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Effect of Notch2 Pathway Mediated by miR-146a-5p On Differentiation of Human Dental Pulp Stem Cells into Cementoblasts

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KEYWORDS MicroRNA-146a-5p. Notch2 Pathway. Human Dental Pulp Stem Cells. Cementoblast Differentiation. Cementogenesis

ABSTRACT This work explored the potential regulatory role of the miR-146a-5p/Notch2 signaling axis in directing the cementoblast-like differentiation of mesenchymal stem cells originating from human dental pulp. Cells received transfection with miR-146a-5p mimics or inhibitors, their respective negative controls, or a Notch2-knockdown plasmid, in addition to a non-transfected baseline group. Suppressing miR-146a-5p activity enhanced ALP levels, facilitated matrix mineral deposition, and elevated the expression of cementogenesis-associated genes (Notch2, CAP, BSP, OCN, OPN). In contrast, miR-146a-5p overexpression or interference with Notch2 resulted in reduced ALP activity and diminished mineralization and marker expression. A dual-luciferase assay confirmed that miR-146a-5p directly binds to the 32 UTR of Notch2. Collectively, reducing miR-146a-5p levels releases Notch2 from repression and accelerates cementoblastic commitment, highlighting the miR-146a-5p/Notch2 pathway as a promising target for periodontal cementum regeneration.

INTRODUCTION

The periodontium comprises coordinated mineralized and soft components that ensure tooth support while safeguarding neurovascular structures and defending against microbes (Cho et al. 2021). In adults, periodontal disease is a leading contributor to tooth loss and constitutes a major public-health concern (Jiao et al. 2021). Epidemiological data from China indicate prevalences of 52.8 percent among individuals aged 35–44 years, 69.3 percent in those 55–64 years, and 64.6 percent in adults over 64 years. Among periodontal diseases, chronic periodontitis is highly prevalent globally and is characterized by progressive damage to the cementum, periodontal ligament, and surrounding alveolar bone. Conventional periodontal therapies (for example, scaling and root planning/SRP) mechanically eliminate infectious sources in clinical practice. However, they frequently lead to long junctional epithelial attachment on root surfaces and rarely accomplish functional restoration of damaged periodontal tissues (Liang et al. 2020).

Regenerating periodontal tissues represents a promising therapeutic avenue for periodontal disease. Numerous strategies have been investigated to regenerate functional periodontal tissues. Researchers have assessed diverse biomaterials, including chitosan, nanoparticle-based scaffolds, and calcium phosphate–derived substitutes like hydroxyapatite and CPCs. Moreover, a variety of bioactive molecules—such as TGF, BMP family members, and FGF—along with stem cells from multiple sources have also been applied to enhance periodontal repair. Nonetheless, outcomes from these modalities often fail to translate robustly to patients with periodontitis. A key bottleneck is the scarcity of seed cells capable of supporting effective tissue regeneration within periodontal lesions (Kulakauskiene et al. 2020). Consequently, it is essential to identify appropriate seed cells that can reliably undergo cementoblastic differentiation.

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Dental pulp stem cells, serving as odontogenic mesenchymal stem cells with multiple differentiation potentials, can differentiate into dentin and osteoblasts (Bar et al. 2021). Cementum is a kind of bone-like tissue surrounding the surface of tooth roots. Traditionally, mesenchymal stem cells are considered as an important source of cementoblasts (Cao et al. 2020). Research indicates that DPSCs can differentiate into cementum periodontal complex components, making them potential candidates for periodontal regeneration (Chang et al. 2020). However, the mechanism guiding their differentiation into cementoblasts remains unclear.

MicroRNA (miRNA) play key regulatory roles in the proliferation, self-renewal, and lineage-specific differentiation of odontogenic mesenchymal stem cells. Earlier studies have indicated that miR-146a-5p is closely associated with the ability of dental pulp stem cells to undergo osteogenic differentiation (Cao et al. 2022). However, it remains unclear whether miR-146a-5p also participates in directing dental pulp stem cells toward cementoblastic differentiation. Notch2 signaling has been identified as an essential pathway that governs the osteogenic differentiation process in mesenchymal stem cells. Evidence further indicates that the Notch2 contributes to controlling the osteoblastic differentiation process in dental pulp stem cells (Manokawinchoke et al. 2017). However, its role in cementoblast differentiation remains unexplored. Previous studies have pointed out the targeting effect of miR-146a-5p on Notch2 (Yan et al. 2022). It remains unclear whether this targeting relationship contributes to guiding dental pulp stem cells toward a cementogenic lineage, and additional investigation is required. Accordingly, this study sought to clarify how the miR-146a-5p–Notch2 regulatory axis functions and the mechanisms through which it influences the cementoblast-like differentiation of human DPSCs.

