Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Elucidating the Role of HIF-1α/YAP Signaling Pathway in Regulating Inflammation in Human Periodontal Stem Cells: An in vitro Study

Hui-Wei Zhao[1], Shu-Tai Liu[2], Xiang-Jin Wang[1], Xue-Mei Zhang[1], Xiang Ma[1]

[1]Department of Periodontology, The Affiliated Yantai Stomatological Hospital, Binzhou Medical University, Yantai, 264000, People's Republic of China; [2]Department of Periodontology, Shenzhen Stomatology Hospital, Shenzhen, 518000, People's Republic of China

Correspondence: Xiang Ma, Department of Periodontology, The Affiliated Yantai Stomatological Hospital, Binzhou Medical University, 148 North Road, YanTai, People's Republic of China, Email MaXiang900218@163.com

Background and Objective: Periodontitis is a chronic inflammatory disease caused by dental plaque accumulation, leading to damage of periodontal tissues and potential tooth loss. Understanding the mechanisms of periodontitis, particularly the role of hypoxia in inflammation, is critical for identifying novel therapeutic strategies. This study investigated the effects of the prolyl hydroxylase (PHD) inhibitor DMOG on pro-inflammatory cytokine expression in human periodontal ligament stem cells (hPDLSCs) and examined the involvement of the HIF-1α/YAP signaling path ay in modulating inflammation.

Materials and Methods: hPDLSCs were cultured and treated with lipopolysaccharide (LPS) to induce inflammation, followed by DMOG treatment. Cell proliferation was assessed using the CCK-8 assay, while ELISA and RT-qPCR evaluated the expression levels of HIF-1α, IL-1β, TNF-α, and YAP. YAP expression was knocked down using siRNA transfection to examine its effects on inflammatory cytokines.

Results: DMOG significantly increased HIF-1α expression while reducing IL-1β and TNF-α levels in LPS-treated hPDLSCs. 0.1 mmol/L DMOG inhibited cell proliferation after 72 hours ($P < 0.001$). ELISA results showed that HIF-1α concentrations in the LPS + DMOG group were significantly higher than in the LPS group ($P < 0.01$), while IL-1β and TNF-α levels were significantly reduced ($P < 0.01$). RT-qPCR confirmed these trends, showing reduced mRNA levels of IL-1β and TNF-α and increased YAP expression in the LPS + DMOG group ($P < 0.0001$). YAP knockdown via siRNA transfection reversed these effects, increasing IL-1β and TNF-α levels ($P < 0.01$) while significantly reducing HIF-1α expression ($P < 0.05$).

Conclusion: This study demonstrated that DMOG reduces inflammatory cytokine expression in hPDLSCs by stabilizing HIF-1α and activating the YAP signaling pathway. These findings provide a mechanistic basis for targeting the HIF-1α/YAP axis to control periodontal inflammation and support the potential of PHD inhibitors as therapeutic agents for periodontitis.

Keywords: periodontitis, HIF-1α, YAP, DMOG, inflammation

Introduction

Periodontitis is a chronic, multifactorial inflammatory disease induced by dental plaque accumulation, primarily affecting the gingiva, alveolar bone, cementum, and periodontal ligament. If left untreated, it can eventually lead to tooth mobility, displacement, and even tooth loss, making it one of the leading causes of tooth loss in adults worldwide.[1] Beyond its local effects on the periodontium, periodontitis may also influence systemic health through direct bacterial infection, dissemination of bacterial products into the bloodstream, and host immune responses.[2,3] Epidemiological studies have demonstrated an association between severe periodontitis and at least 43 systemic diseases. Furthermore, as global populations age and life expectancy increases, the burden of periodontal disease is expected to rise significantly. Therefore, understanding the mechanisms underlying periodontitis and identifying effective and safe therapeutic approaches are urgent clinical challenges.

Received: 20 November 2024
Accepted: 20 January 2025
Published: 6 February 2025

In recent years, the relationship between hypoxia and inflammation has been extensively studied in various chronic diseases. Hypoxia is not only a consequence of inflammation but also exacerbates the inflammatory response, leading to further tissue damage.[4,5] Periodontitis is similarly characterized by hypoxia, where the inflamed periodontal tissues experience a state of ischemia and oxygen deficiency due to increased metabolic activity and disrupted microcirculation, thereby worsening disease progression.[6] Hypoxia-inducible factor (HIF), a key transcription factor in the body's adaptation to low oxygen environments, plays a critical role in regulating gene expression under hypoxic conditions and modulating inflammatory responses.[7] Prolyl hydroxylase (PHD), a rate-limiting enzyme in the degradation of HIF, controls HIF stabilization and is a pivotal target in regulating inflammation. PHD inhibitors have shown anti-inflammatory potential in various inflammatory diseases by stabilizing HIF-1α.[8–11] However, the specific role of the PHD/HIF axis in periodontitis remains unclear.

In parallel, the Hippo-YAP signaling pathway has been implicated in the regulation of cell growth, differentiation, and inflammatory responses.[12,13] YAP interacts with classical inflammatory signaling pathways such as NF-κB and JNK, playing a critical role in controlling cell proliferation, differentiation, and inflammation.[14,15] Studies have suggested that YAP modulates inflammation in a variety of inflammatory diseases by suppressing the expression of inflammatory cytokines. Nevertheless, the exact role of YAP in periodontitis and its potential interaction with HIF remain to be fully elucidated.[16]

Therefore, this study aims to investigate the effects of the PHD inhibitor DMOG on pro-inflammatory cytokine expression in human periodontal ligament stem cells (hPDLSCs) under lipopolysaccharide (LPS)-induced inflammatory conditions. Given the critical role of hypoxia in exacerbating periodontal inflammation, we hypothesize that DMOG can stabilize HIF-1α and modulate inflammatory responses via the HIF-1α/YAP signaling pathway. This research seeks to elucidate the interactions between HIF-1α and YAP in inflammation regulation, providing a basis for targeting this pathway as a therapeutic strategy. Ultimately, we aim to determine whether DMOG and other PHD inhibitors hold potential as novel treatments for periodontitis by reducing inflammation and promoting tissue regeneration.

Materials and Methods

Cell Culture and Passage

Human periodontal ligament stem cells (hPDLSCs) (iCell; Shanghai, China) were cultured at a density of 1 × 10^4 cells per well in DMEM (Thermo Fisher Scientific; USA) supplemented with 10% FBS, under conditions of 37 °C and 5% CO2. For all experiments, cells were used between passages 3 and 5 (P3-P5) to ensure consistency and avoid potential effects of senescence or dedifferentiation. Cells that showed abnormal morphology, contamination, or poor viability were excluded from further experiments.

