PERK-eIF2α-ATF4 pathway mediated by endoplasmic reticulum stress response is involved in osteodifferentiation of human periodontal ligament cells under cyclic mechanical force

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A B S T R A C T

To prevent excess accumulation of unfolded proteins in endoplasmic reticulum (ER), eukaryotic cells have signaling pathways from the ER to the cytosol or nucleus. These processes are known as the endoplasmic reticulum stress (ERS) response. Protein kinase R like endoplasmic reticulum kinase (PERK) is a major transducer of the ERS response and it directly phosphorylate α-subunit of eukaryotic initiation factor 2 (eIF2α), resulting in translational attenuation. Phosphorylated eIF2α specifically promoted the translation of the activating transcription factor 4 (ATF4). ATF4 is a known important transcription factor which plays a pivotal role in osteoblast differentiation and bone formation. Furthermore, ATF4 is a downstream target of PERK. Studies have shown that PERK-eIF2α-ATF4 signal pathway mediated by ERS was involved in osteoblastic differentiation of osteoblasts. We have known that orthodontic tooth movement is a process of periodontal ligament cells (PDLCs) osteodifferentiation and alveolar bone remodeling under mechanical force. However, the involvement of PERK-eIF2α-ATF4 signal pathway mediated by ERS in osteogenic differentiation of PDLCs under mechanical force has not been clearly understood. In our study, we applied the cyclic mechanical force at 10% elongation with 0.5 Hz to mimic occlusal force, and explored whether PERK-eIF2α-ATF4 signaling pathway mediated by ERS involved in osteoblastic differentiation of PDLCs under mechanical force. Firstly, cyclic mechanical force will induce ERS and intensify several osteoblast marker genes (ATF4, OCN, and BSP). Next, we found that PERK overexpression increased eIF2α phosphorylation and expression of ATF4, furthermore induced BSP, OCN expression, thus it will promote osteodifferentiation of hPDLCs; mechanical force could promote this effect. However, PERK−/− cells showed the opposite changes, which will inhibit osteodifferentiation of hPDLCs. Taken together, our study proved that PERK-eIF2α-ATF4 signaling pathway mediated by ERS involved in osteoblast differentiation of PDLCs under cyclic mechanical force.

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1. Introduction

As we know, the endoplasmic reticulum (ER) is one of the most important cellular compartments in eukaryotic cells [1]. ER participated in processing various cellular signals and localizing the secreted and transmembrane proteins, and its dysfunction would be lethal to cells. When cells are subjected to hypoxia, calcium imbalance and other stimuli, unfolded or misfolded proteins would accumulate in ER and Ca2+ concentration changed, which cause the imbalance in the structure and function of ER, then the relative signaling pathways will be activated to avoid excess accumulation of unfolded proteins in the ER, triggering endoplasmic reticulum stress (ERS) [2,3]. ERS is mainly mediated by three major transducers-protein kinase R like endoplasmic reticulum kinase (PERK) [4], activating transcription factor 6 (ATF6) [5], and inositol requiring enzyme 1 (IRE1) [6]. The activation of PERK is in the center position in ERS; When PERK was activated, it would cause the phosphorylation of the α-subunit of eukaryotic initiation factor 2 (eIF2α), which inhibits the synthesis of some proteins [4,7]. However, unlike most proteins, activating transcription factor 4 (ATF4) avoided translational attenuation by eIF2α phosphorylation because ATF4 has upstream open reading frames (ORFs) in its 5′-untranslated region. Under normal conditions, these upstream ORFs prevent translation of true ATF4, but only when eIF2α is phosphorylated, they are ignored and ATF4 translation occurs [8,9].
ATF4, one of the transcription factor family members of CAMP response element binding (CREB), could regulate osteoblast differentiation and bone formation [10,11]. As transcriptional targets of ATF4, osteocalcin (OCN) and bone sialoprotein (BSP) are also osteoblast-specific and important markers for osteoblast differentiation. Previous studies have shown that mechanical force increased the expression of osteogenesis-related genes and proteins in PDLCs including ATF4, OCN, BSP, etc [12-17]. It is reported that ATF4-deficient mice revealed a marked reduction or delay in bone mineralization [18]. These studies clearly proved that ATF4 is an important transcription factor for osteoblast differentiation and bone formation.

