MicroRNA-155 Expression is Associated with Pulpitis Progression by Targeting SHIP1

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Research Article

Keywords: Pulpitis, MicroRNA-155, SHIP1, PI3K/AKT

Posted Date: November 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-735774/v2

Abstract

Pulpitis is a commonly seen oral inflammation condition in clinical practice, it can cause much pain for the patient and may induce infections in other systems. Much is still unknown for the pathogenic mechanism of pulpitis. In this work, we discovered that the expression of miR-155 was associated with dental pulpal inflammation both *in vivo* and *in vitro*. Experiments on odontoblast cell line MDPC-23 showed miR-155 could act as a positive regulator by increasing the production of pro-inflammatory cytokines IL-1β and IL-6 during inflammatory responses, whereas knockdown of miR-155 can reverse the effects. Bioinformatics analysis demonstrated that SHIP1 is a direct target of miR-155 in odontoblasts, this result was further verified at both mRNA and protein level. Inhibition of miR-155 resulted in the downregulation of inflammation factors, while co-transfection of si-SHIP1 and miR-155 inhibitor promoted the inflammatory responses. Treatment with miR-155 mimic or si-SHIP1 up-regulated the protein level of p-PI3K and p-AKT. By contrast, miR-155 inhibitor exerted the opposite effects. miR-155 mimics could upregulate the gene expression of IL-1β and IL-6. Co-transfection of LY294002 and miR-155 mimic attenuated the inflammatory responses. Consistent with *in vitro* results, miR-155$^{-/-}$ mice could alleviate inflammatory response, as well as decrease the activation of p-PI3K and p-AKT, whereas increase the activation of SHIP1. In conclusion, these data revealed a novel role for miR-155 in regulation of dental pulpal inflammatory response by targeting SHIP1 through PI3K/AKT signaling pathway.

Introduction

Pulpitis is a prevalent chronic inflammation in the dental pulp. Bacterial infection into the pulp tissue is one of the primary causes, cells in the pulp under the stimulation of bacterial components can release a variety of inflammatory factors, leading to severe pain [1]. Pulpitis can induce infections and other conditions in distant systems if not treated properly in time [2]. Researchers have studied many aspects of inflammatory mechanism in the dental pulp, which including pathogen factors, related signal pathways and the response of pulp cells like fibroblasts and odontoblasts [3]. Among them, reports have been made about the possible involvement of microRNAs in the process of pulpitis [4].

MicroRNAs are small non-coding RNAs that regulate gene expression by binding to complementary sequences found in the 3'UTR of target mRNAs, thus repressing translation, or degrading the expression of mRNA. Varies of microRNAs are expressed in dental pulp and periodontal tissues, it has been reported that microRNAs can work as factors in oral inflammations [5, 6]. Researches on miR-155 have found it is a multifunctional factor which regulates inflammation in cancers, lung disease and coronary heart diseases [7, 8]. Previous studies have revealed that miR-155 has been recognized as an important biomarker in oral diseases. As it can regulate the proliferation, cell cycle and apoptosis of oral cancer [9]. It can also participate in the immune process of oral lichen planus and periodontal and peri-implantation diseases [10, 11]. Yue *et al*. have demonstrated that miR-155 may play an important role in apical periodontitis progression, which indicates that miR-155 is implicated in the regulation of apical periodontal inflammation [12]. For the mechanism of miR-155 regulation, previous studies have demonstrated that the miR-155 can downregulate SHIP1 in macrophages [13] and reduce collagen

production and endothelia-mesenchymal transition in lung fibrosis [14]. However, for the progression of pulpitis, the mechanism of miR-155 modulates dental specific cells like odontoblasts on pulpal inflammatory responses is yet to be testified.

In this study, we explored the role of miR-155 in pulpal inflammation, attempting to find its interaction with other factors and signal pathways for the regulation. Results show that miR-155 could act as a positive role in regulation of inflammatory response by repressing SHIP1 through enhancing PI3K/AKT signaling activation.

Materials And Methods

Ethical declaration

All animal experiments were conducted under the protocols approved by and in accordance with the guidelines of the Institute Animal Care and Use Committee of the University (approval number: GY2018-096).

Induction of Pulpal Lesion on Mice

In this study, Pulpitis model was established as our previous work described [15]. In brief, C57BL/6 mice were acquired from the Guangdong Medical Laboratory Animal Center. Exposed pulp group (n=10) mice were anesthetized with intra-abdominal injection of pentobarbital sodium (50mg/kg). Their dental pulp of maxillary first molars were opened on the occlusal surface. This procedure was carried out with #1/4 dental round diamond burs on high-speed handpieces and operated under a surgical microscope. Mice without treatment were set to control group (n=10). Animals were sacrificed at time points of 1, 6, 12 and 24 hours after operation. Five samples were used for histopathological and immunohistochemical analysis, while the other 5 maxillae sections were prepared for analysis of IL-6, IL-1β and miR-155. Study of gene knockout animals was set with fifty miR-155$^{-/-}$ mice, which were purchased from the Jackson Laboratory, USA.

