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ORIGINAL RESEARCH

Role of Long Non-Coding RNA GUSBP11 in Chronic Periodontitis Through Regulation of miR-185-5p: A Retrospective Cohort Study

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Purpose: Previous studies have shown that long non-coding RNA GUSBP11 is abnormally expressed in patients with periodontitis, but the specific mechanism remains to be investigated. The purpose of this study was to explore the role of GUSBP11/miR-185-5p in chronic periodontitis (CP) and its potential mechanism, so as to provide a basis for elucidating the pathogenesis of CP.

Patients and Methods: The expression trends of GUSBP11 and miR-185-5p in gingival crevicular fluid of CP patients and control group were analyzed by RT-qPCR. Human gingival fibroblasts (HGF) induced by 10μg/mL LPS were used to construct CP cell models in vitro. The level of intracellular gene expression is regulated by cell transfection. The cell viability of HGF was evaluated by CCK-8 method, and the expression of HGF inflammatory factors was evaluated by ELISA. The targeting relationship between GUSBP11 and miR-185-5p was confirmed by luciferase reporter gene. The target genes of miR-185-5p were predicted using an online database, and the intersection target genes were obtained by constructing Venn diagram. Then GO analysis and KEGG pathway enrichment analysis were performed.

Results: Compared with the control group, the expression levels of GUSBP11 and miR-185-5p in gingival crevicular fluid of CP patients were up-regulated and down-regulated, respectively ($P < 0.001$). The levels of GUSBP11 and miR-185-5p increased and decreased with the severity of CP, respectively ($P < 0.01$). LPS induces the decrease of HGF activity and the activation of inflammatory response, and the decrease of GUSBP11 may prevent the adverse effect of LPS on HGF ($P < 0.001$). Dual luciferase reporter genes showed that miR-185-5p interacts with GUSBP11. The increase of miR-185-5p also significantly improved the negative effect of LPS induction on HGF ($P < 0.001$).

Conclusion: GUSBP11 promotes the inflammatory response and proliferation inhibition of human gingival fibroblasts induced by LPS by down-regulating miR-185-5p, thus promoting the development of CP.

Keywords: chronic periodontitis, inflammation, GUSBP11, miR-185-5p

Introduction

Chronic periodontitis (CP) is a chronic infectious disease that occurs in the periodontal supporting tissue. It is one of the most common oral diseases. Progressive attachment loss and alveolar bone destruction caused by periodontitis can lead to tooth loosening, displacement, or even loss, affecting chewing and pronunciation.[1] More importantly, CP is a risk factor for a variety of systemic diseases, such as diabetes, rheumatoid arthritis, and cardiovascular diseases.[2,3] Therefore, it is urgent to explore the pathogenesis of periodontitis so as to prevent and overcome this disease.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNA molecules with transcripts longer than 200nt that regulate gene expression at multiple levels, usually in the form of RNA-protein complexes.[4] Studies have shown that lncRNAs participate in a variety of important regulatory processes, and are closely related to almost all physiological and pathological processes such as cell proliferation, regulation, and immune regulation. Glucuronidase beta pseudogene 11 (GUSBP11) is located on human chromosome 22 and is known to play a role as an oncogene in a variety of human cancers, such as nasopharyngeal cancer, triple-negative breast cancer and lung cancer.[5–7] Recent studies have found that

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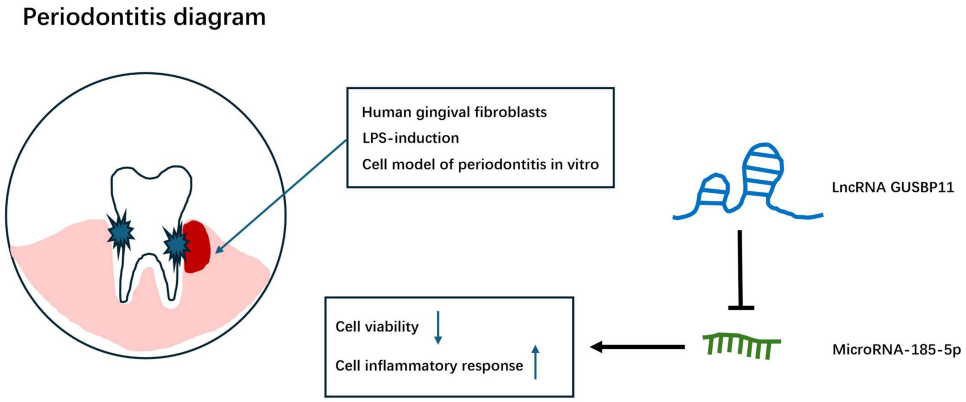
Graphical Abstract



GUSBP11 is involved in the regulation of the occurrence and development of immune-inflammatory diseases. For example, Zhao et al observed that the abnormal expression of GUSBP11 in dermatomyositis was closely related to immune cell infiltration.[8] A study of acute liver failure showed that abnormally increased levels of more than a dozen lncRNAs, including GUSBP11, were associated with increased levels of CRP, an indicator of inflammation in the acute phase of the disease.[9] Wu et al reported that three lncRNAs used to regulate immune response, including GUSBP11, were abnormally upregulated in CP, and the expression level of these lncRNAs was positively correlated with the proportion of plasma cells in periodontitis, which would help to explain the immune mechanism theory in the occurrence of periodontitis.[10] However, the action mechanism of GUSBP11 in CP still needs to be clarified.