MATERIAL AND METHODS

Experimental Reagents

The experimental reagents used in the study include the following. Mouse anti-human fluorescein isothiocyanate (FITC)-labelled CD45 (CD45 FITC) antibody, STRO-1 FITC antibody, PE labelled CD34 (CD34 PE) antibody, CD73 PE antibody (purchased from Santa Cruz Biotechnology, batch number sc-19597 FITC, sc-47733 FITC, sc-7324 PE and sc-398260 PE), mouse anti-human PE-labelled CD166 antibody (purchased from Shanghai Hengfei Biotechnology Co. Ltd., batch number 130-106-575), plasmids carrying miR-146a-5p mimics (synthetic sequence: positive-sense chain 52 -UGAGAACUGAAUUCCAUGGGUU-32 , antisense chain 52 -CCCAUGGAAUUCAGUUCUCAUU-32), plasmids carrying miR-146a-5p inhibitors (synthetic sequence: positive-sense chain 52 -AACCCAUG GAAUUCAGUUCUCA-32 , antisense chain 52 -UCGCGGAUCGGACUGAAUUUCA-32), plasmid carrying miR-146a-5p inhibitor negative control (NC inhibitor, synthetic sequence: positive-sense chain 52 -CUAGGCGAUUCUAGAGUUGCAC-32 , antisense chain 52 -UCGCGGAUCGGA CUGAAUUUCA-32), miR-146a-5p mimics negative control (miR-NC, synthetic sequence: positive-sense chain 52 -UUCUCCGAACGUGUCACGU-32 , antisense chain 52 -ACGUGACACGUUCGGAGAA-32), plasmids carrying Notch2 inhibitor (synthetic sequence: positive-sense chain TTCGCGTUAGGCTUGCTA-32 , antisense chain TGUAUAGGCTCTTATAGCU-32), wild-type and mutant pmirGLO-Notch2 reporter plasmids (constructed and synthesised by Guangzhou Editgene Technology Co. Ltd.), lipofectamine transfection kit (purchased from Thermo Fisher, batch number L3000150), BCIP/NBT ALP chromogenic kit (purchased from Shanghai LMAI Bio Engineering Co. Ltd., batch number LM-0324), human ALP activity detection kit (supplied by Nanjing Jiancheng Bioengineering Institute, batch number A059-2-2), alizarin red staining solution (purchased from Cyagen (Guangzhou) Biotechnology Co. Ltd., batch number ALIR-10001), miR-146a-5p, Notch2, CAP, cementoblast differentiation related bone sialoprotein (BSP), OCN, OPN, phosphate dehydrogenase (GAPDH) polymerase chain reaction (PCR) (commissioned Hepeng (Shanghai) Biotechnology Co. Ltd.), Trizol reagent (supplied by Beijing Solarbio Science and Technology Co. Ltd., batch number 15596-018), total protein extraction kit (purchased from Amyjet Technology Co. Ltd., batch number AMJ-KT0007), mouse anti-human Notch2, CAP, BSP, OCN, OPN, GAPDH monoclonal antibodies (primary antibody) (purchased from Santa Cruz Biotechnology, batch number sc-518169, sc-53947, sc-73634, sc-390877, sc-21742 and sc-47724), goat anti-mouse horseradish

peroxidase (HRP)-labelled Notch2, CAP, BSP, OCN, OPN, GAPDH polyclonal antibody (secondary antibody) (purchased from Abcam, UK, batch number ab98659, ab97040, ab97265, ab97230, ab98693, ab97040), and dual luciferase reporter assay kit (purchased from Wuxi Yuncui Biotechnology Co. Ltd., batch number YAD0010).

