Article

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Upregulated ATF1 Promotes Lipopolysaccharide Induced Inflammatory Response and Inhibits Osteogenic Differentiation of Human Periodontal Ligament Cells by Regulating NF-κB Pathway

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Background: Periodontitis is a chronic inflammatory disease resulting from bacterial plaque infection. While the involvement of activating transcription factor 1 (ATF1) has been extensively explored in various human diseases, its specific role in periodontitis remains unclear. This study aims to elucidate the expression and biological function of ATF1 in the context of periodontitis.
Methods: Primary human periodontal ligament cells (hPDLCs) were procured from clinical samples and subsequently characterized. Following treatment with *P. gingivalis* lipopolysaccharide (LPS, 10 µg/mL), hPDLCs underwent transfection with either ATF1 vector or siRNA. The expression levels of ATF1 in LPS-treated hPDLCs or transfected cells were evaluated through real-time quantitative polymerase chain reaction (RT-qPCR) and western blot assay. Inflammatory factors, including interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β), were quantified using Enzyme-linked Immunosorbent Assay (ELISA). The assessment of osteogenic proteins, such as runt-related transcription factor 2 (Runx2), osteopontin (OPN), and osteoprotegerin (OPG), as well as noncanonical nuclear factor-kappaB (NF-κB) pathway-related proteins (p65, p-p65, IkBα, p-IkBα), was conducted using western blot assay. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and flow cytometry assays were employed to detect cell viability.
Results: LPS induced an inflammatory response and hindered the osteogenic differentiation of hPDLCs ($p < 0.05$, $p < 0.01$). Furthermore, ATF1 silencing enhanced cell proliferation and suppressed apoptosis in LPS-stimulated hPDLCs ($p < 0.05$, $p < 0.01$). ATF1 silencing not only restrained the inflammatory response but also promoted the osteogenic differentiation of LPS-stimulated hPDLCs ($p < 0.05$, $p < 0.01$). Importantly, ATF1 silencing effectively blocked the LPS-induced activation of the NF-κB signaling pathway ($p < 0.05$, $p < 0.01$, $p < 0.001$).
Conclusions: ATF1 emerges as a promising treatment option, inhibiting the osteogenic differentiation of hPDLCs and mitigating the inflammatory response by preventing the phosphorylation of the NF-κB signaling pathway.

Keywords: ATF1; periodontitis; inflammatory; NF-κB; human periodontal ligament cells

Introduction

Periodontitis, a prevalent chronic infectious oral disease, is instigated by bacteria in dental plaque invading periodontal tissue [1]. Common symptoms include red and swollen gums, bleeding gums, loose teeth, weakened chewing, pain, and potential tooth loss [2]. Although periodontitis can manifest at any age, it predominantly affects adults, with a noticeable increase in prevalence after the age of 35 [3]. The primary goal of periodontitis treatment is to alleviate inflammation, impede disease progression, and avert recurrence. The regeneration of periodontal tissue assumes paramount significance in safeguarding patients' gums and teeth [4]. Consequently, there is an imperative need to investigate factors that can prevent inflammation and facilitate the regeneration of periodontal tissue for effective periodontitis treatment.

In recent studies, several influential factors such as long non-coding ribonucleic acids (lncRNAs), microRNAs (miRNAs), and genes have been identified as participants in the pathogenesis of periodontitis. For instance, the down-regulation of lncRNA maternally expressed gene 3 (lncRNA MEG3) has been associated with the suppression of osteogenic differentiation in periodontal ligament stem cells (PDLCs) during periodontitis [5]. MicroRNA-214 has been found to inhibit the osteogenic differentiation of human PDLCs by targeting activating transcription factor 4 (ATF4) [6]. Furthermore, knockdown of enhancer of zeste homolog 2 (EZH2) has been shown to hinder osteoclast formation and mitigate periodontitis by upregulating miR-101 [7].

In the current study, the role of activating transcription factor 1 (ATF1) in periodontitis was elucidated.

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ATF1's function has been extensively explored in various human diseases. For instance, the ATF1/miR-214-5p/integrin subunit alpha 7 (ITGA7) axis has been reported to promote osteoclastogenesis, influencing ovariectomized (OVX)-induced bone absorption [8]. The down-regulation of ATF1 has been linked to early neuroectoderm differentiation of human embryonic stem cells through SRY-box transcription factor 2 (SOX2) [9]. Simultaneously, ATF1 has been implicated in promoting metastasis and regulating the expression of matrix metalloproteinase 2 (MMP2) in gastric cancer [10]. However, the regulatory mechanism of ATF1 in periodontitis remains unknown.

The human inflammatory response following infection necessitates the initiation of the NF-κB signaling pathway [11]. Prolonged activation of the noncanonical nuclear factor-kappaB (NF-κB) pathway can impact the expression of inflammatory factors, including interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-1beta (IL-1β) [12]. The NF-κB signaling pathway has been implicated in the pathogenesis of periodontitis. For instance, the down-regulation of long non-coding RNA antisense non-coding RNA in the INK4 locus (ANRIL) has been linked to the suppression of osteogenic differentiation in PDLCs through the NF-κB pathway [13]. However, the regulatory mechanism of ATF1/NF-κB has not been elucidated in the context of periodontitis.