MicroRNAs (miRNAs) are a class of endogenous, highly conserved, single-stranded non-coding RNAs with a size of about 22 nucleotides.[11] With the continuous development of RNA omics and bioinformatics prediction, more than 500 miRNAs have been found in the human body, which play an important role in the development of human diseases. miR-185-5p is located on human chromosome 22q11.2 and has been shown to have anti-inflammatory effects in many diseases, such as neuropathic pain and atherosclerosis.[12,13] Recent bioinformatics studies have found that miR-185-5p may be related to the progression of periodontitis, and the mechanism of action involves the abnormal migration of periodontal membrane cells and the driving of inflammatory response.[14,15] StarBase database predicted that miR-185-5p was the target gene of GUSBP11, so we speculated that GUSBP11/miR-185-5p might play a vital role in CP regulation.

In this study, we determined the relationship between GUSBP11 and miR-185-5p through clinical and cellular experiments. It was found that GUSBP11 increased significantly in CP patients compared to healthy individuals. In addition, cytokines and plaque index of CP patients were significantly positively correlated with GUSBP11 expression, so it was speculated that GUSBP11 level might be correlated with CP severity. The results of in vitro studies showed that inhibition of GUSBP11 alleviated LPS-induced cellular inflammation and activity inhibition by increasing the expression of miR-185-5p, thereby alleviating the inflammatory damage of human gum fibroblasts induced by LPS.

Materials and Methods

Populations and Samples

The study included 65 patients diagnosed with CP for the first time. The inclusion criteria are as follows: 1) meet the diagnostic criteria of CP; 2) first diagnosis and no treatment; 3) complete clinical data. Exclusion criteria: 1) medical or surgical treatment within 6 months; 2) suffering from diseases such as diabetes and malignant tumors. In addition, 50 individuals who were examined in this hospital at the same time and confirmed to have no periodontal disease were selected as the control group. The project complies with the Declaration of Helsinki. The conduct of this study has been approved by the Ethics Committee of Affiliated Hospital of Nantong University, and all subjects have given informed consent.

Acquisition of Gingival Crevicular Fluid

The research team selected a tooth with the most severe CP from the maxillary and mandibular first and second molar teeth as the test tooth to collect gingival crevicular fluid. The right maxillary and mandibular first molars were selected as the test teeth in the control group. Samples should be collected 24 hours after periodontal examination to prevent the components of gingival crevicular fluid from being interfered with by periodontal examination. Before sampling, subjects gargled with clean water. The doctor cleaned the tooth surface thoroughly with probing, and then inserted each filter paper tip into the gingival groove at four sites with 20g force, removed it 30s later, and quickly placed it into the EP tube. If the filter paper is contaminated by saliva and blood, it should be discarded quickly and sampled again after 10min.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA from gingival crevicular fluid samples and cell samples was extracted using TRIzol reagent provided by Invitrogen Corp. (Carlsbad, CA, USA). The total RNA was reverse-transcribed into cDNA by PrimeScript RT Reagent kit (TaKaRa, Shiga, Japan), and then amplified by Roche LightCycler480 fluorescence quantitative PCR system (Roche Molecular Systems Inc., Basel, Switzerland) using the SYBR Premix Ex Taq II kit (Takara Bio, Inc., Dalian China) followed by the kit instructions for reaction procedures. GAPDH and U6 were selected as internal controls for GUSBP11 and miR-185-5p, respectively, and the relative expression levels of target genes were calculated by $2^{-\Delta\Delta Ct}$ method. The primer sequence is as follows:

Genes	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
GUSBP11	GCTCTTATCCCCGTAGATGC	CAACGTGCAGCCATACAACA
miR-185-5p	CGCTGGAGAGAAAGGCAGT	GTGCAGGGTCCGAGGT
GAPDH	GAAATCCCATCACCATCTTCCA	CATGTGGGCCATGAGGTCCACCAC
U6	CTCGCTTCGGCAGCACA	AACGCTTCAGGAATTTGCGT

Cell Culture and Transfection

Human gingival fibroblasts (HGF) were obtained from Shanghai Honsun Biological Technology Co., Ltd (Shanghai, China). HGF is suitable for growth in dulbecco's modified eagle medium (DMEM, Gibco, Carlsbad, CA, USA) containing 1% penicillin/streptomycin and 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA) and cultured in a humid atmosphere containing 5%CO_2 at 37°C. HGF at logarithmic growth stage was taken, cells were counted, inoculated into 24-well plates at a density of 5×10^4 cells/well, and cultured for 24h until the cell confluence exceeded 70%. The transfection process was conducted in strict accordance with the instructions of the Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA). Cell culture was performed for 24h after transfection.

Determination of LPS Concentration

HGF at logarithmic growth stage was inoculated into 6-well plates at a density of 2×10^5 cells/well and cultured for 24h. The P. gingivalis LPS (P.g LPS) was obtained from Sigma (St. Louis, MO, USA). The HGF was stimulated with different concentrations of P.g LPS solution (0, 0.1, 1.0, 10.0μg/mL) for 6h and 12h, respectively. The expression level of GUSBP11 was detected by RT-qPCR (Roche Molecular Systems Inc., Basel, Switzerland).

Cell Counting Kit-8 Assay

The statically transfected cells were inoculated into the 96-well plate (3×10^4 cells/well), and after treatment according to the experimental procedure, the upper culture medium was removed. At the pre-set time point (0h, 24h, 48h, 72h), 20μL of CCK-8 reagent (Sigma-Aldrich, St. Louis, MO, USA) was added into cells and incubated for another 1h, and the optical density (OD) value at 450nm was detected.