CCK-8 Assay

To evaluate the effects of LPS and DMOG on cell proliferation, hPDLSCs in the logarithmic growth phase were seeded at a density of 1 × 10^4 cells per well in three 96-well plates. After 24 hours, the medium was replaced, and the cells were divided into the following groups: Control, LPS (100 ng/mL), LPS + DMOG (100 ng/mL LPS + 0.1 mmol/L DMOG), and DMOG (0.1 mmol/L). Each experimental group included six technical replicates (wells) to minimize random error and ensure the accuracy and reliability of the data. The cells were then cultured for 24, 48, and 72 hours, followed by the addition of 10 μL of CCK-8 solution to each well, and further incubation for 2 hours at 37 °C. The absorbance at 450 nm was measured using a microplate reader SpectraMax M2 (Molecular Devices; USA).

ELISA

To assess the impact of DMOG on the expression of HIF-1α, IL-1β, TNF-α, and YAP, hPDLSCs were seeded at 5 × 10^4 cells per well, and the medium was changed according to the Control, LPS, and LPS + DMOG groups, with three replicates for each group. After 24 and 72 hours of culture, the supernatants were collected and analyzed using Human TNF-α ELISA Kit and Human IL-1β ELISA Kit (Abbkine; Wuhan, China). Absorbance values were measured at 450 nm, and standard curve data were used for linear regression to calculate the concentrations of IL-1β and TNF-α in the

samples. Following a similar protocol, cells were lysed and centrifuged to obtain supernatants for protein concentration measurement using a BCA protein assay kit (Abbkine; Wuhan, China). Absorbance was measured at 562 nm to create a standard curve and determine sample concentrations, which were diluted to a fixed concentration with PBS. Human HIF-1α ELISA Kit and Human YAP1 ELISA Kit were purchased from Mlbio (Shanghai, China). Pre-coated ELISA microplates for YAP and HIF-1α (HIF-1α, YAP: Mlbio, China) were then used according to the instructions, and absorbance at 450 nm was measured to calculate the protein concentrations of HIF-1α and YAP.

RT-PCR

To evaluate the effects of DMOG on the expression of HIF-1α, IL-1β, TNF-α, and YAP mRNA, hPDLSCs were seeded at 1 × 10^5 cells per well, and the medium was changed according to the Control, LPS, and LPS + DMOG groups, with three replicates for each group. Total RNA was extracted using the AG RNAex Pro RNA extraction kit after 24 and 72 hours of culture. The concentration and purity of the RNA were assessed, and cDNA was synthesized via reverse transcription. PCR reaction mixtures were prepared, and the PCR program was set as follows: initial denaturation at 95 °C for 30 seconds; denaturation at 95 °C for 5 seconds; annealing at 60 °C for 30 seconds, repeated for 40 cycles. After the reaction, amplification and melt curves were analyzed, and GAPDH was used as the internal control. The relative expression levels of IL-1β, TNF-α, YAP, and HIF-1α mRNA were calculated using the 2^-ΔΔCt method.

Primers:

GAPDH-F: 5'-TGACATCAAGAAGGTGGTGAAGCAG-3'
GAPDH-R: 5'-GTGTCGCTGTTGAAGTCAGAGGAG-3'
IL-1β-F: 5'-GGACAGGATATGGAGCAACAAGTGG-3'
IL-1β-R: 5'-TCATCTTTCAACACGCAGGACAGG-3'
TNF-α-F: 5'-AGCCCTGGTATGAGCCCATCTATC-3'
TNF-α-R: 5'-TCCCAAAGTAGACCTGCCCAGAC-3'
YAP-F: 5'-CAGAACCGTTTCCCAGACTACCTTG-3'
YAP-R: 5'-GCAGACTTGGCATCAGCTCCTC-3'
HIF-1α-F: 5'-CCATTAGAAAGCAGTTCCGCAAGC-3'
HIF-1α-R: 5'-GTGGTAGTGGTGGCATTAGCAGTAG-3'

Knockdown of YAP in hPDLSCs

YAP expression in hPDLSCs was knocked down via transfection to examine its effects on HIF-1α, IL-1β, and TNF-α expression. hPDLSCs were seeded at 1.8 × 10^5 cells per well, and when the cell confluence reached 70%-80%, lipid-based transfection reagents were used to introduce varying concentrations of siRNA. After 24 hours, transfection efficiency was evaluated using a fluorescence inverted microscope (DmiB manual, Leica, Germany), and the concentration of siRNA with the highest transfection efficiency was selected for further experiments. Following a second round of transfection, three experimental groups were established with different siRNA sequences (YAP-siRNA#1, YAP-siRNA#2, YAP-siRNA#3), along with a blank control group (without transfection reagents and siRNA), a negative control siRNA group (NC group), and a transfection reagent control group. After 48–72 hours of culture, RT-qPCR was used to assess the knockdown efficiency of YAP, and the siRNA group with the most significant knockdown effect was selected for subsequent experimental operations. The protein expression of YAP post-transfection was also evaluated. Finally, IL-1β, TNF-α, and HIF-1α protein levels were measured 72–96 hours after successful transfection.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 9.0, GraphPad Software, San Diego, CA, USA). Data were expressed as mean±standard deviation (SD) unless otherwise specified. Comparisons between two groups were conducted using an unpaired two-tailed Student's *t*-test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test for pairwise comparisons. Normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated with Levene's test. For data that did not meet normality or variance homogeneity assumptions, non-parametric tests (Mann–Whitney *U*-test

or Kruskal–Wallis test) were applied. A p-value of < 0.05 was considered statistically significant. All experiments were repeated at least three times independently to ensure reproducibility.

Results

Effects of LPS and DMOG on Cell Proliferation

CCK-8 assays demonstrated that treatment with 100 ng/mL LPS significantly promoted cell proliferation after 24 hours (Figure 1). No significant difference in cell proliferation rates was observed between the Control and DMOG groups after 24 hours, indicating that 0.1 mmol/L DMOG did not significantly affect cell proliferation in this timeframe. After 48 and 72 hours, the proliferation rates of the LPS and Control groups were comparable. As illustrated in Figure 1, although 100 ng/mL LPS exhibited a slight promoting effect on cell proliferation, the difference was not statistically significant. In contrast, the proliferation rate in the Control group compared to the DMOG group revealed a significant inhibition of cell proliferation by 0.1 mmol/L DMOG (Figure 1). These results suggest that low concentrations of LPS (100 ng/mL) can initially promote cell proliferation; however, this effect diminishes over time. Conversely, 0.1 mmol/L DMOG significantly inhibits cell proliferation, and this inhibitory effect becomes more pronounced with extended exposure.

Verification of the Inflammatory Environment

ELISA assays indicated that the secretion levels of IL-1β and TNF-α were significantly higher in the LPS group compared to the Control group (Figure 2A and B). Additionally, RT-qPCR results demonstrated that IL-1β mRNA expression was markedly elevated following LPS induction (Figure 2C), and TNF-α mRNA expression also significantly increased (Figure 2D), confirming the successful establishment of an inflammatory environment.