Cell culture and treatment

Mice odontoblast-like cell lines MDPC-23 cells (Cell Bio, Shanghai, China) were cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Sigma, MO, USA) containing 10% fetus bovine serum (FBS) and 1% of penicillin/streptomycin mixture at 37ºC and 5% CO_2. We observed the cells growth to cover 70-80% of the flask surface as an indication for LPS activation. The original medium was discarded and additional 1μg/mL LPS (Sigma) was used to stimulate cell inflammation for 3 hours, 6 hours, 12 hours, and 24 hours in different groups.

Cell transfection

MDPC-23 cells were transfected with miR-155 mimic, miR-155 inhibitor, miRNA negative control (miR-NC) and siRNA (RiboBio, Guangzhou, China) for 48hours using the riboFECT CP transfection reagent (RiboBio) following the manufacturer's protocol. After transfection, the stably transfected cells were treated with medium containing 1 μg/mL LPS for 12 hours [16].

Histopathological Analysis

The murine maxillae were dissected and fixed in 4% phosphate-buffered paraformaldehyde for 48 hours at room temperature. Then the samples were rinsed with PBS and decalcified with ethylene diamine tetra acetic acid (EDTA) solution for two weeks. After that, the samples were embedded in paraffin and cut into 4mm thick serial sections. Representational sections of each group were stained with hematoxylin-eosin (H&E) for further analysis.

Immunohistochemical Analysis

Animal samples were performed to immunohistochemical analysis according to a previously described protocol [16]. The primary antibodies were anti-PI3K p85 (1:300, Abcam, MA, USA), anti-AKT (1:300, Abcam) and SHIP1(1:300, Abcam), following by biotinylated secondary antibody. In each section, we chose three random different visions (× 400 magnification). Acquired photographs were analyzed with Image Pro Plus 6.0 software.

RNA isolation and quantitative real time-PCR （qRT-PCR）

Total RNA was extracted from cells or tissues using the Trizol reagent (Invitrogen, CA, USA) following the manufacturer's protocol. Conversion into cDNA were performed with PrimeScript RT Master Mix and Mir-X miRNA First-Strand Synthesis Kit (Takara, Dalian, China) for mRNA and miRNA determination respectively. qRT-PCR analysis for mRNA and miRNA were conducted by using SYBR Premix Ex Taq II solution (Takara) with relative quantification method. The mRNA level of U6 and GAPDH were used as normalization controls.

The following are the primer sequences: miR-155: forward ACACTCCAGCTGGGTTAATGCTAATTGTG and reverse CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACCCCTAT; IL-6: forward TCACAGAAGGAGTGGCTAAGGACC and reverse ACGCACTAGGTTTGCCGAGTAGAT; IL-1β: forward ACGGCCTTCCCTACTTC and reverse GCTGGACTGTTTCTAATGC; SHIP1: forward GTACAACTTGCCGTCCTGGT and reverse TGTGACTCCTGCTTCAAACG; GADPH: forward AGAAGGTGGTGAAGCAGGCATC and reverse AGAAGGTGGTGAAGCAGGCATC; U6: forward CTCGCTTCGGCAGCACA and reverse AACGCTTCACGAATTTGCGT.

Western Blot

The proteins were extracted with RIPA lysis buffer (Thermo Fisher, IL, USA). Protein concentration was determined with bicinchoninic acid (BCA) quantitative detection reagent kit following manufacturer's protocol (Epizyme Biotech, Shanghai, China). Western Blot was carried out in following steps: Proteins were separated with 10% SDS-PAGE electrophoresis and transferred on PVDF membranes, following manufacturer's protocol (Epizyme). We then probed proteins with primary antibodies: anti-PI3K p85 (1:2000, Abcam), anti-AKT (1:2000, Abcam), anti-SHIP1 (1:1000, Abcam, Cambridge, UK) and GAPDH (1:6000, Abcam) in 4°C overnight. Then the membranes were incubated with secondary antibody of goat anti-mouse IgG (Proteintech, IL, USA) carrying alkaline phosphatase for 1h at room temperature. We detected the membranes using BCIP/NBT kit. (Beyotime, Shanghai, China) following the protocol. Quantification was calculated with Image Pro Plus 6.0 software.

Luciferase reporter assay

The SHIP1 3'UTR target site and the luciferase reporter constructs carrying a putative miR-155-binding site were amplified by PCR (Promega, WI, USA). Cells were cultured on 48-well plates and co-transfected with psiCHECK-2 luciferase reporter plasmid, miR-155 control were transfected with double luciferase reporter vector plasmid. Plates were incubated in a CO_2 incubator at 37°C for 24~96 hours. Dual Luciferase Reporter Assay (Promega) was performed to calculate relative luciferase activity after 24 h transfection following manufacturer's protocol.

In brief, the pre-configured Luciferase Assay Reagent was added to the samples, we measured the luciferase reaction intensity (hLuc fluorescence value) and the internal reference Renilla fluorescence value (hRluc fluorescence value). Every sample was performed with three times repeat. The hLuc/hRluc ratio and the ratio of the control wells were statistically analyzed for verification of the accuracy of miRNAs' target sites. This procedure was set to determine whether the predicted binding site mutations will change the effects of miRNAs.