Enzyme-Linked Immunosorbent Assay (ELISA)

After the cells were treated according to the experimental procedure, the levels of IL-6, IL-1β and TNF-α in the supernatant of cell culture were determined according to the instructions of the ELISA kit (Beyotime, Shanghai, China) of IL-6, IL-1β and TNF-α, respectively. In short, the biotinylated antibody working solution was added to the culture plate and incubated at 37°C for 30min.Then, after washing with PBS for three times, each well was added with 100μL enzyme binding solution and continued incubation for 10min. The reaction was terminated by adding 100μL color developing solution into the hole for 15min, and then the stop buffer was added to stop the reaction. Finally, OD values at 450nm were detected by microplate reader, and cytokine concentrations were calculated by standard curve.

Dual-Luciferase Reporter Gene

The binding region of miR-185-5p and GUSBP11 was predicted by ENCORI database (https://rnasysu.com/encori/ceRNA.php?source=lncRNA), and wild type (WT) pmir-GLO plasmid and mutant (MUT) pmir-GLO plasmid of GUSBP11 were constructed. The above recombinant plasmids were transfected into HGF with miR-185-5p mimic or inhibitor using Lipofectamine 2000, respectively (6-well culture plate, density $1.0\times10^{6}cm^{-2}$). After 48h of incubation, the luciferase activity of cells in each group was detected and analyzed by dual-luciferase reporter gene detection system (Promega Corp., Madison, WI, USA).

Target Gene Prediction and Functional Annotation of miR-185-5p

Four online databases, TargetScan (https://www.targetscan.org/vert_80/), ENCORI (https://rnasysu.com/encori/ceRNA.php?source=lncRNA), miRPathDB (https://mpd.bioinf.uni-sb.de/overview.html) and EVmiRNA (https://guolab.wchscu.cn/EVmiRNA//#!/), were used to predict the target genes of miR-185-5p, and then the above four groups of target gene data were imported into Veney online tool to obtain Venn diagram and intersection target genes. The DAVID database was used to perform Gene ontology (GO) functional annotation analysis of the predicted intersection target genes of miR-185-5p, including biological processes (BP), cell components (CC) and molecular function (MF) enrichment analysis. Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed on intersecting target genes to obtain signal transduction pathways and disease pathways involved in target genes.

Data Analysis

SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad, La Jolla, CA, USA) statistical software were used to analyze the results. The results are presented as mean ± standard deviation (SD). Independent sample *t* test was used for the comparison of two groups, and one-way analysis of variance (ANOVA) was used for the comparison of multiple groups. Pearson correlation coefficient was used to evaluate the correlation between GUSBP11 and clinical indicators of abnormal expression in CP patients. Each experiment was repeated at least three times, and $P < 0.05$ means significant difference.

Results

Comparison of Baseline Information

The baseline information of CP patients and control population is summarized in Table 1. The differences in gender, age and BMI between the two groups were not statistically significant, so they were comparable ($P > 0.05$). In addition, compared with the control group, significant differences in PLI, GI, PD, pro-inflammatory cytokines IL-6, IL-8 and anti-inflammatory cytokines IL-10 were observed in CP patients ($P <0.001$).

Expression Profile of GUSBP11 in CP

Through the analysis of GUSBP11 expression level in gingival crevicular fluid, it can be observed that the GUSBP11 levels in CP patients is higher than that in control group (Figure 1A, $P < 0.001$). CP patients were further divided into three groups according to the severity of the disease: mild (n = 22), moderate (n = 25) and severe (n = 18) groups. It was observed that GUSBP11 gradually enhanced with the increase of the disease severity (Figure 1B, $P < 0.001$).

Table 1 Baseline Data for the Subject Population

Characteristics	Controls (n = 50)	CP (n = 65)	*P*
Sex (male/female)	27/23	38/27	0.657
Age (years)	43.62±6.49	44.87±5.76	0.336
BMI (kg/m^2)	22.34±2.29	22.61±1.66	0.419
PLI	0.54±0.50	2.37±0.63	<0.001
GI	0.98±0.71	2.86±0.35	<0.001
PD	1.92±0.54	6.12±1.78	<0.001
IL-6 (pg/mL)	7.63±2.84	19.37±4.42	<0.001
IL-8 (pg/mL)	37.26±14.91	126.53±22.18	<0.001
IL-10 (pg/mL)	18.19±10.06	5.74±2.33	<0.001

Abbreviations: CP, chronic periodontitis; BMI, body mass index; PLI, plaque index; GI, gingival index; PD, probing depth; IL-6, interleukin-6. $P < 0.05$ was considered significant difference.

Correlation Analysis Between GUSBP11 and Abnormal Index

Pearson correlation analysis was conducted between GUSBP11 and clinical indicators with significant differences in Table 1. The results showed that PLI, GI, PD, IL-6 and IL-8 levels in CP patients were positively correlated with GUSBP11 expression ($P < 0.01$), while IL-10 levels were negatively correlated with GUSBP11 expression (Table 2, $P < 0.001$).

Effect of GUSBP11 on HGF Induced by LPS

The effect of different concentrations of LPS on GUSBP11 expression in HGF cells was detected. As shown in Figure 2A, after 6h induction, only 10μg/mL LPS could significantly increase GUSBP11 expression level in HGF cells ($P < 0.001$). When treated with LPS for 12h, both 1 and 10μg/mL LPS could significantly increase the expression of GUSBP11 compared with 0μg/mL LPS, while GUSBP11 tended to be higher at 10μg/mL ($P < 0.001$). Therefore, 10μg/mL LPS was selected as the cell-induced concentration for subsequent experiments. In HGF, transfection of si-GUSBP11 can significantly down-regulate the expression level of GUSBP11 (Figure 2B, $P < 0.001$). GUSBP11 was significantly upregulated in LPS-induced HGF, and pre-transfection of si-GUSBP11 significantly inhibited LPS-induced GUSBP11 upregulation (Figure 2C, $P < 0.001$). Further evaluation of cell function and inflammation showed that the reduction of GUSBP11 alleviated the inhibitory effect of LPS treatment on cell viability and the acceleration effect on inflammatory response (Figure 2D–E, $P < 0.001$).

