Circ_0062491 alleviates LPS-induced apoptosis and inflammation in periodontitis by regulating miR-498/SOCS6 axis

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Abstract

Periodontitis is a prevalent chronic inflammatory disease. Circular RNAs (circRNAs) have been revealed to play roles in the inflammatory response. Hence, this work aimed to explore the role and mechanism of circ_0062491 in periodontitis progression. Human periodontal ligament cells (PDLCs) were isolated from the periodontal ligament (PDL) of the healthy teeth with orthodontic requirement after tooth extraction. *In vitro* experiments were conducted by cell counting Kit-8 (CCK-8) assay, flow cytometry, Western blot, and ELISA to determine cell viability, apoptosis, and inflammatory response. The binding between miR-498 and circ_0062491 or SOCS6 was confirmed using dual-luciferase reporter and RNA immunoprecipitation (RIP) assays. Circ_0062491 expression was decreased in periodontitis and LPS-induced PDLCs. Restoration of circ_0062491 attenuated LPS-induced apoptosis and inflammation in PDLCs *in vitro*. Mechanistically, circ_0062491 functioned as a sponge for miR-498, and miR-498 directly targeted SOCS6. Rescue experiments showed that miR-498 up-regulation reversed the protective action of circ_0062491 on PDLCs under LPS treatment. Moreover, silencing of miR-498 protected PDLCs from LPS-induced apoptosis and inflammation, which were abolished by SOCS6 knockdown. Circ_0062491 protected PDLCs from LPS-induced apoptosis and inflammation, suggesting a new target for the amelioration of periodontitis patients.

Introduction

Periodontitis is a complex infectious disease characterized by gingival inflammation and pathologic loss of the periodontal ligament and alveolar bone, which can lead to sore, bleeding gums, painful chewing problems, and even tooth loss in later stages, impacting the quality of life.[1,2] Besides that, periodontitis has been identified to be interrelated with systemic conditions through increasing the risk of many diseases, including cancer, cardiovascular diseases, autoimmune disease, and respiratory diseases, relying on its low systemic inflammatory burden.[3–5] Therefore, further clarification on the mechanisms involving periodontitis is imperative for the prevention of periodontitis.

Circular RNAs (circRNAs) are a class of RNA molecules formed by covalently closed loop structures without terminal 5′ caps and 3′ polyadenylated tails.[6] CircRNAs are more abundant, specific, and highly organized in comparison to linear RNAs.[7] Recently, the dys-regulation of circRNAs has been identified in different diseases, importantly, circRNAs are implicated in a wide range of cellular crucial biological processes related to carcinogenesis, apoptosis, and inflammatory response, indicating that circRNAs may act as one of the potential biomarkers for diagnosis and therapy.[8–10] Additionally, chronic inflammation is implicated in the genesis and progression of periodontitis, emerging evidence has revealed that circRNAs have roles in the

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progression and management of the inflammatory response by transcriptionally regulating some inflammation-related mediators.[11,12] Thus, circRNAs may be involved in the inflammation-related alterations of periodontitis. Circ_0062491 is derived from its host gene MIR650, it locates at chr22: 23063339-23180200 with the length of 116861 bp. A recent study showed that circ_0062491 was decreased in periodontitis-gingival tissues relative to the healthy tissues, suggesting the potential association with periodontitis.[13,14] Healthy periodontal ligament cells (PDLCs) are the main basis of periodontal repair and regeneration,[15] they cannot only differentiate into osteoblasts and cementoblasts to construct cementum and alveolar bone, but also contribute to the formation of new main fibres.[16,17] Thus, we hypothesized that circ_0062491 might be involved in the pathogenesis of periodontitis by affecting the apoptosis and inflammation of PDLCs.

Herein, this study used LPS-induced PDLCs to mimic the growth environment of PDLCs under the inflammatory condition of periodontitis *in vitro*, thereby investigating the potential role and mechanism of circ_0062491 in periodontitis progression.

Materials and methods

Tissue collection

Human periodontal ligament (PDL) tissues were collected from diseased teeth with chronic periodontitis ($n = 21$, 11 males, 10 females, median age: 32 ± 7 yr old), or sex- and age-matched healthy teeth with orthodontic requirement ($n = 21$, 12 males, 9 females, median age: 30 ± 8 yr old) after tooth extraction. Diagnostic criteria of periodontitis were as follows: 1) redness and swelling of the gingiva on the surface of 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. All subjects did not receive antibiotic therapy and clinical specimens were immediately stored at −80°C until used. This work was approved by the Ethics Committee of the Fourth People's Hospital of Haikou City and written informed consent has been obtained from all subjects before specimen collection.

Cell culture and treatment

Normal PDLCs were isolated from PDL tissues scraped from the middle third of the root surface of three healthy donors. PDL tissues were minced into small pieces of 1 mm³. Then the small tissue cubes (combined) were incubated with a solution of 1.5 mg/ml collagenase (type I) with 5 mg/ml dispase in α-minimum essential medium (α-MEM, Hyclone, South Logan, UT, USA) for 30 min. Single-cell suspensions obtained after enzymic digestion and a strainer filtration were cultured in α-MEM containing 10% FBS (Hyclone), 2 mmol/L-glutamine and 1% penicillin/streptomycin (all from Sigma-Aldrich, St Louis, MO, USA) with 5% CO2 at 37°C. PDLCs were used at 3 to 6 passages. 293T cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), and then grown in DMEM (Hyclone) containing 10% FBS (Hyclone) and 1% penicillin/streptomycin (Sigma-Aldrich) at 37°C with 5% CO2.

In functional experiments, PDLCs were treated with 10 µg/ml *Porphyromonas gingivalis* LPS (Invitrogen, Carlsbad, CA, USA) for 3 h to imitate the growth environment of PDLCs under the inflammatory condition of periodontitis *in vitro*. Untreated cells were used as negative control.