As mentioned, PERK is one of the major transducers of ERS. PERK directly cause eIF2α phosphorylation and phosphorylated eIF2α promotes the translation of ATF4. ATF4 is a translational target of activated PERK. Some researchers found that up-regulation of ATF4 under mechanical stress may involve PERK in the cardiovascular disease, kidney and other tissues [1,19,20]. Furthermore, studies have shown that ERS occurred during osteoblast differentiation and PERK-eIF2α-ATF4 signal pathway mediated by ERS was involved in osteoblastic differentiation of osteoblasts [21]. As we know, orthodontic tooth movement is also a process of periodontal ligament cells (PDLCs) osteodifferentiation and alveolar bone remodeling under mechanical stimuli [12,22,23]. Osteodifferentiation of PDLCs plays a crucial role in periodontal tissue remodeling and preserving periodontal homeostasis [24]. However, whether PERK-eIF2α-ATF4 signal pathway mediated by ERS involved in periodontal remodeling and osteogenic differentiation of PDLCs during orthodontic tooth movement or not and the potential function of ERS is still not well understood. To explore this point, we examined the expression of ATF4 by loss and over-expression of PERK in vitro followed by the promotion of gene expression essential for osteogenesis. Then, we studied the effect of mechanical force in activating ERS. Finally, we will detect the biological function of PERK-eIF2α-ATF4 signaling in osteogenic differentiation of PDLCs when they are submitted to mechanical force.

2. Materials and methods

2.1. Cell culture

Human PDLCs (hPDLCs) were cultured with tissue culture method. Primary PDL tissues were isolated from healthy premolar teeth of teenagers (12–16 years old) undergoing tooth extraction for orthodontic reasons. Informed consent was given to all patients before extraction. The PDL tissue was scraped from the mid-third of the root surface, then cut into small pieces, and placed in 35-mm tissue culture flasks. The cells from explants were cultured in alpha minimum essential medium (α-MEM; Hyclone, USA) containing 100 U/ml penicillin, 100 mg/ml streptomycin, and 15% (v/v) fetal bovine serum (FBS; Gibco, USA). Cells were cultured in 37 °C in a humidified atmosphere of 95% air and 5% CO2. The medium was replaced every 2 to 3 days when cells began to grow from the explants. Once achieving fusion, the cells were separated with 0.25% trypsin and recultured until confluence. PDLCs of passages three and four were used in this experiment.

2.2. Application of cyclic mechanical force to hPDLCs

hPDLCs were seeded into six-well culture plates with 35-mm silicone membrane coated on the bottom. After the cultures reached nearly 80% confluences for 2–3 days, cells were serum-deprived for 24 h. Then, cyclic mechanical force was applied at 10% deformation and 0.5 Hz (30 cycles/min) for 1 h, 3 h, 6 h, 12 h and 24 h, using Flexcell® FX-5000™ Tension System (Flexcell International Corporation). Control cells not submitted to mechanical force were cultured on the similar six-well plate and in the same incubator for the maximum stretching period. Mechanical loading was conducted for three independent times, and the mean data of each time would be collected as the final data for statistical analysis.