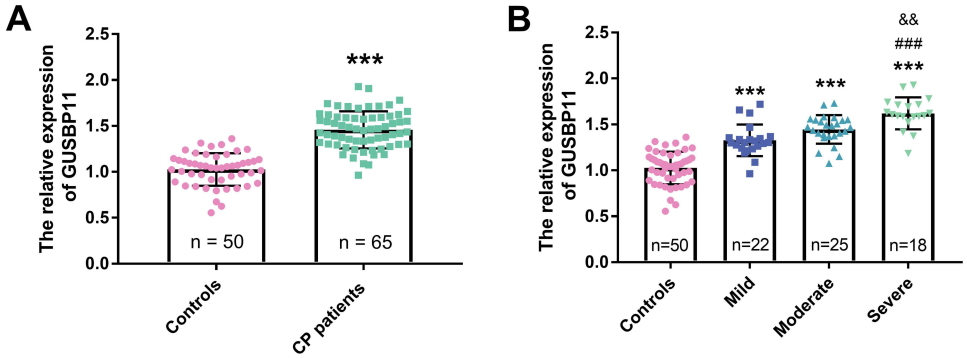


Figure 1 Expression level of GUSBP11 in gingival crevicular fluid of patients with chronic periodontitis (CP) and patients with different degrees of disease. (**A**). The expression level of GUSBP11 in gingival crevicular fluid of CP patients was upregulated compared with that of control group. (**B**). The expression of CUSBP11 increased progressively with the severity of the disease. $^{***}P < 0.001$ vs Controls, $^{\#\#\#}P < 0.001$ vs Mild group, $^{\&\&}P < 0.01$ vs Moderate group.

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Table 2 Pearson Method Analyzed the Correlation Between Clinical Indexes and GUSBP11 Expression

Indicators	r	*P*
PLI	0.622	<0.001
GI	0.403	0.001
PD	0.705	<0.001
IL-6 (pg/mL)	0.474	<0.001
IL-8 (pg/mL)	0.698	<0.001
IL-10 (pg/mL)	−0.822	<0.001

Notes: $P < 0.05$ was considered significant difference.
Abbreviations: PLI, plaque index; GI, gingival index; PD, probing depth; IL-6, interleukin-6.

miR-185-5p is Directly Target of GUSBP11

The complementary sites of miR-185-5p and GUSBP11 were predicted by StarBase V2.0, as shown in Figure 3A. The luciferase activity of wild-type GUSBP11 vector was evaluated using luciferase reports. As shown in Figure 3B, it was found that miR-185-5p mimic could significantly improve the luciferase activity of wild-type vector ($P < 0.001$), but had no effect on the activity of mutant vector ($P > 0.05$). Moreover, it was observed in gingival crevicular fluid samples that the expression level of miR-185-5p in CP patients was significantly lower than that in the control group (Figure 3C, $P < 0.001$). Further analysis showed that the expression of miR-185-5p decreased with the increase of CP severity (Figure 3D, $P < 0.01$). Pearson correlation analysis showed that the level of miR-185-5p was negatively correlated with GUSBP11 in clinical samples of CP patients (Figure 3E, $P < 0.001$).