Reverse transcription and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated by using the Trizol reagent (Invitrogen). Then isolated RNAs were reverse-transcribed to cDNA by using the PrimeScript RT Reagent Kit (Takara, Dalian, China), and qRT-PCR was conducted by the SYBR Green PCR Kit (Takara). The relative fold changes were calculated by the $2 - \Delta\Delta CT$ method and normalized to GAPDH or U6. The primer sequences:

circ_0062491: F 5′-GACAGGGCTGTGAGAGCTAA-3′, R 5′-TGTCTCCTTGGCATGTGATCC-3′;

SOCS6: F 5′-CGCTAGCCAGTGACTTTGGA-3′, R 5′-TCTGTTTTGCAGAAAGCCGC-3′;

miR-498: F 5′-GCCGAGTTTCAAGCCAGGGGG-3′, R 5′-CTCAACTGGTGTCGTGGAG-3′;

GADPH: F, 5′-GACAGTCAGCCGCATCTTCT-3′ and R, 5′-GCGCCCAATACGACCAAATC-3′;

U6: F 5′-CTCGCTTCGGCAGCACA-3′, R 5′-AACGCTTCACGAATTTGCGT-3′.

Cell transfection

The pCD5-ciR circ_0062491 overexpression vector (oe-circ_0062491) and empty pCD5-ciR negative control (vector), SOCS6 specific siRNAs (si-SOCS6) and non-target siRNA negative control (si-NC) were procured from Genema (Shanghai, China). The miR-498 mimic, inhibitor, and negative control (mimic NC or inhibitor NC) were provided by Ribobio (Guangzhou, China). Thereafter, Lipofectamine 2000 proved by Invitrogen was applied to conduct transient transfection with 50 nM of siRNAs, 100 ng of plasmids, or 40 nM of miRNA mimics

or inhibitors in PDLCs. After the confirmation of transfection efficiency using qRT-PCR or Western blot, cells were treated with 10 μg/ml LPS for 3 h for subsequent analyses.

Cell counting Kit-8 (CCK-8) assay

PDLCs were placed into each well of 96-well plates with 10% FBS-contained medium overnight. After 2 h of incubation with 10 μl of CCK-8 (5 mg/ml) (Solarbio, Shanghai, China), the absorbance at 490 nm was read to assess cell viability.

Flow cytometer

After washing with cold-PBS, harvested PDLCs were re-suspended in 200 μl binding buffer and stained with an FITC-conjugated anti-Annexin V Ab and PI (BD Biosciences, San Diego, CA, USA) away from light. Cell apoptosis was analysed by flow cytometry after 15 min of incubation.

Caspase3/7 activities analysis

The Apo-ONE homogenous caspase 3/7 activity assay kit (Promega, Madison, WI, USA) was applied for the detection of caspase 3/7 activities as per the manufacturer's protocol. Lastly, a microplate reader was used to detect the results with excitation at 485 nm and emission at 530 nm.

Western blot

Equal amounts of protein isolated using RIPA buffer (Solarbio) were separated by 10% SDS-PAGE, transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and followed by blocking for 2 h at room temperature in 3% skimmed milk. Subsequently, primary Ab against Bax (1:2000, ab32503), Bcl-2 (1:2000, ab692), Cleaved-caspase 3 (1:1000, ab2302), pro-caspase3 (1:1000, ab32150), SOCS6 (1:1000, ab197335) and GAPDH (1:10000, ab181602), all obtained from Abcam (Cambridge, UK), were added to the membrane, and incubated overnight at 4°C. After interaction with a goat anti-rabbit HRP-conjugated Ab (ab6721, 1:5000, Abcam), the signal intensity was detected by the enhanced chemiluminescence system (Beyotime, Beijing, China).

ELISA

The levels of IL-6, IL-1β or TNF-α in cell culture supernatant of PDLCs were analysed following the manufacturer's instructions of the corresponding ELISA kits (Abcam).

Dual-luciferase reporter assay

The fragments of circ_0062491 and SOCS6 3′UTR covering wild type (WT) binding sites in miR-498 were amplified and inserted into the pGL3 basic luciferase reporter vector (Promega). The mutated (MUT) sequences in miR-498 binding sites were provided by Invitrogen. Thereafter, 293T cells transfected with miR-320b mimic or mimic NC at 50 nM were infected with 50 ng PGL3 vector reporter and 10 ng pRL-TK Renilla vector. The luciferase activities were examined by after 48 h of transfection.

RNA immunoprecipitation (RIP) assay

The EZMagna RIP Kit (Millipore) was employed to carry out RIP assay. In short, 293T cells were lysed using RIP lysis buffer. Human ani-Ago2 Ab or anti-IgG Ab (Abcam) was conjugated with magnetic beads, and then incubated with cell lysates. Finally, the co-precipitated RNAs were purified, and subjected to qRT-PCR analysis.

Statistical analysis

The result data were manifested as mean ± standard deviation. Student's *t* test (two-sided) or analysis of variance was used to compare the difference. The correlation analysis was performed by Pearson's correlation analysis. All statistical significance was conducted on GraphPad Prism 6 software (GraphPad, San Diego, CA, USA) with $P < 0.05$ as significant differences.

Results

Circ_0062491 expression was decreased in periodontitis and LPS-induced PDLCs

To determine the expression pattern of circ_0062491 in periodontitis, PDL tissues from diseased teeth with chronic periodontitis and healthy teeth with orthodontic requirement were collected. The results of qRT-PCR suggested that circ_0062491 expression was lower in PDL tissues of periodontitis than that of healthy PDL tissues (Figure 1A). Thereafter, LPS was used to imitate the inflammatory condition of periodontitis in PDLCs, and it was observed that the expression of circ_0062491 was time-dependently down-regulated in PDLCs by 10 μg/ml LPS exposure at 0, 3, 6, and 12 h (Figure 1B). Circ_0062491 is derived from its host gene MIR650, it locates at chr22: 23063339-23180200 with the length of 116861 bp (Figure 1C). Based on the above results, dysregulation of circ_0062491 might be associated with the process of periodontitis.

