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Original Article

Expression level and clinical significance of NEAT1 in patients with chronic periodontitis

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KEYWORDS
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Abstract *Background/purpose:* Previous studies have shown that lncRNA nuclear autosomal transcript 1 (NEAT1) is abnormally expressed in periodontitis patients. However, the pathological mechanism of NEAT1 regulating periodontitis is still not clear. This study attempted to explore the expression of NEAT1 in periodontitis patients and its effect on periodontitis cell model and inflammatory response.

Materials and methods: The expressions of NEAT1 and miR-205-5p in gingival crevicular fluid (GCF) and cell samples were analyzed by qRT-PCR. The diagnostic value of NEAT1 in periodontitis was evaluated by constructing an ROC curve. The efficacy of NEAT1 on cell function and inflammatory response were assessed in LPS-induced PDLSCs. Luciferase reporter gene assay verified the targeting relationship between miR-205-5p and NEAT1.

Results: In the clinical section of this study, it was observed that NEAT1 expression was increased in GCF of periodontitis patients, and NEAT1 was found to be useful for periodontitis diagnosis. Besides, in vitro experiments suggested that inhibition of NEAT1 could improve cell viability and attenuated cell apoptosis and generation of inflammatory factors. Subsequently, luciferase reporter gene assay revealed that miR-205-5p was the target gene of NEAT1, and was negatively regulated by NEAT1.

Conclusion: High expression of NEAT1 has diagnostic value for periodontitis, and NEAT1 knockdown may reduce LPS-induced cell damage by increasing the level of miR-205-5p, which may provide a new breakthrough for the diagnosis and treatment of periodontitis.

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Introduction

According to the statistics, oral diseases affected 3.5 billion people around the world, accounting for almost half of the world population.[1] Major oral diseases include dental caries, gingival disease, and oral cancer.[2] Periodontitis is formed by the further expansion of gingivitis to deep periodontal tissues.[3] The early clinical manifestations of periodontitis are non-conspicuous, but the symptoms such as loose teeth, weak occlusion and repeated periodontal abscess may appear in the later stage of the disease, which is one of the main causes of tooth loss.[4] Therefore, periodontitis was once considered an important public health problem due to its high incidence.

Long non-coding RNA (lncRNA) is a kind of non-coding transcription material with nucleotide sequence greater than 200, which plays crucial regulatory role in maintaining the steady state of cells and tissues.[5] In recent years, increasing data show that lncRNA is closely related to periodontitis. For example, lncRNA MEG family was found to be closely related to osteogenic differentiation of periodontal ligament stem cells (PDLSCs).[6] PDLSCs is a kind of mesenchymal stem cells, which can differentiate into adipocytes and fibroblasts in vitro, and its differentiation process is regulated by many factors.[7] According to Liu et al.,[8] PTCSC3 improves the progression of periodontitis by regulating the proliferation of PDLSCs and the expression of TLR4. NEAT1 (nuclear autosomal transcript 1), also known as VINC, participates in the regulation of immune response and gene expression.[9] Sayad et al.[10] declared that NEAT1 expression was increased in gingival tissues of periodontitis patients but decreased in serum of periodontitis patients. Besides, Huang et al.[11] found that the expression profile of lncRNA in PDLSCs changed under pressure, among which the level of NEAT1 increased significantly. These studies have expanded our understanding of lncRNA associated with periodontitis. Accumulating data suggested that microRNA (miRNA) is crucial in regulating pathophysiological processes, such as cell differentiation, carcinogenesis, and cancer suppression. Maryta et al.[12] found that *P.* gingivalis reduced the level of miR-205-5p in gingival epithelial cells by upregulation of ZEB1 promoter activity, thereby promoting the occurrence of oral squamous cell carcinoma (OSCC). In a previous report of a large sample of gingival tissue, miR-205-3p was found to be down-regulated.[13] As such, it can be imagined that miRNA abnormalities are common in oral tissues, but how miR-205-5p is involved in the regulation of periodontitis in this study is still worth further investigation. In the current study, we examined the expression level of NEAT1 in gingival crevicular fluid (GCF) of all objects, and we evaluated the clinical diagnostic significance of NEAT1 in periodontitis. In addition, the in vitro periodontitis cell model was established to estimate the effect of abnormal expression of NEAT1 on cell model and inflammatory response, and to further analyze the potential mechanism of NEAT1 in periodontitis.

