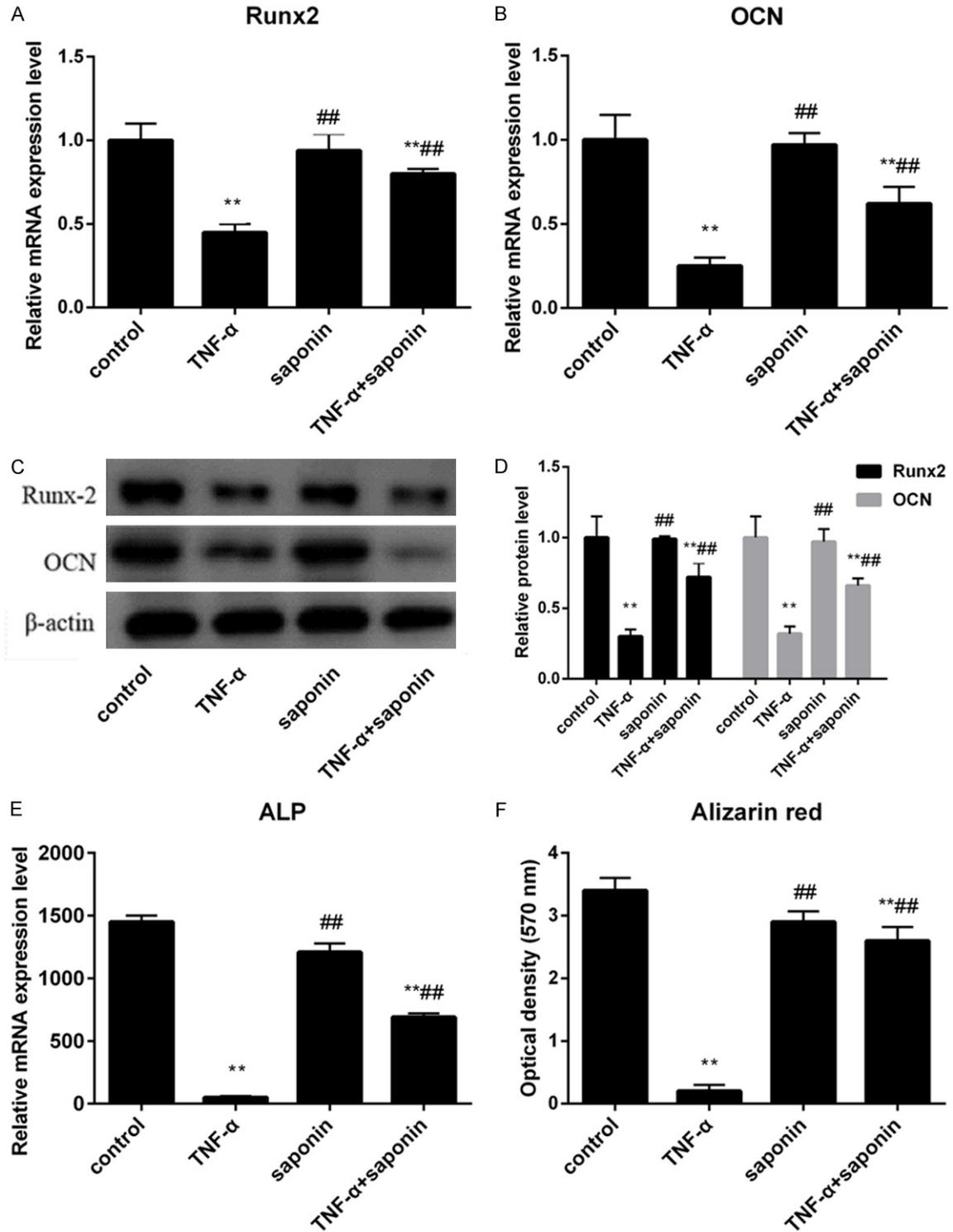


Figure 2. Effect of Asperosaponin VI on osteogenic differentiation of PDLSCs. A. Quantitative Real-time PCR was used to detect Runx-2; B. Quantitative Real-time PCR was used to detect OCN; C. Western-blotting was used to detect the protein changes of Runx-2 and OCN; D. Western-blotting was used to detect the protein changes of Runx-2 and OCN; E. Quantitative Real-time PCR was used to detect ALP mRNA (mol/L); F. Quantitative determination of Alizarin red staining. **P < 0.01 stands for comparison with control group; ##P < 0.01 stands for comparison with 10 ng/ml TNF-α.