2.3. Cell transfection mediated by lentiviral vector

Overexpression vector of human PERK (PERK+/−) and PERK knock-out (PERK−/−) were constructed by Genechem (Shanghai Genechem Co., Ltd.). Empty virus vector GFP served as a negative control respectively. To obtain optimal value of multiplicity of infection (MOI), we first conducted a preliminary experiment. HPDLCs were cultured to reach 40–60% confluence and were transfected by lentivirus at MOI at 50, 60, 80, 100, 150. The transfection efficiency was evaluated by fluorescence microscope (Nikon, Japan). In the formal experiment, the appropriate amounts of lentivirus were added according to the appropriate MOI values (PERK+/−: MOI = 100, PERK−/−: MOI = 100, empty vector: MOI = 60). The effects of PERK+/− and PERK−/− were assessed by quantitative real-time PCR and Western blotting. In order to improve the transfection efficiency, enhanced infection solution (ENi-S) and 10 μg/ml polybrene were added to select positively-transduced cells. Six hours later, the medium was changed and the cells were cultured in standard α-MEM with no additions, containing 10% FBS, for 2–3 days.

2.4. Quantitative real-time RT-PCR

HPDLCs were collected at the indicated time, and the total RNA was isolated from each sample by means of Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA). After RNA isolation, 500 ng RNA was reverse transcribed into cDNA by SYBR-PrimeScript RT-PCR Kit (Takara). 2.0 μl of the cDNA sample was subjected to real-time PCR using SYBR Premix Ex Taq (Takara) in a thermal cycler (Light Cycler 480; Roche, Basel, Switzerland). The real-time PCR protocol was as follows: 30 s of initial denaturation at 95 °C; 40 cycles comprising 5 s of denaturation at 95 °C and 20 s of annealing at 60 °C. The ΔΔCt method was used to calculate relative mRNA levels of all genes that normalized to housekeeping gene GAPDH. The results were performed in three times and expressed as a relative fold change in gene expression compared to the control. Specific primers used for amplification were synthesized based on the GenBank database and were shown in Table 1.

2.5. Western blot analysis

The PDLCs were collected and solubilized with lysis buffer that consisted of 50 mM Tris-HCL, 1 mM EDTA, 1% TritonX-100, 150 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride (PMFS, proteasine inhibitor). The supernatant was obtained by centrifugation at 12,000g for 15 min. Protein concentration was measured using a BCA Protein Assay Reagent Kit (Sunbio Biological Technology Co LTD., Beijing, China). Proteins were separated by electrophoresis on 10% SDS-PAGE gels using a constant voltage of 80 V for 2 h, and then transferred electrophoretically onto polyvinylidenefluoride (PVDF) membranes.
(Amersham Biosciences, Uppsala, Sweden). Membranes were blocked with 5% skimmed milk in tris-buffered saline tween-20 (TBST) for 1 h at room temperature, and then incubated at 4 °C overnight with primary antibodies. Next, the membrane was washed with TBST for 10 min for 3 times, followed by incubation at room temperature for 2 h with HRP-conjugated goat anti-mouse secondary antibody. Immunoreactivity was observed using the enhanced chemiluminescence (ECL) plus Detection Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions, and the densitometric value of each band was determined by LabWorks Image (Scion Corporation, Frederick, MD, USA). The following primary antibodies were used: anti-PERK (Santa Cruz Biotechnology; 1:500), anti-eIF2α (Cell Signaling; 1:1000), anti-phospho-eIF2α (Stress Gen; 1:1000), anti-ATF4 (Abcam; 1:500), anti-OCN (Santa Cruz Biotechnology; 1:1000), anti-BSP (COSMO BIO; 1:1000), anti-GAPDH (1:3000).

2.6. Alizarin red staining

The hPDLCs of non-transfection, transfected with PERK+/+, PERK−/−, and LV-GFP were cultured for 3 weeks. The cells were washed with PBS three times and fixed with 70% ethanol for 15 min on the ice. Then, the cells were stained with 2% Alizarin red solution (pH 4.2) for 10 min at room temperature. Next, the cells were washed with distilled water for 3–5 times and photographed. Images of stained cells were obtained using a digital camera (Canon, Japan) and microscope (Olympus, Tokyo, Japan). Alizarin red staining is an indicator of Ca deposition in cellular matrix and to evaluate the degree of mineralization.

