Circ_0138960 contributes to lipopolysaccharide-induced periodontal ligament cell dysfunction

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Abstract

Background: Periodontitis is a common oral inflammatory disease, and lipopolysaccharide (LPS) is a key risk factor in periodontitis pathology. Here, we used LPS-induced periodontal ligament cells (PDLCs) to explore the molecular mechanism of periodontitis.

Methods: Cell viability, proliferation, and apoptosis were analyzed by Cell Counting Kit-8, 5-ethynyl-20-deoxyuridine (EDU), and flow cytometry assays, respectively. Apart from that, their targeting relationship was validated using dual-luciferase reporter and RNA-pull down.

Results: Circular RNA_0138960 (circ_0138960) was notably upregulated in periodontitis sufferers (p < .001) and LPS-disposed PDLCs (p < .05). LPS exposure dampened PDLC proliferation, and promoted apoptosis and inflammation (p < .05). Circ_0138960 acted as a microRNA sponge for miR-518a-5p to affect histone deacetylase 6 (HDAC6) expression. Circ_0138960 absence-mediated protective effects in LPS-induced PDLCs were largely abrogated via silencing miR-518a-5p or HDAC6 overexpression (p < .05).

Conclusion: Circ_0138960 promoted LPS-induced dysfunction in PDLCs by targeting miR-518a-5p/HDAC6 axis, which provided novel potential therapeutic targets for periodontitis.

KEYWORDS
circ_0138960, HDAC6, lipopolysaccharide, miR-518a-5p, periodontal ligament cells, periodontitis

1 | INTRODUCTION

As a progressive disease of periodontal tissue, Periodontitis is ascribed to the accumulation of subgingival pathogens. The pathology of periodontitis is associated with environmental factors (bad living habits and smoking) and genetic factors. Current research revealed that clarification on the mechanisms involving periodontitis is of great significance for the prevention of periodontitis. Lipopolysaccharide (LPS) is highly toxic to the periodontal ligament and can induce cellular dysfunction, thus contributing to periodontitis progression. Hence, LPS-induced periodontal ligament cells (PDLCs) are generally utilized as periodontitis cell models in vitro.

Circular RNAs (circRNAs) with conserved stable loop structures highlighted their potential as biomarkers for human diseases. CircRNAs play important regulatory roles in periodontitis pathology.
downregulated in patients with periodontal disease, and circ_0081572 overexpression attenuates LPS-induced dysfunction in PDLCs. Here, we focus on an abnormally upregulated circRNA, circ_0138960, in patients with periodontal disease, but its biological role and mechanism in periodontitis pathology remain to be clarified.

Recent reports have described that circRNAs act as microRNA (miRNA) sponges to indirectly modulate gene expression, thus affecting cell behaviors. This study first investigated the role of circ_0138960 in LPS-triggered PDLC dysfunction. Subsequently, we explored the downstream miRNA/Messenger RNA (mRNA) axis of circ_0138960 to uncover its working mechanism in periodontitis pathology.

2 | MATERIALS AND METHODS

2.1 | Gingival tissues

After being signed written informed consent, human gingival tissues were collected from patients with periodontal disease (n = 37) or from normal controls (n = 25) who received gingivectomy during orthodontic treatment at Dongying Shengli Oilfield Central Hospital. Diagnostic criteria of periodontitis were presented as follows: (1) redness and swelling of the gingiva on the surface of the periodontal pocket or bleeding after probing; (2) probe depth > 3 mm and attachment loss > 1 mm; (3) X-ray showed horizontal or vertical resorption of alveolar bone. Exclusion criteria included patients with systemic diseases and antibiotic intake within 1 month before the surgery. Approval to perform this project was acquired from the Ethics Committee of Dongying Shengli Oilfield Central Hospital. NO. S2020623. Table 1 displayed the clinico-pathologic characteristics of periodontitis sufferers.

2.2 | Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

RNA samples were isolated from gingival tissues and PDLCs with Trizol reagent (Invitrogen). RNA was reversely transcribed to complementary DNA (cDNA) using an M-MLV kit (Invitrogen) and miRNA cDNA Synthesis Kit (GeneCopoeia). Then, qPCR was carried out using the Power SYBR-Green PCR master mix (Applied Biosystems). After normalization with β-actin or U6, the obtained results were processed using the $2^{-\Delta\Delta C_t}$. All primers were shown in Table 2.