Effects of DMOG on HIF-1α, IL-1β, TNF-α, and YAP Expression

ELISA results presented in Figure 3 indicated that the HIF-1α concentration in the LPS + DMOG group was significantly higher than that in the LPS group (Figure 3A). Concurrently, the expression levels of IL-1β and TNF-α in the LPS + DMOG group were significantly decreased (Figure 3B and C), while YAP expression was notably increased (Figure 3D). These findings suggest that DMOG can upregulate HIF-1α expression in an inflammatory environment while concurrently reducing LPS-induced IL-1β and TNF-α secretion from hPDLSCs, and further activating YAP.

RT-qPCR results displayed in Figure 4 reveal that HIF-1α mRNA expression in the LPS group was significantly higher than that in the Control group (Figure 4A). In contrast, the LPS + DMOG group exhibited a significant decrease in

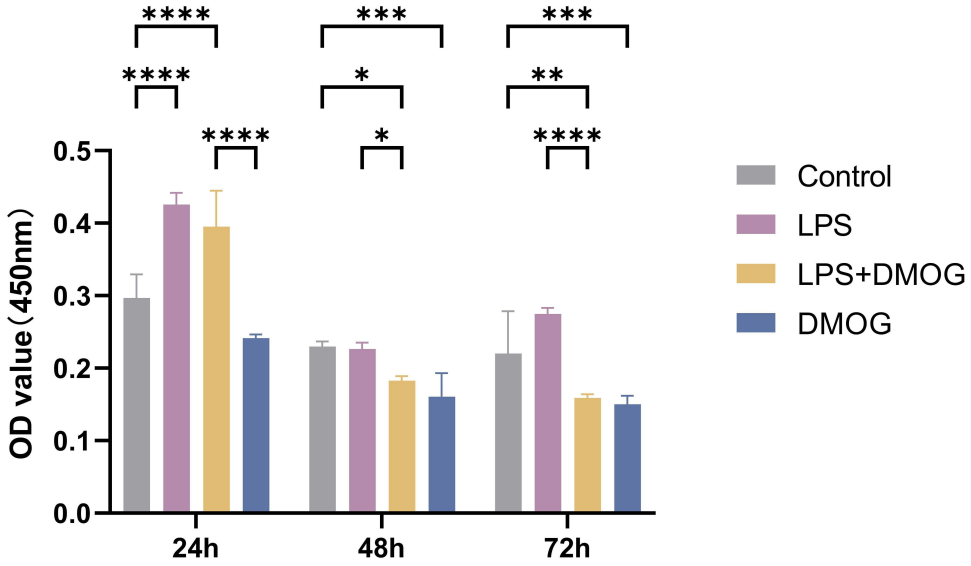

Figure 1 CCK-8 assay demonstrating cell proliferation in different groups. Comparison between groups: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

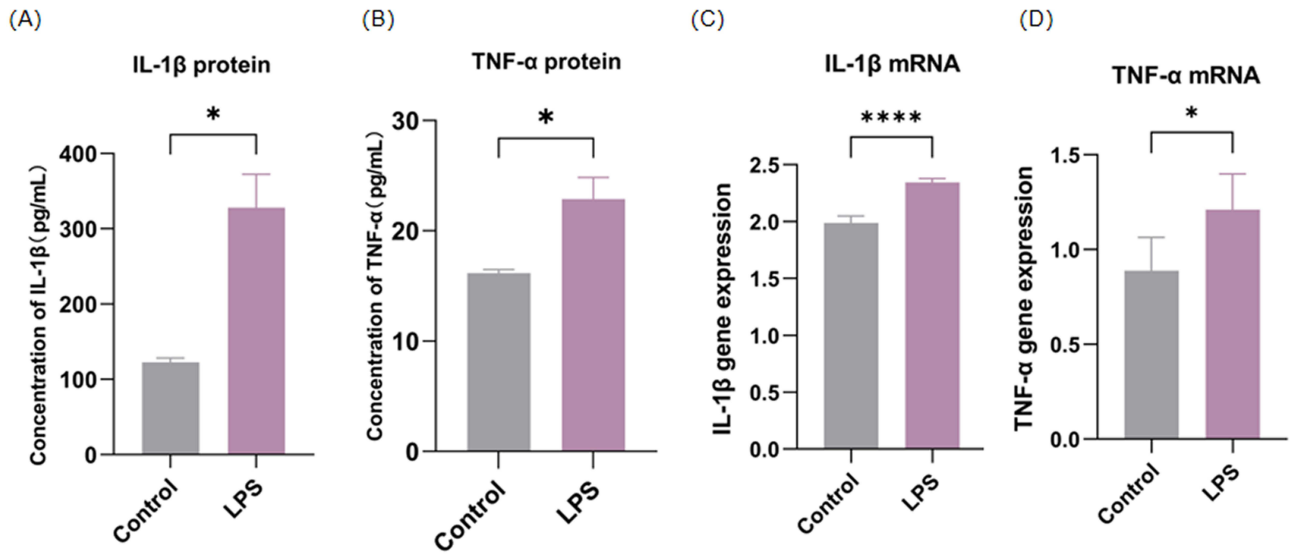

Figure 2 LPS up-regulated the expression of pro-inflammatory factors (IL-1β and TNF-α) in hPDLSCs (**A**) ELISA data showed that IL-1β concentration was increased in LPS group compared with Control group (P = 0.023). (**B**) ELISA data showed that TNF-α concentration was increased in LPS group compared with Control group (P = 0.019). Data are expressed as mean ± SD, n = 3 wells per group. (**C**) RT-qPCR analysis confirming the trends in mRNA expression levels of IL-1β (P < 0.0001). (**D**) RT-qPCR analysis confirming the trends in mRNA expression levels of TNF-α (P = 0.035). Data are expressed as mean ± SD, n = 6 wells per group. Comparison between groups: *P < 0.05, ****P < 0.0001.

HIF-1α mRNA expression compared to the LPS group (Figure 4A). Additionally, the expression levels of IL-1β and TNF-α mRNA were significantly reduced in the LPS + DMOG group compared to the LPS group (Figure 4C and D). Notably, the expression of YAP mRNA was significantly increased in the LPS + DMOG group compared to the LPS group (Figure 4B).

Establishment and Validation of Transfection Conditions

The transfection efficiency of fluorescently labeled siRNA at different concentrations was observed using an inverted fluorescence microscope. As shown in Figure 5, the transfection efficiency was highest at a concentration of 50 nM. Therefore, this concentration was selected for subsequent experiments.

RT-qPCR results demonstrating the silencing effect of different siRNA sequences on YAP expression are presented in Figure 6. No significant differences in YAP gene expression were observed among the blank control, transfection reagent control, and negative control groups. Both the YAP-siRNA#1 and YAP-siRNA#2 groups significantly inhibited YAP mRNA expression (Figure 6A), with no notable difference in inhibition efficiency. The YAP-siRNA#3 group also suppressed YAP mRNA expression (Figure 6A), but the inhibition efficiency was significantly lower compared to the YAP-siRNA#1 group (Figure 6A). Consequently, YAP-siRNA#1 was selected for further experimental procedures. ELISA results indicated that YAP-siRNA#1 significantly reduced YAP protein expression in hPDLSCs compared to the untransfected group (Figure 6B).