2.7. Statistical analysis

Quantitative data were expressed as mean ± SD. Significant differences were determined by using Student's t-test and one-way ANOVA. The differences between groups were statistically significant at *P < 0.05 and **P < 0.01, ***P < 0.005 and ###P < 0.001.

3. Results

3.1. Effects of mechanical force on the expressions of ERS-related genes

The primary hPDLCs growing from the scraped PDL tissue in culture after 10 day (Fig. 1A1). Mechanical force resulted in cellular re-orientation of hPDLCs along with the direction of stretch (Fig. 1A3). While the non-loaded hPDLCs presented randomly orientation (Fig. 1A2). During the application of mechanical stress, we examined the expression of ERS-related genes, including Bip, Xbp1, PERK, total-eIF2α (t-eIF2α), phospho-eIF2α (p-eIF2α), using GAPDH as a control variable to quantify and normalize the results. The data indicated that the mRNA level of Bip did not start to elevate until 6 h, and gradually rising from 12 to 24 h. There were some fluctuations in the expression of Xbp1, it decreased after 3 h and 6 h but increased after 12, 18, and 24 h (Fig. 1B). Meanwhile, the results suggested that mechanical force significantly augmented the expressions of PERK and p-eIF2α in a time-dependent manner, while had very little effect on the expressions of t-eIF2α (Fig. 1C, D). The level of PERK peaked after 3 h and 6 h of mechanical force, and slightly decreased but remained at a relatively stable level after 12, 18, and 24 h. Expressions of p-eIF2α have not obvious change in early period, then increased sharply after 6 h, and maintained at a higher level after 12, 18, and 24 h. These results indicated that mechanical stimulation would induce ERS.

3.2. Expressions of osteogenic genes in hPDLCs stimulated by mechanical force

The effect of mechanical force on ATF4, OCN, and BSP mRNA expressions was analyzed by real-time PCR. As shown in Fig. 2A, immediately after 1 h of mechanical stimulation, the mRNA levels of ATF4 increased significantly to a peak, then decreased rapidly at 6 h, and started to increase from 12 h to 24 h. The level of OCN mRNA increased markedly after 6 h and peaked at 24 h. Furthermore, the levels of BSP presented a stable increase up to 24 h. Western blot results depicted the protein of ATF4 under mechanical force (Fig. 2B, C). Exposure to mechanical force led to a significant up-regulation of ATF4 protein at 1 h, then it reduced sharply at 3 h, rose again from 6 h to 24 h. Overall, these results proved that mechanical stimulation up-regulated the osteogenic gene expressions of hPDLCs in a time-dependent manner.

3.3. PERK+/+ promoted the osteoblast differentiation of hPDLCs, while PERK−/− suppressed the osteoblast differentiation of hPDLCs

hPDLCs were transfected by lentivirus containing PERK (PERK+/+), PERK knockout (PERK−/−), and Empty lentivirus vector (no PERK insert) green fluorescent protein (LV-GFP). The transfection efficiency of

Fig. 1. Effects of mechanical force on the expressions of ERS-related genes. (A) hPDLCs (arrows) were growing out of the scraped PDL tissue (A1). The cellular re-orientation along with the direction of mechanical force (A2 control cells; A3 cells were exposed to mechanical force for 12 h); (B) Bip and Xbp1 mRNA expressions of hPDLCs after 1, 3, 6, 12, 18, and 24 h of stretch were evaluated. GAPDH expression was used as internal control. (C) Western blotting exhibited the expression of PERK, eIF2α and p-eIF2α in hPDLCs after 1, 3, 6, 12, 18, and 24 h of stretch. GAPDH was used as the loading control. (D) The densitometric analysis of PERK, eIF2α and p-eIF2α expression. Data were showed as the ratio to GAPDH and presented as mean ± SD. All the experiments were repeated at least three times. *P < 0.05, **P < 0.01, ***P < 0.005, ###P < 0.001.
Similarly, mechanical force also enhanced the expression of p-eIF2α-ATF4, OCN, and BSP in PERK+/+ infected hPDLCs and the downregulated expressions of PERK mRNA and protein in PERK−/− infected hPDLCs, and there were no differences between LV-GFP transfected and non-transfected hPDLCs (Fig. 3B, C). For the mineralization analysis, Alizarin red staining was performed according to the standard protocols. Both camera and microscope pictures revealed that PERK+/++CMF group, PERK+/+ group and the rest of the three groups were similar in the levels of ATF4 expression. (See Fig. 5.)