Hence, this study delved into the regulatory mechanism of ATF1/NF-κB in periodontitis. Furthermore, it examined the impact of ATF1 on the cell viability of lipopolysaccharide (LPS)-stimulated human periodontal ligament cells (hPDLCs) and the levels of inflammatory factors. This research aims to provide a more comprehensive understanding of the pathogenesis of periodontitis.

Materials and Methods

Isolation of hPDLCs and Culture

Primary human periodontal ligament cells (hPDLCs) were obtained through enzymatic digestion of healthy periodontal ligament tissue extracted from the root third of molar teeth during orthodontic treatment [14]. The isolated cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (12800017, Gibco, Carlsbad, CA, USA) under standard incubation conditions (20% fetal bovine serum, 5% CO_2, and 37 °C). Following 7–10 days of primary culture, cell morphology was observed using an inverted microscope (37X-2, CSOIF, Shanghai, China). All cells underwent mycoplasma testing and were verified to be uncontaminated. Cell lines were identified through short tandem repeat (STR) analysis, and cells at the third passage of culture were utilized in this study. Informed consent was obtained from all patients, and the study protocol received approval from the ethics committees of The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine (No.2023-KY-022). The study adhered to the principles of the Declaration of Helsinki (2013).

Cell Treatment and Transfection

The third passage of hPDLCs was subjected to treatment with *P. gingivalis* lipopolysaccharide (LPS, 10 µg/mL, InvivoGen, San Diego, CA, USA) for 12, 24, or 48 hours, respectively. The blank control comprised hPDLCs without LPS treatment. For lentivirus production and transfection, the full-length cDNA of ATF1 was subcloned into the pLVX-3FLAG-PGK-Puro vector (ATF1 vector), while the empty pLVX-3FLAG-PGK-Puro vector (Vector-NC) served as a control. The ATF1 vector, Vector-NC, ATF1 siRNA, and negative control plasmid (si-NC) were procured from Shanghai GenePharma Company (GenePharma, Shanghai, China). Subsequently, the ATF1 vector, Vector-NC, ATF1 siRNA, and si-NC were transfected into LPS-stimulated hPDLCs using Lipofectamine 2000 (11668-027, Invitrogen, Waltham, MA, USA). The sequences of the siRNA were as follows: ATF1 siRNA: 5′-CGAACUACACCUUCAGCUA-3′; si-NC: 5′-UUCUCCGAACGUGUCACGU-3′.

The coding sequence of Hum-ATF1: ATGACACA AGGGCGTCTGTACCCTTTGGGATGGGCGACCCAG CCGAGGTTGATTATGGAAGATTCCCACAAGAGTA CCACGTCAGAGACAGCACCTCAACCTGGTCAGCA GTTCAGGGAGCTCACATTTCTCATATTGCTCAAC AGGTATCATCTTTATCAGAAAGTGAGGAGTCCCA GGACTCATCCGACAGCATAGGCTCCTCACAGAAA GCCCACGGGATCCTAGCACGGCGCCCATCTTACA GAAAAATTTTGAAAGACTTATCTTCTGAAGATAC ACGGGGCAGAAAAGGAGACGGAGAAAATTCTGG AGTTTCTGCTGCTGTCACTTCTATGTCTGTTCCAA CTCCCATCTATCAGACTAGCAGCGGACAGTACAT TGCCATTGCCCCAAATGGAGCCTTACAGTTGGCA AGTCCAGGCACAGATGGAGTACAGGGACTTCAG ACATTAACCATGACAAATTCAGGCAGTACTCAGC AAGGTACAACTATTCTTCAGTATGCACAGACCTC TGATGGACAGCAGATACTTGTGCCCAGCAATCAG GTGGTCGTACAAACTGCATCAGGAGATATGCAAA CATATCAGATCCGAACTACACCTTCAGCTACTTCT CTGCCACAAACTGTGGTGATGACATCTCCTGTGA CTCTCACCTCTCAGACAACTAAGACAGATGACCC CCAATTGAAAAGAGAAATAAGGTTAATGAAAAA CAGAGAAGCTGCTCGAGAATGTCGCAGAAAGAA GAAAGAATATGTGAAATGCCTGGAAAACCGAGT TGCAGTCCTGGAAAATCAAAATAAAACTCTAATA GAAGAGTTAAAAACTTTGAAGGATCTTTATTCCA ATAAAAGTGTTTGA.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA isolation was carried out using TRIzol reagent (15596026, Gibco, Carlsbad, CA, USA). RT-qPCR was conducted utilizing the Prime-Script miRNA cDNA Synthesis kit (TaKaRa, Tokyo, Japan), ABI 7500 fluorescence Quantitative PCR system (ABI 7500, Life Technologies, Carlsbad, CA, USA), and appropriate

primers. The normalization of ATF1 expression was performed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and quantified using the $2^{-\Delta\Delta Ct}$ method. The primer sequences employed were as follows: ATF1 forward, 5′-TTCTGGAGTTTCTGCTGCTGT-3′, reverse, 5′-CCATCTGTGCCTGGACTTG-3′; GAPDH forward, 5′-GGTATCGTGGAAGGACTCATGAC-3′, reverse, 5′-ATGCCAGTGAGCTTCCCGT TCAGC-3′.