Effects of YAP Knockdown on IL-1β and TNF-α Expression

ELISA results illustrated in Figure 7 show that the concentrations of IL-1β and TNF-α were significantly increased in the YAP-siRNA#1 group compared to the LPS + DMOG group (Figure 7A and B). RT-qPCR results confirmed that IL-1β and TNF-α mRNA levels were significantly higher in the YAP-siRNA#1 group compared to the LPS + DMOG group (Figure 7C and D), indicating that knockdown of YAP expression leads to upregulation of pro-inflammatory cytokines IL-1β and TNF-α.

Effects of YAP Knockdown on HIF-1α Expression

ELISA results in Figure 8A show that HIF-1α concentrations were significantly lower in the YAP-siRNA#1 group compared to the LPS + DMOG group. Additionally, RT-qPCR results in Figure 8B indicated that HIF-1α mRNA levels

https://doi.org/10.2147/JIR.S504793

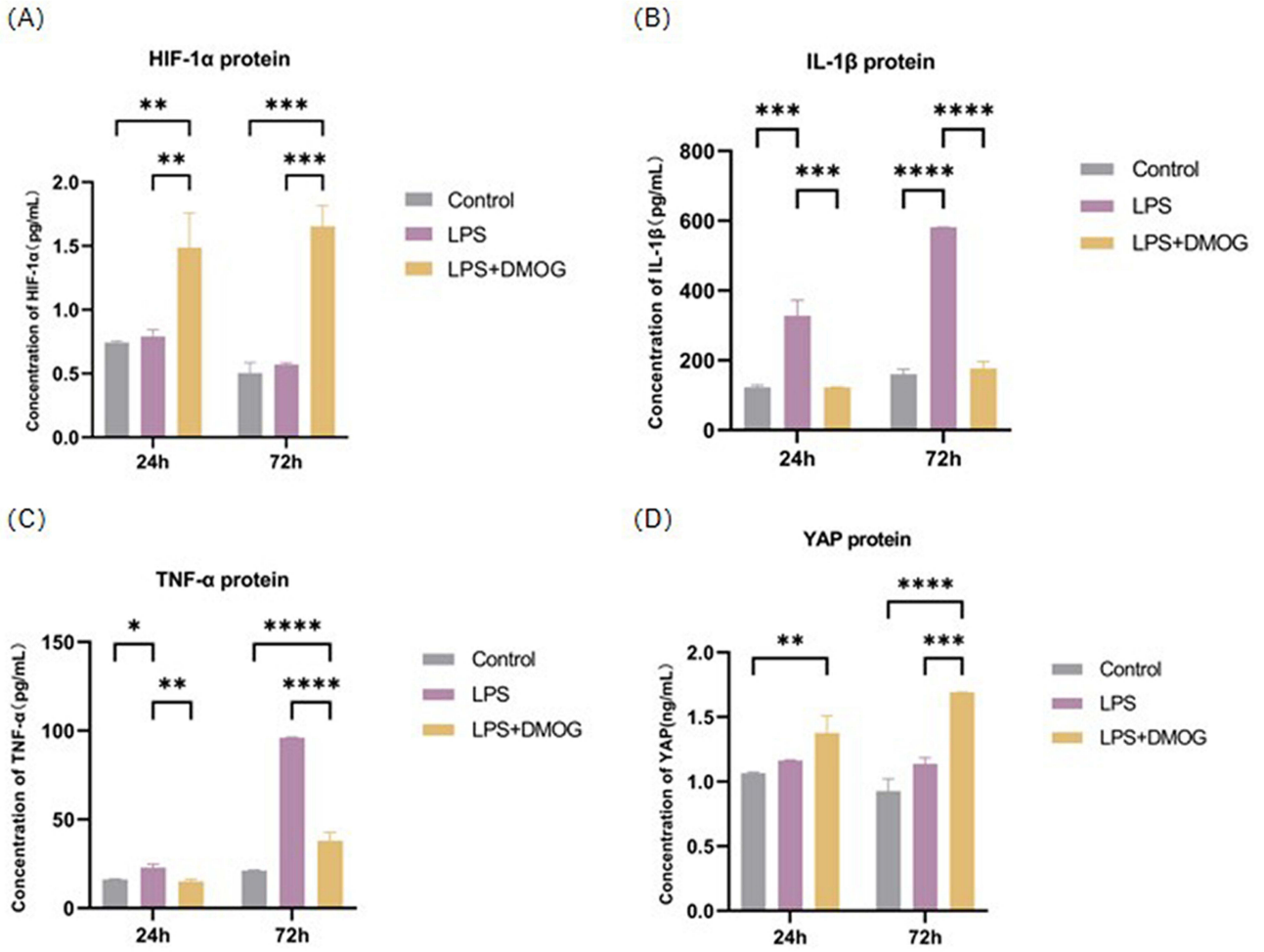

Figure 3 DMOG upregulates HIF-1α protein expression while reducing pro-inflammatory cytokines (IL-1β and TNF-α) in hPDLSCs. hPDLSCs were treated with LPS to induce inflammation, followed by DMOG treatment. (**A**) ELISA data showed that HIF-1α protein concentration in LPS + DMOG group was higher than that in LPS group at 24h ($P = 0.003$) and 48h ($P < 0.001$). (**B**) IL-1β protein expression level in LPS + DMOG group was higher than that in LPS group at 24h ($P < 0.001$) and 48h ($P < 0.0001$) (**C**) TNF-α protein expression level in LPS +DMOG group was higher than that in LPS group at 24h ($P = 0.006$) and 48h ($P < 0.0001$) (**D**) YAP protein expression level in LPS + DMOG group was higher than that in LPS group at 48h ($P < 0.001$). Data are expressed as mean ± SD, n = 3 wells per group. Comparison between groups: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

were significantly reduced in the YAP-siRNA#1 group compared to the LPS + DMOG group. This indicates that knockdown of YAP expression downregulates HIF-1α levels, suggesting a positive correlation between HIF-1α and YAP expression.

Discussion

This study investigated whether DMOG could modulate inflammatory responses in hPDLSCs by stabilizing HIF-1α and regulating the downstream HIF-1α/YAP signaling pathway, which may influence the expression of key pro-inflammatory cytokines such as IL-1β and TNF-α under inflammatory conditions. We anticipated that DMOG would have minimal impact on these pathways under both normal and inflammatory conditions. However, our findings demonstrate that DMOG exerts significant anti-inflammatory effects in hPDLSCs by engaging this pathway.