Experimental Instruments

The instruments used in the study include the following. CX33 fluorescence microscope, CKX41 inverted microscope (purchased from Olympus, Japan), M3 microplate reader (purchased from Molecular Devices, USA), Attune NxT flow cytometer (purchased from Thermo Fisher), T100 PCR instrument (purchased from BIO-RAD, USA), and CSL-IEF-KIT-MAXIPLUS electrophoresis apparatus (purchased from Cleaver, UK)

METHODOLOGY

Human Dental Pulp Stem Cells' Isolation, Culture and Identification

Human dental pulp stem cells were obtained through a modified tissue explant technique as previously described (Xin et al. 2020). Following extraction of healthy, caries-free teeth at the orthodontic department of the researchers' hospital, the pulp was disinfected, the apical third removed, rinsed in PBS supplemented with penicillin and streptomycin, and subsequently cut into small fragments.The tissue explants were maintained in DMEM supplemented with 20 percent fetal bovine serum. Once the cells migrated out from the explants, the medium was refreshed every three days, and subculture was performed when cellular confluence reached approximately 80 percent. Third-passage cells in good condition were harvested, digested with trypsin, adjusted to 1×10v /mL, and incubated with anti-human CD45 FITC, CD34 PE, STRO-1 FITC, CD73 PE, and CD166 PE antibodies for staining; surface antigen expression was then assessed by flow cytometry. The findings verified that the cultured cells displayed characteristic features typical of human dental pulp stem cells.

Cell Transfection and Grouping

Human dental pulp stem cells at the logarithmic growth stage were used for subsequent assays. When cultures reached approximately 70 to 80 percent confluence, the cells were subjected to transfection with miR-146a-5p mimics, inhibitors, their respective negative controls, or a Notch2-silencing plasmid, according to the instructions provided with the liposome-based transfection reagent. Accordingly, the experimental groups were designated as miR-146a-5p overexpression, miR-146a-5p suppression, down-regulation control, up-regulation control, and Notch2 knockdown groups. Untransfected cells served as the blank control, with six replicate wells prepared for each group. 48 hours after transfection, the transfection efficiency was detected by fluorescence microscope. The transfection efficiency was verified to be greater than 80 percent.

ALP Staining and Detection of ALP Activity

At 48 hours post-transfection, cells were treated with mineralisation induction medium, rinsed with PBS, fixed, and then incubated with 2 mL ALP staining solution (according to the kit protocol) for 30 minutes at ambient temperature while protected from light. After terminating the reaction with distilled water, ALP staining was visualised using an inverted microscope. During the detection of ALP activity, after adding cell lysate, the process included inoculating it into 96-well plates, adding buffer and matrix solution, standing at room temperature for 15 minutes, adding chromogenic agent, mixed well, detecting the absorbance value at 520 nm by microplate reader, and calculating the ALP activity (Fayyaz et al. 2024).

Alizarin Red Staining Used to Observe Cell Mineralisation

Forty-eight hours after transfection, the cells were transferred to mineralization-inducing medium, then washed with PBS, fixed, and stained with alizarin red for 10 minutes. Following rinsing with double-distilled water, the mineralized nodules were visualized using an inverted microscope, and their areas were quantified with Image Pro Plus 6.0.

Real time quantitative polymerase chain reaction (RT-qPCR) was employed to detect the expression of miR-146a-5p, Notch2, CAP, BSP, OCN and OPN mRNA.

At 48 hours post-transfection, total RNA was isolated using TRIzol reagent, converted into

complementary DNA (cDNA), and subsequently amplified by PCR. The PCR program consisted of an initial denaturation at 95°C for 30 seconds, followed by 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds, with a final step at 50°C for 30 seconds. The primer sequences are shown in Table 1, and the relative expression of genes were analysed through $2^{-\Delta\Delta Ct}$ (Zhang et al. 2023).

The protein expressions of Notch2, CAP, BSP, OCN and OPN were detected by Western blot (WB) 48 hours after transfection, the total protein of cells in each group was extracted, and the protein expressions of Notch2, CAP, BSP, OCN, OPN in cells were detected (Yang et al. 2020).

A dual-luciferase reporter system was employed to determine if Notch2 is directly regulated by miR-146a-5p. miRWalk analysis indicated that Notch2 is one of the predicted target genes of this microRNA. Guangzhou Editgene Technology Co. Ltd. was entrusted to construct wild-type and mutant pmirGLO-Notch2 reporter plasmids, and introduced them into human dental pulp stem cells together with the miR-146a-5p mimic, inhibitor, or corresponding negative control. Seventy-two hours post-transfection, luciferase activity was measured using the dual-luciferase reporter system. The relative luciferase value was calculated as the ratio of firefly to Renilla luciferase signal.