Enzyme-Linked Immunosorbent Assay (ELISA)

Supernatants from both treated and untreated hPDLCs were employed to assess inflammatory cytokines. ELISA kits from Cusabio Wuhan Huamei Biotech Co., Wuhan, China, were utilized to analyze fresh cell culture supernatants, measuring the concentrations of IL-1β (70-EK201), IL-8 (70-EK108), tumor necrosis factor-alpha (TNF-α) (WM-YX10110), and IL-6 (CSB-E04638h). The alterations in cytokine levels in the culture supernatant were evaluated 24 hours after the therapy.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) Assay

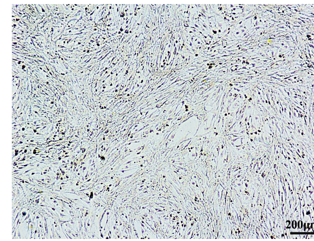
Treated and untreated hPDLCs cells (2×10^4 cells) were seeded into 96-well plates. Following incubation for 24, 48, 72, or 96 hours, each well received 20 µL of MTT solution and was incubated at 37 °C for 4 hours. Subsequently, 150 µL of dimethyl sulfoxide (DMSO) was added and oscillated at room temperature for 10 minutes. Finally, a Microplate Reader (IX31, Olympus, Tokyo, Japan) was employed to measure the absorbance at a wavelength of 490 nm.

Flow Cytometry Assay

A flow cytometry assay was employed to assess the impact of ATF1 on hPDLCs cell apoptosis and surface marker expression. For apoptosis evaluation, both treated and untreated hPDLCs were collected and washed in phosphate-buffered saline (PBS). Subsequently, 300 µL of 1× Binding Buffer was added to suspend the cells. The hPDLCs were then incubated with FITC Annexin V and PI (5 µL, CA1020, Solarbio, Beijing, China) at room temperature in the dark. After 1 hour, apoptotic rates were determined using flow cytometry (CA1020, Solarbio, Beijing, China).

Regarding the assessment of surface markers, 5×10^5 cells were washed with PBS and labeled with PE-conjugated antibodies against CD34, CD45, CD73, CD90, and CD105 (1:10; BD Biosciences, Franklin Lakes, NJ, USA). Fluorescent intensities were measured using a BD Accuri C6 (BD Biosciences) immediately after staining, and flow cytometry was employed for analyses. The PE-conjugated antibodies (CD34 (11-0349-42), CD45 (17-0459-42), CD73 (17-0739-42), CD90 (11-0909-42), and CD105 (12-1057-42)) were procured from eBiosciences (San Diego, CA, USA).

A



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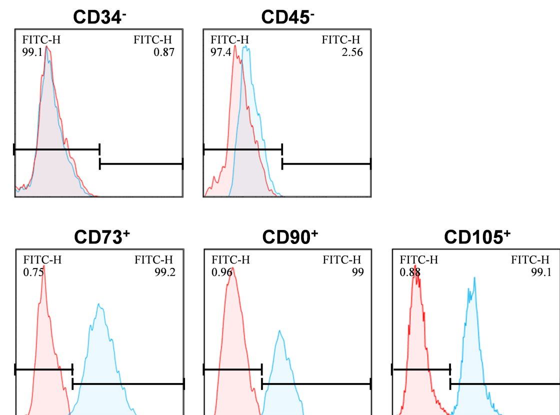

Fig. 1. Characteristics of human periodontal ligament cells (hPDLCs). (A) Morphological observation of hPDLCs under inverted microscope. Scale bar, 200 µm. (B) Cell surface markers including CD105, CD73, CD90, CD34, and CD45 of hPDLCs were detected by flow cytometry.

Western Blot Assay

Protein extraction was carried out using radioimmunoprecipitation assay (RIPA) buffer (89901, Thermo, Shanghai, China). Subsequently, protein samples were separated by 10% SDS-PAGE and transferred onto a PVDF membrane (IPVH00010, Millipore, MA, USA). The membrane was then incubated with primary antibodies targeting osteoprotegerin (OPG), osteopontin (OPN), runt-related transcription factor 2 (Runx2), p65, p-p65, IkBα, p-IkBα, and GAPDH at 4 °C overnight, following blocking with 5% non-fat milk. Afterward, the membrane was incubated with the corresponding goat polyclonal anti-rabbit IgG H&L secondary antibody (1:10,000, 31460, Invitrogen, Shanghai, China) for 1 hour at room temperature. Protein bands were developed using the ECL system (E412-02, Vazyme, Jiangsu, China) and quantified using Image J software (V1.8.0, National Institutes of Health, Bethesda, MD, USA).