The primary findings of this study demonstrate that DMOG exerts anti-inflammatory effects in hPDLSCs by stabilizing HIF-1α and reducing the expression of key pro-inflammatory cytokines, including IL-1β and TNF-α. Specifically, we observed that DMOG significantly upregulated HIF-1α expression under both normal and LPS-induced inflammatory conditions, while concurrently decreasing the levels of IL-1β and TNF-α in the inflammatory environment. These results highlight the dual role of DMOG in modulating hypoxia-responsive pathways and reducing

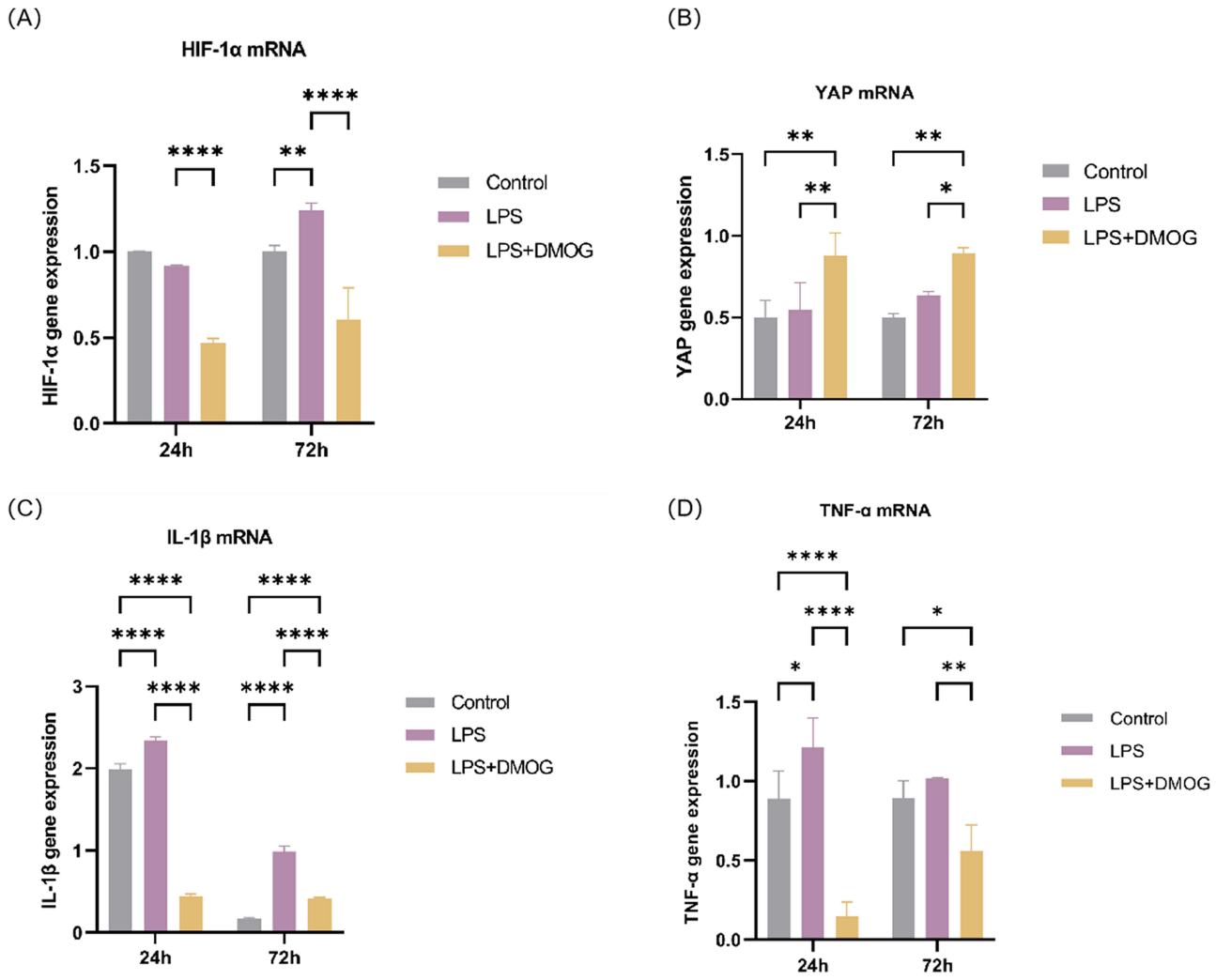

Figure 4 Relative mRNA expression levels of HIF-1α\YAP\IL-1β and TNF-α in hPDLSCs under different experimental conditions. hPDLSCs were treated with LPS to induce inflammation, followed by DMOG treatment. Gene expression levels were quantified by RT-qPCR and normalized to GAPDH. (**A**) RT-qPCR data showed that HIF-1α gene expression concentration in LPS + DMOG group was lower than that in LPS group at 24h and 72h ($P < 0.0001$). (**B**) YAP gene expression level in LPS + DMOG group was higher than that in LPS group at 24h ($P = 0.004$) and 72h ($P = 0.019$). (**C**) IL-1β gene expression level in LPS + DMOG group was lower than that in LPS group at 24h and 72h ($P < 0.0001$). (**D**) TNF-α gene expression level in LPS + DMOG group was lower than that in LPS group at 24h ($P < 0.0001$) and 72h ($P = 0.004$). Data are expressed as mean ± SD, n = 6 wells per group. Comparison between groups: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

inflammation in hPDLSCs. Furthermore, these findings align with previous studies, which demonstrated the critical role of HIF-1α stabilization in modulating inflammatory responses under hypoxic or inflammatory conditions.[17,18]

Based on these findings, we hypothesize that the HIF-1α/YAP signaling axis plays a crucial role in regulating the inflammatory response of hPDLSCs. Specifically, DMOG may inhibit the release of pro-inflammatory factors by stabilizing HIF-1α, while YAP may function as a co-regulator in this process. Our data indicate a similar expression pattern between YAP and HIF-1α, suggesting that these two proteins may form a regulatory network during the inflammatory response.[19–21] Furthermore, the downregulation of YAP is associated with increased expression of TNF-α and IL-1β, which may subsequently activate the NF-κB signaling pathway, exacerbating the inflammatory response. This observation is consistent with previously reported findings.[22–24]

Our interpretation is corroborated by other research, which has demonstrated that DMOG can exert anti-inflammatory effects in various inflammatory diseases through the stabilization of HIF-1α. Additionally, the positive correlation between YAP and HIF-1α expression under inflammatory conditions aligns with observations by Li et al, who noted increased YAP expression following the stabilization of HIF-1α with CoCl□.[19] However, the precise mechanisms of interaction between YAP and HIF-1α in hPDLSCs remain unclear and warrant further investigation. We propose that