3.4. PERK-eIF2α-ATF4 signaling pathway mediated by ERS involved in PDLCs osteodifferentiation under mechanical force

We applied cyclic mechanical force on the hPDLCs after they were transfected by lentivirus. HPDLCs were divided into five groups: control group; PERK+/+ cells loaded by cyclic mechanical force (PERK+/+ + CMF); PERK+/+ cells without application of cyclic mechanical force (PERK+/+), PERK−/− cells loaded by cyclic mechanical force (PERK−/− + CMF); PERK−/− cells without application of cyclic mechanical force (PERK−/−). As shown in Fig. 4A, p-eIF2α, ATF4, OCN, and BSP levels were elevated by overexpression of PERK and mechanical force greatly enhanced this augmentation. Moreover, the group of PERK+/+ cells loaded by mechanical force exhibited the highest expressions of these genes among all groups. The PERK+/+ cells with no mechanical loading also presented high expressions of p-eIF2α, ATF4, OCN, and BSP. There were significant differences among PERK+/+ + CMF group, PERK−/− group and the rest of the three groups in the levels of those genes (Fig. 4B). (See Fig. 5.)

However, the PERK−/− group without application of mechanical force exhibited the lowest expressions of these genes. The levels of p-eIF2α, ATF4, OCN, and BSP decreased significantly in PERK−/− group. Similarly, mechanical force also enhanced the expression of p-eIF2α, ATF4, OCN, and BSP in PERK−/− group. The expressions of OCN and BSP were slightly increased in PERK−/− + CMF group compared to the PERK−/− group (Fig. 4B). There were significant differences between PERK−/− + CMF group compared to the PERK−/− group in the levels of those genes. Nevertheless, there were no significant differences between PERK−/− + CMF group and control group in the levels of ATF4 and OCN. Additionally, between PERK+/+ group and PERK−/− groups, those genes expressions were always higher in cells loaded by CMF than that of non-loading cells. Overall, these results demonstrated that mechanical force not only activated PERK-eIF2α-ATF4 pathway and further promoted hPDLCs osteodifferentiation. Overexpression of PERK and mechanical stimulation have a synergistic effect on the promotion of osteogenic differentiation.

4. Discussion

In recent years, many scholars have focused on the cellular behaviors of hPDLCs and the response of periodontal tissues to mechanical force. The mechanisms underlying the stress-induced osteoblast differentiation of PDLCs are extremely complex and are influenced by the interaction of several factors. Therefore, different kinds of mechanical stimulation have been applied in vitro to explore this issue. These comprise a centrifugal force [15,25], a tensile force [16,26], a compressive force [27,28], and cyclic stretch [17,29]. In our study, we applied cyclic mechanical force at 10% elongation with 0.5HZ to hPDLCs, it was able to represent the occlusal force more and explain the molecular mechanism of osteodifferentiation in hPDLCs [30,31].