Materials and methods

Participant recruitment and sample collection

This research plan was carried out after being approved by the Ethics Committee of Dongying Hospital of Traditional Chinese Medicine (approval number: 20191201-1). All subjects have agreed to collect information and body fluid samples and have signed informed consent. A total of 178 subjects, including 85 periodontitis patients and 93 healthy controls, were enrolled in this study. The diagnostic criteria for periodontitis follow the EFP/AAP criteria issued in 2017.[14] The specific inclusion criteria are as follows: 1) Healthy periodontal tissues (healthy controls); 2) Periodontitis patients with generalized periodontitis at stage III or IV; 3) Probing pocket depth (PPD) $\geq$ 5 mm. Exclusion criteria: 1) Pregnant and lactating women; 2) Periodontal treatment or implant or prosthesis in the past year; 3) Received systemic antibiotic treatment in the past year; 4) Suffering from systemic or chronic diseases affecting periodontal tissues and requiring long-term medication.

GCF samples from all subjects were collected. In brief, plaque from gums was removed with cotton swabs and the sampling point was allowed to dry naturally to avoid saliva contamination. The filter paper was inserted gently into the gingival groove and left for 30s to obtain the GCF samples. Then, the filter paper strips contaminated by saliva or blood should be discarded, and the uncontaminated samples should be put into the marked sterile tubes. The volume of GCF was determined using moisture metre (Periotron 8000, IDE Interstate, Amityville, NY, USA). The filter papers were placed in a sterile tube containing 0.01 M sodium phosphate buffer, pH 7.4, and mixed in a vortex for 40s. Subsequently, after centrifugation for 10min, supernatant was taken and stored in -80 °C refrigerator for further experiments. In addition, general clinical indicators, including gender, age, and body mass index (BMI), as well as clinical pathological indicators such as probing pocket depth (PPD) were collected and recorded for subsequent analysis.

Cell culture and cell transfection

Human Periodontal Ligament Stem Cells (PDLSCs) were acquired from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultivated in DMEM (Gibco, Grand Island, NY, USA) with 10% FBS (Gibco), plus 1% Penicillin/streptomycin (Gibco). In this study, the cell model of periodontitis in vitro was established according to the previously published literature.[15] In brief, cells were inoculated in a culture plate, and 100 ng/mL *Porphyromonas gingivalis* LPS test solution was added to the cells at the logarithmic growth phase, followed by incubation for 48 h.

According to the usage method specified in the product manual, Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used for cell transfection. The cells were inoculated in 6-well plates and cultured overnight, and then transfected with si-NEAT1 and si-NC purchased from GenePharma. Company (Suzhou, Zhejiang, China). After transfection for 24 h, LPS was added to the cells and incubated for another 48 h.

RNA extraction and RT-qPCR

Total RNAs were extracted from GCF and PDLSCs using TRIzol (Invitrogen), and cDNA was synthesized using PrimeScript RT Reagent kit (TaKaRa, Dalian, China) and Mir-X miRNA First-Strand Synthesis Kit (TaKaRa). Subsequently,

amplification of cDNA was performed on the PCR instrument (Applied Biosystems, Foster City, CA, USA) according to the instructions of the SYBR Green PCR Kit (Takara). GAPDH and U6 expression were used as the endogenous reference of NEAT1 and miR-205-5p, respectively. The relative expressions of targeted genes were normalized to housekeeping genes GAPDH and U6 and were calculated by $2^{-\Delta\Delta Ct}$ method. The primer sequences used in this study were as follows:

NEAT1 primers:
Forward primer: 5′-TCCTCCCTTTAACTTATCCATTC-3′
Reverse primer: 5′-TCTCCTTGCCAAGCTTCCTTC-3′
miR-205-5p primers:
Forward primer: 5′-TCCTTCATTCCACCGGAGTCTG-3′
Reverse primer: 5′-GCGAGCACAGAATTAATACGAC-3′
GAPDH primers:
Forward primer: 5′-AGAAGGCTGGGGCTCATTTG-3′
Reverse primer: 5′-GGTCTGGGATGGAAACTGTG-3′
U6 primers:
Forward primer: 5′-CTCCGATAGATCTGCCCTCTT-3′
Reverse primer: 5′-AACGCTTCACGAATTTGCGT-3′

Cell viability assay

PDLSCs were inoculated into 96-well plate for cell counting kit-8 (CCK-8) assay. At 0 h, 24 h, 48 h and 72 h after cell transfection and LPS treatment, add 10 μL CCK-8 working solution to cell plate. Next, after placing the cell culture plate in an incubator for 4 h, the OD value at 450 nm was detected by a microplate reader (Bio-Tek, Indianapolis, IN, USA).