https://doi.org/10.2147/JIR.S504793

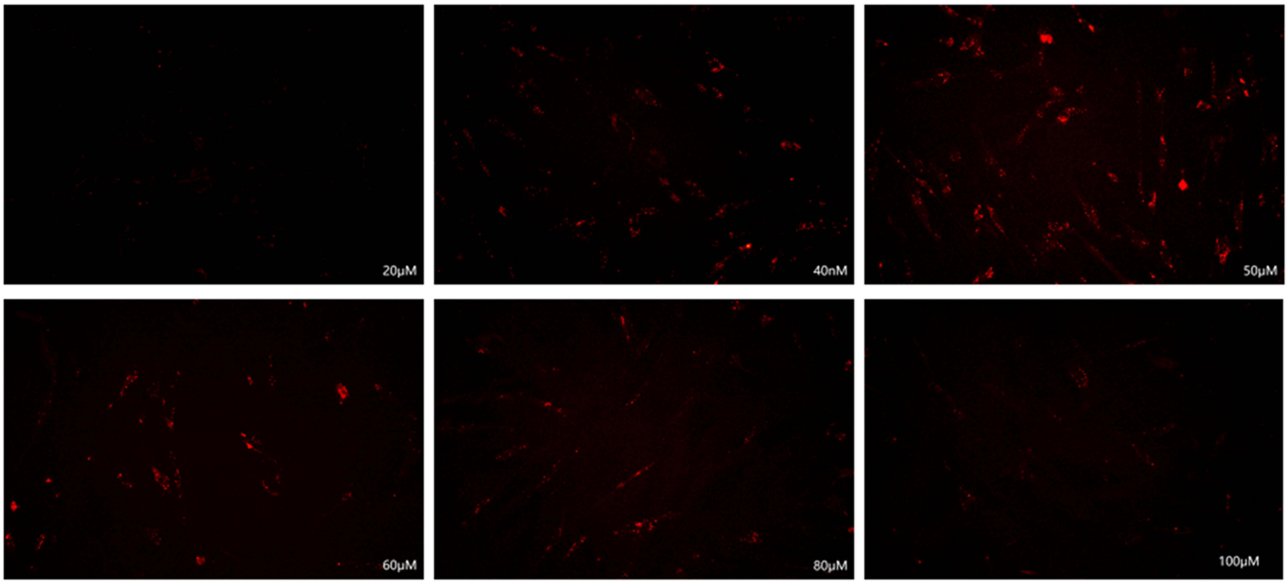

Figure 5 Transfection efficiency observed under an inverted fluorescence microscope.

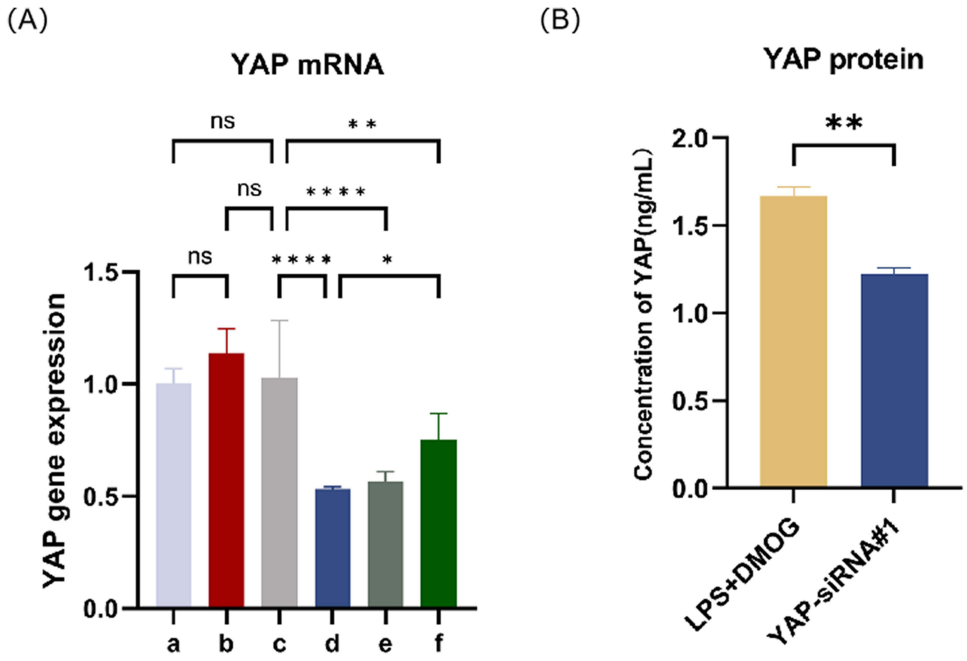

Figure 6 Detection of the YAP gene silencing effect (**A**)The relative expression of YAP mRNA in hPDLSCs was detected by RT-qPCR under different experimental conditions. Group a: blank control group; Group b: transfection reagent control group; Group c: transfection negative control group; Group d: YAP-siRNA#1 group; Group e: YAP-siRNA#2 group; Group f: YAP-siRNA#3 group. Data are expressed as mean ± SD, n = 6 wells per group. (**B**) ELISA data showed that the expression level of YAP protein in YAP-siRNA#1 group was lower than LPS + DMOG group (P = 0.009). Data are expressed as mean ± SD, n = 3 wells per group. Comparison between groups: *P < 0.05, **P < 0.01, ****P < 0.0001, ns: no significance.

YAP may enhance the stability of HIF-1α, or conversely, that HIF-1α may promote YAP expression, thereby providing protective effects in inflammatory environments.

Our findings shed light on the potential role of the HIF-1α/YAP signaling pathway in modulating inflammatory responses in periodontal tissues. By demonstrating that DMOG can stabilize HIF-1α and reduce pro-inflammatory cytokines such as IL-1β and TNF-α in LPS-treated hPDLSCs, this study provides mechanistic insights into how this pathway may influence periodontal inflammation. These results suggest that targeting the HIF-1α/YAP axis might be