Overexpression of circ_0062491 reversed LPS-evoked apoptosis and inflammation in PDLCs in vitro
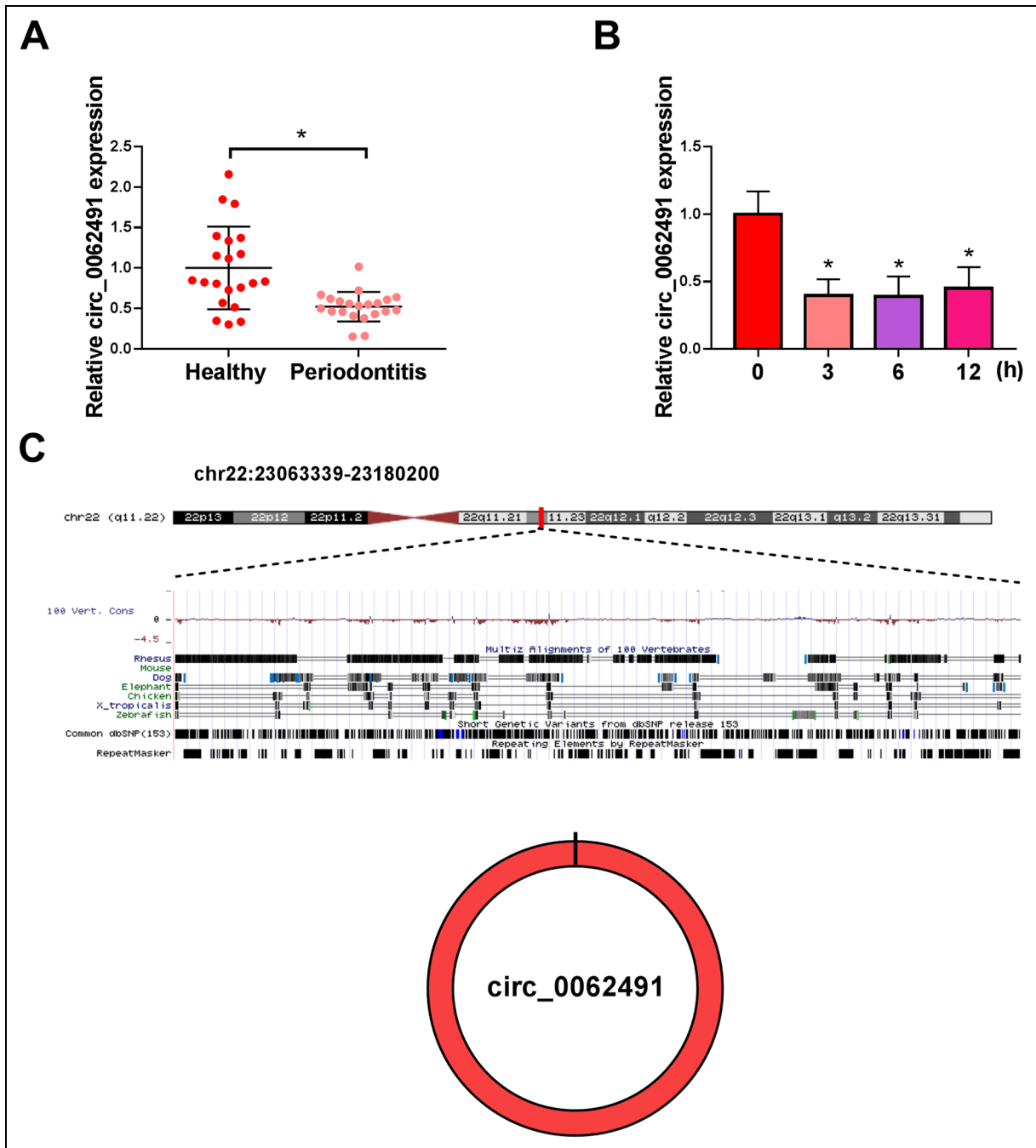
Figure 1. Circ_0062491 expression was decreased in periodontitis and LPS-induced PDLCs. (A) qRT-PCR analysis of circ_0062491 expression in PDL tissues from diseased teeth with chronic periodontitis and healthy teeth with orthodontic requirement. (B) qRT-PCR analysis of circ_0062491 expression in PDLCs exposed to 10 µg/ml LPS at 0, 3, 6, and 12 h. (C) The schematic exhibited the circularizing of MIR650 into the form of circ_0062491 by head-to-tail. *$P < 0.05$.