Statistical Analysis

Data analysis was performed using SPSS version 22.0 software. The data were expressed in ($\bar{x} \pm s$). Differences between two groups were analyzed using Student's t-tests, while one-way ANOVA was applied for comparisons among multiple groups. A P-value below 0.05 was regarded as statistically significant, and values under 0.01 indicated a stronger level of significance.

RESULTS

ALP Staining and ALP Activity of Cells in Each Group

Relative to the blank, down-regulation control, and up-regulation control groups, the miR-146a-5p overexpression group displayed weaker ALP staining with markedly reduced activity ($P<0.01$), while the inhibition group presented stronger staining and elevated ALP activity ($P<0.01$) (Fig. 1). Similarly, the Notch2 down-regulation group had lighter ALP staining and significantly lower ALP activity ($P<0.01$) compared to the blank, down-regulation control, and up-regulation control groups. Moreover, in comparison with the miR-146a-5p overexpression group, the inhibition group exhibited markedly deeper ALP staining and a two- to threefold elevation in ALP activity ($P<0.01$) (Fig. 2). These findings suggest that increased miR-146a-5p expression, together with reduced Notch2 levels, leads to suppression of ALP expression.

Note: Comparing to the blank group, $^{*}P<0.05$, $^{**}P<0.01$; comparing to the down-regulation control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; comparing to the up-

Table 1: Primer sequences of PCR reaction

Gene(s)	*Primer sequences*	*Length of amplified product (bp)*
miR-146a-5p	Upstream primer 52 -GGGTGAGAACTGAA TTCCA-32 Downstream primer 52 -CAGTGCGTGTCGTGGAGT-32	73
U6	Upstream primer 52 -CTCGCTTCGGCAGCACA-32 Downstream primer 52 -AACGCTTCACGAATTTGCGT-32	103
Notch2	Upstream primer 52 -CACTGAGAGCTCCTGTTTCAA-32 Downstream primer 52 -CAGGCCATCAACACACGTTC-32	160
CAP	Upstream primer 52 -CCTGGCTCACCTTCTACGAC-32 Downstream primer 52 -CCTCAAGCAAGGCAAATGTC-32	156
BSP	Upstream primer 52 -TCCTTTATCACACCCCCTACC-32 Downstream primer 52 -TTAGCCAAATTGTTCCCTGC-32	189
OCN	Upstream primer 52 -CCCTCCTGCTTGGACACAA-32 Downstream primer 52 -ATGGGCCATTCCAGTTCCTC-32	112
OPN	Upstream primer 52 -ATCACCTGTGCCATACC-32 Downstream primer 52 -ACTCCTCGCTTTCCAT-32	433
GAPDH	Upstream primer 52 -CTCAGACACCATGGGGAAGGTGA-32 Downstream primer 52 -ATGATCTT2GAGGCTGTTGTCATA-32	450

Fig. 1. ALP staining of cells in each group (PNPP Staining, ×100, Scale Bar: 100ìm)

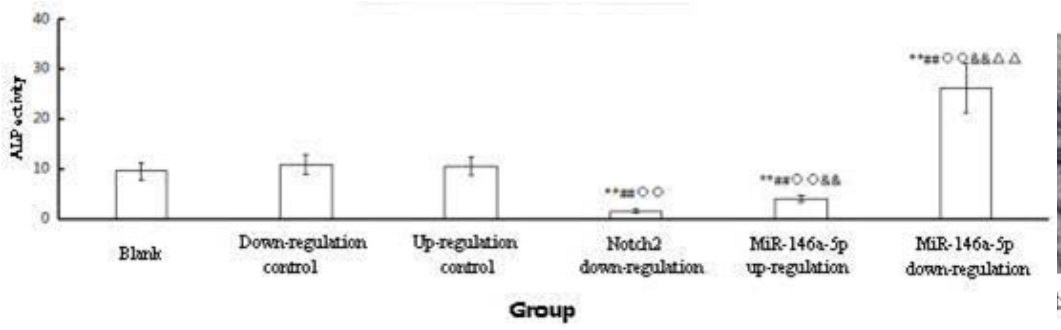
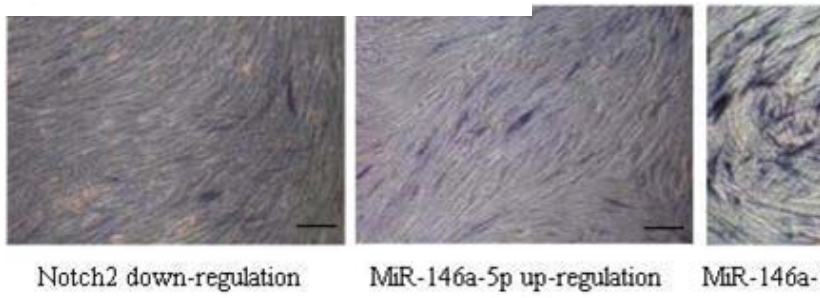