The primary antibodies used were as follows: anti-OPG (1:1000, ab93876, Abcam, Cambridge, MA, USA), anti-OPN (1:500, ab13418, Abcam, Cambridge, MA, USA), anti-ATF1 (1:1000, 5232, CST, Boston, USA), anti-RUNX2 (1:1000, 8486, CST, Boston, USA), anti-IkBα (1:1000, 10268-1-AP, Proteintech, Wuhan, China), anti-p-IkBα (Ser32/36) (1:1000, 9426, CST, Boston, USA), anti-p65 (1:1000, 3034, CST, Boston, USA), anti-p-

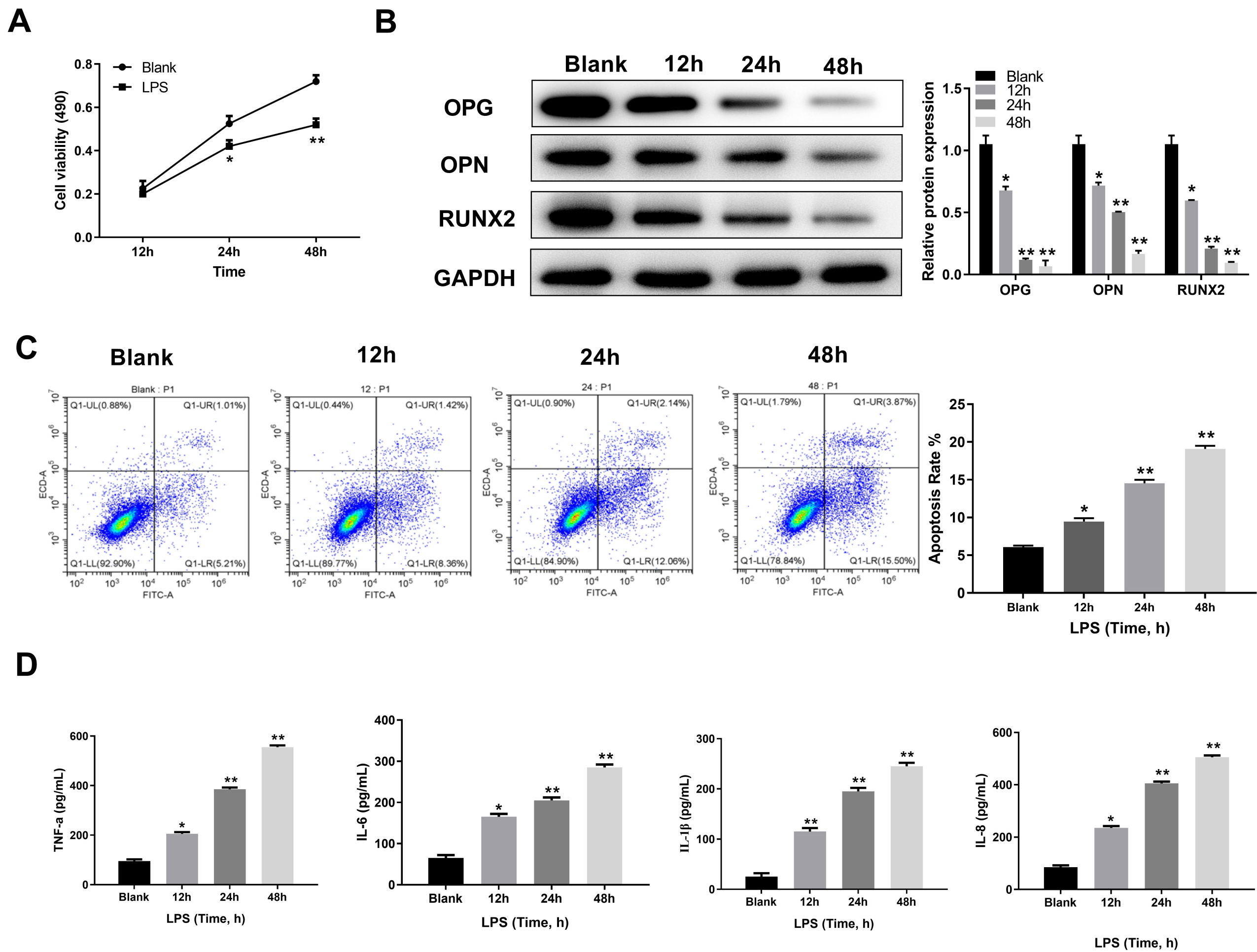

Fig. 2. Lipopolysaccharide (LPS) induces inflammatory response and inhibits osteogenic differentiation of hPDLCs. (A) LPS treatment resulted in the suppression of cell proliferation in hPDLCs (n = 3). (B) LPS affected the expression of osteoprotegerin (OPG), osteopontin (OPN), and runt-related transcription factor 2 (RUNX2) proteins in hPDLCs (n = 3). (C) Flow cytometry revealed the induction of cell apoptosis at different time points after LPS treatment (n = 3). (D) LPS influenced the concentrations of inflammatory factors (tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-8) in hPDLCs (n = 3). *$p < 0.05$, ** $p < 0.01$.

p65 (Ser536) (1:1000, 3033, CST, Boston, USA), and anti-GAPDH (1:10,000, 10494-1-AP, Proteintech, Wuhan, China).

Statistical Analysis

Data analysis was conducted using GraphPad Prism 6.0 (version 6; GraphPad Software, San Diego, CA, USA). The differences between two groups were assessed using Student's *t*-tests and the chi-squared test. For multiple groups, data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. The results are presented as the mean $\pm$ standard deviation (SD). A *p*-value less than 0.05 was considered statistically significant.