Enzyme-linked immunosorbent assay (ELISA)

The culture medium collected from the cells of different groups were measured directly by ELISA to quantify the production of IL-1β and IL-6 according to the manufacturer's protocol (R&D Systems, MN, USA). The results were analyzed with an enzyme - labelled meter (Thermo Fisher Scientific, MA, USA)

Statistical analysis

Data is presented as mean ± standard deviation (SD) for at least three independent experiments. Differences between groups were subjected to *t*-test or one-way ANOVA using SPSS statistics 16.0 (SPSS

Inc, Chicago, IL, USA). The values of $p < 0.05$ was considered to be statistically significant.

Results

miR-155 is a related regulator in pulpal inflammation animal model

In this study, we first explored miR-155 involvement in pulpitis with a C57BL/6 mice model. Experimental pulpal exposure C57BL/6 mice was established, and time points were set at 1, 6, 12 and 24 hours. The expressions of inflammatory factor IL-1β and IL-6 were both gradually increased in pulpal tissues (Fig.1 A). To discover the role of miR-155 in pulpal inflammation, we measured the miR-155 expression by qPCR. Results showed that miR-155 expression was lower in the pulpitis group compared to the control group, but its expression increased as the inflammation progressed, although there was no statistical significance (Fig.1 B). These results suggested that miR-155 is an involving factor of pulpitis in mice.

miR-155 is involved in the reaction of MDPC-23 cells to LPS treatment

The role of miR-155 on the regulation of LPS-treated MDPC-23 cells was evaluated to assess expression of miR-155 *in vitro*. Cells were stimulated with 1 μg/ml LPS for 3h, 6h, 12h and 24h time periods. Results showed IL-1β and IL-6 expression was increased in MDPC-23 cells (Fig.1 C). qPCR results of LPS-treated MDPC-23 cells showed that miR-155 was obviously down-regulated in LPS-stimulated cells compared to those at control group (Fig. 1 D).

miR-155 down-regulation in tissues and LPS-stimulated cells suggest that miR-155 may be an involving factor of pulpal inflammation in odontoblasts.

miR-155 modulates LPS-induced inflammation in MDPC-23 cells

MDPC-23 cells were transfected with miR-155 mimic or control, cells were stimulated with LPS as previous mentioned. Pro-inflammatory cytokines including IL-1β and IL-6 were measured by qRT-PCR and ELISA assay. qRT-PCR was performed to assess the efficiency of transfection (Fig.2 A). qRT-PCR and ELISA results showed that overexpression of miR-155 can increase the mRNA and protein levels of IL-6 and IL-1β after exposure to LPS (1 μg/mL) for 12h. In addition, we use miR-155 inhibitor to investigate whether down regulation of miR-155 could induce opposite impacts. As expected, the data confirmed that the expression and production of IL-6 and IL-1β were decreased compared with control group (Fig.2 B). Taken together, these results demonstrated that miR-155 plays a positive regulative role in LPS-induced inflammatory productions.

miR-155 deficiency significantly alleviates pulpal inflammatory responses *in vivo*

To further examine the role of miR-155 *in vivo,* experimental pulpal animal models was established with C57BL/6 mice and miR-155$^{-/-}$ mice. Results demonstrated that the miR-155$^{-/-}$mice exhibited mild damage of acute inflammatory cells compared with the C57BL/6 mice as measured by H&E staining assays (Fig.2 C). Additionally, qRT-PCR analysis also confirmed that the expression level of IL-6 and IL-1β was decreased in miR-155$^{-/-}$ mice at 6 hours and 12 hours (Fig.2 D), indicating that miR-155$^{-/-}$ mice results in lower susceptibility to pulpitis in animal model.

miR-155 increase inflammatory responses through directly targeting SHIP1 3'UTR

To explore how miR-155 regulated LPS-induced dental pulp inflammation, we use three publicly available algorithms (miRBase, TargetScan and miRanda) to identify potential target genes of miR-155. These results plus with the support of earlier reports [17] showed that SHIP1 is highly likely to be the target gene of miR-155 (Fig.3 A). To confirm the association of miR-155 and SHIP1 in odontoblasts, 3'UTR regions of mouse SHIP1 containing putative binding site for miR-155 was constructed in psiCHECK2 by using Dual-Luciferase Reporter assays. As shown in Figure 3 B, overexpression of miR-155 repressed the luciferase activity in wild-type 3'UTR, whereas no difference was observed when cells were transfected with the SHIP1 mutant 3'UTR. Moreover, the data showed that the expression of SHIP1 was significantly decreased in miR-155-overexpressing cells at both the mRNA and protein levels, whereas knockdown of miR-155 can increase the expression of SHIP1 (Fig.3 C, D). Hence, these results demonstrate that miR-155 directly interacts with 3' UTR of SHIP1.

miR-155 regulates LPS-stimulated inflammatory responses by inhibiting SHIP1 and the activation of PI3K/AKT pathway

Investigation was performed to determine whether SHIP1 directly contributes to miR-155 function in pulpitis. MDPC-23 cells were co-transfected with miR-155 inhibitor combined with si-SHIP1. Our data suggested that the down regulation of inflammation mediated by miR-155 inhibitor was abolished by the knockdown of SHIP1 (Fig.4 A). In summary, these data further confirm that miR-155 regulates LPS-induced dental pulp inflammatory reactions by mediating SHIP1.

