Scientific Research Report

DUXAP8 Promotes LPS-Induced Cell Injury in Pulpitis by Regulating miR-18b-5p/HIF3A

Ying Dai, Guihong Xuan, Min Yin *

Department of Stomatology, Shaoxing People's Hospital, Shaoxing, China

ARTICLE INFO

Article history:
Received 3 July 2022
Received in revised form
7 November 2022
Accepted 20 November 2022
Available online 14 December 2022

Key words:
DUXAP8
Pulpitis
mir-18b-5p
HIF3A

ABSTRACT

Background: The dysregulated long noncoding RNAs (lncRNAs) are implicated in progression of various diseases, including pulpitis. Double homeobox A pseudogene 8 (DUXAP8) has been found to be upregulated in pulpitis. Herein, the functional mechanism of DUXAP8 in lipopolysaccharide (LPS)-induced pulpitis was explored.

Material and methods: DUXAP8, microRNA-18b-5p (miR-18b-5p), or hypoxia-inducible factor 3A (HIF3A) levels were examined through reverse transcription-quantitative polymerase chain reaction assay. Cell behaviours were determined by Cell Counting Kit-8 assay for cell viability, Ethynyl-2′-deoxyuridine (EdU) assay for cell proliferation, and flow cytometry for cell apoptosis. Protein levels were measured using western blot. Inflammatory reaction was analysed via enzyme-linked immunosorbent assay. Oxidative stress was assessed by commercial kits. Dual-luciferase reporter assay, RNA immunoprecipitation assay, and pull-down assay were used for validation of interaction between targets.

Results: Cell apoptosis, inflammatory reaction, and oxidative stress were induced by LPS in human dental pulp cells (HDPCs). DUXAP8 upregulation and miR-18b-5p downregulation were found in pulpitis. LPS-induced cell injury was relieved after downregulation of DUXAP8. DUXAP8 interacted with miR-18b-5p. The regulation of DUXAP8 was related to miR-18b-5p sponging function in LPS-treated HDPCs. HIF3A served as a target of miR-18b-5p. MiR-18b-5p protected against LPS-induced cell injury through targeting HIF3A. DUXAP8 targeted miR-18b-5p to regulate HIF3A level.

Conclusions: Results demonstrated that LPS-induced cell injury in pulpitis was promoted by DUXAP8 through mediating miR-18b-5p/HIF3A axis.

Introduction

Pulpitis is a typical inflammatory disease with acute and intense pain of dental pulp tissues.[1,2] Lipopolysaccharide (LPS) can induce dental inflammation and immune response in pulpitis.[3] Epigenetic pathways are involved in dental pulp inflammation, such as methylation and acetylation of histones or dysregulation of noncoding RNAs (ncRNAs).[4] Long ncRNAs (lncRNAs) and small microRNAs (miRNAs) exhibit important regulation in oral inflammation.[5,6] It is important to research the pathogenic mechanism of pulpitis associated with lncRNAs and miRNAs.

lncRNAs are absent from specific open reading frames without protein-coding ability, and they are crucial players in a variety of pathologic and physiologic processes in human diseases.[7] lncRNAs are known to regulate gene level via functioning as miRNA sponges to construct lncRNA/miRNA/mRNA axis in disease regulation[8] as well as in pulpitis.[9] Double homeobox A pseudogene 8 (DUXAP8) was aberrantly overexpressed in pulpitis tissues,[9] but its regulatory function is unclear in pulpitis. In addition, Du et al[10] found that miR-18b-5p was downregulated in LPS-induced periodontitis, indicating the important role of miR-18b-5p in dental diseases. The potential function of miR-18b-5p in pulpitis remains to be identified. Chen et al[11] stated that hypoxia-inducible factor 3A (HIF3A) promoted the progression of periodontitis, and MALAT1 resulted in upregulation of HIF3A by controlling miR-769-5p. The involvement of HIF3A in pulpitis requires further research.

Herein, DUXAP8 was hypothesised to induce a sponge effect on miR-18b-5p to regulate the level change of HIF3A. DUXAP8/

* *Corresponding author*. Min Yin, Department of Stomatology, Shaoxing People's Hospital, NO. 568, Zhongxing North Road, Shaoxing 312000, China.
E-mail address: sxyinmin@163.com (M. Yin).

https://doi.org/10.1016/j.identj.2022.11.011

miR-18b-5p and miR-18b-5p/HIF3A target relationships were affirmed in this study. The current purpose was to discover the molecular regulation of DUXAP8/miR-18b-5p/HIF3A in LPS-induced pulpitis.

Materials and methods

Human specimens

Twenty-seven patients were diagnosed with pulpitis by 2 experienced stomatologists. The pulpitis tissues (n = 27) were collected from patients with pulpitis, and normal pulp tissues (n = 23) were acquired from patients with other tooth diseases. Inflamed pulp tissues were extracted from teeth diagnosed with irreversible pulpitis in the late stage according to the endodontic diagnosis system from the American Association of Endodontists. The inclusion criteria were as follows: (1) patients older than 18 years; (2) those without any other inflammatory disease; and (3) patients who had not received endodontic treatment or any related therapeutic treatment. The exclusion criteria were as follows: (1) patients without complete clinical treatment history; (b) patients with more than a single indicated tooth, (c) patients with any periodontal disease; (d) patients without the complete treatment. Normal pulp tissues were collected from healthy third molars extracted for orthodontic purposes. Then specimens were conserved in liquid nitrogen at Shaoxing People's Hospital, until extraction of total RNA or protein. The protocols were based on the Declaration of Helsinki and were approved by the Ethics Committee of Shaoxing People's Hospital.