2.3 | Cell isolation, culture, and identification

Teeth were collected from premolar teeth extracted from healthy patients (mean age: 14) for orthodontic

| Characteristics     | Number | circ_0138960 expression | Odds ratio | p Value |
|---------------------|--------|-------------------------|------------|---------|
|                     |        | Low (N = 18) | High (N = 19) |         |
| **Age (years)**     |        |            |            |         |
| <65                 | 20 (54%)| 10          | 10         | .8584   |
| ≥65                 | 17 (46%)| 8           | 9          | .6301   |
| **Sex**             |        |            |            |         |
| Male                | 16 (43%)| 9           | 8          | .6301   |
| Female              | 21 (57%)| 9           | 11         |         |
| **Order of severity**|       |            |            |         |
| Slight + medium     | 24 (65%)| 16          | 8          | .0029   |
| Severe              | 13 (35%)| 2           | 11         |         |
| **Grade of periodontitis** |     |            |            |         |
| Level 0 + 1         | 24 (65%)| 15          | 9          | .0220   |
| Level 2             | 13 (35%)| 3           | 10         |         |

Note: Level 0, individuals with a healthy periodontium and up to one proximal site with loss of attachment ≥ 3 mm; Level 1, presence of proximal attachment and loss ≥ 3 mm in ≥2 nonadjacent teeth; Level 2, presence of proximal attachment loss ≥ 5 mm in ≥30% of teeth.
treatment. Pretreated extracted teeth need to be disinfected with 1% iodine and 72% alcohol. After extraction, these samples from the middle third of the periodontal membrane root were washed and managed with α-MEM. After being acquired from the root surface, PDL tissues at 37°C were incubated with the medium plus 1% penicillin/streptomycin. Meanwhile, the fourth to seventh generations of PDLCs were used for the following research. PDLCs were identified using the avidin-biotin complex immunohistochemistry.

2.4 | LPS treatment

PDLCs were stimulated with Porphyromonas gingivalis LPS (Sigma) at the dose of 2.5, 5, or 10 ng/μl for 12 h. Finally, PDLCs were treated with 10 ng/μl LPS for 12 h to induce the periodontitis-like injury, which was used for further analysis.

2.5 | Cell Counting Kit-8 (CCK8) assay

2 × 10^3 PDLCs were incubated with CCK8 solution (Sigma) for 4 h. The optical density at 450 nm was examined under a microplate reader (NYW-96M; Nuoyawei Biotech).

2.6 | 5-ethyl-20-deoxyuridine (EDU) assay

PDLCs were incubated with 100 μl 50 μM EDU reagent (Ribobio) for 2 h at 37°C. Then, cells were immobilized with 4% paraformaldehyde for 30 min at 25°C and incubated with glycine for 4 h at 37°C. Subsequently, PDLCs were mixed with Apollo reaction cocktail and Triton X-100 solution for 20 min. Then, the nucleus of PDLCs was stained with DAPI (Sigma) at 37°C. The fluorescence photographs of PDLCs were captured under a fluorescence microscope (Olympus).

2.7 | Western blot assay

PDLCs were disrupted with RIPA reagent (Beyotime), and analyzed using a BCA kit (Thermo Fisher Scientific). Proteins were denatured in a boiling water bath for 10 min. After being subjected to 10% separating gel, samples were shifted to PVDF membranes, which then were mixed with primary antibodies (Abcam) at 4°C overnight: anti-CDK6 (ab124821; 1:8000), anti-proliferating cell nuclear antigen (PCNA) (ab29; 1:5000), anti-Cyclin D1 (ab16663; 1:3000), anti-Bax (ab32503; 1:8000), anti-Cleaved-casp3 (ab32042; 1:5000), anti-Bcl2 (ab32124; 1:3000), anti-IL-1β (ab216995; 1:5000), anti-IL-18 (ab234091; 1:3000), anti-TNF-α (ab133493; 1:2000), and anti-β-actin (ab8226; 1:20000). The visualization of signals was implemented according to an ECL Kit, after secondary antibody (ab6721/ab6789; 1:5000) incubation.