Previous studies have identified miR-155 remarkably promoted pro-inflammatory secretions through PI3K/AKT activation [18]. To investigate whether miR-155 increases LPS-induced inflammatory factor expression through PI3K/AKT pathway, MDPC-23 cells were transfected with miR-155 mimic or inhibitor with LPS stimulation to examine the expression of p-PI3K and p-AKT by western blot. As shown in Figure 4 B, the transfection with the miR-155 mimic obviously up-regulated the protein level of p-PI3K and p-AKT.

In contrast, knockdown of miR-155 markedly inhibited the p-PI3K and p-AKT in protein level. Furthermore, the results suggested that the protein level of p-PI3K and p-AKT were up regulated by the knockdown of SHIP1. In addition, the results showed that co-transfection of LY294002(PI3K/AKT inhibitor) and miR-155 mimic attenuated LPS-induced inflammatory effect of miR-155 mimic in MDPC-23 cells (Fig.4 C). Taken together, these findings provide evidence that miR-155 plays a positive role in LPS-induced inflammatory responses of MDPC-23 by inhibiting SHIP1 via PI3K/AKT pathway, thus promoting inflammation mediator production.

miR-155 deficiency significantly alleviates pulpal inflammatory responses leads to increase SHIP-1 expression by decreasing the activation of PI3K/AKT signaling in experimental pulpitis mice.

To compare the results *in vitro*, we then evaluated whether the change in miR-155 could modulate pulpal inflammation through PI3K/AKT signaling pathway *in vivo*. An expression analysis of SHIP1, p-PI3K and p-AKT was performed by using Immunohistochemistry and found that SHIP1 was significantly increased in pulp tissue of miR-155$^{-/-}$ mice compared to control group, whereas p-PI3K and p-AKT were decreased at 6 hours compared with C57BL/6 group, suggesting that the knockdown of miR-155 could contributes to relieve the inflammatory responses in experimental pulpitis (Fig.5 A, B).

Discussion

Recent studies have identified miR-155 as critical regulators in various physiological and pathologic processes, including hematopoietic stem-progenitor cells differentiation, inflammation, infections, and immune process of cancer formation [19, 20, 21]. The different functions of miR-155 in different tissues, is calling for studies to identify its role in particular circumstances. Emerging evidence has indicated that miR-155 have a direct impact on the expression of inflammatory cytokines in biological processes of periodontal disease [6]. The close relationship between miRNAs and periodontal disease, meaning that miR-155 could be considered as possible novel biomarkers for periodontal disease [22, 23]. Reports have indicated that in inflammatory cells like the macrophages and lymphocytes, the expression of miR-155 is regulated by LPS/ IFN-γ or NFκB, while miR-155 can inhibit SHIP1 expression, which leads to the activation of PI3K/Akt, mTOR and other factors downstream [24, 25]. The role and mechanism of miR-155 has attracted the attention of scholars about the prospection of a potential diagnosis marker and targets of therapeutic methods [26]. The pathogenesis of pulpitis is characterized by its inflammation environment, which resembles those oral diseases mentioned above. Due to the similarity, miR-155 involvement in the pathologic processes of pulpitis, along with its detailed mechanism, is worthwhile for exploration.

In our study, we utilize previously established experimental pulpitis animal models to observe whether miR-155 is also a biomarker in pulpitis [15]. The results showed that the expression of miR-155 was down-regulated in experimental pulpitis, which indicates that miR-155 could be recognized as a related factor in pulpal inflammation. By comparing with previous reports of miR-155's involvement in inflammation [27, 28], which generally state that miR-155 a promotor of inflammation, we deduced that miR-155 also acts as a pro inflammation factor in dental pulp, and our further experiments below proved it. For the explanation of the downregulation of miR-155 in animal model, the reasonable deduction can be that its expression was suppressed by inflammatory factors such as IFN-γ or NFκB [29]. There were also reports about other possible negative regulation mechanism of miR-155 in the initial stage of inflammation, as literatures have reported [30, 31].

During the progress of inflammation in pulpal tissues, odontoblasts are considered to be one of the first cell populations to sense the gram-negative bacterial stimulation [32]. Therefore, we chose MDPC-23 cells for our *in vitro* study. Lipopolysaccharide (LPS) is an important component of the gram-negative bacteria, participates in the inflammatory cytokine-mediated pathogenesis of pulpitis [33, 34]. The expression levels of pro-inflammatory cytokines are identical biomarkers for inflammation [35]. LPS can induce cells to express the pulpal inflammatory factor IL-6 and IL-1β, which are the criteria for the diagnosis of pulpal inflammation [36, 37]. In LPS-induced MDPC-23 cells, miR-155 was down-regulated. It was found that the expression of miR-155 and inflammatory factors were not in a linear relationship according to the development of inflammation but fluctuating one. miR-155 first decreased compared to the control group, and then showed a trend to growth according to the timeline. These results shared a similar trend with our murine pulp exposure model. This may indicate that the expression of pro-inflammatory factors may be regulated by a variety of factors. miR-155 was firstly suppressed and mainly activated at the later development stage of inflammation. Our observations *in vitro* and *in vivo* suggested that there was a close link between miR-155 and pulpal inflammation.