Next, the potential functions of circ_0062491 in periodontitis were investigated. Circ_0062491 overexpression vector was designed and transfected into PDLCs to up-regulate circ_0062491 expression level (Figure 2A). After subjecting to 10 µg/ml LPS exposure for 3 h, it was demonstrated that circ_0062491 overexpression attenuated LPS-triggered reduction of cell viability (Figure 2B) and promotion of cell apoptosis (Figure 2C) in PDLCs. The activity of caspase3/7 was also evaluated, the results suggested that the activity of caspase3/7 was enhanced in PDLCs under LPS exposure which was reduced by circ_0062491 overexpression
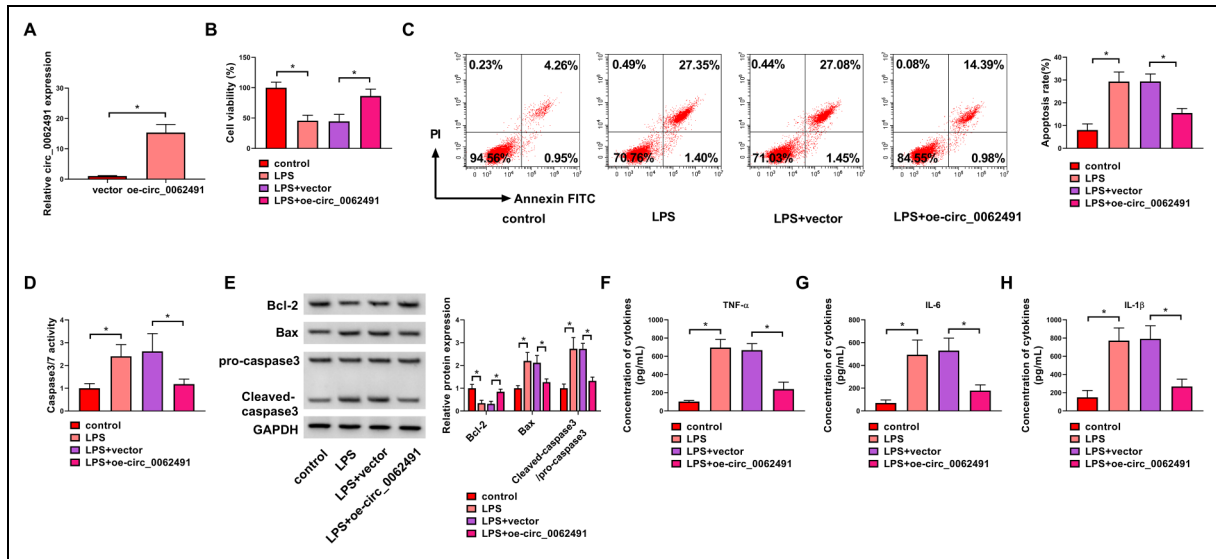
Figure 2. Overexpression of circ_0062491 reversed LPS-evoked apoptosis and inflammation in PDLCs *in vitro*. (A) qRT-PCR analysis of circ_0062491 expression in PDLCs transfected with vector or oe-circ_0062491. (B-H) Transfected PDLCs were subjected to 10 μg/ml LPS exposure for 3 h. (B) CCK-8 assay for PDLC viability. (C) Flow cytometry for PDLC apoptosis. (D) Measurement of the activity of caspase3/7 using ELISA in PDLCs. (E) Western blot analysis of Bcl-2, Bax, Cleaved-caspase3 and pro-caspase3 protein levels in PDLCs. (F-H) ELISA analysis for the levels of TNF-α, IL-6 and IL-1β in PDLCs. *$P < 0.05$.

(Figure 2D). Moreover, Western blot analysis showed that LPS treatment led to a decrease of Bcl-2 protein and an increase of Bax protein as well as Cleaved-caspase3 in PDLCs, while circ_0062491 overexpression reversed this condition (Figure 2E). Besides that, the increases of TNF-α, IL-6 and IL-1β in PDLCs caused by LPS treatment were abolished by circ_0062491 up-regulation (Figure 2F-H). Taken together, circ_0062491 protected PDLCs from LPS-evoked apoptosis and inflammation *in vitro*.

MiR-498 is a target of circ_0062491

According to the prediction of Circinteractome database, circ_0062491 was found to have a putative binding site in miR-498 (Figure 3A). The results of dual-luciferase reporter assay showed that miR-498 overexpression significantly reduced the luciferase activity in the target sites of WT circ_0062491 luciferase reporter but not the mutated type circ_0062491 vector with point mutations in 293T cells (Figure 3B). Furthermore, RIP assay exhibited that miR-498 and circ_0062491 were enriched preferentially in anti-Ago2 immuno-precipitates in comparison to anti-IgG immuno-precipitates (Figure 3C), further verifying the binding between miR-498 and circ_0062491. MiR-498 expression was discovered to be increased in PDL tissues with periodontitis (Figure 3D), which was negatively correlated with circ_0062491 expression (Figure 3E). Also, its expression was elevated in LPS-induced PDLCs in a time-dependent manner (Figure 3F). Therefore, we confirmed

that circ_0062491 targeted miR-498 and negatively regulated its expression.

Circ_0062491 reversed LPS-evoked apoptosis and inflammation in PDLCs via miR-498

To explore whether miR-498 mediated the protective effects of circ_0062491 on PDLCs under LPS, the elevation efficiency of miR-498 mimic was first investigated in PDLCs with the significant increase of miR-498 level in cells (Figure 4A). Then PDLCs were co-transfected with oe-circ_0062491 and miR-498 mimic, the results of qRT-PCR showed that miR-498 mimic rescued circ_0062491-induced decrease of miR-498 in PDLCs (Figure 4B). Subsequently, transfected cells were treated with 10 μg/ml LPS exposure for 3 h, we observed that miR-498 mimic reversed circ_0062491 overexpression-mediated promotion on cell viability (Figure 4C), inhibition on cell apoptosis (Figure 4D-F), and reduction of inflammatory cytokines TNF-α, IL-6 and IL-1β (Figure 4G-I) in PDLCs under LPS treatment. In all, we demonstrated that circ_0062491/miR-498 was responsible for LPS-evoked PDLC apoptosis and inflammation.