Fig. 2. ALP activity of cells in each group (, n=6)

regulation control group; $^{Ë\%}P<0.05$, $^{Ë\%Ë\%}P<0.01$; comparing to Notch2 down-regulation group, $^{\&}P<0.05$, $^{\&\&}P<0.01$; comparing to miR-146a-5p up-regulation group, $^{\%}P<0.05$, $^{\%\%}P<0.01$

Cell Mineralisation in Each Group

Compared with the blank group as well as the up- and down-regulation controls, the miR-146a-5p mimic group showed a pronounced decrease in mineralized nodule formation ($P < 0.01$), while the inhibitor group exhibited a significant increase in



miR-146a-5p combined with Notch2 suppression attenuated ALP expression and associated mineralization activities.

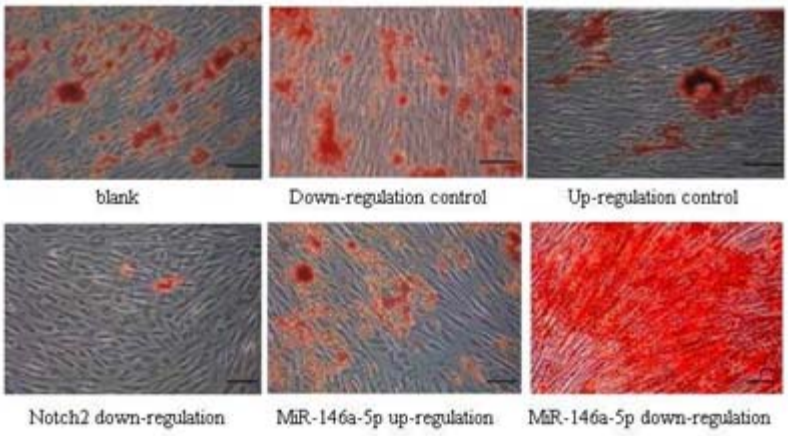


Fig. 3. Cell mineralisation in each group (Alizarin Red Staining, ×100, Scale Bar: 100ìm)

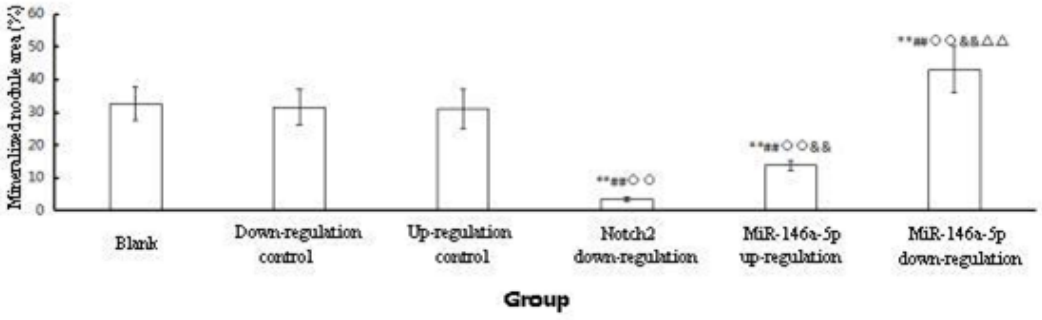


Fig. 4. Mineralised nodule area of cells in each group

Note: Comparing to the blank group, $^{*}P<0.05$, $^{**}P<0.01$; comparing to the down-regulation control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; comparing to the up-regulation control group; $^{É\%}P<0.05$, $^{É\%É\%}P<0.01$; comparing to Notch2 down-regulation group, $^{\&}P<0.05$, $^{\&\&}P<0.01$; comparing to miR-146a-5p up-regulation group, $^{\%}P<0.05$, $^{\%\%}P<0.01$

MiR-146a-5p Expression, Notch2, CAP, BSP, OCN, OPN mRNA Expression in Cells of Each Group