Our experiments *in vitro* showed that overexpression of miR-155 can increased the production of IL-1β and IL-6. Furthermore, knockdown of miR-155 decreased IL-1β and IL-6 at mRNA levels. The experimental pulpitis model with gene knockout animals was established to further investigate the biological function of miR-155. Consistent with the finding *in vitro*, miR-155$^{-/-}$ mice inhibited the expression of IL-6 and IL-1β compared with the miR-155 C57BL/6 group. Our findings have illustrated that miR-155 modulates pulpal inflammation by promoting the expression of IL-6 and IL-1β *in vitro* and *in vivo*.

For the downstream regulatory mechanism of miR-155, SHIP1 has been reported as an important factor [17]. In the searching for the most prominent targets of miR-155 in odontoblast, our bioinformatics results confirmed that SHIP1 was one of the most important factors in dental inflammation. Many reports have stated that SHIP1 is a key factor for miR-155 regulation in inflammation, fibrosis, and other immune processes [14, 21, 38]. The regulatory axis of SHIP1-PI3K/Akt-mTOR is a prominent signal pathway in inflammations [39]. We verified that miR-155 directly interacted and regulated the expression of SHIP1 in pulpal inflammation. Overexpression of miR-155 led to an obvious decrease of SHIP1 at mRNA and protein level. Furthermore, when co-transfected miR-155 inhibitor and si-SHIP1, the pro-inflammatory

cytokines were notably upregulated compared with the miR-155 inhibitor group at mRNA levels. That means the inhibition of inflammatory effects induced by the knockdown of miR-155 was reversed by si-SHIP1. All above evidence demonstrate that miR-155 regulates pulpal inflammatory reaction by targeting SHIP1.

The expression of SHIP1 is associated to PI3K and AKT phosphorylation expression [40]. PI3K is an upstream molecule of the AKT/mTOR signaling pathway and is a class of specific kinases that catalyze phosphatidylinositol lipids [41, 42]. According to relevant studies, miR-155 can mediate inflammatory responses by modulating PI3K/AKT pathway [13, 43]. Study have discovered that PI3K/AKT pathways worked as an effector of miR-155 and SHIP1 interaction in fibrosis of macrophages [13]. Studies have stated that PI3K/AKT can be a pro-inflammation effector in oral diseases like periodontitis [44]. They can also affect the process of different kinds of oral squamous cancers [45]. However, in pulpitis and dental pulp specific cells like the odontoblasts, their relationship still needs experimental evidence. In our study, further investigations demonstrated that overexpression of miR-155 or SHIP1 silence could enhance PI3K phosphorylation and AKT phosphorylation, whereas knockdown of miR-155 demonstrated an opposite trend in PI3K and AKT phosphorylation. LY294002 is a synthetic molecule, known to inhibit the activation of PI3K/AKT signaling pathway. It was used to further confirm the interaction of miR-155 and PI3K/AKT signaling pathway [46]. Our results demonstrated that LY294002 could reversed the inflammatory responses caused by miR-155 mimic. Similarly, in experimental pulpitis animal model, miR-155$^{-/-}$ mice exhibited lower positive staining of p-PI3K and p-AKT than the mice in C57BL/6 groups. The protein expression in this part of the results showed a non-linear fluctuation, which may be related to the rapid progression of pulpitis in mice. The sharp destruction of inflammation in the pulp tissue of mice reduces the secretion of cells in the pulp tissue, resulting in decreased expression of related proteins.

According to the above results, miR-155 contributes to pulpitis by suppressing SHIP1 Meanwhile, the activation of PI3K/AKT signaling pathway is involved with miR-155-regulated pulpal inflammatory development. The regulation of the miR-155-SHIP1-PI3K/AKT axis might set a novel mechanism for dental inflammation and for odontoblasts specifically.

In summary, our work has showed that miR-155 is a positive regulator in the process of dental pulpal inflammation. miR-155 promotes the progression of pulpitis through downregulated SHIP1, and controls the downstream PI3K/AKT pathway, which in turn enhances the expression of IL-6 and IL-1β. Therefore, the inhibition of miR-155 could effectively alleviate pulpitis and cytokine secretion. These results showed the potential of miR-155 as a clinical diagnosis marker, and the possibility of manipulating its expression to confine the inflammation of dental pulp in clinical practice.

Declarations

Acknowledgments

This work was supported by the Guangzhou Health Commission (No. 20191A011108), Science and Technology Research Projects of Universities in Guangzhou (No. 1201610402). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

All animal experimental procedures were approved by the Animal Ethical Committee in the School of Stomatology, Guangzhou Medical University, Guangzhou, China. Experiments were proceeded according to the guidelines and regulations (approval number: GY2018-096).

Informed Consent.

Not applicable as human participant is not involved in this study.

Consent to Participate.

Not applicable as human participant is not involved in this study.

Consent for Publication.

The manuscript is approved by all authors for publication. Human participant consent is not applicable as this study did not contain data from individual human samples.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Mr. Baishun Li: performed experiments and analyzed data. Dr. Liyang Guo: analyzed data and drafted the manuscript. Ms. Ying He: assisted experiments. Mr. Xingran Tu: assisted experiments. Ms. Jialin Zhong: assisted experiments. Prof. Hongbing Guan: revised manuscript. Prof. Qianzhou Jiang: designed and

administrated project. Prof. Yiguo Jiang: administrated project. All authors read and approved the final manuscript.