SOCS6 is a target of miR-498

The downstream targets of miR-498 were then investigated. Using the Starbase3.0 database, miR-498 was predicted to have a complementary sequence on SOCS6 (Figure 5A). Further dual-luciferase reporter assay manifested that
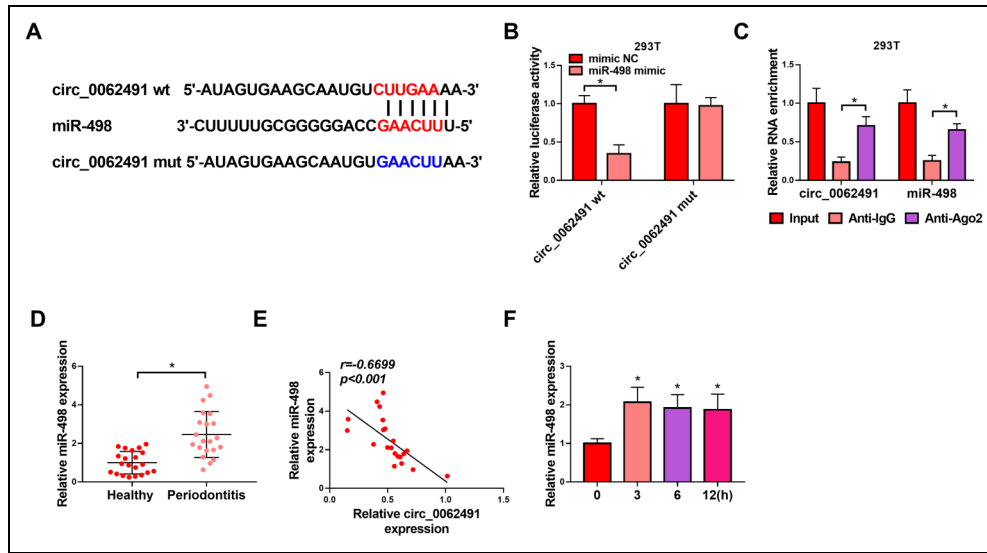
Figure 3. MiR-498 is a target of circ_0062491. (A) A putative binding site between miR-498 and circ_0062491. (B) Dual-luciferase reporter assay for the luciferase activity of wild and mutated circ_0062491 reporter after miR-498 overexpression in 293T cells. (C) Anti-Ago2 RIP assay was used in 293T cells to determine miR-498 and circ_0062491 RNA enrichment in immuno-precipitate complexes. (D) qRT-PCR analysis of miR-498 expression in PDL tissues from diseased teeth with chronic periodontitis and healthy teeth with orthodontic requirement. (E) Correlation analysis was performed between miR-498 and circ_0062491 expression in PDL tissues of periodontitis by Pearson's correlation analysis. (F) qRT-PCR analysis of miR-498 expression in PDLCs exposed to 10 μg/ml LPS at 0, 3, 6, and 12 h. $*P < 0.05$.

miR-498 mimic significantly reduced the luciferase activity with only WT 3′UTR of SOCS6 in PDLC cells but not the mutated one in 293T cells (Figure 5B), indicating the binding between SOCS6 and miR-498. SOCS6 was observed to be down-regulated in PDL tissues with periodontitis (Figure 5C), and a negative correlation between SOCS6 and miR-498 expression in PDLs was discovered (Figure 5D). Moreover, SOCS6 expression was time-dependently decreased by LPS in PDLCs (Figure 5E). Thus, we verified that miR-498 targeted
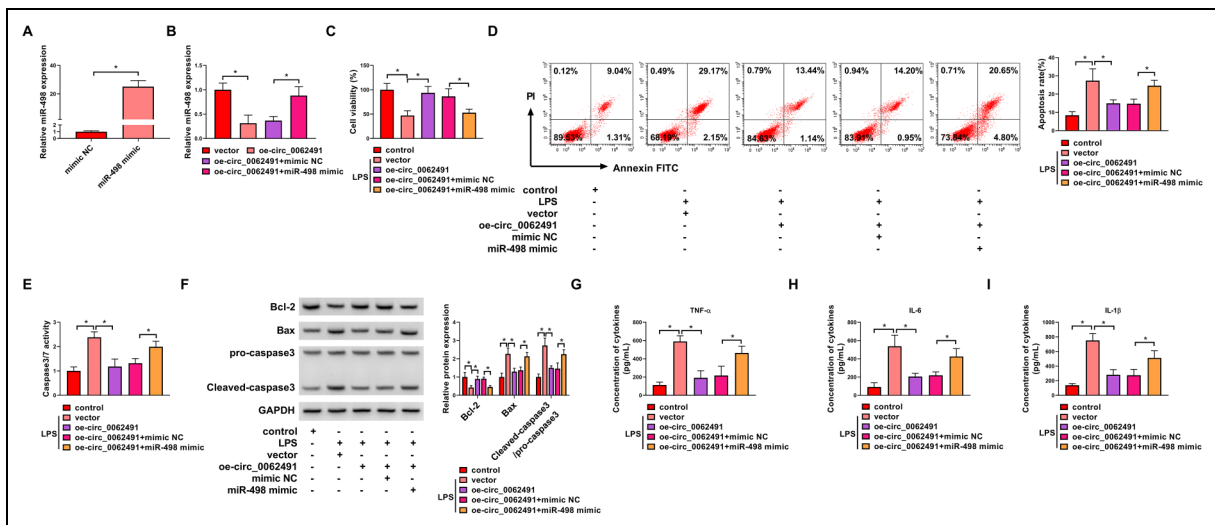