Results

Characterization of hPDLCs

The cells derived from primary hPDLCs, cultured up to the third passage, exhibited a star-shaped or long spindle-shaped morphology under high magnification microscopy, accompanied by round or oval nuclei—typical characteristics of fibroblasts (Fig. 1A). Further observation revealed that the treated hPDLCs expressed markers associated with mesenchymal stem cells (MSCs), such as CD105, CD73, and CD90, while remaining negative for CD34 and CD45 (Fig. 1B). Collectively, these findings affirm that the isolated and cultured hPDLCs cells adhere to both morphological and MSC characteristics, suggesting their origin in the mesoderm.

LPS Induces Inflammatory Response and Inhibits Osteogenic Differentiation of hPDLCs

To establish the periodontitis cell model, hPDLCs were treated with LPS. As depicted in Fig. 2A, cell proliferation in the LPS group was significantly inhibited compared to the blank control group ($p < 0.05$, $p < 0.01$). Lower expression levels of osteogenic proteins RUNX2, OPN, and OPG were observed in the LPS group compared to the blank

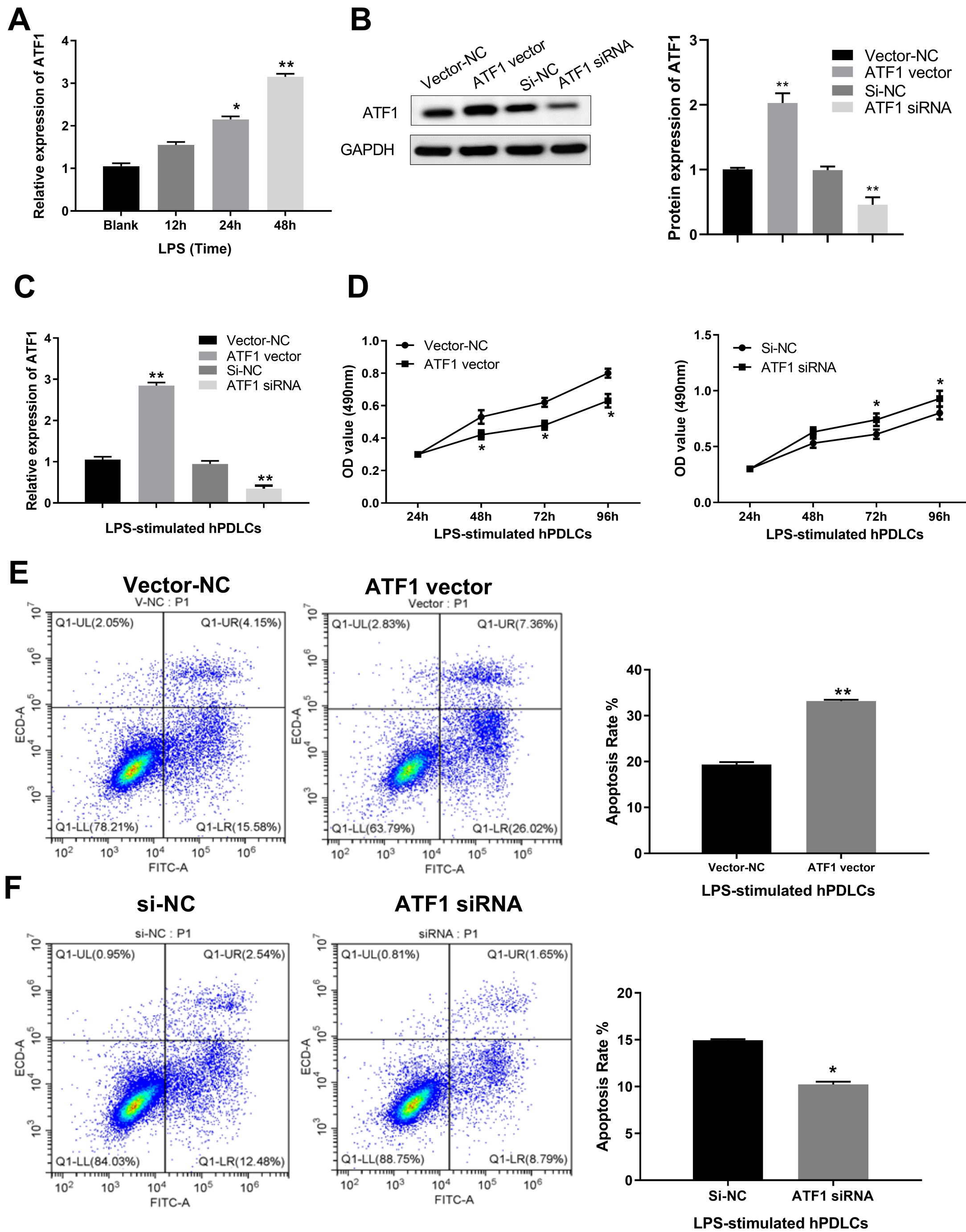

Fig. 3. Activating transcription factor 1 (ATF1) silencing promotes cell proliferation and restrains apoptosis in LPS-stimulated hPDLCs. (A) ATF1 expression was detected in LPS-stimulated hPDLCs at different times (n = 3). (B) ATF1 expression was detected in LPS-stimulated hPDLCs with ATF1vector or siRNA (n = 3). (C–F) Cell proliferation and apoptosis were detected in LPS-stimulated hPDLCs with ATF1vector or siRNA (n = 3). *$p < 0.05$, **$p < 0.01$.