2.8 | Flow cytometry

Annexin-V-FITC/PI dye liquor was prepared using 500 μl binding buffer, 10 μl Annexin-V-FITC, and 10 μl PI (BD Biosciences). PDLCs were incubated with the Annexin-V-FITC/PI dye liquor, away from light. Fifteen min later, the apoptotic rate was analyzed on the FC-500 flow cytometer.
2.9 | Cell transfection

In this study, PDLCs were further cultured into 96-well plates overnight to reach 70%—0% confluence. Meanwhile, GenePharma and Ribobio offer circ_0138960-specific siRNA (si-circ_0138960), siRNA of HDAC6 (si-HDAC6), miR-518a-5p mimics/inhibitor (miR-518a-5p/anti-miR-518a-5p), HDAC6 plasmid (HDAC6), and controls (si-NC, miR-NC, anti-miR-NC, and plasmid cloning DNA[pcDNA]). Then, Lipofectamine™ 3000 reagent (Invitrogen) was utilized for transient transfection.

2.10 | Establishment of circRNA/miRNA/mRNA axis

The interacted miRNAs of circ_0138960 and the interacted mRNAs of miR-518a-5p were predicted by Circinteractome (https://circinteractome.irp.nia.nih.gov) and targetscan (http://www.targetscan.org/vert_71) databases, respectively. All possible miRNA targets of circ_0138960 predicted by Circinteractome database were shown in Table 3.

2.11 | Dual-luciferase reporter assay

A partial sequence of circ_0138960 or HDAC6 3’ untranslated region (UTR) was amplified by qPCR. Meanwhile, their mutant sequence was synthesized via site-directed mutation. The constructed reporter plasmids were termed circ_0138960WT/MUT and HDAC6-3’UTRWT/MUT. PDLCs were cotransfected with small RNAs and constructed plasmids, followed by analysis according to Dual-Luciferase Reporter Assay kit (Promega) after transfection for 24 h.

2.12 | RNA-pull down assay

In short, Biotin-labeled miR-518a-5p probe (Bio-miR-518a-5p) and Bio-miR-NC were constructed by GenePharma. Cell lysates were simultaneously incubated with the probe and streptavidin agarose magnetic beads (Invitrogen). RNA enrichment was analyzed by RT-qPCR.

Furthermore, cell extracts were simultaneously incubated with Biotin-labeled circ_0138960 probe (circ_0138960 probe) or oligo probe (GenePharma) and streptavidin agarose magnetic beads (Invitrogen). miR-1205, miR-1299, miR-518a-5p, and miR-527 expressions were examined using RT-qPCR.

2.13 | Statistical analysis

The mean between groups was compared using Student’s t-test or one-way analysis of variance followed by Tukey’s test. GraphPad Prism 7.0 software (GraphPad) was used for statistical analysis. p < .05 was regarded as the threshold of significance.

3 | RESULTS

3.1 | Circ_0138960 expression is elevated in patients with periodontal disease

Circ_0138960 (366 nt) is derived from the back-splicing of the gene GDA (Figure 1A). Meanwhile, our data displayed that circ_0138960 was only amplified using divergent primers in the cDNA group, rather than gDNA (Figure 1B). The expression of circ_0138960 in periodontitis was analyzed. Circ_0138960 was notably upregulated in patients with periodontal disease (n = 37) versus normal controls (n = 25) (Figure 1C, p < .001). To
identify the association of circ_0138960 expression with the clinicopathologic characteristics, the 37 patients with periodontitis disease were then classified in Table 1. Result displayed that circ_0138960 expression was associated with Order of severity and Grade of periodontitis ($p < .05$). Overall, circ_0138960 was associated with periodontitis pathology.