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Figures

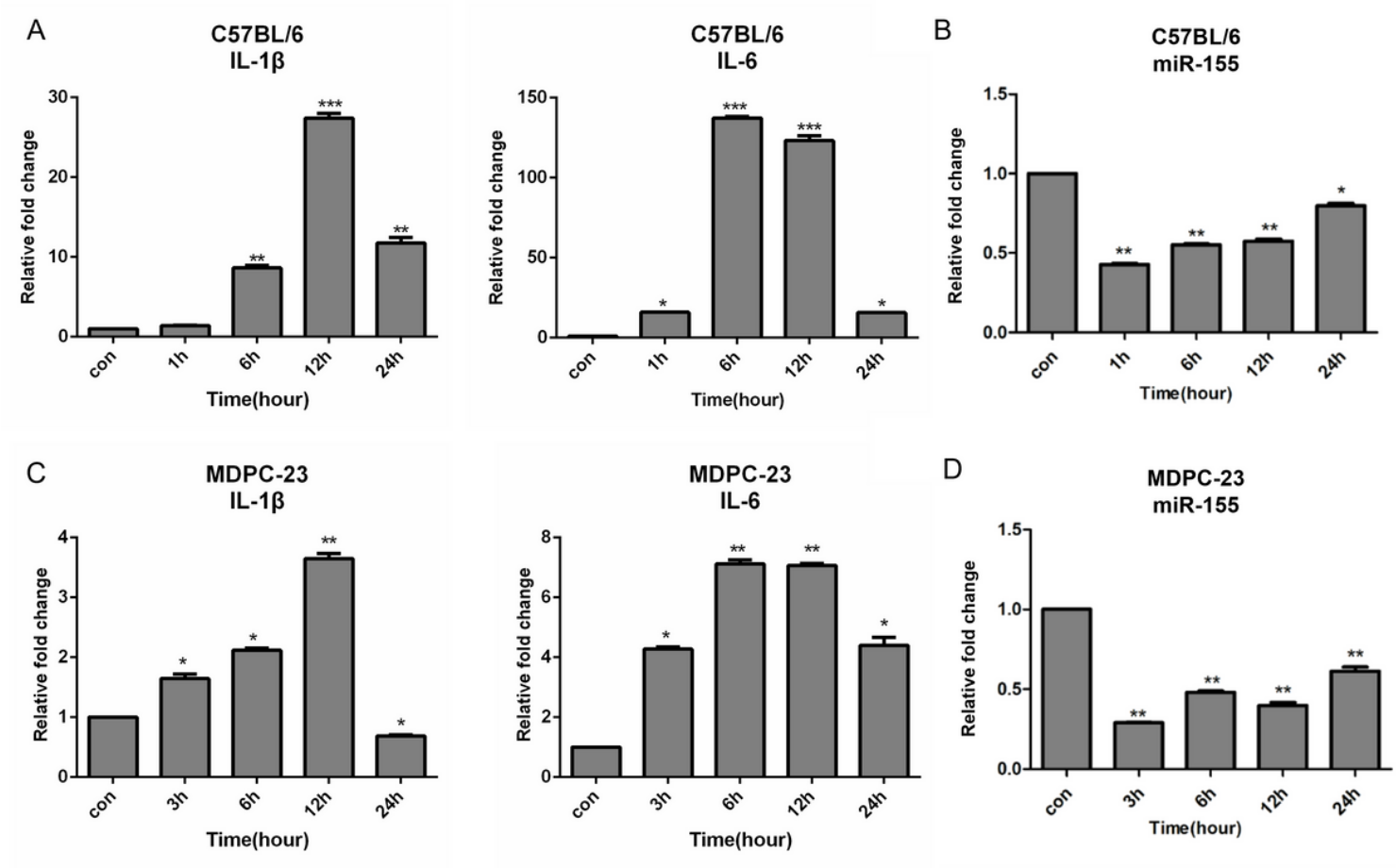

Figure 1

Down-regulation of miR-155 in mice pulpitis model and LPS-treated MDPC-23 cells. (A, B) The relative fold change of IL-1β, IL-6 and miR-155 of pupal inflammation in C57BL/6 mice at each time point. (C, D) MDPC-23 cells were treated with LPS (1μg/ml) at each time point. The level of IL-1β, IL-6 and miR-155 were measured by qRT-PCR. GAPDH and U6 was used as an internal control. Data are shown as mean ± SD of at least three independent experiments. *p<0.05, **p <0.01, ***p <0.001.