LPS-induced cell model

Human dental pulp cells (HDPCs) were purchased from American Tissue Culture Collection, followed by cell incubation with Dulbecco's modified Eagle's medium (Sigma) containing 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 units/mL penicillin (Sigma). HDPCs were cultured in a humidified 5% CO_2 incubator at 37 °C, and cell passage was performed every 2 to 3 days. The third-passaged cells were used for subsequent research. LPS is usually used for mimicking a cell model of pulpitis.[12,13] HDPCs were stimulated with LPS (Sigma) of different concentrations (0 μg/mL, 0.5 μg/mL, 1 μg/mL, 2 μg/mL) for 24 hours, and 1 μg/mL was used as a final use concentration of LPS.

Cell counting kit-8 (CCK-8) assay

Cell viability determination was performed using CCK-8 assay. HDPCs in 96-well plates were incubated with 10 μL/well CCK-8 solution (Beyotime), and then cell absorbance was assayed via microplate reader after 2 hours. Cell viability was defined as percentage of viable cells.

Ethynyl-2′-deoxyuridine (EdU) assay

EdU Detection Kit (RIBOBIO) was applied to assess proliferation. Briefly, HDPCs were performed with labeling of EdU solution and staining of diamidino phenylindole (DAPI; Beyotime). EdU and DAPI merged cells were counted as EDU-positive cells with proliferation ability.

Flow cytometry

Apoptosis phenomenon was analysed through Annexin V-FITC Apoptosis Detection Kit (Beyotime). Moreover, 1×10^6 HDPCs were dyed with Annexin V-fluorescein isothiocyanate (Annexin V-FITC; 5 μL) and propidium iodide (PI; 10 μL) for 20 minutes, following the user's instruction book. Then, fluorescence detection was performed on a flow cytometer (BD Biosciences), and Annexin V-FITC$^+$/PI$^-$ + Annexin V-FITC$^+$/PI$^+$ cells were calculated as apoptotic cells.

Western blot

After lysis of tissues and cells by radioimmunoprecipitation assay buffer (Sigma), sample concentration was examined via BCA Protein Assay Kit (Sigma). Then, 40 μg proteins were used for western blot analysis.[14] Antibodies were bought from Abcam: anti-Cyclin D1 (ab134175, 1:1000), anti-Bcl2 associated X (anti-Bax; ab182733, 1:1000), anti-HIF3A (ab10134 1:1000), anti-glyceraldehyde-phosphate dehydrogenase (anti-GAPDH; ab9485, 1:2000), and goat-anti rabbit immunoglobulin G (IgG) H&L (HRP) secondary antibody (ab205718, 1:5000). Electrochemiluminescence Substrate Kit (Abcam) was used for exhibiting back blots on the membranes, then the expression level was analysed via Image J software (NIH).

Enzyme-linked immunosorbent assay (ELISA)

Inflammatory factors were measured through ELISA. The supernatant cells were harvested, then interleukin-6 (IL-6) and interleukin-1beta (IL-1β) concentrations were determined according to human IL-6 ELISA Kit and IL-1β ELISA Kit (Sangon). The concentrations were expressed as pg/mL.

Superoxide dismutase (SOD) and malondialdehyde (MDA) assays

SOD activity and MDA level were detected to evaluate oxidative injury. The operating procedures were in accordance with guidelines of SOD Assay Kit and Lipid Peroxidation (MDA) Assay Kit (Sigma). Cell absorbance was read at 450 nm for SOD assay and 530 nm for MDA assay.

Cell transfection

RNAs were synthesised by RIBOBIO, including small interfering RNA for DUXAP8 (si-DUXAP8) and matched control (si-NC), mimic for miR-18b-5p (miR-18b-5p) and relative control (miR-NC), and inhibitor for miR-18b-5p (anti-miR-18b-5p) and corresponding control (anti-miR-NC). Additionally, pcDNA, pcDNA-DUXAP8 (DUXAP8), and pcDNA-HIF3A (HIF3A) vectors were obtained from GENESEED. RNAs or vectors were diluted with Opti-MEM® Reduced Serum Medium (Gibco), followed by transfection with Lipofectamine 3000 reagent (Invitrogen) into HDPCs at 37 °C.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

Total RNA isolation by TRIzol Reagent (Invitrogen) and complementary DNA (cDNA) synthesis by ReadyScript cDNA Synthesis Mix (Sigma) were performed following manufacturers' specifications. The amplified reaction was implemented using SYBR Green One-Step qPCR Kit (Invitrogen) and specific primers (Sangon), as shown in Supplementary Table 1. U6 small nuclear RNA (U6) or glyceraldehyde-phosphate dehydrogenase (GAPDH) was selected as references for expression normalisation, then data analysis was performed through the $2^{-\Delta\Delta Ct}$ method.[15] In addition, nuclear and cytoplasmic RNAs were isolated from HDPCs via PARIS Kit (Invitrogen). Then DUXAP8, U6, and GAPDH levels were examined to identify the localisation of DUXAP8.

Dual-luciferase reporter assay

Luciferase expression plasmid pmirGLO (Promega) was cloned with DUXAP8 or HIF3A 3′UTR sequence. The recombinant plasmids containing miR-18b-5p binding sites were regarded as wild-type (WT) plasmids: WT-DUXAP8 and WT-HIF3A 3′UTR. Binding sites in DUXAP8 and HIF3A 3′UTR sequences were mutated, followed by construction of mutant-type (MUT) plasmids MUT-DUXAP8 and MUT-HIF3A 3′UTR. HDPCs were collected after WT- and MUT-DUXAP8 or -HIF3A plasmids were co-transfected with miR-18b-5p or miR-NC for 48 hours. Dual-Luciferase Reporter System (Promega) was employed for detection of luciferase activity in each group.

RNA immunoprecipitation assay

Further identification of target interaction was carried out using Imprint RNA Immunoprecipitation Kit (Sigma). HDPCs were incubated with antibody Argonaute 2 (Ago2)-coupled magnetic beads and antibody IgG acted as a negative control. Total RNAs on beads were purified, then RNA levels (DUXAP8, miR-18b-5p, HIF3A) were assayed via RT-qPCR.