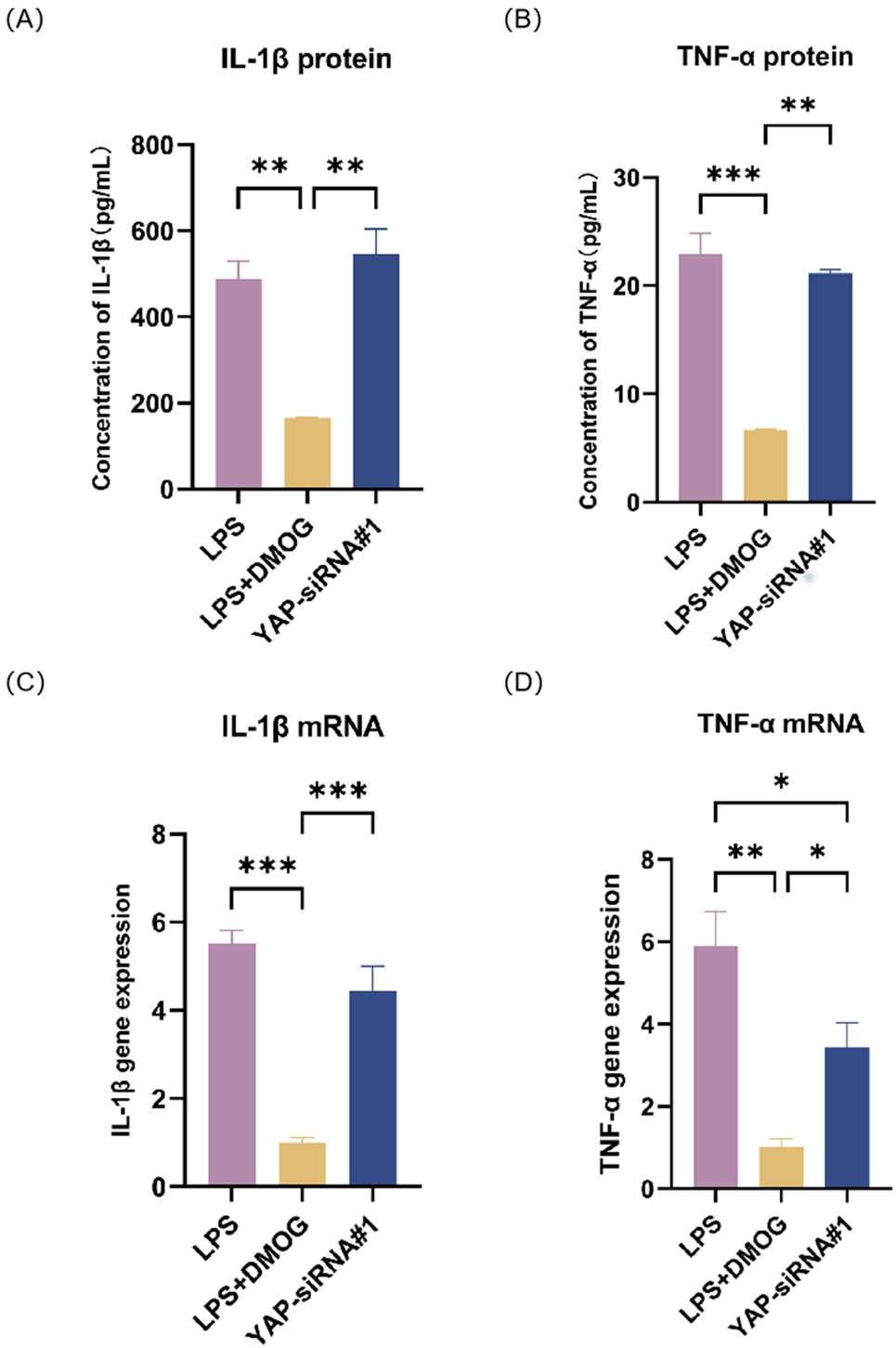

Figure 7 Effects of YAP knockdown on the expression of IL-1β and TNF-α in hPDLSCs. (**A**) ELISA data showed that IL-1β concentration was increased in YAP-siRNA#1 group compared with LPS + DMOG group (P = 0.002). (**B**) ELISA data showed that TNF-α concentration was increased in YAP-siRNA#1 group compared with LPS+DMOG group (P = 0.001). Data are expressed as mean ± SD, n = 3 wells per group. (**C**) RT-qPCR data showed that IL-1β concentration was increased in YAP-siRNA#1 group compared with LPS + DMOG group (P < 0.001). (**D**) RT-qPCR showed that TNF-α concentration was increased in YAP-siRNA#1 group compared with LPS + DMOG group (P = 0.016). Data are expressed as mean ± SD, n = 6 wells per group. Comparison between groups: *P < 0.05, **P < 0.01, ***P < 0.001.

a promising direction for future therapeutic exploration. However, it is important to note that these findings are based on in vitro experiments, and further studies are required to validate their translational relevance in vivo and in clinical settings.

https://doi.org/10.2147/JIR.S504793

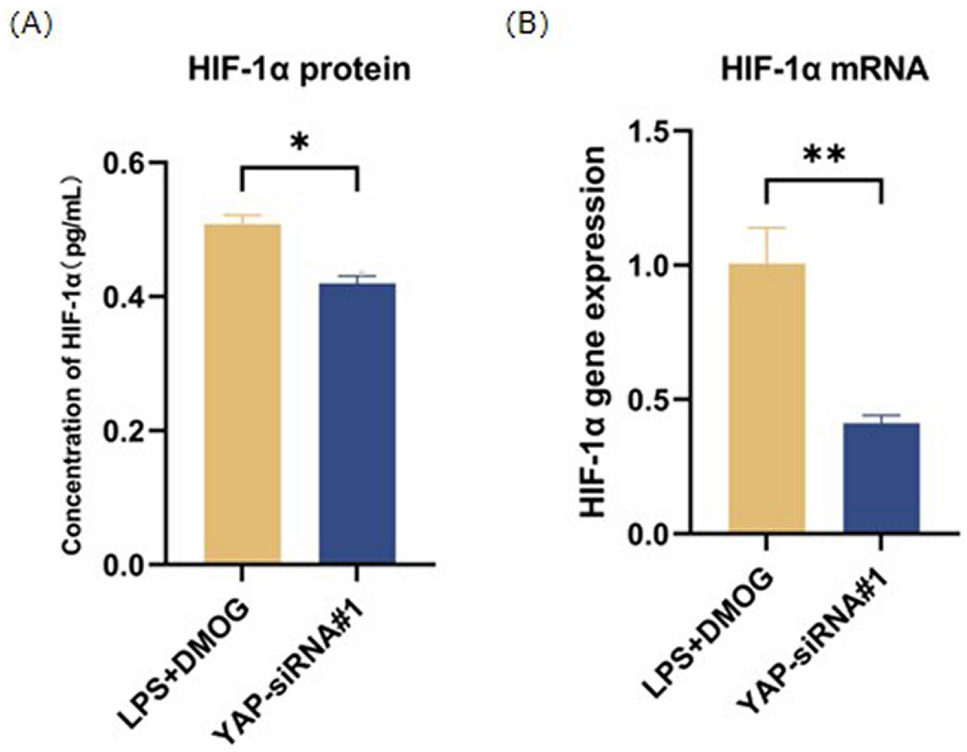

Figure 8 Effects of YAP knockdown on the protein (**A**) and gene expression (**B**) of HIF-1α in hPDLSCs. (**A**) ELISA data showed that HIF-1α protein concentration was decreased in YAP-siRNA#1 group compared with LPS + DMOG group (P = 0.011). Data are expressed as mean ± SD, n = 3 wells per group (**B**) RT-qPCR showed that HIF-1α gene concentration was decreased in YAP-siRNA#1 group compared with LPS + DMOG group (P = 0.009). Data are expressed as mean ± SD, n = 6 wells per group. Comparison between groups: *P < 0.05, **P < 0.01.

Nevertheless, some limitations of this study must be acknowledged. First, our primary focus was on the changes in pro-inflammatory cytokine expression, without investigating the interplay between pro-inflammatory and anti-inflammatory factors, which could provide a more comprehensive understanding of the inflammatory process. Second, while we identified YAP as a regulator of pro-inflammatory cytokines, the underlying mechanisms remain unclear. Future studies should explore the involvement of key pathways such as NF-κB and JNK to elucidate the interactions between HIF-1α/YAP and these downstream regulators. Addressing these limitations will help provide a more complete understanding of the signaling networks involved in periodontal inflammation and the therapeutic potential of targeting these pathways.