In our study, we found that after application of mechanical force, the mRNA expressions of some osteogenic genes like ATF4, OCN, and BSP, were time-dependently up-regulated. This was consistent with our previous studies, which demonstrated that the levels of osteogenic genes increased in hPDLCs under centrifugal force [15,32]. In addition, osteogenic differentiation resulted from a complex molecular interplay involving the action of various transcription factors such as Runx2, Osx, and ATF4. ATF4 is a member of CREB protein family [10,18], which is also required for preserving mature osteoblast function including synthesis of collagen [18,33,34]. Moreover, ATF4-deficient mice exhibited a marked reduction or delay in mineralization of bones including frontal and parietal bones, clavicles, and long bones [35]. Furthermore, OCN and BSP are transcriptional targets of ATF4, which are osteoblast-specific and a marker for the late stage of osteoblast differentiation [36,37]. As mentioned, loss of function mutations of PERK in humans and mice cause several neonatal developmental defects, including diabetes, growth retardation, and multiple skeletal dysplasia [38–42]. PERK−/− mice displayed severe osteopenia, which is caused by a deficiency in the number of mature osteoblasts and impaired osteoblast differentiation [42]. The phenotypes observed in bone tissues of PERK−/− mice are very similar to those of ATF−4−/− mice. As known that ATF4 is a translational target of activated PERK and it is a downstream molecule. Thus, it is possible that bone phenotypes in PERK−/− mice are due to the loss of ATF4 activity, which is involved in osteoblast terminal differentiation and bone formation. Furthermore, a previous report demonstrated that ERS occurs during osteoblast differentiation, indicating that the PERK-eIF2α-ATF4 pathway may be involved in bone formation or osteoblast differentiation mediated by ERS [43].
Additionally, recent studies concerned ERS mediated by PERK-eIF2\(\alpha\)-ATF4 pathway is involved in osteoblast differentiation induced by bone morphogenic protein 2 (BMP2). Their findings proved that PERK-eIF2\(\alpha\)-ATF4 signaling pathway plays a role in BMP2-induced osteoblast differentiation and osteogenesis in culture [21]. In our study, we explored the effect of the PERK-eIF2\(\alpha\)-ATF4 signaling pathway on osteodifferentiation of hPDLCs under mechanical force. The results we got suggested that this signaling pathway is involved in osteodifferentiation of hPDLCs as follows. (1) ATF4 protein levels were up-regulated in PERK\(^+/+\) hPDLCs and down-regulated in PERK\(^{-/-}\) hPDLCs. (2) OCN and BSP, targets of ATF4, presented the same changes as ATF4 in PERK\(^+/+\) and PERK\(^{-/-}\) hPDLCs. (3) Mechanical force further promoted the expression of ATF4, OCN and BSP in PERK\(^+/+\) hPDLCs, and mechanical forces also have the same effect of their expressions in

Fig. 3. PERK\(^+/+\) promoted the osteoblast differentiation of hPDLCs, while PERK\(^{-/-}\) suppressed the osteoblast differentiation of hPDLCs. (A) Green fluorescence could be detected 72 h after transfection in the stably transduced cells without significant morphological changes (A1, 2 transfection efficiency of LV-GFP at MOI [60]; A3, 4 transfection efficiency of PERK\(^+/+\) at MOI [100]; A5, 6 transfection efficiency of PERK\(^{-/-}\) at MOI [100]) (B) PERK protein expression was evaluated by Western blot after transfection. GAPDH was used as the loading control. (C) PERK mRNA was measured by real-time PCR after transfection. GAPDH was used as a housekeeping gene. (D) hPDLCs were cultured for 3 weeks. Cells were stained with Alizarin red (D1, 2 present the normal group; D3, 4 present the PERK\(^+/+\) group; D5, 6 present the PERK\(^{-/-}\) group; D7, 8 present the LV-GFP group. Date were presented as mean ± SD, *\(P < 0.05\), **\(P < 0.01\).
ERS plays a role in bone remodeling during orthodontic tooth movement. To investigate the involvement of ERS, we chose to apply mechanical force on the hPDLCs to induce ERS, then interfered with and over-expressed the PERK gene (a major transducer of ERS), followed by detecting the related factors in term with PERK-eIF2α-ATF4 signaling in osteogenic differentiation of PDLCs. Our results indicated that mechanical stimulation could induce ERS and PERK-eIF2α-ATF4 pathway will be activated when ERS occurred. Overexpression of PERK promotes eIF2α phosphorylation and the expression of ATF4, furthermore, induce BSP, OCN expression, it will promote osteodifferentiation of hPDLCs; Overexpression of PERK and mechanical stimulation have a synergistic effect on the osteodifferentiation of PDLCs. Conversely, knockout of PERK revealed the opposite changes, which will inhibit osteodifferentiation of hPDLCs (Fig. 4). However, it is unknown whether ERS actually occurs in osteodifferentiation of hPDLCs under mechanical load in vivo or not. Additionally, the function of PERK-eIF2α-ATF4 signaling and their association with osteogenic differentiation in osteogenesis biology in vivo are not clearly defined. A detailed understanding of the significance of ERS signaling, including the PERK pathway, on osteodifferentiation of hPDLCs under mechanical force is necessary.