RNA pull-down assay

Biotin-coupled miR-18b-5p (bio-miR-18b-5p) and negative control (bio-miR-NC) were acquired from RIBOBIO, and then HDPCs were transfected for 48 hours. Then cells were incubated with streptavidin magnetic beads (Thermo Fisher Scientific) at 4 °C overnight, followed by quantification of DUXAP8 and HIF3A by RT-qPCR.

Statistical analysis

Data were collected after experiments were repeated 3 times, and then data were revealed as mean ± standard deviation (SD). Linear relationships were determined via Pearson correlation coefficient. Data analysis was carried out through SPSS 22.0 (SPSS Inc.), and difference comparison was performed using Student t test or analysis of variance followed by Tukey test. Difference was significant if $P < .05$, statistically.

Results

DUXAP8 was upregulated and miR-18b-5p was downregulated in pulpitis

Viable cells were detected after treatment with LPS in HDPCs. CCK-8 data showed that cell viability was reduced in 0.5-μg/mL, 1-μg/mL, and 2-μg/mL LPS groups relative to the 0-μg/mL LPS group (Supplementary Figure 1A). Further, 1 μg/mL was used as a final concentration of LPS in subsequent assays. Then 1 μg/mL LPS was found to reduce cell proliferation but evoke cell apoptosis, inflammatory response, and oxidative stress in HDPCs (Supplementary Figure 1B). This evidence identified that LPS induced cell injury in HDPCs. Expression detection was performed using RT-qPCR. Compared to normal tissues, DUXAP8 was highly expressed in pulpitis tissues with significant change (Figure 1A). DUXAP8 upregulation was detected in HDPCs with 0.5-μg/mL, 1-μg/mL, or 2-μg/mL LPS treatment in contrast with the control group (Figure 1B). Localisation analysis indicated that DUXAP8 was enriched in cytoplasm, with U6 and GAPDH as nuclear and cytoplasmic controls (Figure 1C). Additionally, miR-18b-5p level was reduced in pulpitis samples relative to controls (Figure 1D), and DUXAP8 was negatively associated with miR-18b-5p ($r = -0.8278$, $P < .001$) in pulpitis tissues (Figure 1E). Also, the obvious downregulation of miR-18b-5p was validated in LPS-treated HDPCs (Figure 1F). The above data revealed abnormal expression levels of DUXAP8 and miR-18b-5p in pulpitis.

DUXAP8 inhibition attenuated cell injury in LPS-treated HDPCs

Transfection with siRNA was used for expression knockdown, and RT-qPCR demonstrated that DUXAP8 level was significantly downregulated in the LPS+DUXAP8 group compared with the LPS+si-NC group (Figure 2A). Cell viability reduction (Figure 2B), proliferation suppression (Figure 2C), and apoptosis acceleration (Figure 2D–E) caused by LPS were evidently alleviated by si-DUXAP8 in HDPCs. Furthermore, CyclinD1 and Bax protein levels affirmed the mitigation of si-DUXAP8 for LPS-induced effects on proliferation and apoptosis (Figure 2F). IL-6 and IL-1β concentrations were reduced after knockdown of DUXAP8 in LPS-treated HDPCs (Figure 2G–H). SOD activity (Figure 3I) and MDA level (Figure 2J) showed that LPS-aroused oxidative stress was relieved by DUXAP8 downregulation. In addition, si-DUXAP8 attenuated ROS level and Fe2+ level in LPS-treated HDPCs (Supplementary Figure 2). DUXAP8 knockdown inhibited oxidative stress and ferroptosis in LPS-induced pulpitis. The upregulation of DUXAP8 aggravated LPS-induced inhibition of cell growth and promotion of apoptosis, inflammation, and oxidative stress (Supplementary Figure 3). Thus, DUXAP8 contributed to cell injury in LPS-induced pulpitis.

DUXAP8 regulated cell injury in LPS-treated HDPCs via targeting miR-18b-5p

Starbase prediction and reverted assays suggested that DUXAP8 directly interacted with miR-18b-5p (Supplementary Figure 4). LPS-treated HDPCs were transfected with