Cell apoptosis assay

Cell apoptosis was investigated by FITC Annexin V/PI kit (Invitrogen) and flow cytometry. Briefly, PDLSCs were inoculated into a 6-well plate for cell transfection and LPS treatment. Subsequently, after the cells were treated for 48 h, cells were gathered and resuspended with 100 μL binding solution to prepare a cell suspension. 10 μL FITC solution and 5 μL PI solution were added to cell suspension and incubated in the dark for 20 min. Finally, 300 μL binding solution was added to the cells, and apoptosis was measured by flow cytometry (BD Biosciences, San Jose, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

The inflammatory factors such as TNF-α, IL-6, and IL-8 in cell supernatant were determined by ELISA kit (R&D System, Minneapolis, MN, USA). After the cells were treated according to the experimental requirements, cell supernatants were collected, and the TNF-α, IL-6, and IL-8 level were measured by Human TNF-α ELISA Kit (Catalog number: cat # MTA00B), Human IL-6 ELISA Kit (Catalog number: cat # M6000B) and Human IL-8 ELISA Kit (Catalog number: cat # M8000B) according to the product description, respectively.

Luciferase reporter gene assay

The potential complementary sequence of miR-205-5p and NEAT1 was predicted by online program StarBase. The relationship between miR-205-5p and NEAT1 was confirmed by luciferase reporter gene analysis. Firstly, the predicted binding sequence of miR-205-5p in NEAT1 was cloned into pmirGLO vector to construct NEAT1 wild-type luciferase reporter vector (NEAT1-WT). Then, the NEAT1 mutant luciferase reporter vector (NEAT1-MUT) was constructed by mutating the seed site of miR-205-5p. Subsequently, PDLSCs were co-transfected with NEAT1-WT or NEAT1-MUT and miR-205-5p mimic/inhibitor, or mimic/inhibitor-NC using Lipofectamine 2000 (Invitrogen). The luciferase activity was assessed by dual-luciferase reporter system (Promega, Madison, WI, USA) after 48 h of cell transfection. Renilla luciferase was deemed as the internal reference.

Data analysis

Data analysis was conducted using SPSS 21.0 software packages. Data were expressed as mean ± SD. Student t test and one-way ANOVA were used for two or multiple group comparisons. ROC curve was conducted for the diagnostic value assessment of NEAT1 for periodontitis. Multiple regression analysis was carried out to estimate the independent influence of variables related to NEAT1. A p value less than 0.05 was considered as significantly different. The experiment was repeated at least three times in parallel.

Results

Demographic characteristics and clinicopathological indicators

The baseline data and pathological indicators are shown in Table 1. It was obvious from the results that there were no statistically significant differences in age, gender, body mass index (BMI), and smoking history between the two groups ($P > 0.05$). However, the two groups showed significant differences in IL-6, IL-8, TNF-α, bleeding on probing (BOP), plaque control record (PCR), probing pocket depth (PPD), clinical attachment level and other indicators ($P < 0.001$).

Expression of NEAT1 in GCF of periodontitis patients

The expression level of NEAT1 in GCF was measure by RT-qPCR. As illustrated in Fig. 1, the expression level of NEAT1 in periodontitis patient increased significantly compared with healthy control group, which suggested that abnormal expression of NEAT1 might be related to periodontitis ($P < 0.001$).

Diagnostic value of NEAT1 or miR-205-5p in periodontitis

The diagnostic value of NEAT1 or miR-205-5p in periodontitis was evaluated using ROC curve analysis. The results in Fig. 2A manifested that the AUC value of NEAT1 was 0.903, with the sensitivity of 82.4% and the specificity of 88.2%, respectively. Similarly, Fig. 2B also showed that miR-205-5p

Table 1 Clinical data of the study subjects.