Figure 4. Circ_0062491 reversed LPS-evoked apoptosis and inflammation in PDLCs via miR-498. (A) qRT-PCR analysis of miR-498 expression in PDLCs transfected with mimic NC or miR-498 mimic. (B) qRT-PCR analysis of miR-498 expression in PDLCs transfected with vector, oe-circ_0062491, oe-circ_0062491 + mimic NC, or oe-circ_0062491 + miR-498 mimic. (C-I) Transfected PDLCs were subjected to 10 μg/ml LPS exposure for 3 h. (C) CCK-8 assay for PDLC viability. (D) Flow cytometry for PDLC apoptosis. (E) Measurement of the activity of caspase3/7 using ELISA in PDLCs. (F) Western blot analysis of Bcl-2, Bax, Cleaved-caspase3 and pro-caspase3 protein levels in PDLCs. (G-I) ELISA analysis for the levels of TNF-α, IL-6 and IL-1β in PDLCs. $*P < 0.05$.
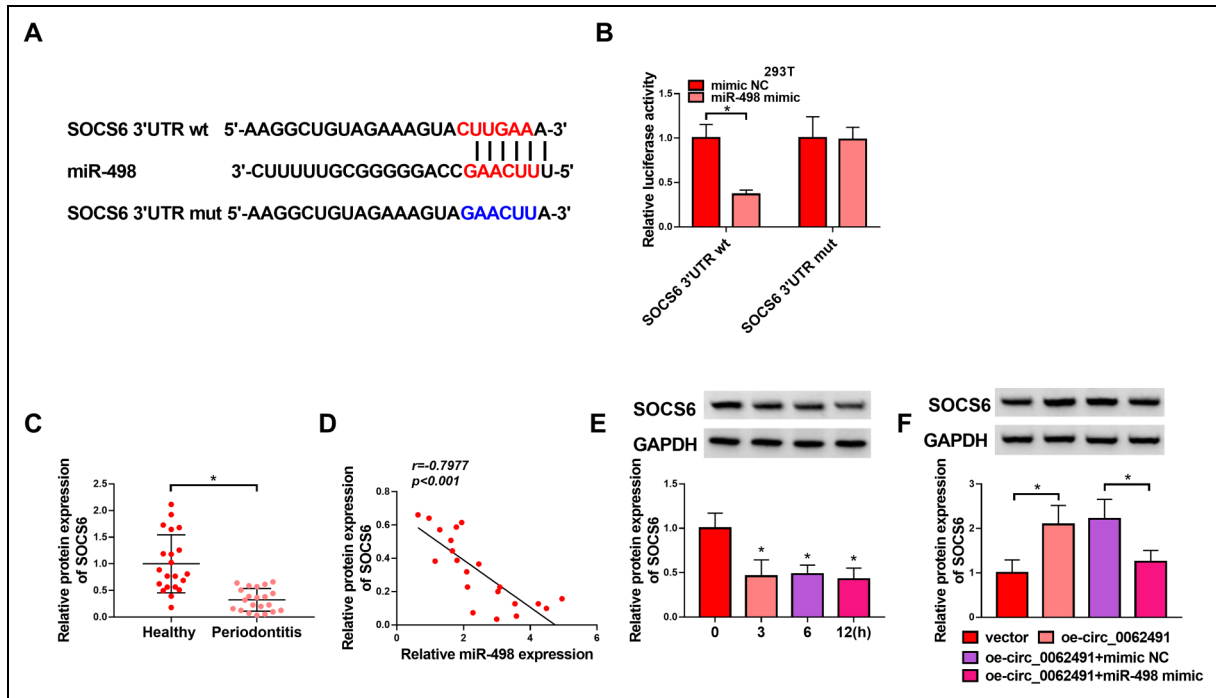
Figure 5. SOCS6 is a target of miR-498. (A) A putative binding site between miR-498 and SOCS6. (B) Dual-luciferase reporter assay for the luciferase activity of wild and mutated SOCS6 reporter after miR-498 overexpression in 293T cells. (C) qRT-PCR analysis of SOCS6 RNA expression in PDL tissues from diseased teeth with chronic periodontitis and healthy teeth with orthodontic requirement. (D) Correlation analysis was performed between miR-498 and SOCS6 expression in PDL tissues of periodontitis by Pearson's correlation analysis. (E) Western blot analysis of SOCS6 expression in PDLCs exposed to 10 μg/ml LPS at 0, 3, 6, and 12 h. (F) Western blot analysis of SOCS6 expression in PDLCs transfected with vector, oe-circ_0062491, oe-circ_0062491 + mimic NC, or oe-circ_0062491 + miR-498 mimic. *$P < 0.05$.

SOCS6 and negatively modulated its expression. Besides that, Western blot analysis also showed that circ_0062491 overexpression elevated SOCS6 expression in PDLCs, which was reversed by miR-498 up-regulation (Figure 5F), indicating the circ_0062491/miR-498/SOCS6 axis in PDLCs.

MiR-498 silencing reversed LPS-evoked apoptosis and inflammation in PDLCs through SOCS6

Subsequently, the biological functions of SOCS6 in PDLCs function were investigated. Following the transfection of si-SOCS6#1, si-SOCS6#2, si-SOCS6#3, SOCS6 expression was reduced in PDLCs (Figure S1A). Then we found that si-SOCS6#3 introduction re-inforced LPS-evoked cell viability arrest (Figure S1B), apoptosis (Figure S1C-E) and inflammation (Figure S1F-H) in PDLCs, indicating that SOCS6 might protect against periodontitis. After that, it was studied whether miR-498/SOCS6 axis was responsible for LPS-evoked PDLC injury. The si-SOCS6#3 (named as si-SOCS6 in following experiments) was used in following functional assays on account of its better transfection efficiency. The knockdown efficiency of miR-498 inhibitor or SOCS6 siRNA was first validated by