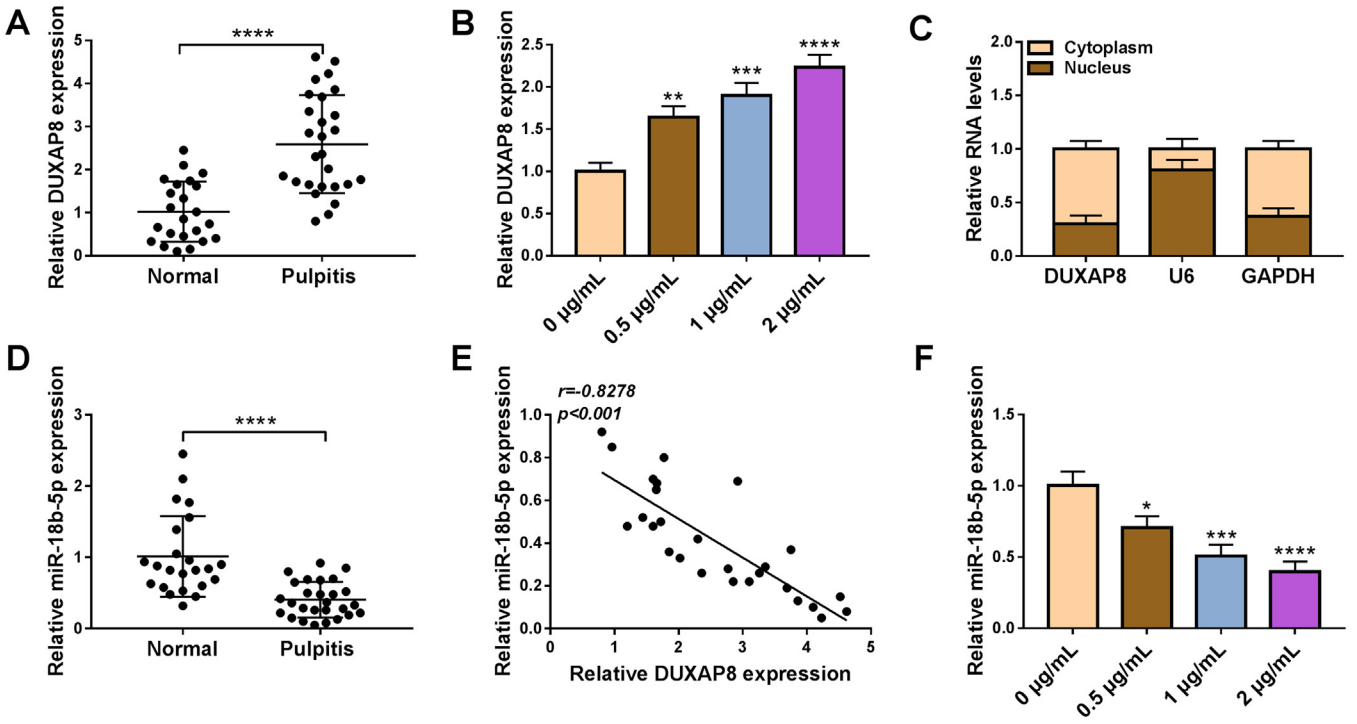

Fig. 1 – Double homeobox A pseudogene 8 (DUXAP8) was upregulated and microRNA-18b-5p (miR-18b-5p) was downregulated in pulpitis. DUXAP8 expression was determined through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in pulpitis tissues and normal controls (A) as well as in lipopolysaccharide (LPS)-treated human dental pulp cells (HDPCs) and control cells (B). C, DUXAP8, U6, and glyceraldehyde-phosphate dehydrogenase (GAPDH) levels were analysed by RT-qPCR in nucleus and cytoplasm. D, RT-qPCR was performed for miR-18b-5p quantification in pulpitis and normal tissues. E, The relationship between DUXAP8 and miR-18b-5p was analysed using Pearson correlation coefficient. F, The miR-18b-5p level was detected via RT-qPCR after LPS treatment in HDPCs. Experiments were repeated 3 times with 3 parallels, and data are expressed as mean ± SD. *P < .05; **P < .01; ***P < .001; ****P < .0001.