control group (Fig. 2B, $p < 0.05$, $p < 0.01$). Additionally, the findings indicated that LPS treatment markedly promoted the apoptosis of hPDLCs in a time-dependent manner (Fig. 2C, $p < 0.05$, $p < 0.01$). Furthermore, inflammatory cytokines TNF-α, IL-6, IL-8, and IL-1β were released in hPDLCs treated with LPS (Fig. 2D, $p < 0.05$, $p < 0.01$), and notably, these results exhibited time-dependent variations. Taken together, these findings demonstrate that LPS induces inflammatory damage and inhibits the osteogenic differentiation of hPDLCs.

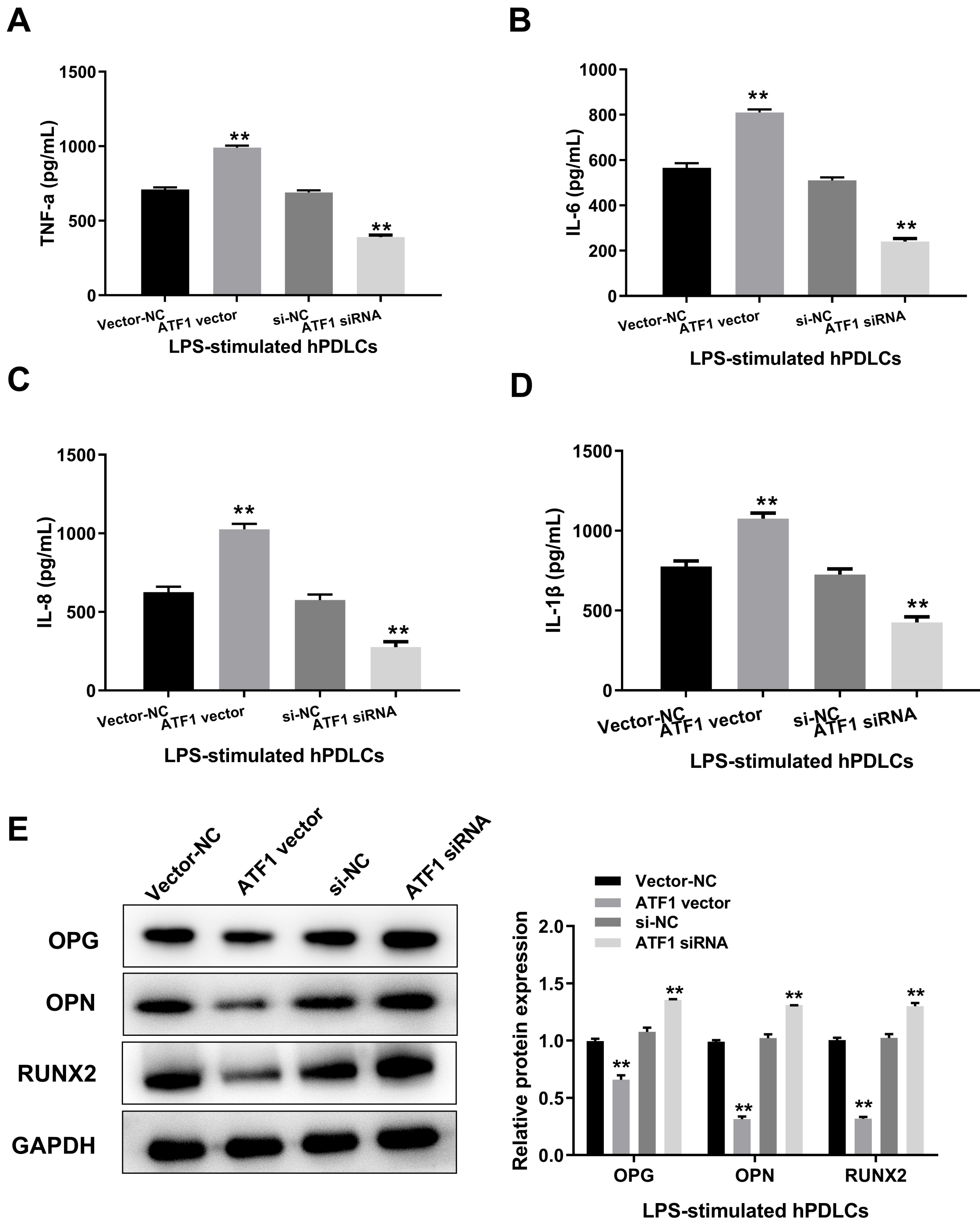

Fig. 4. ATF1 silencing inhibits the inflammatory response and promotes the osteogenic differentiation of LPS-stimulated hPDLCs. (A–D) Inflammatory factors (TNF-α, IL-1β, IL-6 and IL-8) concentrations were measured in LPS-stimulated hPDLCs with ATF1vector or siRNA (n = 3). (E) OPG, OPN and RUNX2 protein expressions were examined in LPS-stimulated hPDLCs with ATF1vector or siRNA (n = 3). ** $p < 0.01$.