3.2 LPS treatment suppresses PDLC proliferation and induces apoptosis and inflammation

Furthermore, the periodontitis cell model was established by stimulating PDLCs with LPS at the dose of 2.5, 5, or 10 ng/μl for 12 h. We found that PDLC cell viability was reduced upon LPS treatment in a dose-dependent manner (Figure 2A). LPS exposure gradually diminished PDLC proliferation, evidenced by a reduced percentage of EDU+ cells (Figure 2B). Three proliferation-associated markers (CDK6, PCNA, and Cyclin D1) were downregulated in PDLCs by LPS stimulation in a concentration-dependent way (Figure 2C–F). LPS exposure induced the apoptosis of PDLCs in a dose-dependent manner (Figure 2G). Proapoptotic proteins (Bax and Cleaved-casp3) were elevated via LPS stimulation, while Bcl2 (an antiapoptotic marker) exhibited an opposite effect (Figure 2H–K). LPS gradually induced the release of proinflammatory cytokines (IL-1β, IL-18, and TNF-α) (Figure 2L–O). Overall, LPS suppressed PDLC proliferation and boosted cell apoptosis and inflammation in a dose-dependent manner.

3.3 LPS-induced dysfunction in PDLCs can be largely reversed by silencing circ_0138960

LPS improved circ_0138960 content in PDLCs in a dose-dependent manner (Figure 3A). To analyze the biological role of circ_0138960 in LPS-induced PDLCs, we performed loss-of-function experiments. LPS-induced circ_0138960 enhancement in PDLCs was reversed via
si-circ_0138960 introduction (Figure 3B). LPS exposure dampened PDLC proliferative ability, which was rescued via si-circ_0138960 (Figure 3C–H). LPS-triggered PDLC apoptosis was largely offset via silencing circ_0138960 (Figure 3I–M). Beyond that, LPS-mediated proinflammatory cytokines (IL-1β, IL-18, and TNF-α) increasing were also effectively counteracted via circ_0138960 absence (Figure 3N–Q). Overall, circ_0138960 interference protected PDLCs from LPS-induced dysfunction.

3.4 | Circ_0138960 acts as a molecular sponge for miR-518a-5p

It is widely accepted that circRNAs can sponge miRNAs to regulate cell physiological and pathological processes.12 Using the Circinteractome database, all possible miRNA targets of circ_0138960 were predicted (Table 3), and four miRNAs with a high binding score of 99 were selected, including miR-1205, miR-1299, miR-518a-5p,
and miR-527. Subsequently, RNA-pull down assay showed that miR-518a-5p and miR-527 could be pulled down by the circ_0138960 probe, especially miR-518a-5p (Supporting Information: Figure 1A). As displayed in Figure 4A, putative binding sites were found between circ_0138960 and miR-518a-5p. We found that miR-518a-5p expression was prominently decreased in patients with periodontal disease (Figure 4B). LPS exposure reduced miR-518a-5p expression in PDLCs in a dose-dependent manner (Figure 4C). Dual-luciferase reporter assay exhibited that circ_0138960WT luciferase activity was conspicuously dwindled via miR-518a-5p overexpression, while the mutant group (circ_0138960MUT) remained unchanged (Figure 4D). In parallel, circ_0138960 was pulled down when using Bio-miR-518a-5p (Figure 4E). Overall, circ_0138960 sequesters miR-518a-5p in PDLCs.

3.5 | Circ_0138960/miR-518a-5p regulated LPS-induced PDLC injury

Knockdown efficiency of anti-miR-518a-5p in PDLCs was validated (Figure 5A). The silence of miR-518a-5p reduced PDLC viability and suppressed proliferation again (Figure 5B–G). In addition, we found that anti-miR-518a-5p induced apoptosis and proinflammatory cytokines (IL-1β, IL-18, and TNF-α) (Figure 5H–P). These findings together demonstrated that circ_0138960 absence attenuated LPS-induced PDLC dysfunction partly via miR-518a-5p.