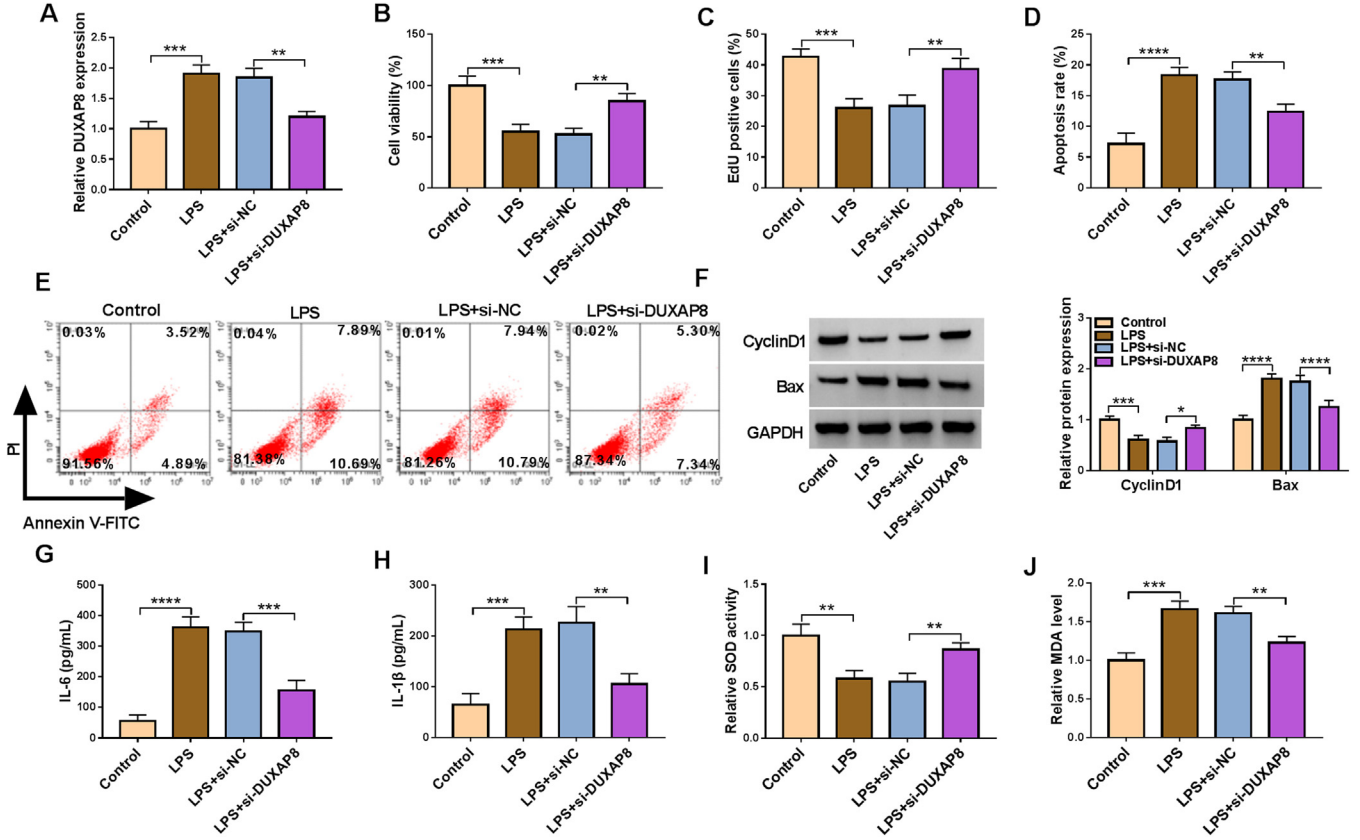

Fig. 2 – Double homeobox A pseudogene 8 (DUXAP8) inhibition attenuated cell injury in lipopolysaccharide (LPS)-treated human dental pulp cells (HDPCs). HDPCs were divided into control, LPS, LPS+si-NC, and LPS+si-DUXAP8 groups. A, Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was applied to determine DUXAP8 level. B, Cell counting kit-8 assay was used for cell viability analysis. C, Ethynyl-2′-deoxyuridine (EdU)-positive cells were measured using EdU assay. D–E, Cell apoptosis rate was examined via flow cytometry. F, CyclinD1 and Bax protein detection was performed by western blot. G–H, Inflammatory cytokines were detected by enzyme-linked immunosorbent assay. Oxidative stress was evaluated by superoxide dismutase activity (I) and malondialdehyde level (J). Experiments were repeated 3 times with 3 parallels, and data are expressed as mean ± SD. *P < .05; **P < .01; ***P < .001; ****P < .0001.

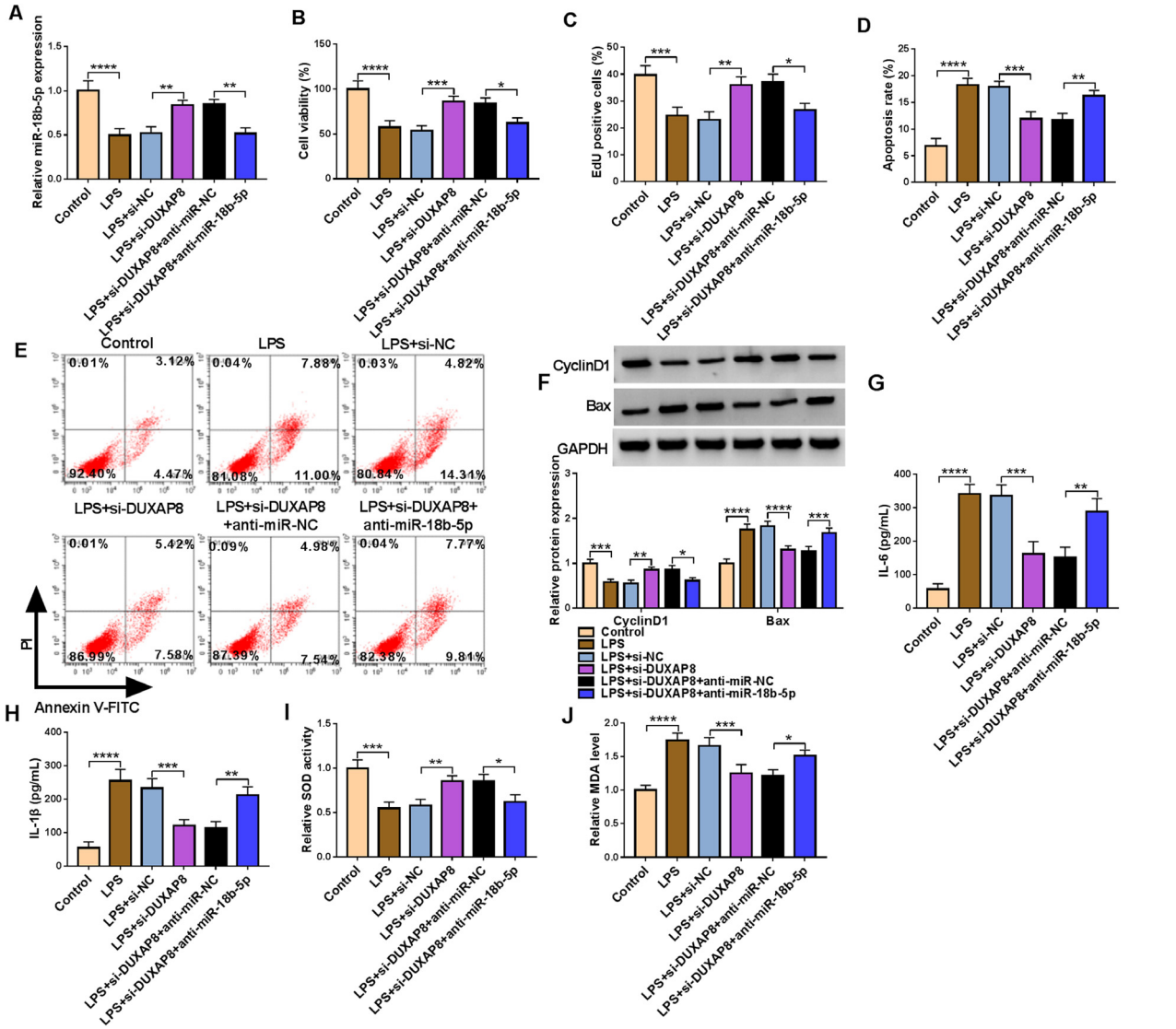

Fig. 3 – Double homeobox A pseudogene 8 (DUXAP8) regulated cell injury in lipopolysaccharide (LPS)-treated human dental pulp cells (HDPCs) via targeting microRNA-18b-5p (miR-18b-5p). HDPCs were treated with LPS, LPS+si-DUXAP8, LPS+si-DUXAP8+anti-miR-18b-5p, and there were corresponding control groups. A, The expression of miR-18b-5p was examined via reverse transcription-quantitative polymerase chain reaction. B, Cell counting kit-8 assay was performed to determine cell viability. C, EdU assay was performed for assessment of proliferation. D–E, Flow cytometry was applied to detect cell apoptosis. F, Western blot was used for protein detection of CyclinD1 and Bax. G–H, Enzyme-linked immunosorbent assay was used for examination of inflammatory cytokines. Superoxide dismutase activity (I) and malondialdehyde level (J) were measured by commercial kits. Experiments were repeated 3 times with 3 parallels, and data are expressed as mean ± SD. *P < .05; **P < .01; ***P < .001; ****P < .0001.