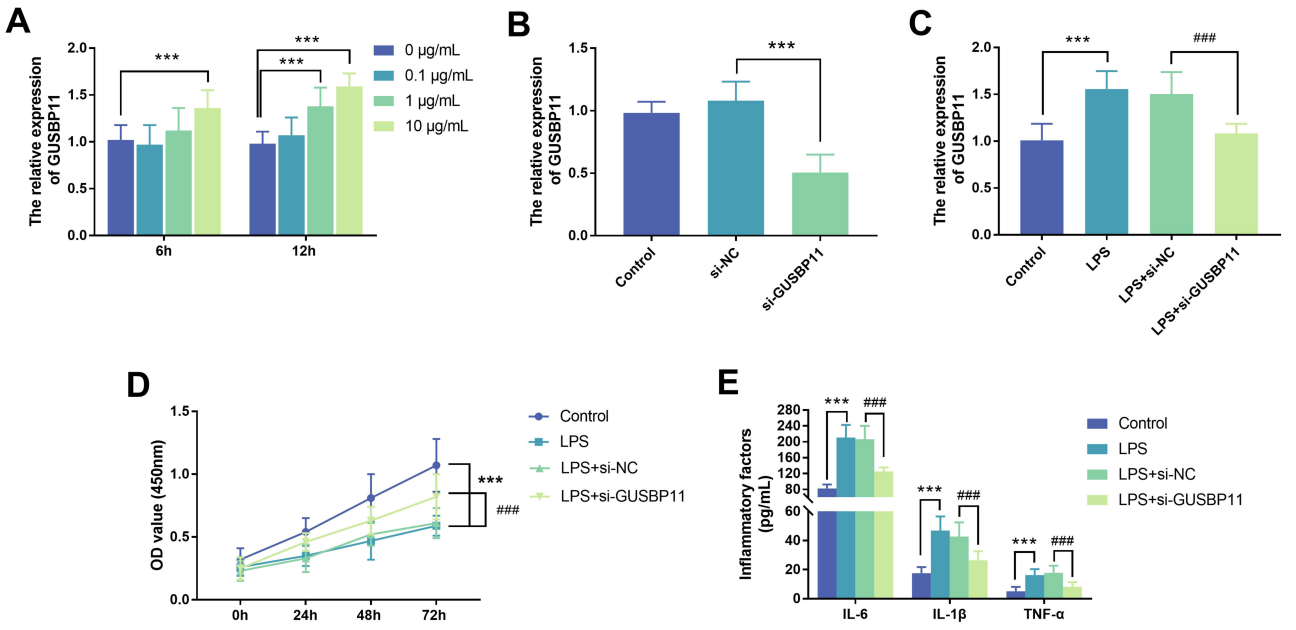

Figure 2 The regulatory effect of GUSBP11 on the biological function of periodontitis cell model. (**A**). Screening of LPS modeling concentration. (**B**). Effect of transfection of si-GUSBP11 on GUSBP11 expression in human gingival fibroblasts. (**C**). Effect of transfection of si-GUSBP11 on GUSBP11 expression in periodontitis cell model. (**D**). Down-regulation of GUSBP11 can enhance the viability of LPS-induced cells. (**E**). Inhibition of GUSBP11 alleviates the LPS-induced cellular inflammatory response. ***$P < 0.001$ vs 0 μg/mL group or si-NC group or Control group, ###$P < 0.001$ vs LPS+si-NC group.

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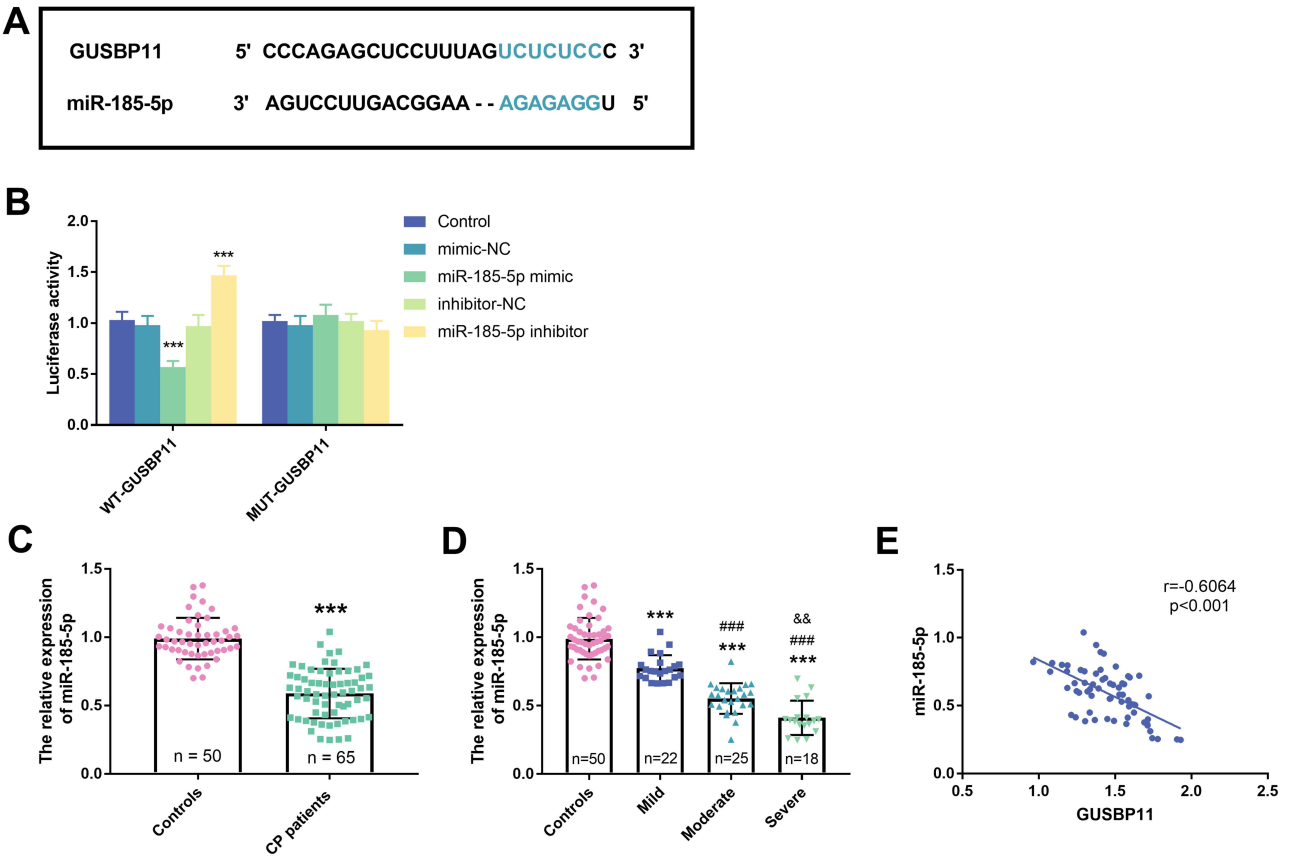

Figure 3 Prediction and validation of GUSBP11 target gene. (**A**). Complementary sequence of GUSBP11 and miR-185-5p. (**B**). Dual-luciferase reporter gene assay. (**C**). miR-185-5p expression decreased in gingival crevicular fluid in patients with chronic periodontitis. (**D**). The expression of miR-185-5p decreased progressively with the severity of the disease. (**E**). The expression level of miR-185-5p in clinical samples was negatively correlated with GUSBP11. $^{***}P < 0.001$ vs Controls, $^{\#\#\#}P < 0.001$ vs Mild group, $^{\&\&}P < 0.01$ vs Moderate group.