ATF1 Silencing Promotes Cell Proliferation and Restrains Apoptosis in LPS-Stimulated hPDLCs

Furthermore, ATF1 expression was found to be upregulated by LPS in PDLCs in a time-dependent manner (Fig. 3A, $p < 0.05$, $p < 0.01$). Subsequently, hPDLCs treated with LPS (10 µg/mL) for 48 hours were selected for further experiments. To investigate the role of ATF1 in periodontitis, ATF1 vector or siRNA was transfected into LPS-stimulated hPDLCs. RT-qPCR demonstrated that ATF1 vector upregulated its expression, while ATF1 siRNA downregulated its expression in LPS-stimulated hPDLCs (Fig. 3B,C, $p < 0.01$). Functionally, ATF1 overexpression restrained the proliferation of LPS-stimulated hPDLCs (Fig. 3D, $p < 0.05$). In contrast, the proliferation of LPS-

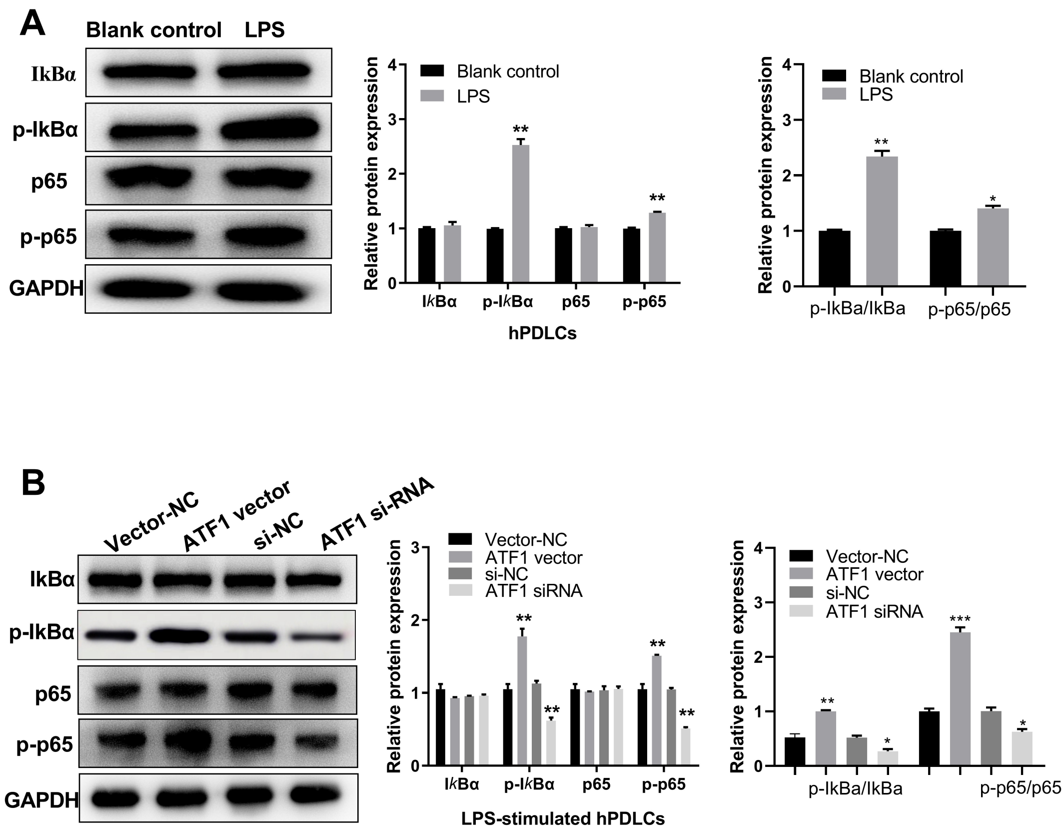

Fig. 5. ATF1 silencing can block LPS-induced activation of nuclear factor-kappaB (NF-κB) signal pathway. (A) The protein expression of p65, p-p65, IkBα, and p-IkBα was affected by LPS in hPDLCs (n = 3). (B) The protein expression of p65, p-p65, IkBα, and p-IkBα were measured in lipopolysaccharide (LPS)-stimulated human periodontal ligament cells (hPDLCs) with activating transcription factor 1 (ATF1) vector or siRNA (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

stimulated hPDLCs was enhanced by ATF1 knockdown (Fig. 3D). Additionally, the apoptosis of LPS-stimulated hPDLCs was induced by ATF1 upregulation and inhibited by ATF1 silencing (Fig. 3E,F, $p < 0.05$, $p < 0.01$). Collectively, ATF1 silencing can alleviate LPS-stimulated hPDLCs injury by increasing cell viability.