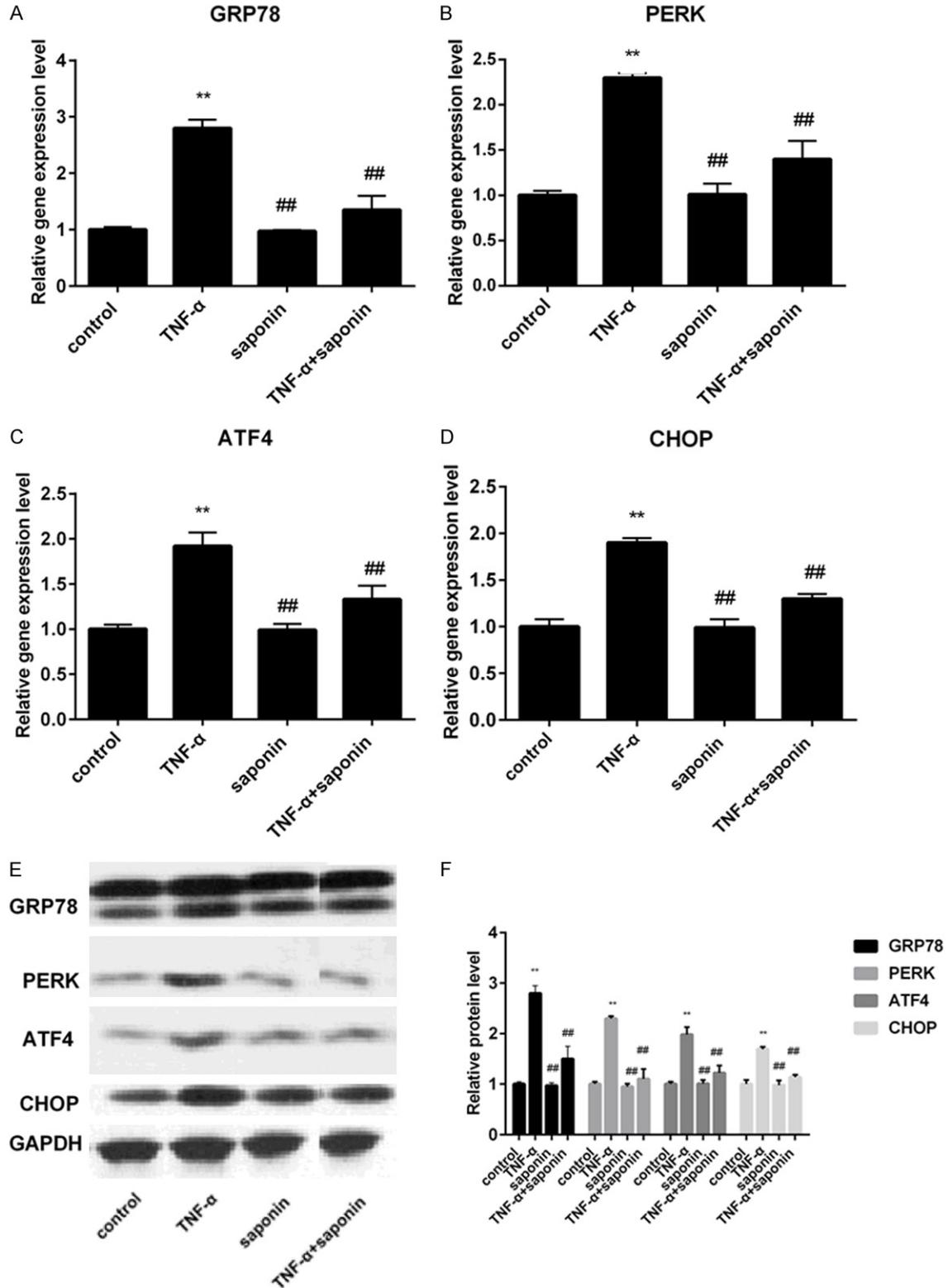


Figure 3. Effect of Asperosaponin VI on the activation of PERK pathway. A. Quantitative real-time PCR was used to detect GRP-78; B. Quantitative real-time PCR was used to detect PERK; C. Quantitative real-time PCR was used to detect ATF4; D. Quantitative real-time PCR was used to detect CHOP; E, F. Western-blotting was used to detect the protein levels of GRP-78, PERK, ATF4 and CHOP. **P < 0.01 stands for comparison with control group; ##P < 0.01 stands for comparison with 10 ng/ml TNF- α .