USBP11 Regulates the Function and Inflammation of CP Cell Models by Targeting miR-185-5p

The expression of miR-185-5p was reduced in LPS treated HGF, while pre-transfection of miR-185-5p mimic could significantly up-regulate the level of miR-185-5p in LPS models (Figure 4A, $P < 0.001$). Additionally, the addition of miR-185-5p mimics can significantly improve the cell viability of LPS cell models and inhibit the production of cellular inflammatory factors (Figure 4B and C, $P < 0.01$). Further, the up-regulation effect of miR-185-5p induced by transfection of si-GUSBP11 was offset by the addition of miR-185-5p inhibitors (Figure 4D, $P < 0.001$). Similarly, in terms of cell viability and inflammation, the positive effects of transfection of si-GUSBP11 on LPS cell models were offset by the reduction of miR-185-5p, which was characterized by decreased cell viability and increased production of inflammatory factors (Figure 4E and F, $P < 0.01$).

Prediction and Functional Analysis of miR-185-5p Target Genes

TargetScan, ENCORI, miRPathDB and EVmiRNA were used to predict the downstream target genes of miR-185-5p, and the predicted genes were mapped with Venn diagram, and the target genes in the overlap of the four databases were selected for follow-up study. The Venn diagram is shown in Figure 5A, at least 90 target genes were predicted by the four databases together. GO functional annotation analysis of these 90 target genes showed that these target genes were widely involved in biological processes such as cell adhesion, intracellular transport, pinocytosis, NMDA receptor activity regulation, and histone modification, and were mainly enriched in the nuclear membrane cavity and ribosomes. At the same time, these target genes also enriched the molecular functions of lipoprotein particle receptor activity, telomerase

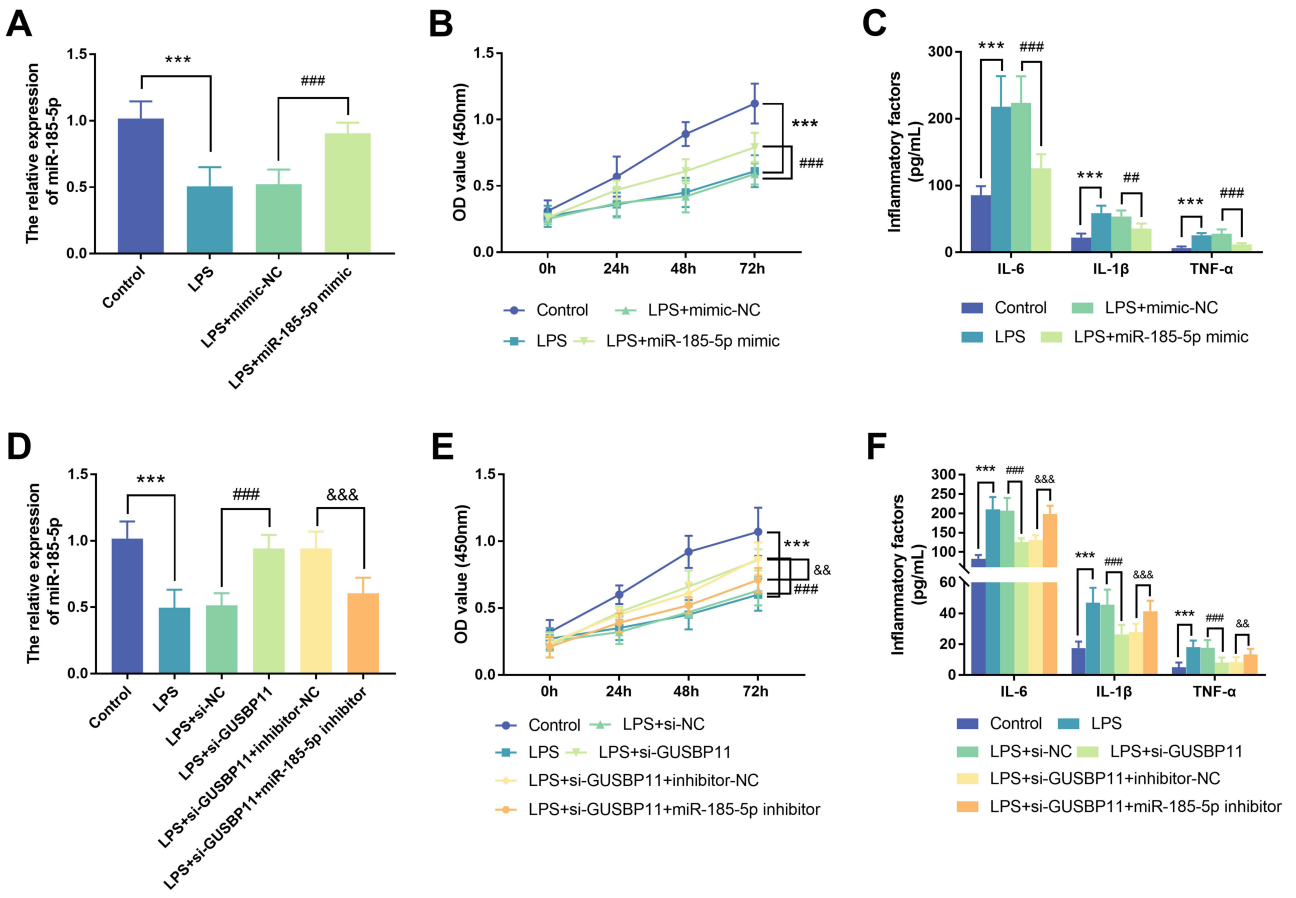

Figure 4 Regulation of the function of miR-185-5p in periodontitis cell models. (**A**). Transfection of miR-185-5p can up-regulate the expression of LPS-induced intracellular miR-185-5p. (**B**). Up-regulation of miR-185-5p increased the activity of cell models. (**C**). The increase of miR-185-5p inhibited the LPS-induced inflammatory response. ***P < 0.001 vs Controls, ##P < 0.01, ###P < 0.001 vs LPS+mimic-NC group. (**D**). Transfection of miR-185-5p can reverse the promotion of miR-185-5p induced by GUSBP11 suppression. (**E**). The reduction of miR-185-5p negated the promoting effect of transfection with si-GUSBP11 on cell viability. (**F**). Inhibition of miR-185-5p can significantly reverse the inhibitory effect of si-GUSBP11 on inflammatory response. ***P < 0.001 vs Control group, ###P < 0.001 vs LPS+si-NC group, &&P < 0.01, &&&P < 0.001 vs LPS+si-GUSBP11+inhibitor-NC group.