Parameter	Healthy individuals (n = 93)	Patients with periodontitis (n = 85)	*P*-value
Age	57.72 ± 4.18	56.92 ± 4.25	0.206
Gender (n/%)			0.995
Male	47 (50.54)	43 (50.59)	
Female	46 (49.46)	42 (49.41)	
BMI (kg/m^2)	21.50 ± 0.85	21.42 ± 0.90	0.541
Smoking (n/%)			0.128
Yes	43 (46.24)	49 (57.65)	
No	50 (53.76)	36 (42.35)	
Interleukin 6 (pg/ml)	179.49 ± 37.48	400.59 ± 86.13	<0.001
Interleukin 8 (pg/ml)	175.91 ± 37.56	513.22 ± 118.25	<0.001
Tumor necrosis factor alpha (pg/ml)	187.80 ± 39.99	566.21 ± 139.76	<0.001
BOP (%)	2.59 ± 1.73	15.52 ± 11.63	<0.001
PCR (%)	20.88 ± 15.53	35.98 ± 21.42	<0.001
PPD (mm)	1.71 ± 0.19	6.80 ± 1.78	<0.001
CAL (mm)	0.95 ± 0.086	3.01 ± 1.10	<0.001

Footnotes: Data are expressed as n or mean ± standard deviation. BMI = body mass index; PPD = probing pocket depth; CAL = clinical attachment level; BOP = bleeding on probing; PCR = plaque control record.

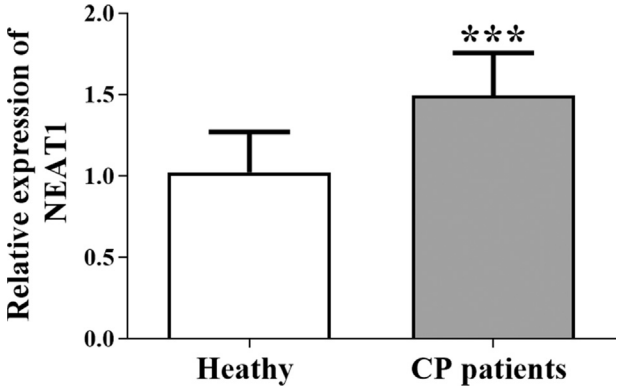

Figure 1 qRT-PCR analysis. The expression level of NEAT1 in periodontitis patients was enhanced compared with healthy control group. ***$P < 0.001$.

had high clinical diagnostic accuracy in periodontitis. These results preliminary indicate that NEAT1 has high clinical diagnostic value for periodontitis.

Effect of NEAT1 on proliferation, apoptosis, and inflammatory response of periodontitis cell model

In vitro periodontitis cell model was established by LPS stimulation of PDLSCs, and the efficacy of NEAT1 on the cell model was evaluated via cell viability and cell apoptosis. In Fig. 3A, it can be observed that the NEAT1 level in LPS-treated cell models is significantly enhanced compared with the control group. Meanwhile, transfection of si-NEAT1 can down-regulate the increase of NEAT1 caused by LPS-stimulation ($P < 0.001$). The induction of Fig. 3B demonstrated that LPS-stimulation reduced the cell viability, while the transfection of si-NEAT1 significantly enhanced the cell viability in a time-dependent manner ($P < 0.001$). Cell apoptosis assay also showed that LPS-stimulation increased the number of apoptotic cells, while si-NEAT1 transfection at the same time relatively down-regulated the level of cell apoptosis (Fig. 3C, $P < 0.001$). In addition, for the levels of inflammatory cytokines, Fig. 3D suggested that the relative levels of inflammatory cytokines in cell supernatant after LPS treatment were significantly increased, indicating that LPS stimulation activated the inflammatory response, and it also proved the successful establishment of in vitro cell models of periodontitis. Furthermore, it was observed that down-regulation of NEAT1 suppressed the generation of inflammatory cytokines ($P < 0.001$).