3.6 | HDAC6 is a target of miR-518a-5p

We predicted the interacted mRNAs of miR-518a-5p using the targetscan database. Among them, five mRNAs that play regulatory roles in periodontitis pathology, including PTEN, SOCS6, HDAC6, NR2F2, and RORA. We analyzed the expression of these five mRNAs in miR-518a-5p-silenced PDLCs and found that SOCS6 and HDAC6 could be negatively regulated by miR-518a-5p, especially HDAC6 (Supporting Information: Figure 1B). Hence, HDAC6 was selected as the follow-up subject. Their putative binding sequence was exhibited in Figure 6A. HDAC6 expression was upregulated in the gingival tissues of patients with periodontal disease (Figures 6B, C). LPS exposure upregulated the protein level of HDAC6 in PDLCs in a dose-dependent manner (Figure 6D). The luciferase intensity of HDAC6-3’UTRWT was conspicuously reduced via miR-518a-5p overexpression (Figure 6E), rather than mutant group (Figure 6E), indicating their direct interaction. MiR-518a-5p interference augmented HDAC6 protein level in PDLCs (Figure 6F). Circ_0138960 absence reduced HDAC6 content in PDLCs, and anti-miR-518a-5p largely
recovered the phenomenon (Figure 6G), suggesting that circ_0138960 could act as a miR-518a-5p sponge to positively regulate HDAC6 content in PDLCs.

### 3.7 HDAC6 overexpression reverses circ_0138960 interference-mediated impacts in PDLCs upon LPS treatment

We analyzed the biological role of HDAC6 in LPS-induced PDLCs by loss-of-function experiments. Knockdown efficiency of si-HDAC6 in PDLCs was validated (Supporting Information: Figure 2A). LPS-induced PDLC dysfunction was effectively diminished via silencing HDAC6 (Supporting Information: Figure 2B–2Q), indicating that HDAC6 knockdown protected PDLCs from LPS-induced dysfunction.

Western blot assay confirmed the overexpression efficiency of HDAC6 plasmid in PDLCs (Figure 7A). The protective action of cell viability and proliferation of LPS-induced PDLCs caused by circ_0138960 depletion was reversed by the overexpression of HDAC6 (Figure 7B–G). Circ_0138960 silencing suppressed LPS-induced apoptosis in PDLCs, and the addition of HDAC6 plasmid triggered cell apoptosis again (Figure 7H–L). The addition of HDAC6 plasmid also induced the inflammation of PDLCs (Figure 7M–P). Taken together, a protective role of circ_0138960 absence on LPS-induced PDLCs was partly mitigated via HDAC6 downregulating.

### 4 DISCUSSION

Inflammation is considered to be a major factor in periodontitis pathology. Therefore, alleviating inflammation-induced PDLCs injury is important for the treatment of periodontitis. LPS is widely utilized in
inflammation-associated cell models in vitro. In this study, LPS-induced PDLCs were used as a periodontitis cell model in vitro. Our study confirmed that LPS stimulation reduced PDLC proliferation and induced apoptosis and inflammation in a dose-dependent manner. Noncoding RNAs, including circRNAs, are identified as promising targets for inflammation-associated diseases such as periodontitis. Here, we found that circ_0138960 was upregulated in patients with periodontal disease, which was consistent with a
previous study.9 LPS exposure gradually upregulated circ_0138960 content in PDLCs. In addition, we found that circ_0138960 depletion relieved LPS-induced PDLC dysfunction. Accumulating evidence has suggested that circRNAs play important regulatory roles in multiple human diseases by acting as miRNA sponges.23,24 For instance, circ_0003204 restrains endothelial cell growth, migration, and angiogenesis in atherosclerosis by sponging miR-370-3p.25 Serving as a miR-31-5p sponge, circ-BPTF might aggravate bladder cancer development.26 Using bioinformatics analysis, miR-518a-5p appeared as a target of circ_0138960 in PDLCs. miR-518a-5p expression was significantly reduced in patients with periodontal disease. Meanwhile, we uncovered that LPS treatment decreased the miR-518a-5p level in PDLCs in a dose-dependent manner. A previous study reported that lncRNA_01126 contributes to periodontitis pathogenesis by sponging miR-518a-5p,27 indicating that miR-518a-5p played a protective role in periodontitis. We found miR-518a-5p downregulation might reverse circ_0138960 absence-provoked protective action in LPS-stimulated PDLCs, indicating that circ_0138960 silencing protected PDLCs against LPS-caused damage largely via miR-518a-5p.
It is well established that miRNAs can accomplish their biological function via interacting with mRNAs.\(^{28,29}\) Our findings uncovered that miR-518a-5p is directly bound to the 3'UTR of HDAC6 in PDLCs. HDAC6 is a member of the histone deacetylase family, and it regulates multiple cellular processes through its histone deacetylase activity.\(^{30}\) A previous study found that IL-1β elevates the mRNA expression of HDAC6 in periodontal ligament fibroblasts,\(^{16}\) implying its pivotal role in periodontitis pathology. We confirmed that HDAC6 was elevated in periodontitis sufferers and LPS-stimulated PDLCs. In addition, we uncovered circ_0138960 positively modulated HDAC6 content via sponging miR-518a-5p in PDLCs. HDAC6 knockdown protected PDLCs from LPS-induced dysfunction. Beyond that, compensation experiments disclosed that circ_0138960 silencing-induced protective influences in LPS-stimulated PDLCs were largely offset by HDAC6.
accumulation, suggesting that circ_0138960 interference played protective impacts in LPS-stimulated PDLCs largely by downregulating HDAC6.