si-DUXAP8, si-DUXAP8+anti-miR-18b-5p, and control groups. The miR-18b-5p expression was downregulated in LPS+si-DUXAP8+anti-miR-18b-5p group relative to the LPS+si-DUXAP8+anti-miR-NC group (Figure 3A). The alleviative effects of si-DUXAP8 on cell viability (Figure 3B), proliferation (Figure 3C), and apoptosis (Figure 3D–E) were all abolished after miR-18b-5p level inhibition in HDPCs. CyclinD1 upregulation and Bax downregulation by si-DUXAP8 were also counteracted by anti-miR-18b-5p in LPS-treated HDPCs (Figure 3F). In addition, miR-18b-5p inhibitor reversed suppressive regulation of si-DUXAP8 in inflammatory reaction (Figure 3G–H) and oxidative stress (Figure 3I–J). The function of DUXAP8 in LPS-induced cell injury was related to negative regulation of miR-18b-5p.

Overexpression of miR-18b-5p protected from LPS-induced cell injury via downregulating HIF3A

Starbase prediction and rescued assays showed that HIF3A was a downstream gene of miR-18b-5p (Supplementary Figure 5). Then, miR-18b-5p function was explored in LPS-treated HDPCs. Transfection of miR-18b-5p reduced HIF3A

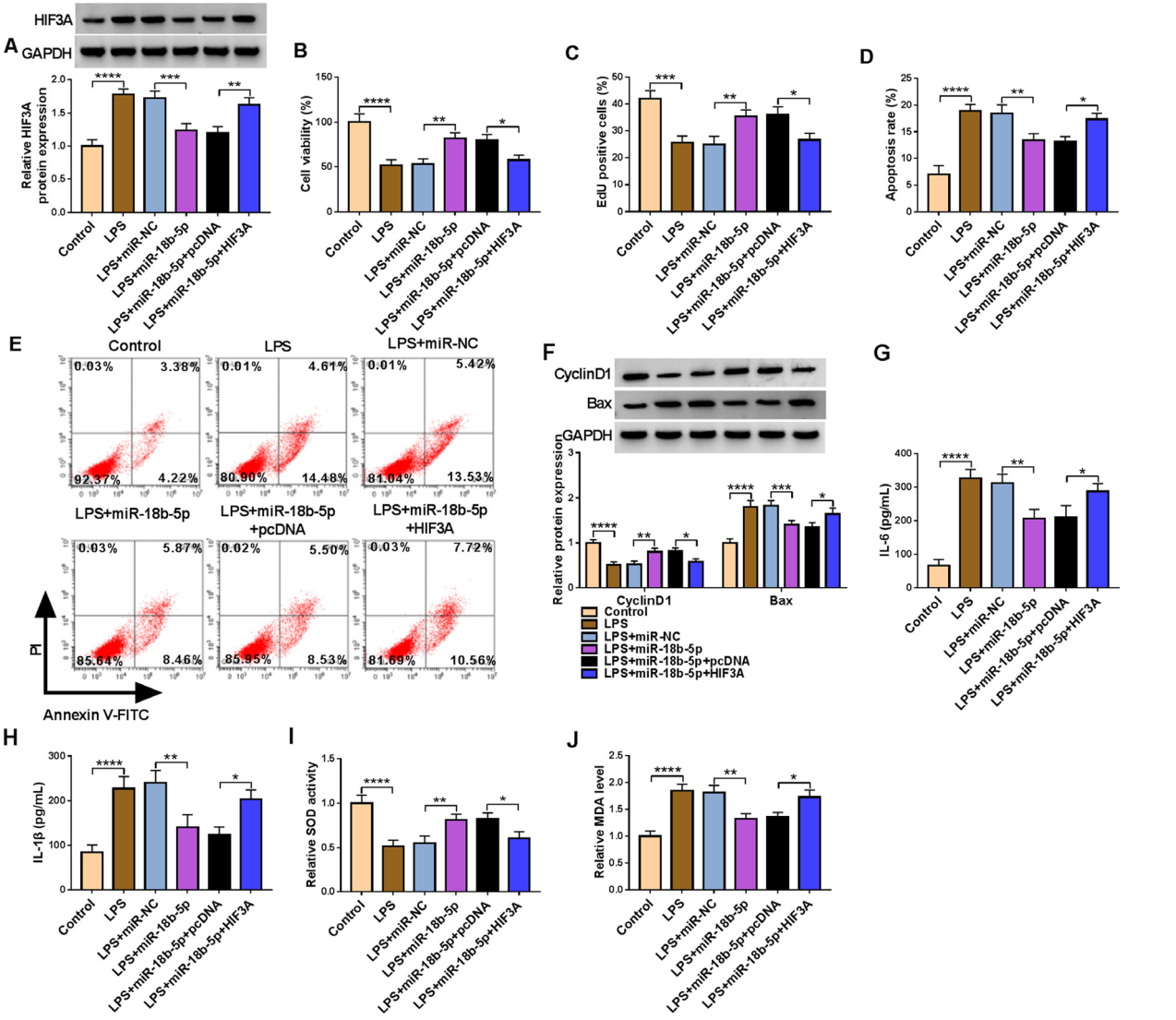

Fig. 4 – Overexpression of microRNA-18b-5p (miR-18b-5p) protected from lipopolysaccharide (LPS)-induced cell injury via downregulating hypoxia-inducible factor 3A (HIF3A). Human dental pulp cells (HDPCs) were treated with LPS, LPS+miR-18b-5p, and LPS+miR-18b-5p+HIF3A, and there were matched control groups. A, HIF3A protein detection was carried out by western blot. B, Cell viability examination was conducted via cell counting kit-8 assay. C, Cell proliferation determination was performed using EdU assay. D–E, Cell apoptosis was evaluated through flow cytometry. F, Protein levels of CyclinD1 and Bax were detected using western blot. G–H, Inflammatory response was assessed via enzyme-linked immunosorbent assay. Superoxide dismutase activity (I) and malondialdehyde level (J) were used to analyse oxidative stress. Experiments were repeated 3 times with 3 parallels, and data are expressed as mean ± SD. *P < .05; **P < .01; ***P < .001; ****P < .0001.