Verification of interaction between NEAT1 and miR-205-5p

In order to explore the potential mechanism of NEAT1 in periodontitis cell model, we identified the complementary sequence of NEAT1 and miR-205-5p through Starbase database (Fig. 4A). And next, their relationship was verified by constructing luciferase reporter vector. We found that the addition of miR-205-5p mimic resulted in a significant reduction in luciferase activity in NEAT1-WT group, while there was no significant increase or decrease in NEAT1-MUT group under the same conditions (Fig. 4B, $P < 0.001$). In the GCF samples of periodontitis patients, the level of miR-205-5p revealed a decreasing trend, contrary to the trend of NEAT1 (Fig. 4C, $P < 0.001$). As shown in Fig. 4D, Pearson correlation coefficient showed that miR-205-5p expression was negatively associated with NEAT1 ($r = -0.6504$, $P < 0.0001$). Besides, we also detected the miR-205-5p level in periodontitis cell model. It was observed that miR-205-5p expression attenuated significantly after LPS-stimulation, whereas miR-205-5p expression level augmented after NEAT1 silence (Fig. 4E, $P < 0.001$). According to the above results, we also verified the effect of transfection of miR-205-5p on NEAT1 through cell transfection experiment. The results showed that transfection of miR-205-5p mimic had no effect on the expression level of NEAT1 in cell model (Fig. 4F). Furthermore, Fig. 4G illustrated that LPS-induced inflammatory cytokines were down-regulated after transfection with miR-205-5p mimic ($P < 0.001$). The above data all verified that miR-205-5p was the downstream target gene of NEAT1, and its expression was negatively regulated by NEAT1. *Correlation of the NEAT1 or miR-205-5p levels with clinical characteristics.*

After adjusting for confounding factors (age, sex, BMI, and smoking), multiple regression analysis was used to evaluate the relationship between NEAT1 or miR-205-5p expression and other confounding factors (IL-6, IL-8, TNF-α, BOP, PCR, PPD and CAL) which may be related to the severity of periodontitis. Table 2 revealed that the expressions of NEAT1 were correlated with levels of IL-6, IL-8, TNF-α, BOP, PCR, PPD and CAL, respectively. NEAT1

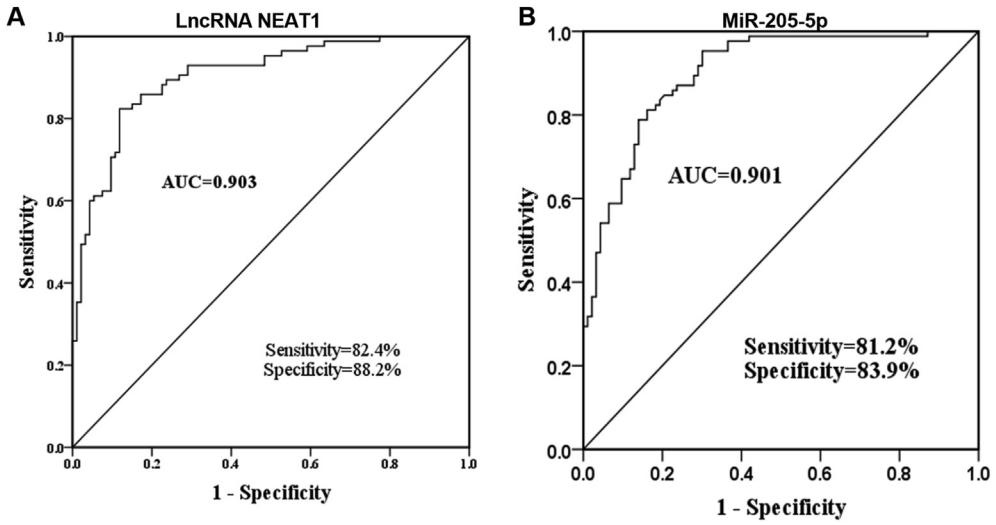

Figure 2 ROC curve analysis. **(A)** The AUC of NEAT1 was 0.903, with sensitivity of 82.4% and specificity of 88.2%. **(B)** The AUC of miR-205-5p was 0.901, with sensitivity of 81.2% and specificity of 83.9%.