RNA binding, protein tyrosine kinase activity and so on (Figure 5B–D). Further, KEGG pathway analysis of the above target genes showed that the target genes of miR-185-5p were widely involved in the p53 signaling pathway, ErbB signaling pathway, and GnRH signaling pathway (Figure 5E).

Discussion

The results of this study showed that compared with the control group, the expression of GUSBP11 and miR-185-5p in gingival crevicular fluid in CP group showed an increasing trend and a decreasing trend, respectively. With the increase of disease severity, the expression of GUSBP11 showed a progressive increase, while the expression of miR-185-5p showed a progressive decrease. These results suggest that GUSBP11/miR-185-5p may be involved in the pathogenesis of CP. In the LPS-induced HGF cell model, up-regulated and down-regulated GUSBP11 and miR-185-5p were also observed, which was consistent with the results in clinical samples. It was found that miR-185-5p is a target gene of GUSBP11 and is negatively regulated by the latter. In the HGF model, inhibition of GUSBP11 expression can improve the viability and inflammatory response of LPS-induced HGF by upregulating miR-185-5p. In the study, the expression levels of GUSBP11 and miR-185-5p have been verified, which are consistent with the results of previously published literature studies. These provide a new way for us to further explore the pathogenesis of CP.

As an important cellular component in periodontal tissue, HGF can secrete a variety of inflammatory cytokines and participate in the occurrence and development of periodontitis.[16] Pathogenic microorganism infection is the initiating factor of CP. The infection of pathogenic microorganism is the initial factor of periodontitis. LPS is the most important

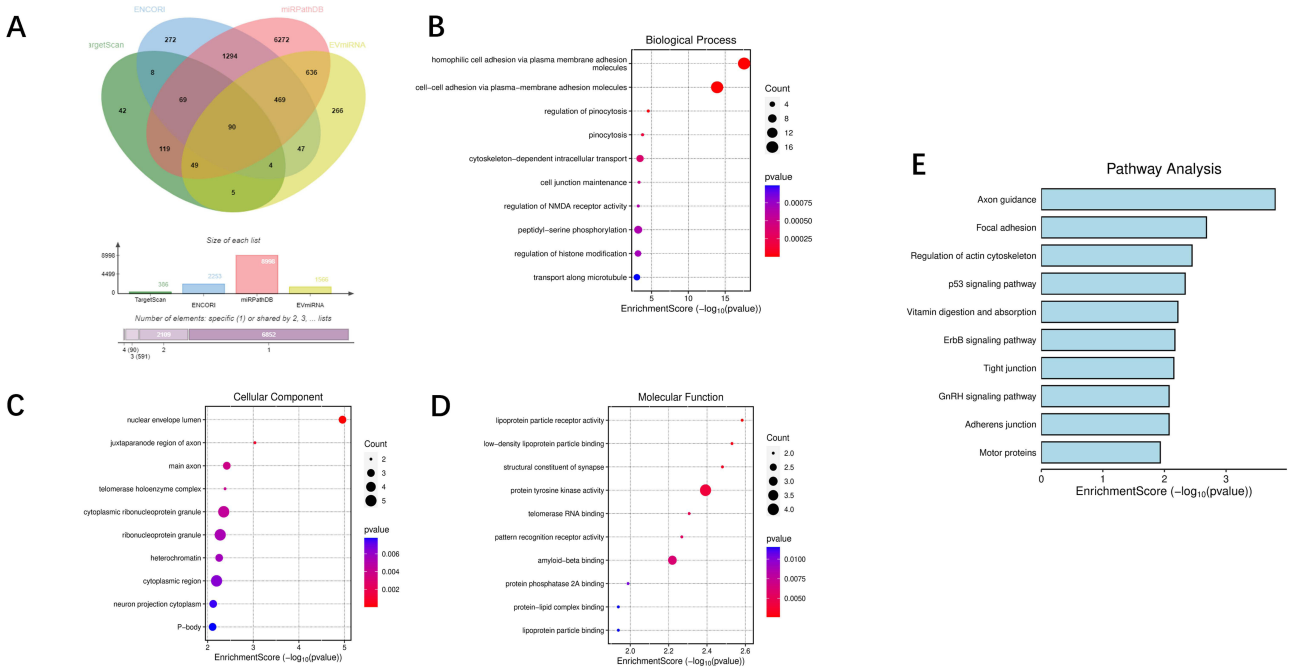

Figure 5 Bioinformatics analysis of miR-185-5p. (**A**). Venn diagram of downstream target genes of miR-185-5p predicted by four online databases. (**B**). The biological process of main enrichment of target genes in GO analysis results. (**C**). The cellular component of main enrichment of target genes in GO analysis results. (**D**). The molecular function of main enrichment of target genes in GO analysis results. (**E**). KEGG pathway enrichment analysis.

pathogenic factor on the surface of gram-negative bacteria, which can stimulate a variety of cells to secrete cytokines and induce inflammation.[17] P.g is the main pathogen of CP, therefore, to explore the role and potential mechanism of GUSBP11 and miR-185-5p in CP, we used P.g LPS-induced HGF as the cell model of CP in vitro. In cell studies, we found that GUSBP11 is a target molecule of miR-185-5p. A great number of studies have found that lncRNA, as a sponge/bait for miRNA, affects the level and function of the latter. These lncRNAs are also known as competitive endogenous RNAs (ceRNAs). For example, in the development of gastric cancer, a high level of PVT1 acts as a sponge for miR-16, elevates CCND1, and enhances the invasion and migration ability of gastric cancer cells.[18] Similarly, in this study, we observed that the decrease of GUSBP11 can correspondingly up-regulate the expression of miR-185-5p, further elucidate the targeting relationship between GUSBP11 and miR-185-5p.