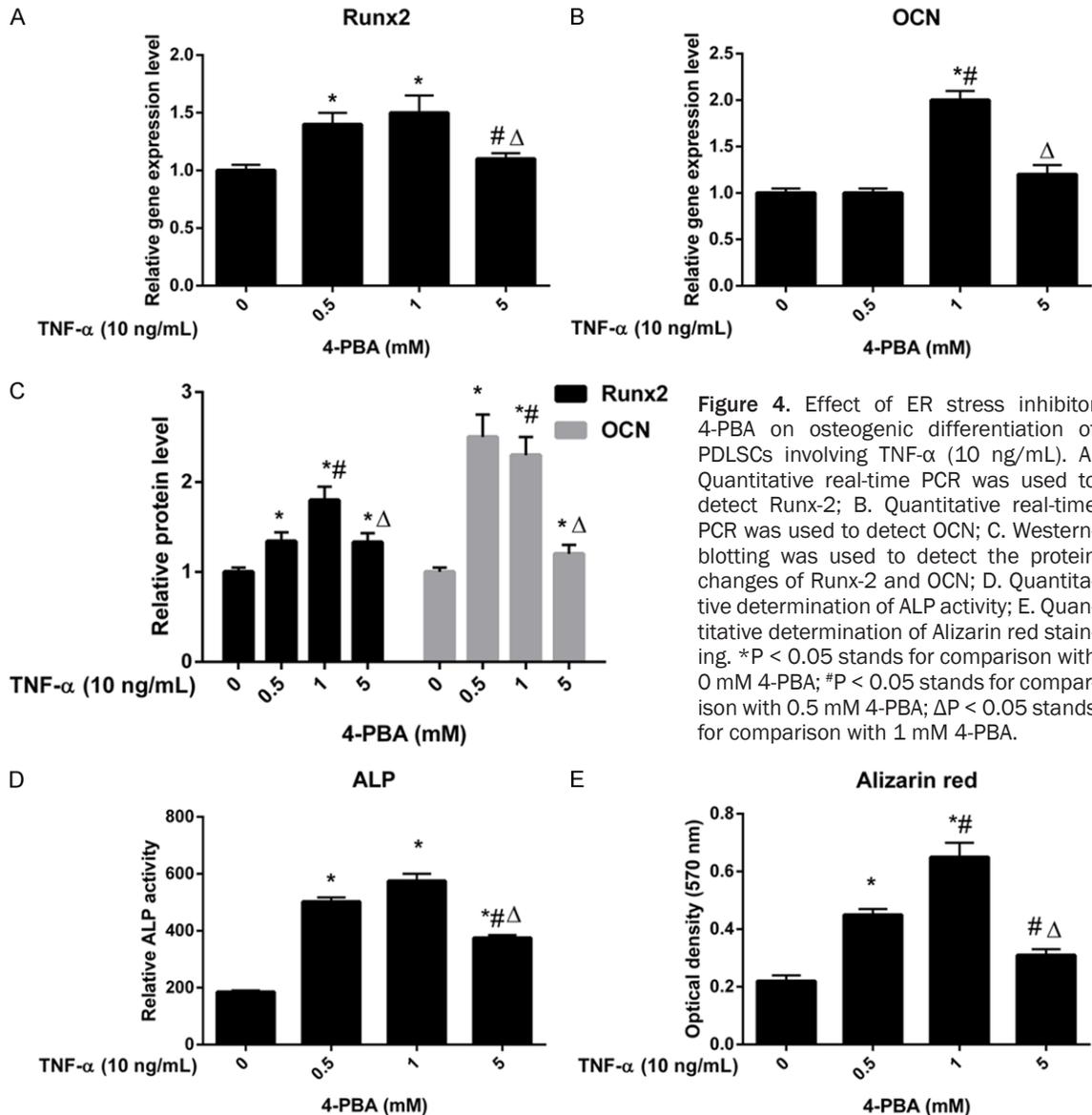


Figure 4. Effect of ER stress inhibitor 4-PBA on osteogenic differentiation of PDLSCs involving TNF-α (10 ng/mL). A. Quantitative real-time PCR was used to detect Runx-2; B. Quantitative real-time PCR was used to detect OCN; C. Western blotting was used to detect the protein changes of Runx-2 and OCN; D. Quantitative determination of ALP activity; E. Quantitative determination of Alizarin red staining. *P < 0.05 stands for comparison with 0 mM 4-PBA; #P < 0.05 stands for comparison with 0.5 mM 4-PBA; ΔP < 0.05 stands for comparison with 1 mM 4-PBA.

was significantly increased by 4-PBA pretreatment. OCN gene expression was restored by 4-PBA with concentration of 1 and 5 mM (Figure 4B). However, 5 mM 4-PBA aggravated the negative effects of TNF-α on osteogenic related factors and ALP staining level.

Effect of PERK siRNA on osteogenic differentiation of PDLSCs involving TNF-α

In this study, whether PERK played a role in TNF-α-induced osteogenic potential damage in PDLSC has been further studied. PERK silencing was induced by treatment of cells with siRNA and 30% knockdown of the RNA level

was confirmed (Figure 5A). The decrease of Runx2 (Figure 5B) and OCN mRNA (Figure 5C) expression and protein (Figure 5D) and ALP staining (Figure 5E, 5F) levels was induced by TNF-α treatment in PERK siRNA reverse transcription (P < 0.05).

Discussion

Irreversible alveolar bone resorption is one of the most difficult diseases in periodontal treatment [16]. The decreased osteogenesis and bone regeneration during chronic inflammatory diseases were thought to be caused by inflammatory microenvironment. The change of