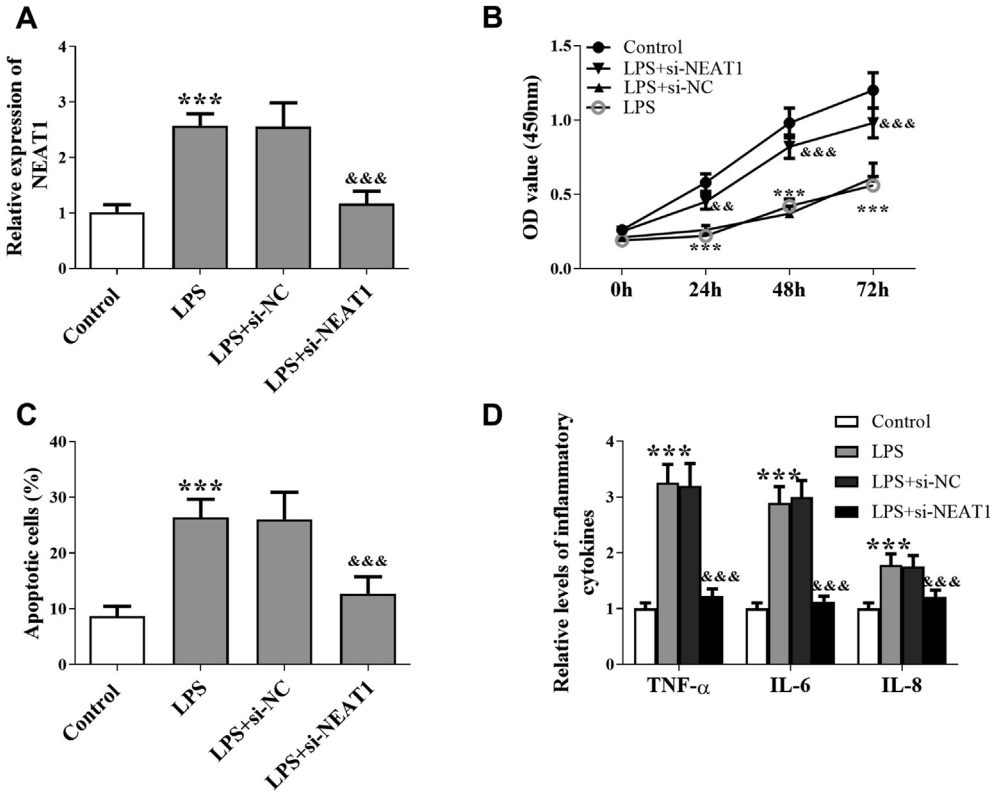

Figure 3 Inhibition of NEAT1 attenuated LPS-induced cell model damage. **(A)** qRT-PCR was used to detect the expression of NEAT1 in LPS-induced periodontitis cell models. **(B)** Inhibition of NEAT1 reversed LPS-induced down-regulation of cell viability. **(C)** Inhibition of NEAT1 reversed LPS-induced up-regulation of cell apoptosis. **(D)** Inhibition of NEAT1 reversed LPS-induced increased production of inflammatory factors. $^{***}P < 0.001$, $^{\&\&\&}P < 0.001$.

negatively related to BOP and PPD, and positively associated with IL-6, IL-8, TNF-α, PCR and CAL ($P < 0.05$). In addition, it was also found that the level of miR-205-5p was correlated with IL-6, IL-8, TNF-α, BOP and CAL, and was negatively correlated with IL-6, IL-8, TNF-α and CAL ($P < 0.05$).

Discussion

Periodontitis is a chronic destructive disease, which occurs in supporting tissues of teeth, including gingiva, cementum, periodontal ligament, and alveolar bone.[16] The most common harm of periodontitis is chewing pain, followed by