protein expression, which was recovered by introduction of HIF3A in LPS-treated HDPCs (Figure 4A). LPS-induced viability reduction (Figure 4B), proliferation inhibition (Figure 4C), and apoptosis promotion (Figure 4D–E) were suppressed by miR-18b-5p, whereas these effects were partly offset by upregulation of HIF3A. CyclinD1 protein upregulation and Bax downregulation by miR-18b-5p were abated after HIF3A was overexpressed in LPS-treated HDPCs (Figure 4F). LPS-released IL-6 and IL-β were relieved by miR-18b-5p, and then HIF3A transfection abolished this regulation (Figure 4G–H). Additionally, HIF3A overexpression counterbalanced miR-18b-5p–mediated SOD activity increase (Figure 4I) and MDA level suppression (Figure 4J) in LPS-exposed HDPCs. Altogether, miR-18b-5p ameliorated LPS-induced cell injury through targeting HIF3A. Moreover, DUXAP8 was identified to upregulate HIF3A via targeting miR-18b-5p (Supplementary Figure 6).

Discussion

DUXAP8 was validated to aggravate LPS-induced cell injury in pulpitis via miR-18b-5p–mediated HIF3A upregulation. The DUXAP8/miR-18b-5p/HIF3A network was implicated in pulpitis progression.

LPS is commonly used for stimulation of inflammatory response in pulpitis.[16] Our data showed that LPS treatment led to cell apoptosis, inflammation, and oxidative damages in HDPCs, revealing that the LPS-induced pulpitis cell model was successfully constructed. Previous studies have indicated that lncRNA dysregulation is associated with pathogenic development of pulpitis.[17] Liu et al[18] stated that MEG3 downregulation prevented the secretion of inflammatory cytokines and facilitated odontogenic differentiation of HDPCs. Wang et al[19] affirmed that NUTM2A-AS1 overexpression contributed to apoptosis and inflammation in LPS-disposed HDPCs in pulpitis. DUXAP8 upregulation was confirmed in pulpitis samples, and LPS-induced damage of HDPCs has been alleviated with DUXAP8 knockdown. The results of the cell model demonstrated that DUXAP8 accelerated the progression of pulpitis.

LncRNAs have sponge function for miRNAs in various kinds of dental diseases. For instance, pPDLSC osteogenesis impairment-related lncRNA (POIR) enhanced osteogenic differentiation in periodontitis through interacting with miR-182 to inhibit miR-182 level.[20] Overexpression of X-inactive specific transcript promoted periodontitis progression via absorbing miR-214–3p.[21] Also, NUTM2A-AS1 served as a molecular sponge for miRNAlet-7c-5p in pulpitis.[19] In this work, DUXAP8 exhibited target relation with miR-18b-5p, and miR-18b-5p downregulation reversed si-DUXAP8–mediated cell injury inhibition in LPS-treated HDPCs. Significantly, DUXAP8 was involved in pulpitis progression through sequestering the level of miR-18b-5p.

In addition to lncRNAs, miRNAs with dysregulated levels are also essential regulators in pulpitis.[22] Upregulation of miR-506 protected against LPS-induced inflammation and apoptosis via reducing SIRT1 expression,[23] and miR-21 played an anti-inflammatory role in LPS-treated HDPCs by targeting TRAF6 or PDCD4.[24] Additionally, the miR-223/NLRP3 axis regulated an LPS-caused inflammatory reaction in pulpitis.[25] The current data manifested that miR-18b-5p level was reduced in pulpitis tissues, and HIF3A acted as a miR-18b-5p target. Moreover, LPS-evoked cell injury was partly mitigated by miR-18b-5p via resulting in downregulation of HIF3A. The pathogenic role of HIF3A was also verified in pulpitis.

More importantly, lncRNAs are known as competing endogenous RNAs by binding to miRNAs to regulate gene expression in dental diseases.[9,26] HIF3A expression was shown to be suppressed by siRNA of DUXAP8, and miR-18b-5p inhibitor abolished this effect in LPS-treated HDPCs. Therefore, DUXAP8 regulated the level of HIF3A through completely sponging miR-18b-5p in LPS-mediated pulpitis progression.

In conclusion, LPS upregulated DUXAP8 to regulate the miR-18b-5p/HIF3A axis to inhibit cell proliferation but induce apoptosis, inflammation, and oxidative stress (Figure 5). This study provided a novel molecular mechanism (DUXAP8/miR-18b-5p/HIF3A) in pulpitis progression that may be useful for further research of pulpitis.

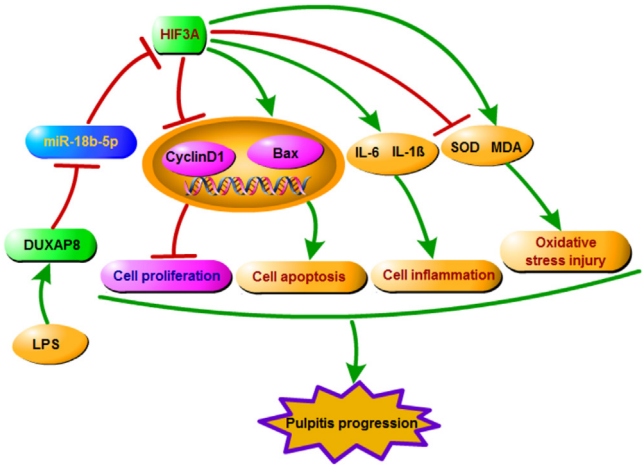

Fig. 5 – Lipopolysaccharide upregulated double homeobox A pseudogene 8 to regulate the microRNA-18b-5p/hypoxia-inducible factor 3A axis to inhibit cell proliferation but induce apoptosis, inflammation, and oxidative stress.