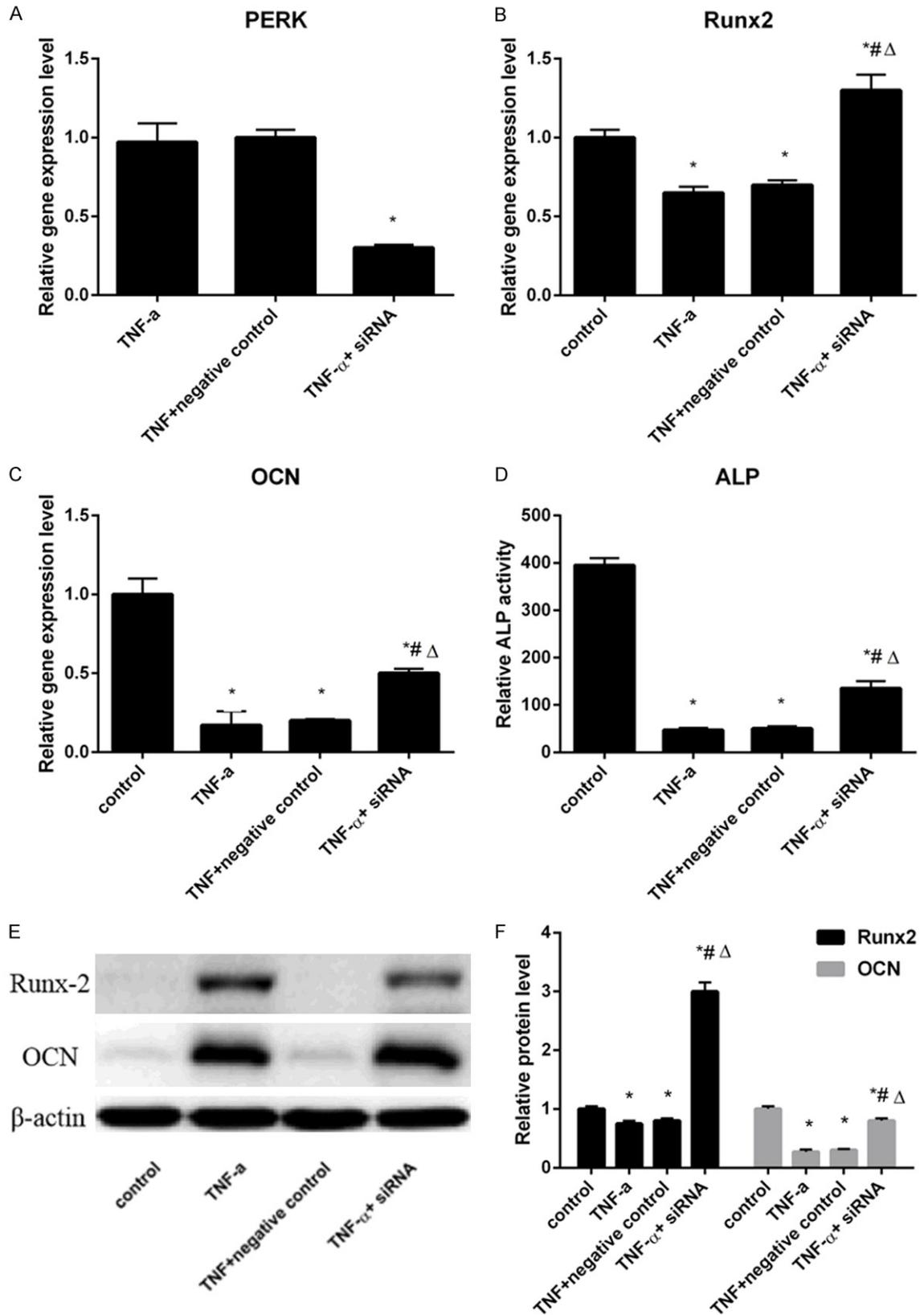


Figure 5. Effect of PERK siRNA on osteogenic differentiation of PDLSCs involving TNF- α . A. Quantitative real-time PCR was used to detect PERK to evaluate the efficiency of siRNA; B. Quantitative real-time PCR was used to detect OCN; C. Quantitative real-time PCR was used to detect Runx-2; D. Western-blotting was used to detect the protein

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changes of Runx-2 and OCN; E, F. Quantitative determination of ALP activity. *P < 0.05 stands for comparison with control; #P < 0.05 stands for comparison with TNF- α + control siRNA; Δ P < 0.05 stands for comparison with TNF + negative control.

osteogenic differentiation ability of MSCs can significantly affect the formation of alveolar bone. However, the role of the inflammatory microenvironment in the differentiation of stem cells with specific functions is unclear. In this study, the mechanism of inflammation affecting osteogenic differentiation defect of MSC was discussed, and the effect of Asperosaponin VI on osteogenic differentiation was observed. It has been reported that TNF- α may inhibit the osteogenic differentiation of PDLSCs by activating the PERK pathway, suggesting that PERK acts downstream of TNF- α to block osteogenic differentiation. Our study found that after treatment with TNF- α at 10 ng/mL, mRNA expression of Runx2, ALP and OCN in PDLSCs was decreased, and the mRNA and protein expression of GRP78, PERK, ATF4 and CHOP were significantly increased. While after the co-culture with 4-PBA, the abundance of ALP-positive cells and mRNA expression of ALP were significantly improved, and the mRNA and protein expression of Runx2 and OCN were also significantly increased. Our results are consistent with the conclusions in above studies. PDLSC can effectively promote the regeneration of periodontal tissue and show multiple differentiation potential [17]. PDLSC shows similar characteristics to MSC, such as: 1) clonal growth; 2) expression of MSC surface markers; 3) multi-lineage differentiation potential for successful separation and purification. TNF- α is the major pro-inflammatory cytokine that causes alveolar bone damage. It is released early in the disease and induces inflammatory response. Generally speaking, lower concentrations of TNF- α can promote or maintain the osteogenic differentiation of PDLSCs, but higher concentrations of TNF- α can inhibit the differentiation [18]. On the contrary, it has been shown that higher concentrations of TNF- α (20 ng/mL) can improve osteogenesis in human MSC in a study [19]. The difference may be attributed to the original cells or experimental conditions. In current study, the results were under the condition of TNF- α at 10 ng/mL, but the effect of TNF- α at 20 ng/mL on osteogenic differentiation was not observed, which will be further observed in the next study.