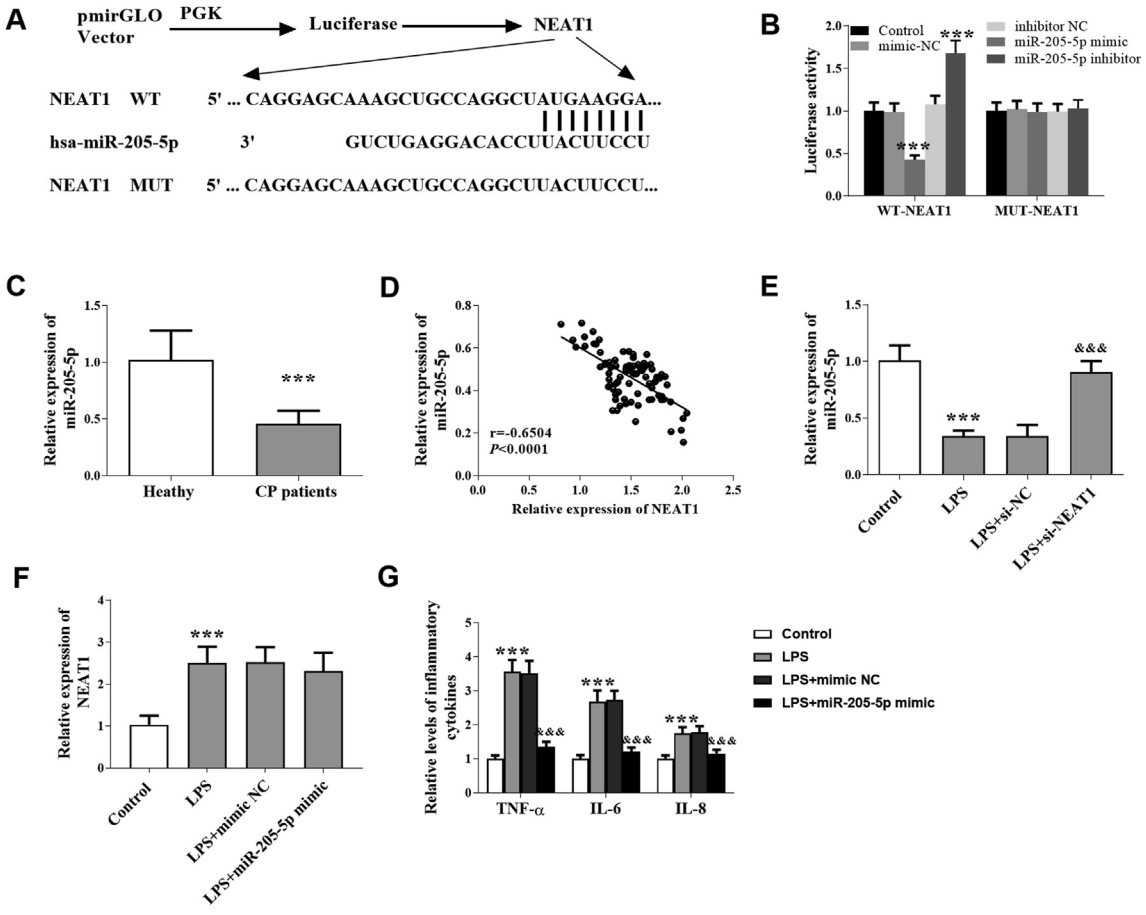

Figure 4 miR-205-5p was a target of NEAT1. **(A)** The complementary sequences of miR-205-5p and NEAT1 was predicted by StarBase. **(B)** The experimental analysis of luciferase reporter gene assay. **(C)** miR-205-5p expressions in periodontitis patients. **(D)** miR-205-5p was negatively correlated with NEAT1. **(E)** qRT-PCR measured miR-205-5p level in LPS-induced periodontitis cell models. **(F)** The relative expression of NEAT1 in LPS-treated PDLSCs. **(G)** The relative levels of inflammatory cytokines after transfection with miR-205-5p mimic. $^{***}P < 0.001$, $^{\&\&\&}P < 0.001$.

Table 2 Multiple linear regression analysis of lncRNA NEAT1 and miR-205-5p with related variables.

Characteristics	LncRNA NEAT1				miR-205-5p			
	Coefficient	Standard error	t	*P-value*	Coefficient	Standard error	t	*P-value*
Interleukin 6 (pg/ml)	0.001	0.000	4.394	<0.001	−0.002	0.000	−13.586	<0.001
Interleukin 8 (pg/ml)	0.001	0.000	3.996	<0.001	0.000	0.000	−4.784	<0.001
Tumor necrosis factor α (pg/ml)	0.001	0.000	7.357	<0.001	0.000	0.000	−2.824	0.006
BOP (%)	−0.002	0.001	−2.130	0.036	0.001	0.001	2.072	0.042
PCR (%)	0.002	0.001	2.291	0.025	−0.001	0.000	−1.636	0.106
PPD (mm)	−0.017	0.008	−2.213	0.037	0.002	0.036	0.397	0.692
CAL (mm)	0.038	0.014	2.768	0.007	−0.108	0.004	−2.643	0.010

Footnotes: PPD = probing pocket depth; CAL = clinical attachment level; BOP = bleeding on probing; PCR = plaque control record.