qRT-PCR and Western blot, respectively (Figure 6A, B). Then PDLCs were transfected with inhibitor NC, miR-498 inhibitor, miR-498 inhibitor + si-NC or miR-498 inhibitor + si-SOCS6, and the results of Western blot showed that miR-498 inhibitor caused an increase of SOCS6 expression, which was reduced by SOCS6 knockdown (Figure 6C). After treatment with 10 μg/ml LPS for 3 h, functional experiments revealed that miR-498 down-regulation promoted cell viability (Figure 6D) and suppressed cell apoptosis (Figure 6E) in PDLCs under LPS exposure, while SOCS6 knockdown reversed these effects. Furthermore, SOCS6 knockdown attenuated miR-498 down-regulation-induced inhibition on caspase3/7 activity in PDLCs in the presence of LPS (Figure 6F). The expression of Bcl-2 protein was increased, while levels of Bax and Cleaved-caspase3 were increased by miR-498 down-regulation in LPS-stimulated PDLCs, while this condition was abated by SOCS6 silencing (Figure 6G). Meanwhile, the levels of TNF-α, IL-6 and IL-1β in LPS-stimulated PDLCs were inhibited by miR-498 down-regulation, which were rescued by SOCS6 knockdown (Figure 6H-J). Altogether, miR-498 silencing had a
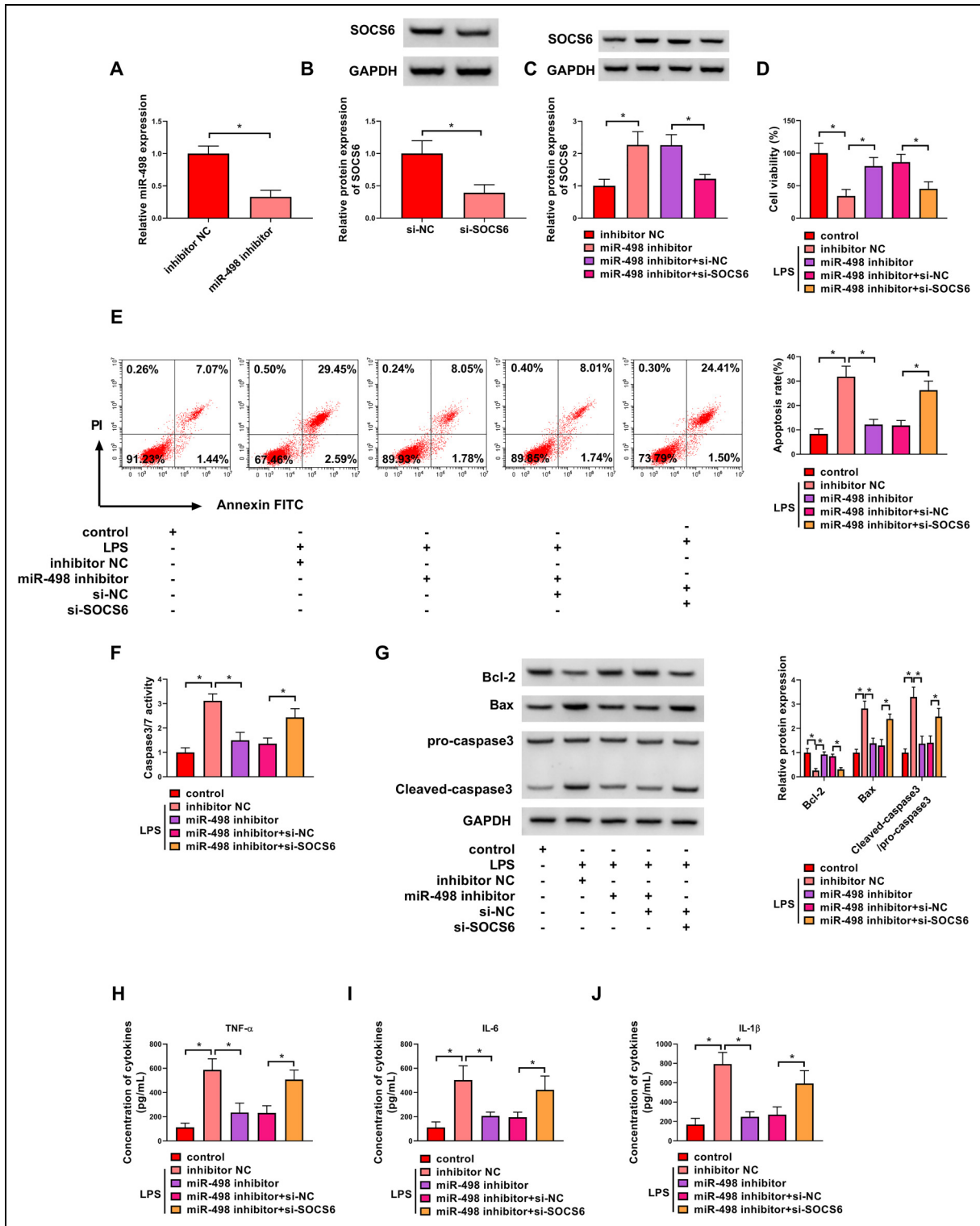
Figure 6. MiR-498 silencing reverses LPS-evoked apoptosis and inflammation in PDLCs through SOCS6. (A) qRT-PCR analysis of miR-498 expression in PDLCs transfected with inhibitor NC or miR-498 inhibitor. (B) Western blot analysis of SOCS6 expression in PDLCs transfected with si-NC or si-SOCS6. (C) Western blot analysis of SOCS6 expression in PDLCs transfected with inhibitor NC, miR-498 inhibitor, miR-498 inhibitor + si-NC or miR-498 inhibitor + si-SOCS6. (D-J) Transfected PDLCs were subjected to 10 μg/ml LPS exposure for 3 h. (D) CCK-8 assay for PDLC viability. (E) Flow cytometry for PDLC apoptosis. (F) Measurement of the activity of caspase3/7 using ELISA in PDLCs. (G) Western blot analysis of Bcl-2, Bax, Cleaved-caspase3 and pro-caspase3 protein levels in PDLCs. (H-J) ELISA analysis for the levels of TNF-α, IL-6 and IL-1β in PDLCs. *$P < 0.05$.
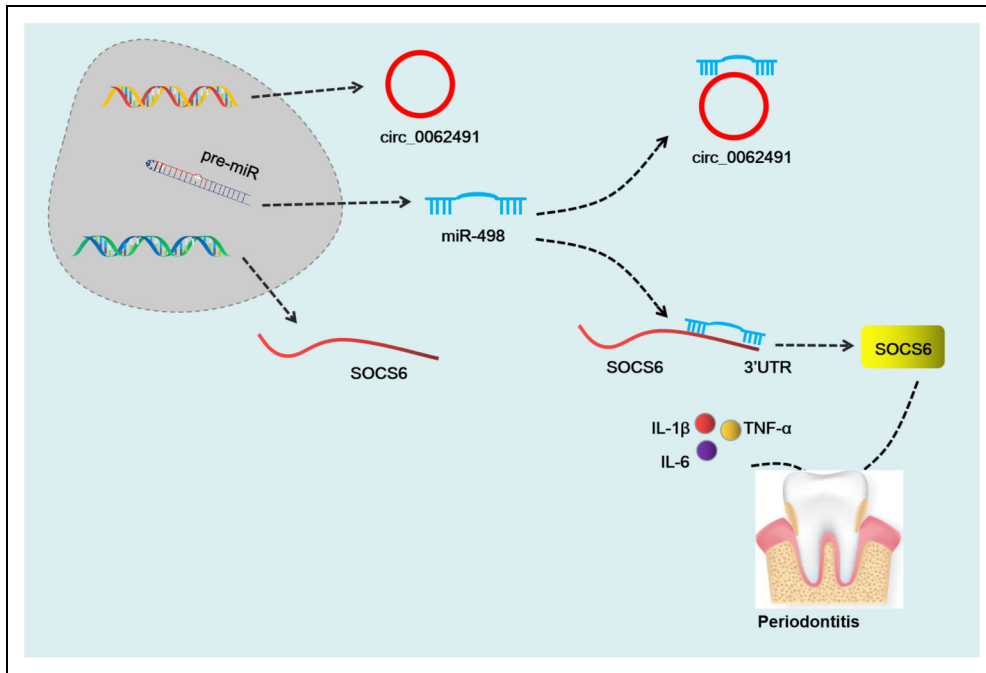
Figure 7. Schematic diagram of circ_0062491 regulates periodontitis progression. Circ_0062491 binds to miR-498 to elevate SOCS6 expression to suppress periodontitis progression.