In summary, our findings confirmed that circ_0138960 contributed to LPS-induced dysfunction in PDLCs by miR-518a-5p/HDAC6 (Figure 8). This new ceRNA mechanism provides novel potential therapeutic targets for periodontitis. However, the current research is limited by the small sample size and the lack of animal model experiments. Hence, our future research will try to include more patients and construct periodontitis animal models to further verify our conclusions. Furthermore, the working mechanism of HDAC6 in periodontitis pathology still needs to be explored.

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REFERENCES
1. Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017;75(1):7-23. doi:10.1007/prd.12221
2. Bartold PM. Lifestyle and periodontitis: the emergence of personalized periodontics. Periodontol 2000. 2018;78(1):7-11. doi:10.1111/prd.12237
3. Choi GE, Hyun KY. Inhibitory effect of Acer tegmentosum maxim extracts on P. gingivalis LPS-induced periodontitis. Arch Oral Biol. 2020;109:104529. doi:10.1016/j.archoralbio.2019.104529
4. Han Y, Wang F, Shao L, Huang P, Xu Y. LncRNA TUG1 mediates lipopolysaccharide-induced proliferative inhibition and apoptosis of human periodontal ligament cells by sponging miR-132. Acta Biochim Biophys Sin (Shanghai). 2019;51(12):1208-1215. doi:10.1093/abbs/gmz125
5. Wang F, Chen X, Han Y, Xi S, Wu G. circRNA CDR1as regulated the proliferation of human periodontal ligament stem cells under a Lipopolysaccharide-Induced inflammatory condition. Mediators Inflamm. 2019;2019:1625381. doi:10.1155/2019/1625381
6. Zhang Z, Yang T, Xiao J. Circular RNAs: promising biomarkers for human diseases. EBioMedicine. 2018;34:267-274. doi:10.1016/j.EBioMed.2018.07.036
7. Jiao K, Walsh LJ, Ivanovski S, Han P. The emerging regulatory role of circular RNAs in periodontal tissues and cells. Int J Mol Sci. 2021;22(9):4636. doi:10.3390/ijms22094636
8. Wu T, Sun Y, Sun Z, et al. Hsa_circ_0042823 accelerates cancer progression via miR-877-5p/FOXM1 axis in laryngeal squamous cell carcinoma. Ann Med. 2021;53(1):960-970. doi:10.1080/07853890.2021.1934725
9. Li J, Xie R. Circular RNA expression profile in gingival tissues identifies circ_0062491 and circ_0095812 as potential treatment targets. J Cell Biochem. 2019;120(9):14867-14874. doi:10.1002/jcb.28748
10. Panda AC. Circular RNAs Act as miRNA sponges. Adv Exp Med Biol. 2018;1087:67-79. doi:10.1007/978-981-13-1426-1_6
11. Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet. 2016;17(5):272-283. doi:10.1038/nrg.2016.20
12. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495(7441):384-388. doi:10.1038/nature11993
13. Fu C, Wei Z, Zhang D. PTEN inhibits inflammatory bone loss in Ligature-Induced periodontitis via IL1 and TNF-α. BioMed Res Int. 2019;2019:6712591. doi:10.1155/2019/6712591
14. Du W, Wang L, Liao Z, Wang J. Circ_0085289 alleviates the progression of periodontitis by regulating let-7f-5p/SOCS6 pathway. Inflammation. 2021;44(4):1667-1619. doi:10.1007/s10753-021-01445-8