tooth loss. In severe cases, it may cause coronary heart disease or stroke.[17] Many studies have reported that lncRNA participates in the occurrence and progression of periodontitis, for example, MAFG-AS1 is involved in regulating the proliferation of PDLSCs[18] and TWIST1 regulates the osteogenic differentiation of PDLSCs.[19] In this study, the results showed that the expression level of NEAT1 was augmented in GCF samples of periodontitis patients and in LPS treated PDLSCs. Highly expressed NEAT1 revealed high diagnostic value for periodontitis. Inhibition of the NEAT1 expression enhanced the cell viability and declined the number of apoptotic cells and the generation of

inflammatory factors in LPS treated PDLSCs. Furthermore, miR-205-5p was the target gene of NEAT1 and was negatively regulated by NEAT1.

NEAT1 is reported to be a cancer-associated lncRNA known to be involved in innate immune responses.[20] Previous studies on NEAT1 focused on tumor regulation, involving tumor proliferation, migration, and apoptosis, which reflected that there was a large space for research on the relationship between NEAT1 and periodontitis, and the related research had important practical significance. Recently, many studies have reported that NEAT1 is disregulated in gingival tissue or peripheral blood of periodontitis patients compared with healthy people.[21] In their study, Sayad et al. found that NEAT1 level in gingival tissues of periodontitis patients was significantly higher than those of healthy controls, indicating that NEAT1 was related to pathogenesis of periodontitis. Our study samples were taken from the GCF of the individuals, and NEAT1 was found to be significantly increased in GCF of periodontitis patients, which was consistent with Sayad' s results. It is worth noting that overexpression of NEAT1 is closely and complex related to inflammation, and it participated in inflammatory regulation of rheumatoid arthritis,[22] sepsis,[23] atherosclerosis,[24] and systemic lupus erythematosus.[25] In our study, the cell viability of PDLSCs decreased significantly, while the number of apoptotic cells and the generated inflammatory factors increased after LPS treatment. Surprisingly, the above phenomenon can be ameliorated with NEAT1 knockdown. Besides, we found that the expression of NEAT1 was positively correlated with IL-6, IL-8, and TNF-α through multiple linear regression analysis, which further indicated that NEAT1 was associated with the degree of inflammation. This result was confirmed in the validation study of sepsis by Huang et al. They found that the expression levels of IL-6, IL-8, and TNF-α were significantly positively correlated with the expressions of NEAT1.[26] All these results suggested that up-regulation of NEAT1 plays an adverse role in periodontitis or LPS-induced PDLSCs.

Previous studies have suggested that lncRNA may be engaged in pathophysiological regulation through sponging miRNAs.[27,28] In our current study, through bioinformatics analysis, we predicted that miR-205-5p is the downstream target of NEAT1 and is inversely regulated by NEAT1. miR-205-5p, also known as miR-205, has relation to the pathophysiologic mechanism of a variety of solid tumors. For example, miR-205-5p was found to promote cisplatin resistance in ovarian cancer by down-regulating the PTEN expression.[29] Nagai et al.[30] reported that miR-205-5p modulated cancer cell proliferation and migration by targeting metalloproteinase inhibitor 2 in oral squamous cell carcinoma (OSCC). Our experimental results suggested that miR-205-5p exhibited a significant down-regulation trend in the GCF of periodontitis patients and in LPS-treated PDLSCs. Interestingly, Stoecklin et al.[13] found that the expression of miR-205 in gingival tissues of periodontitis patients was lower than in healthy gingival tissues. Li et al.[31] reported that miR-205-5p was down-regulated in periodontal ligament tissues of periodontitis patients and involved in the activation of JAK/STAT signaling pathway. The above results manifest that the downregulation of miR-205-5p promoted the occurrence of periodontitis, and we speculate that NEAT1 may promote the progression of periodontitis through targeted regulation of miR-205-5p.

In summary, this study suggested that NEAT1 may promote the progression of periodontitis by sponging miR-205-5p, which was a new mechanism to explain the relationship between NEAT1 and periodontitis. In *in vitro* cell studies, NEAT1 gene knockdown could alleviate LPS-induced inhibitory effect on PDLSCs viability and promoting effect on cell apoptosis, which can provide a new strategy for clinical treatment of periodontitis.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

Not applicable.

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