In summary, ERS is induced by mechanical force and activates the PERK-eIF2α-ATF4 pathway. The expression of ATF4 and its targets gene (OCN and BSP) are influenced by the knock-out and over-expression of PERK. Thus, PERK-eIF2α-ATF4 signaling pathway mediated by ERS may exert an influence on periodontal remodeling and osteogenesis differentiation of hPDLCs during the process of orthodontic tooth movement. This will contribute to a better understanding about the mechanism of mechanical force-induced periodontal remodeling via PERK-eIF2α-ATF4 pathway.

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References


Fig. 4. PERK-eIF2α-ATF4 signaling pathway mediated by ERS involved in PDLCs osteodifferentiation under mechanical force. (A) Western blotting presented the protein expression of ATF4, OCN, BSP, p-eIF2α in control, PERK−/−, PERK−/− + CMF, PERK−/− + CMF groups; GAPDH was used as the loading control. (B) The densitometric analysis of ATF4, OCN, BSP, p-eIF2α expression. All data were showed as the ratio to GAPDH and represented as mean ± SD. All the experiments were repeated at least three times. Data were analyzed by t-test for control group and the other four groups, *P < 0.05; **P < 0.01. Data were analyzed by one-way ANOVA for PERK−/−, PERK+/+, PERK−/− + CMF, PERK−/− /− CMF, *P < 0.05; **P < 0.01. CMF, cyclic mechanical force.

PERK−/− hPDLCs (Fig. 4). These observations supported that PERK-eIF2α-ATF4 signaling pathway takes effect in osteodifferentiation of hPDLCs under cyclic mechanical force (Fig. 5).

To activate the PERK-eIF2α-ATF4 pathway, ER stress in hPDLCs is required. As demonstrated in previous studies, many environmental factors and intracellular factors, such as hypoxia, glucose or amino acids deficiency, viral infection, gene mutation, calcium concentration disorders etc., are likely to induce ERS [44–48]. Under mechanical force, the periodontal tissue was in a hypoxic condition [49]. In this case, inflammation reaction occurred due to hypoxia and vascular changes and circulatory disturbances would happen. As a kind of stress stimulation, hypoxia would induce ERS in periodontal tissue. Thus we speculated that ERS occur in the PDLCs under cyclic mechanical force. Most studies hypothesize that ERS occur in the PDLCs under cyclic mechanical force. Most studies have demonstrated that the biological process of ERS involving in cardiovascular disease, renal disease, diabetes mellitus (DM), hypertensin, fibration etc [19,40,50–52]. However, it remains unclear whether ERS plays a role in bone remodeling during orthodontic tooth movement.

Fig. 5. Schematic drawing outlining PERK-eIF2α-ATF4 Pathway Mediated by ERS is involved in osteodifferentiation of hPDLCs under cyclic mechanical force.


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