IL-1β is an important immunomodulatory and pro-inflammatory factor in the body. Many clinical reports have indicated that the expression of IL-1β in gingival crevicular fluid in patients with CP activity has increased.[19] IL-6 is a cytokine that plays an important role in body defense, immune inflammatory response and hematopoietic processes.[20] Similarly, as a proinflammatory cytokine, TNF-α also plays a role in initiating inflammatory response in periodontitis.[21] In cell studies, the secretion of IL-1β IL-6 and TNF-α in the supernatant of HGF cell culture was increased after induction by LPS, indicating that LPS treatment activated the inflammatory response of HGF.[22,23] Subsequently, we found that the downregulation of GUSBP11 helps to inhibit the secretion of inflammatory factors, while the increase of miR-185-5p is effective in inhibiting the inflammatory response. Although GUSBP11 is an immunomodulation-related lncRNA, its immunomodulatory mechanism has not yet been studied. As previously mentioned, GUSBP11 is significantly correlated with Mi macrophages, activated NK cells, resting NK cells and other immune cells, all of which can induce the production of pro-inflammatory cytokines under certain conditions.[8] Besides, in a study related to childhood obesity, He et al identified related genes and functional regulatory networks through bioinformatics methods and found that the immune microenvironment of the obese group showed higher immune infiltration, and GUSBP11 participated in M1-type macrophage polarization by regulating CXCL2.[24] For miR-185-5p, a recent study demonstrated that the reduction of miR-185-5p is associated with pro-inflammatory activation of macrophages. Under LPS stress, increased miR-185-5p was beneficial to reduce the expression of pro-inflammatory factors in RAW264.7 macrophages.[25] This is

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also similar to what we found. Taken together, GUSBP11/miR-185-5p is involved in the regulation of inflammatory response, but the reactivity of gene expression is also different in different cell types.

In this study, four databases were used to predict the potential target genes of miR-185-5p, and 90 target genes were obtained after intersection, which were widely present in the nuclear membrane cavity, cytoplasmic region, telomerase complex, ribosome and other cell components, and were mainly involved in the regulation of cell adhesion, intracellular transport, histone modification, NMDA receptor activity and other biological processes, and regulate p53, ErbB, GnRH and other signaling pathways. In some related studies, it has been found that these signaling pathways are related to inflammation and immunity. Schumacher et al reported that ErbB signaling can stimulate the apoptosis of proinflammatory macrophages and thus hinder the occurrence of colitis.[26] Gong et al reported that cardamom can increase the expression of p53 in chondrocytes and thus improve the cartilage degeneration induced by osteoarthritis.[27] The function of miR-185-5p target genes still needs to be verified by more studies.

Presently, the clinical treatment for CP includes basic treatment measures such as cleaning, scraping, and root surface leveling. These methods have a positive effect on improving the condition and can remove the plaque in the mouth and the infected tissue of the bag wall to a large extent, but it is still difficult to completely overcome. Therefore, the treatment of inflammatory diseases from the aspect of gene regulation has become one of the hot research topics. Studies on the treatment of periodontitis based on non-coding RNA mainly focus on regulating the differentiation of periodontal osteoclasts and reducing periodontal bone loss. For example, lncRNA TUG1 (taurine up regulated gene 1) is down-regulated in LPS-stimulated hPDLCs, but its overexpression can both reduce proliferation inhibition and apoptosis and mediate Wnt/β-catenin signaling to prevent periodontitis.[28] Just as down-regulation of GUSBP11 in this study can alleviate LPS-induced cellular inflammation and inhibition of cell viability, it is speculated that si-GUSBP11 may be a new treatment method for CP in the future.

As a basic study, the advantage of this study is that the expression levels of GUSBP11 and miR-185-5p were first verified in human body fluids, and then the regulation of periodontitis inflammation by GUSBP11 was investigated in a CP cell model. All in all, our current study provides a new research idea and direction for the exploration of CP mechanism. The limitations of this study are as follows: First, since this study is a single-center study with a small sample size, selection bias may inevitably be introduced in sample inclusion, resulting in the instability of experimental results. Therefore, the follow-up experiment should try to expand the research scale and carry out a multi-center, large-sample prospective study to further verify the results of this experiment. Second, this study is based on in vitro studies, and whether GUSBP11/miR185-5p can also play the regulating function in the animal microenvironment still needs further research and demonstration. Therefore, in the future studies, we will continue to conduct a deeper discussion on this problem.

Conclusion

The present study demonstrates that the alleviating effect of inhibition of GUSBP11 on periodontitis is related to the increase of miR-185-5p. The reduction of GUSBP11 alleviates the inflammatory response and proliferation inhibition of LPS on human gingival fibroblasts by upregulating the level of miR-185-5p, thereby improving the inflammatory damage of CP cell models. However, more relevant studies are still needed to verify these views in the future, such as exploring the target genes involved in the regulation of miR-185-5p in CP and the inflammatory pathways related to GUSBP11/miR-185-5p axis.

Disclosure

The authors report no conflicts of interest in this work.

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