FIGURE 8  Schematic diagram reveals circ_0138960/miR-518a-5p/HDAC6 axis in LPS-induced PDLCs. HDAC6, histone deacetylase 6; LPS, lipopolysaccharide; PDLCs, periodontal ligament cells.
15. Chen H, Lan Z, Li Q, Li Y. Abnormal expression of long noncoding RNA FGD5-AS1 affects the development of periodontitis through regulating miR-142-3p/SECS6/NF-kB pathway. *Artif Cells, Nanomed, Biotechnol.* 2019;47(1): 2098-2106. doi:10.1080/21691401.2019.1620256

16. Memmert S, Nogueira AVB, Damanaki A, et al. Damage-regulated autophagy modulator 1 in oral inflammation and infection. *Clin Oral Investig.* 2018;22(8):2933-2941. doi:10.1007/s00784-018-2381-6

17. Li W, Zhang Z, Li Y, Wang Z. Abnormal hsa_circ_0003948 expression affects chronic periodontitis development by regulating miR-144-3p/NR2F2/PTEN signaling. *J Periodont Res.* 2021;57:316-323. doi:10.1111/jre.12961

18. Wang J, Du C, Xu L. Circ_0081572 inhibits the progression of periodontitis through regulating the miR-378h/RORA axis. *Arch Oral Biol.* 2021;124:105053. doi:10.1016/j.archoralbio.2021.105053

19. Huang N, Li C, Sun W, Wu J, Xiao F. Long non-coding RNA TUG1 participates in LPS-induced periodontitis by regulating miR-498/RORA pathway. * Oral Dis.* 2021;27(3):600-610. doi:10.1111/odi.13590

20. Yoshihara-Hirata C, Yamashiro K, Yamamoto T, et al. Anti-HMGB1 neutralizing antibody attenuates periodontal inflammation and bone resorption in a murine periodontitis model. * Infect Immu.* 2018;86(5):e00111-18. doi:10.1128/iai.00111-18

21. Wilson M. Biological activities of lipopolysaccharides from oral bacteria and their relevance to the pathogenesis of chronic periodontitis. *Sci Prog.* 1995;78(Pt 1):19-34.

22. Marques-Rocha JL, Samblas M, Milagro FI, Bressan J, Martínez JA, Martí A. Noncoding RNAs, cytokines, and inflammation-related diseases. *FASEB J.* 2015;29(9): 3595-3611. doi:10.1096/fj.14-260323

23. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of eRNA crosstalk and competition. *Nature.* 2014;505(7483): 344-352. doi:10.1038/nature12986

24. Hsiao KY, Sun HS, Tsai SJ. Circular RNA—new member of noncoding RNA with novel functions. *Exp Biol Med (Maywood).* 2017;242(11):1136-1141. doi:10.1177/1535370217708978

25. Zhang S, Song G, Yuan J, et al. Circular RNA circ_0003204 inhibits proliferation, migration and tube formation of endothelial cell in atherosclerosis via miR-370-3p/TGFβ2/phospho-SMAD3 axis. *J Biomed Sci.* 2020;27(1):11. doi:10.1186/s12929-019-0595-9

26. Bi J, Liu H, Cai Z, et al. Circ-BPTF promotes bladder cancer progression and recurrence through the miR-31-5p/RAB27A axis. *Aging.* 2018;10(8):1964-1976. doi:10.18632/aging.101520

27. Zhou M, Hu H, Han Y, et al. Long non-coding RNA 01126 promotes periodontitis pathogenesis of human periodontal ligament cells via miR-518a-5p/HIF-1α/MAPK pathway. *Cell Prolif.* 2021;54(1):e12957. doi:10.1111/cpr.12957

28. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by miRNAs. *Annu Rev Biochem.* 2010;79:351-379. doi:10.1146/annurev-biochem-060308-103103

29. O’Brien J, Hayden H, Zayed Y, Peng C. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol.* 2018;9:402. doi:10.3389/fendo.2018.00402

30. Yan GQ, Wang X, Yang F, et al. MicroRNA-22 promoted osteogenic differentiation of human periodontal ligament stem cells by targeting HDAC6. *J Cell Biochem.* 2017;118(7): 1653-1658. doi:10.1002/jcb.25931

**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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