Conclusion

In conclusion, this study provides novel insights into the anti-inflammatory effects of DMOG in hPDLSCs, emphasizing its role in stabilizing HIF-1α and modulating the HIF-1α/YAP signaling axis. By demonstrating the capacity of DMOG to downregulate pro-inflammatory cytokines, our findings suggest that targeting this pathway could represent a promising strategy for controlling periodontal inflammation. These outcomes not only advance our understanding of hypoxia-responsive pathways in inflammatory regulation but also open avenues for exploring the therapeutic potential of PHD inhibitors beyond inflammation control, particularly in the regeneration of periodontal and other hypoxia-sensitive tissues. Future investigations should focus on elucidating the precise molecular mechanisms underlying the HIF-1α/YAP axis and validating these findings in vivo to enable translational applications.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The present study was supported by Yantai Science and Technology Project (grant No. 2022YD099, 2022YD102).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. *Int Dent J*. 2021;71(6):462–476. doi:10.1111/idj.12630
2. Isola G, Polizzi A, Santonocito S, et al. Effect of quadrantwise versus full-mouth subgingival instrumentation on clinical and microbiological parameters in periodontitis patients: a randomized clinical trial. *J Periodontal Res*. 2024;59(4):647–656. doi:10.1111/jre.13279
3. Dibello V, Custodero C, Cavalcanti R, et al. Impact of periodontal disease on cognitive disorders, dementia, and depression: a systematic review and meta-analysis. *Geroscience*. 2024;46(5):5133–5169. doi:10.1007/s11357-024-01243-8
4. Chen Y, Jin Y, Li K, et al. Is there an association between parkinson's disease and periodontitis? a systematic review and meta-analysis. *J Parkinsons Dis*. 2023;13(7):1107–1125. doi:10.3233/JPD-230059
5. Villoria GEM, Fischer RG, Tinoco EMB, Meyle J, Loos BG. Periodontal disease: systemic condition A. *Periodontol 2000*. 2024;96(1):7–19. doi:10.1111/prd.12616
6. Polizzi A, Nibali L, Tartaglia GM, et al. Impact of nonsurgical periodontal treatment on arterial stiffness outcomes related to endothelial dysfunction: a systematic review and meta-analysis. *J Periodontol*. 2024. doi:10.1002/JPER.24-0422
7. Pattamatta M, Chapple I, Listl S. The value-for money of preventing and managing periodontitis: opportunities and challenges. *Periodontol 2000*. 2024. doi:10.1111/prd.12569
8. Takeda K, Ichiki T, Narabayashi E, et al. Inhibition of prolyl hydroxylase domain-containing protein suppressed lipopolysaccharide-induced TNF-alpha expression. *Arterioscler Thromb Vasc Biol*. 2009;29(12):2132–2137. doi:10.1161/ATVBAHA.109.196071
9. Saas P, Fan GC. Editorial: hypoxia and inflammation: a two-way street. *Front Immunol*. 2023;14:1171116. doi:10.3389/fimmu.2023.1171116
10. Song ZC, Zhou W, Shu R, Ni J. Hypoxia induces apoptosis and autophagic cell death in human periodontal ligament cells through HIF-1α pathway. *Cell Prolif*. 2012;45(3):239–248. doi:10.1111/j.1365-2184.2012.00810.x
11. Eltzschig HK, Bratton DL, Colgan SP. Targeting hypoxia signalling for the treatment of ischaemic and inflammatory diseases. *Nat Rev Drug Discov*. 2014;13(11):852–869. doi:10.1038/nrd4422
12. Ogawa C, Tsuchiya K, Maeda K. Hypoxia-inducible factor prolyl hydroxylase inhibitors and iron metabolism. *Int J mol Sci*. 2023;24(3):3037. doi:10.3390/ijms24033037
13. Dvornikova KA, Platonova ON, Bystrova EY. Hypoxia and intestinal inflammation: common molecular mechanisms and signaling pathways. *Int J mol Sci*. 2023;24(3):2425. doi:10.3390/ijms24032425
14. Eckle T, Köhler D, Lehmann R, El Kasmi K, Eltzschig HK. Hypoxia-inducible factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning. *Circulation*. 2008;118(2):166–175. doi:10.1161/CIRCULATIONAHA.107.758516
15. Halligan DN, Khan MN, Brown E, et al. Hypoxia-inducible factor hydroxylase inhibition enhances the protective effects of cyclosporine in colitis. *Am J Physiol Gastrointest Liver Physiol*. 2019;317(2):G90–G97. doi:10.1152/ajpgi.00049.2019
16. Li M, Zhang FJ, Bai RJ. The Hippo-YAP signaling pathway in osteoarthritis and rheumatoid arthritis. *J Inflamm Res*. 2024;17:1105–1120. doi:10.2147/JIR.S444758
17. Yoshii H, Kajiya M, Yoshino M, et al. Mechanosignaling YAP/TAZ-TEAD axis regulates the immunomodulatory properties of mesenchymal stem cells. *Stem Cell Rev Rep*. 2024;20(1):347–361. doi:10.1007/s12015-023-10646-7
18. Heallen T, Morikawa Y, Leach J, et al. Hippo signaling impedes adult heart regeneration. *Development*. 2013;140(23):4683–4690. doi:10.1242/dev.102798
19. Dong T, Sun X, Jin H. Role of YAP1 gene in proliferation, osteogenic differentiation, and apoptosis of human periodontal ligament stem cells induced by TNF-α. *J Periodontol*. 2021;92(8):1192–1200. doi:10.1002/JPER.20-0176
20. Gonçalo RIC, Sousa JT, Costa CSO, et al. Does YAP influence cell proliferation and apoptosis in benign epithelial odontogenic lesions? *Oral Dis*. 2024;30(7):4538–4546. doi:10.1111/odi.14882
21. Hirai K, Furusho H, Hirota K, Sasaki H. Activation of hypoxia-inducible factor 1 attenuates periapical inflammation and bone loss. *Int J Oral Sci*. 2018;10(2):12. doi:10.1038/s41368-018-0015-0
22. Shang L, Kang W, Li S, Ge S. Prolyl hydroxylase inhibitor DMOG suppressed inflammatory cytokine production in human gingival fibroblasts stimulated with Fusobacterium nucleatum. *Clin Oral Investig*. 2019;23(7):3123–3132. doi:10.1007/s00784-018-2733-2
23. Li H, Li X, Jing X, et al. Hypoxia promotes maintenance of the chondrogenic phenotype in rat growth plate chondrocytes through the HIF-1α/YAP signaling pathway. *Int J Mol Med*. 2018;42(6):3181–3192. doi:10.3892/ijmm.2018.3921
24. Ma X, Zhang H, Xue X, Shah YM. Hypoxia-inducible factor 2α (HIF-2α) promotes colon cancer growth by potentiating Yes-associated protein 1 (YAP1) activity. *J Biol Chem*. 2017;292(41):17046–17056. doi:10.1074/jbc.M117.805655