Figure 2

miR-155 is a positive regulator in LPS-induced inflammatory response in MDPC-23 cells and miR-155 deficiency alleviated mouse experimental pulpitis compared with C57BL/6 groups. (A) MDPC-23 cells were transfected with control or miR-155 mimic; control or miR-155 inhibitor. (B) The expression of IL-1β and IL-6 were determined by qRT-PCR, and the production of IL-1β and IL-6 were measured by ELISA. GAPDH and U6 were used as the internal control. Data are shown as mean ± SD of at least three independent experiments. *p<0.05, **p <0.01, ***p <0.001, ****p <0.0001. (C) Representative histological images of pulpal inflammatory tissues from C57BL/6 mice and miR-155-/- mice at each time point with H&E staining, red arrows indicate increased vascular permeability, asterisk indicates lymphocytes infiltration. (Original magnification, a-j, × 50, scale bar for 200 μm; al-j1, × 400, scale bar for 50μm); (D)

Experimental pulpal exposure mice were elevated the mRNA expression of IL-1β and IL-6. GAPDH was used as the internal control. Data are shown as mean ± SD of at least three independent experiments. *p<0.05.

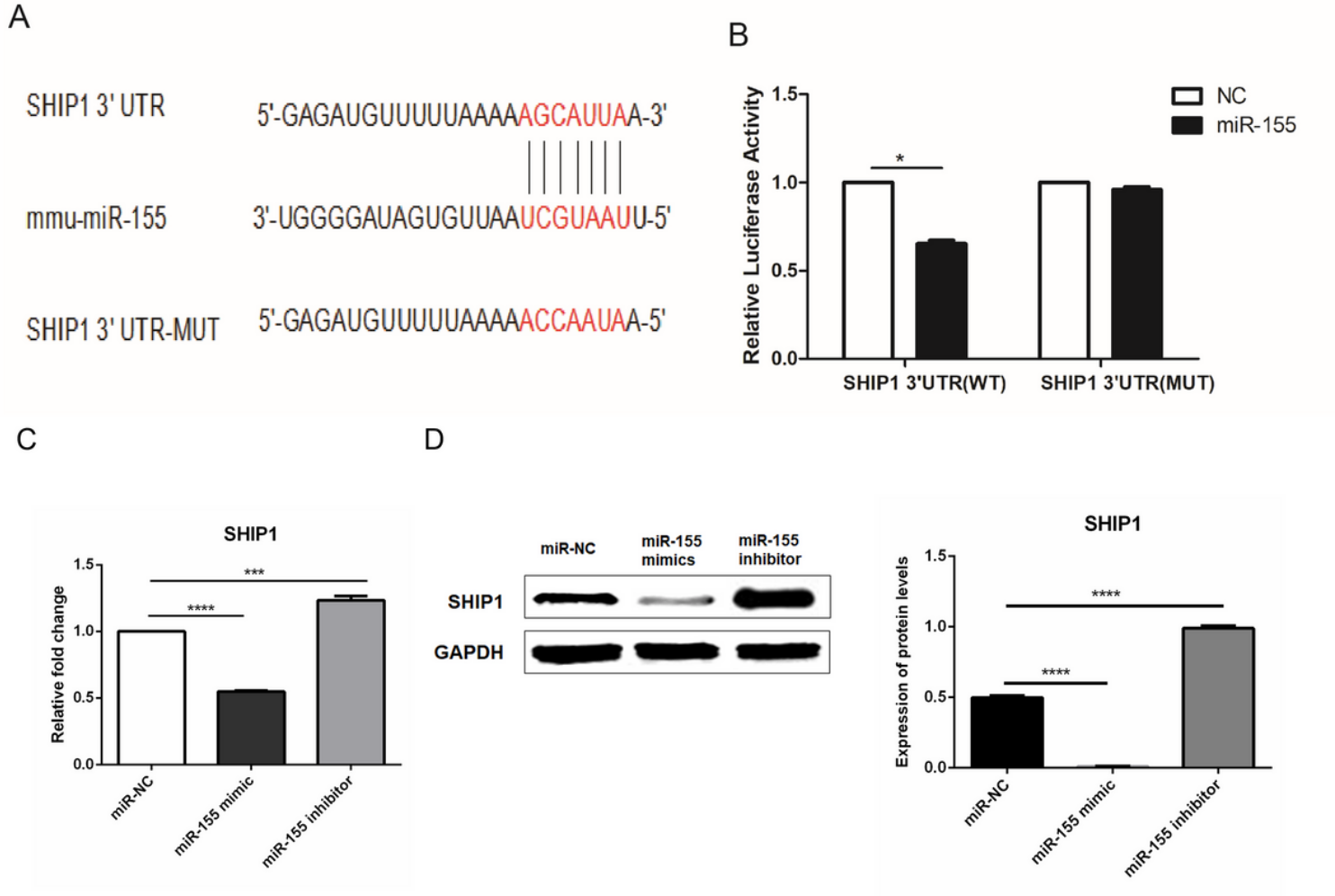

Figure 3

miR-155 directly targets SHIP1. (A) The predicted binding site of miR-155 on SHIP1 and its mutation sequence. (B) Psicheck-SHIP1-wild or mutant type was co-transfected with miR-155 mimic or mimic control. After 48 h, relative luciferase activity was measured. MDPC-23 cells were transfected with miR-155 NC, miR-155 mimic, or miR-155 inhibitors. (C) SHIP1 mRNA levels were measured by qRT-PCR. (D) SHIP1 protein levels were measured by western blot and qualified. GAPDH was used as an internal control. Data are shown as mean ± SD of at least three independent experiments. *p<0.05, ***p <0.001, ****p <0.0001.