It has also been found that 10 ng/mL of TNF- α can reduce the osteogenic differentiation ability of PDLSCs, which can be proved by marker analysis and physiological measurement of differentiation in vitro. It has been proven that ALP can mineralize inorganic phosphates by catalyzing the hydrolysis of organic phosphates. Runx2 is the main osteogenic transcription factor expressed in the early stage of osteoblast differentiation and regulates osteoblast-specific genes, such as ALP, OCN, and the most abundant non-collagen bone matrix protein [20]. After treating PDLSCs with TNF- α , the abundance of ALP-positive cells and the expression of ALP mRNA were significantly decreased. The mRNA and protein levels of Runx2 and OCN were also decreased, which were related to the formation of mineralized nodules (**Figure 2**). The results were consistent with the results of another study, which showed that 10 ng/mL TNF- α treatment impairs the osteogenic differentiation of PDLSCs [3]. When 10 μ mol/L Asperosaponin VI was co-cultured, ALP-positive cell abundance and ALP mRNA expression were significantly improved, and the mRNA and protein levels of Runx2 and OCN were also significantly increased, suggesting that Asperosaponin VI can enhance the osteogenic differentiation ability of impaired PDLSCs stimulated by TNF- α (**Figure 2**).

ER stress is a relatively new subcellular pathological process. UPR is a protective response to ER stress, which is activated by three pathways of protein sensors on ER membrane: 1) PERK/ATF4; 2) inositol kinase 1 α /X-box binding protein 1s (XBP1s); 3) ATF6 [10]. There are few studies confirming the relationship between ER stress and inflammatory microenvironment in periodontitis. The mRNA levels of ATF4, XBP1 and CHOP in gingival tissues of patients with periodontitis were significantly higher than those of patients with gingivitis. The expression levels of GRP78 and XBP1 and alveolar bone resorption were significantly increased in mice treated with porphyromonas gingivalis. In addition, nicotine is a major toxic component in tobacco and also an important risk factor for inducing periodontal disease. It has been proven that nicotine can promote the phosphoryla-

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tion of PERK and α -subunits of eukaryotic initiation factor 2 and increase its role. GRP78 and CHOP protein levels were affected in time-dependent manner [21]. To the author's knowledge, TNF- α can activate PERK pathway in PDLSCs, and the activation of UPR occurs simultaneously with the blockage of osteogenic differentiation.

To further characterize the relationship between UPR and osteogenic differentiation of PDLSCs, chemical chaperone 4-PBA, an ER stress inhibitor inducing cytoprotection, was used to block ER stress response. According to the experience, 4-PBA of various concentrations was tested to determine the best effect on PDLSC. It was found that cell proliferation was not significantly affected by 12-hour 4-PBA treatment and lower concentrations of 4-PBA (0.5, 1 and 2.5 mM) could inhibit the expression of GRP78 and its downstream factors. However, the protein level of CHOP was not down-regulated in parallel with the expression level of mRNA. 12-hour pretreatment may not be sufficient to inhibit the expression of CHOP protein, because it has been shown that CHOP can be induced within 24 hours before 4-PBA treatment of 3T3-L1 preadipocytes and can be reduced within 4 days and 2 days. PBA can treat human gingival fibroblasts [22]. 7.5 mM 4-PBA still induces many other PERK pathway related factors, suggesting that high concentration of 4-PBA may be the trigger of ER stress.

Lower concentration of 4-PBA was sufficient to rescue osteogenic differentiation defects induced by TNF- α . Importantly, it was found that the concentration dependent inhibition of 4-PBA on PERK pathway was positively correlated with the recovery of differentiation ability. It was similar to the present results that the inhibition of UPR by 4-PBA blocked the osteoclastic differentiation of bone marrow macrophages isolated from porphyromonas gingivalis [23]. At the same time, the study also found that the administration of 10 μ mol/L Asperosaponin VI showed a similar trend to 4-PBA, suggesting that Asperosaponin VI may promote the recovery of osteogenic differentiation by inhibiting the activity of PERK pathway.

Previous studies have shown that PERK is a potential regulator of bone development and osteoblast activity [24]. It was suggested that silencing PERK can rescue the osteogenic dif-

ferentiation of PDLSCs in this study, and the Asperosaponin VI has a similar trend. Therefore, it was suggested that PERK may be an important factor in controlling the differentiation of PDNSC downstream of TNF- α . The Asperosaponin VI may promote the recovery of osteogenic differentiation by regulating the activity of the PERK pathway.

In summary, Asperosaponin VI may restore osteogenic differentiation of PDLSCs by inhibiting the activation of the PERK pathway in inflammatory conditions.

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Disclosure of conflict of interest

None.

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