ATF1 Silencing Inhibits the Inflammatory Response and Promotes the Osteogenic Differentiation of LPS-Stimulated hPDLCs

Subsequently, the impact of ATF1 on the inflammatory response and osteogenic differentiation was investigated in LPS-stimulated hPDLCs. The ELISA assay revealed that ATF1 overexpression promoted the release of TNF-α, IL-6, IL-8, and IL-1β in LPS-stimulated hPDLCs (Fig. 4A–D, $p < 0.01$). Conversely, ATF1 silencing lowered the levels of TNF-α, IL-6, IL-8, and IL-1β in LPS-stimulated hPDLCs (Fig. 4A–D, $p < 0.01$). Additionally, knockdown of ATF1 increased the protein expression of RUNX2, OPN, and OPG in LPS-stimulated hPDLCs, while upregulated ATF1 showed the opposite effect on the above proteins (Fig. 4E, $p < 0.01$). These results suggest that ATF1 silencing can mitigate periodontitis injury by inhibiting inflammatory responses and promoting osteogenic differentiation.

ATF1 Silencing can Block LPS-Induced Activation of NF-κB Signal Pathway

The NF-κB signaling pathway is a critical inflammatory pathway. To elucidate the regulatory mechanism of ATF1 in periodontitis, the effect of ATF1 on the NF-κB signaling pathway was investigated in LPS-stimulated hPDLCs. Firstly, it was observed that LPS significantly increased the expression of p-IκBα and p-p65 in hPDLCs compared to the blank control group (Fig. 5A, $p < 0.05$, $p < 0.01$). Additionally, the ATF1 vector also enhanced the expression of p-IκBα and p-p65 in LPS-stimulated hPDLCs (Fig. 5B, $p < 0.01$, $p < 0.001$). Meanwhile, ATF1 siRNA exerted the opposite effect on the expression of p-IκBα and p-p65 in LPS-stimulated hPDLCs (Fig. 5B, $p < 0.05$, $p < 0.01$). Taken together, ATF1 silencing can block LPS-induced activation of the NF-κB signaling pathway in hPDLCs.

Discussion

Numerous genes have been implicated in influencing periodontitis. Examples include *ATF4*, periodontal ligament-associated protein-1 (*PLAP-1*), and retinoid-related orphan receptor alpha (*RORA*), all of which have been reported to regulate PDLCs' proliferation, osteogenic differentiation, and LPS-induced periodontitis [6,15,16]. However, these well-known genes involved in disease development represent only a fraction of the broader picture. In our research, we explored the abnormal expression and function of ATF1 in periodontitis. Our results demonstrated that LPS induced an inflammatory response and inhibited the osteogenic differentiation of hPDLCs. Furthermore, ATF1 silencing promoted proliferation and inhibited apoptosis in LPS-stimulated hPDLCs. ATF1 silencing also mitigated the inflammatory response and promoted the osteogenic differentiation of LPS-stimulated hPDLCs.

Consistent with our findings, previous studies have also reported on the effects of ATF1 on cell proliferation, apoptosis, and differentiation. Specifically, ATF1 has been shown to stimulate oncogenic and differentiation programs in clear cell sarcoma [17]. Moreover, ATF1 was found to enhance the proliferation and invasion of lung cancer cells [18]. Notably, miR-589-3p has been demonstrated to promote the osteogenic differentiation of hPDLCs by targeting ATF1 [19]. In our study, we identified the upregulation of ATF1 in LPS-stimulated hPDLCs, and ATF1 silencing not only inhibited the inflammatory response but also promoted the osteogenic differentiation of LPS-stimulated hPDLCs. These results, particularly the impact on osteogenic differentiation, have not been previously revealed in other studies. Another significant finding is that ATF1 silencing mitigated the damage resulting from periodontitis by inactivating the NF-κB signaling pathway.

It is widely recognized that the activation of the NF-κB pathway can contribute to autoimmune diseases, chronic inflammation, and various cancers [20–23]. Additionally, the NF-κB pathway plays a crucial role in cellular inflammatory responses and immune responses [24]. Moreover, osteoprotegerin is closely linked to the regulation of the NF-κB pathway [25], and the activity of the NF-κB pathway is associated with the phosphorylation of IκBα protein [26].

In the present study, ATF1 upregulation was found to enhance p-IκBα expression in LPS-stimulated hPDLCs. Consistent with our results, lncRNA ANRIL and MEG3 have also been reported to regulate the osteogenic differentiation of hPDLCs through the NF-κB pathway [13,27]. These findings suggest that ATF1 upregulation may exacerbate the damage caused by periodontitis through the activation of the NF-κB signaling pathway. However, it is important to note that the function of ATF1 *in vivo* has not been investigated in this study, and animal experiments are planned for future research.

Conclusions

In summary, our study revealed the upregulation of ATF1 in LPS-stimulated hPDLCs. Elevated ATF1 levels were found to promote LPS-induced inflammatory responses and inhibit the osteogenic differentiation of hPDLCs through the regulation of the NF-κB pathway. These findings suggest that ATF1 can serve as a potential target for the treatment of periodontitis, offering new avenues for therapeutic intervention.

Availability of Data and Materials

All data generated or analyzed during this study are included in the published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Author Contributions

WG designed the research study. SY performed the research. GZ provided help and advice on the ELISA experiments. SY and GZ analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All patients signed the informed consent, and the study protocol was approved by the ethics committees of The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine (No.2023-KY-022). This study was conducted in accordance with the principles of the Declaration of Helsinki (2013).

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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