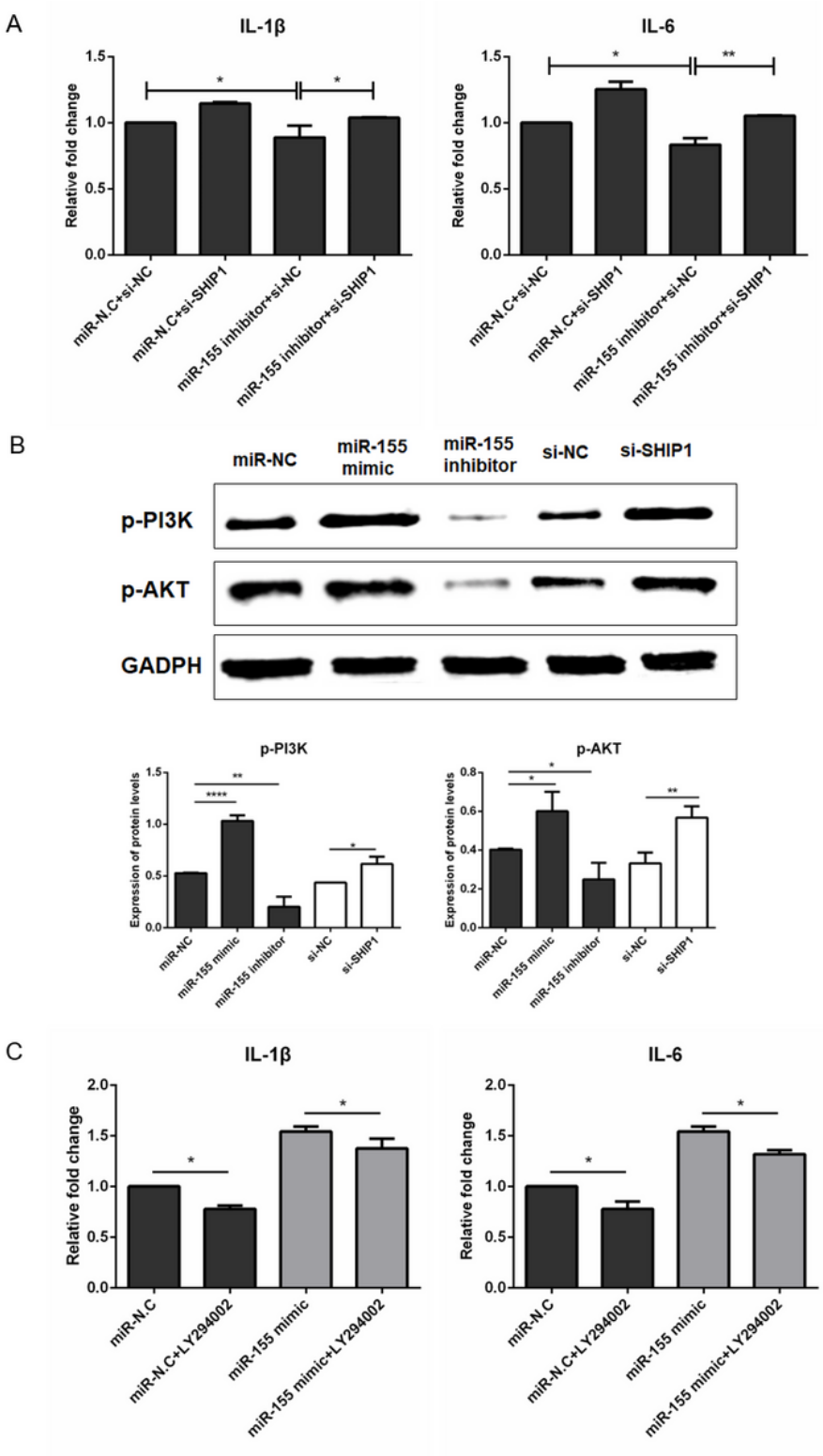

Figure 4

miR-155 regulates LPS-induced inflammatory responses by targeting SHIP1 through PI3K/AKT signaling pathway. (A) MDPC-23 cells were transfected with miR-155 inhibitor combined with si-SHIP1. After that, cells were treated with 1 μg/ml LPS for 12h. The mRNA levels of IL-1β and IL-6 were evaluated by qRT-PCR. (B) MDPC-23 cells were transfected with miR-155 mimic, miR-155 inhibitor, si-SHIP1 and each negative control. The protein levels of p-PI3K and p-AKT were determined by western blot assays. (C)

MDPC-23 cells were transfected with miR-155 mimics combined with LY294002. After that, cells were treated with 1 μg/ml LPS for 12h. The mRNA levels of IL-1β and IL-6 were evaluated by qRT-PCR. GAPDH was used as the internal control. Data are shown as mean ± SD of at least three independent experiments. *p<0.05, **p <0.01, ****p <0.0001.

Figure 5

miR-155 deficiency alleviated mouse experimental pulpitis by regulating the PI3K/AKT signaling pathway. (A) Immunohistochemistry staining for SHIP1, p-PI3K and p-AKT at each time point (n=10; original magnification, a-j, × 400, scale bar for 200μm). (B) Quantification of Immunohistochemistry images. Data are shown as mean ± SD of at least three independent experiments. *p<0.05.