Conflict of interest

None disclosed.

Funding statement

EPO/PCL/COL Electrospun Fibrous Scaffold Releasing Erythropoietin Induces Multi-directional Differentiation of hDPSCs No: Zhejiang Health Science and technology plan (2022KY403).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2022.11.011.

REFERENCES

1. Khorasani MMY, Hassanshahi G, Brodzikowska A, et al. Role(s) of cytokines in pulpitis: latest evidence and therapeutic approaches. Cytokine 2020;126:154896.
2. Fedorowicz Z, van Zuuren EJ, Farman AG, et al. Antibiotic use for irreversible pulpitis. Cochrane Database Syst Rev 2013(12): CD004969.
3. Zhou Y, Zheng L, Zhou X, et al. Dental mesenchymal stem cells in inflamed microenvironment: potentials and challenges for regeneration. Curr Stem Cell Res Ther 2015;10 (5):412–21.
4. Hui T, Wang C, Chen D, et al. Epigenetic regulation in dental pulp inflammation. Oral Dis 2017;23(1):22–8.
5. Zhang K, Qiu W, Wu B, et al. Long noncoding RNAs are novel players in oral inflammatory disorders, potentially premalignant oral epithelial lesions and oral squamous cell carcinoma (weview). Int J Mol Med 2020;46(2):535–45.
6. Naqvi AR, Shango J, Seal A, et al. Herpesviruses and microRNAs: new pathogenesis factors in oral infection and disease? Front Immunol 2018;9:2099.
7. Zhang X, Hong R, Chen W, et al. The role of long noncoding RNA in major human disease. Bioorg Chem 2019;92:103214.
8. Li M, Duan L, Li Y, et al. Long noncoding RNA/circular noncoding RNA-miRNA-mRNA axes in cardiovascular diseases. Life Sci 2019;233:116440.

9. Lei F, Zhang H, Xie X. Comprehensive analysis of an lncRNA-miRNA-mRNA competing endogenous RNA network in pulpitis. PeerJ 2019;7:e7135.
10. Du A, Zhao S, Wan L, et al. MicroRNA expression profile of human periodontal ligament cells under the influence of *Porphyromonas gingivalis* LPS. J Cell Mol Med 2016;20(7):1329–38.
11. Chen Q, Cao M, Ge H. Knockdown of MALAT1 inhibits the progression of chronic periodontitis via targeting miR-769-5p/HIF3A axis. Biomed Res Int 2021;2021:8899863.
12. Liang C, Li W, Huang Q, et al. CircFKBP5 suppresses apoptosis and inflammation and promotes osteogenic differentiation. Int Dent J 2022 Online ahead of print. doi: 10.1016/j.identj.2022.08.001.
13. Xia L, Wang J, Qi Y, et al. Long non-coding RNA PVT1 is involved in the pathological mechanism of pulpitis by regulating miR-128-3p. Oral Health Prev Dent 2022;20(1):263–70.
14. Tian LJ, Wu YP, Wang D, et al. Upregulation of long noncoding RNA (lncRNA) X-inactive specific transcript (XIST) is associated with cisplatin resistance in non-small cell lung cancer (NSCLC) by downregulating microRNA-144-3p. Med Sci Monit 2019;25:8095–104.
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta C(T)) Method. Methods 2001;25(4):402–8.
16. Takimoto K, Kawashima N, Suzuki N, et al. Down-regulation of inflammatory mediator synthesis and infiltration of inflammatory cells by MMP-3 in experimentally induced rat pulpitis. J Endod 2014;40(9):1404–9.
17. Huang X, Chen K. Differential expression of long noncoding RNAs in normal and inflamed human dental pulp. J Endod 2018;44(1):62–72.
18. Liu M, Chen L, Wu J, et al. Long noncoding RNA MEG3 expressed in human dental pulp regulates LPS-induced inflammation and odontogenic differentiation in pulpitis. Exp Cell Res 2021;400(2):112495.
19. Wang X, Sun H, Hu Z, et al. NUTM2A-AS1 silencing alleviates LPS-induced apoptosis and inflammation in dental pulp cells through targeting let-7c-5p/HMGB1 axis. Int Immunopharmacol 2021;96:107497.
20. Wang L, Wu F, Song Y, et al. Long noncoding RNA related to periodontitis interacts with miR-182 to upregulate osteogenic differentiation in periodontal mesenchymal stem cells of periodontitis patients. Cell Death Dis 2016;7(8):e2327.
21. Feng Y, Wan P, Yin L. Long noncoding RNA X-inactive specific transcript (XIST) promotes osteogenic differentiation of periodontal ligament stem cells by sponging microRNA-214-3p. Med Sci Monit 2020;26:e918932.
22. Yi Q, Liu O, Yan F, et al. Analysis of senescence-related differentiation potentials and gene expression profiles in human dental pulp stem cells. Cells Tissues Organs 2017;203(1):1–11.
23. Wang J, Du Y, Deng J, et al. MicroRNA-506 is involved in regulation of the occurrence of lipopolysaccharides (LPS)-induced pulpitis by sirtuin 1 (SIRT1). Med Sci Monit 2019;25:10008–15.
24. Nara K, Kawashima N, Noda S, et al. Anti-inflammatory roles of microRNA 21 in lipopolysaccharide-stimulated human dental pulp cells. J Cell Physiol 2019;234(11):21331–41.
25. Wang D, Sun S, Xue Y, et al. MicroRNA-223 negatively regulates LPS-induced inflammatory responses by targeting NLRP3 in human dental pulp fibroblasts. Int Endod J 2021;54(2):241–54.
26. Li S, Liu X, Li H, et al. Integrated analysis of long noncoding RNA-associated competing endogenous RNA network in periodontitis. J Periodontal Res 2018;53(4):495–505.