protective effect on PDLCs through regulating SOCS6 under LPS treatment.

Discussion

In the present study, the decreased expression of circ_0062491 in PDL tissues of periodontitis patients and LPS-induced PDLCs was investigated. LPS treatment significantly suppressed growth and induced inflammation in PDLCs *in vitro*. Furthermore, gain-of-function experiments revealed that overexpression of circ_0062491 attenuated LPS-evoked injury of PDLCs. Moreover, we also demonstrated that circ_0062491 served its biological functions by miR-498/SOCS6 axis in PDLCs under LPS treatment.

CircRNAs are one type of evolutionarily conserved RNA molecules, research increasingly reveals that circRNAs are emerged as promising non-invasive biomarkers for the therapeutics and diagnosis of different diseases due to their structural stability, high conservation between species, tissue specificity, and accessibility.[18] In periodontitis, some circRNAs have also been identified to be involved in the progression of this disease. For example, Wang et al. confirmed that circRNA CDR1as facilitated the proliferative ability of periodontal ligament stem cells (PDLSCs) isolated from PDL tissues under LPS treatment via activating ERK signal pathway through miR-7.[19] Besides, circ_0081572 was demonstrated to sponge miR-378h to protect PDLCs against LPS-induced injury.[20] Thus, we believed that alteration of

circ_0062491 expression level in PDLCs might be a promising therapeutic strategy for periodontitis.

CircRNA can serve as a competing endogenous RNA (ceRNA) to prevent miRNA-mediated degradation of mRNA through sponging shared miRNA.[21,22] Thus, we further investigated the circ_0062491-miRNA-mRNA network in this study. We confirmed that circ_0062491 directly bound to miR-498, besides, SOCS6 was verified to be a target of miR-498. Moreover, it was validated that circ_0062491 could indirectly regulate SOCS6 expression through sponging miR-498. Thus, the circ_0062491/miR-498/SOCS6 axis was identified. MiRNA species appear to participate in the developmental, physiological, and pathogenic events.[23] MiRNAs are significant modulator in inflammation and immune dys-function is related to the aberrant expression of miRNAs.[24,25] Moreover, recent reports have exhibited that various miRNAs are involved in periodontitis.[26,27] Moreover, Huang et al. showed that miR-498 reversed the inhibitory effects of TUG1 on LPS-induced proliferation arrest and release of inflammatory cytokines via TUG1/miR-498/RORA axis, indicating the potential involvement of miR-498 in periodontitis.[28] SOCS6, a member of the SOCS family of proteins, is a well-known negative regulator of cytokine receptor signalling and induces intrinsic apoptosis by targeting mitochondrial proteins.[29,30] SOCS6 is widely expressed in many of tissues, and reduced SOCS6 expression has been discovered in many human malignancies, in addition, its expression is regulated by several miRNAs.[31-33] In periodontitis, it was

also demonstrated that SOCS6 acted as a target of miRNAs to protect against periodontitis by regulating the function of PDLCs.[34,35] In the present work, we observed an increased miR-498 expression and a decreased SOCS6 expression in PDL tissues of periodontitis patients and LPS-induced PDLCs. Functionally, knockdown of miR-498 reversed LPS-evoked inhibition of cell growth and promotion of inflammation in PDLCs, while this condition was abolished by SOCS6 silencing. Besides that, SOCS6 knockdown enhanced LPS-triggered PDLC dysfunction. Furthermore, we also uncovered that miR-498 overexpression abated the protective effects of circ_0062491 on LPS-triggered PDLC injury.

However, although some interesting results were found in this study, the data presented are based on a limited number of cells *in vitro*. Further research should be carried out in vivo using the animal models with high or low circ_0062491 expression in the future.

In conclusion, this work for the first time revealed that circ_0062491 has a protective role against LPS-induced apoptosis and inflammation in PDLCs through up-regulating SOCS6 via sequestering miR-498 (Figure 7), suggesting the potential involvement of circ_0062491 in periodontitis progression and providing a promising target for periodontitis prevention.

Declaration of conflicting interests

Funding

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Supplemental material

Supplemental material for this article is available online.

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