Relative to the blank, down-regulation control, and up-regulation control groups, the overexpression group exhibited markedly elevated miR-146a-5p levels (2.15±0.35, P<0.01), along with a concomitant decline in Notch2, CAP, BSP, OCN, and OPN mRNA expression (0.64±0.10, 0.52±0.09, 0.81±0.15, 0.75±0.11, P<0.01). Conversely, cells in the miR-146a-5p inhibition group exhibited markedly reduced miR-146a-5p levels (0.42 ± 0.08, P < 0.01), accompanied by a significant rise in the mRNA expression of the related osteogenic genes (P < 0.05 or P < 0.01). In the Notch2-silenced group, transcriptional levels of Notch2, CAP, BSP, OCN, and OPN were substantially diminished compared with the controls (0.25 ± 0.03, 0.41 ± 0.07, 0.65 ± 0.12, 0.52 ± 0.10, 0.34 ± 0.05; all P < 0.01). Furthermore, relative to the miR-146a-5p overexpression group, the inhibition group showed a significant decrease in miR-146a-5p expression (P < 0.01) alongside a pronounced increase in the mRNA levels of these target genes (P < 0.01) (Table 2).

Table 2: MiR-146a-5p expression, Notch2, CAP, BSP, OCN, OPN mRNA expression in cells of each group ($\bar{x} \pm s$, n=6)

Group	*miR-146a-5p*	*Notch2*	*CAP*	*BSP*	*OCN*	*OPN*
Blank group	1.03±0.16	1.03±0.13	0.98±0.14	1.11±0.15	0.99±0.13	1.06±0.15
Down-regulation control group	0.97±0.15	1.09±0.17	0.97±0.12	1.10±0.14	1.05±0.12	1.01±0.13
Up-regulation control group	0.98±0.14	1.06±0.15	0.98±0.12	1.12±0.13	1.06±0.11	1.04±0.14
Notch2 down-regulation group	0.98±0.14	0.25±0.03$^{**\#\#E\%\&E\%}$	0.41±0.07$^{**\#\#E\%\&E\%}$	0.65±0.12$^{**\#\#E\%\&E\%}$	0.52±0.10$^{**\#\#E\%\&E\%}$	0.34±0.05$^{**\#\#E\%\&E\%}$
MiR-146a-5p up-regulation group	2.15±0.35$^{**\#\#E\%\&\&}$	0.64±0.10$^{**\#\#E\%\&\&}$	0.52±0.09$^{**\#\#E\%\&\&}$	0.81±0.15$^{**\#\#E\%\&\&}$	0.75±0.11$^{**\#\#E\%\&\&}$	
0.49±0.07$^{**\#\#E\%\&\&}$						
MiR-146a-5p down-regulation	0.42±0.08$^{**\#\#E\%\&\&\%\%}$	1.44±0.22$^{**\#\#E\%\&\&\%\%}$	1.53±0.26$^{**\#\#E\%\&\&\%\%}$	1.67±0.28$^{**\#\#E\%\&\&\%\%}$	1.47±0.24$^{**\#\#E\%\&\&\%\%}$	
1.39±0.19$^{**\#\#E\%\&\&\%\%}$						
group						
F value	70.268	29.783	41.228	25.369	26.357	50.812
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Comparing to the blank group, $^{*}P<0.05$, $^{**}P<0.01$; comparing to the down-regulation control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; comparing to the up-regulation control group, $^{E\%}P<0.05$, $^{E\%E\%}P<0.01$; comparing to Notch2 down-regulation group, $^{\&}P<0.05$, $^{\&\&}P<0.01$; comparing to miR-146a-5p up-regulation group, $^{\%}P<0.05$, $^{\%\%}P<0.01$

Protein Expression of Notch2, CAP, BSP, OCN and OPN in Cells of Each Group

Besides the mRNA expression, the protein expression also confirmed the signalling pathway. Relative to the blank, down-regulation control, and up-regulation control groups, protein levels of Notch2, CAP, BSP, OCN, and OPN were markedly reduced in the miR-146a-5p overexpression group (0.15±0.03, 0.28±0.04, 0.09±0.01, 0.67±0.11, 0.12±0.02, P<0.01). Conversely, these protein expressions were significantly increased in the miR-146a-5p down-regulation group (0.79±0.12, 1.06±0.14, 0.37±0.06, 1.33±0.21, 0.43±0.07, P<0.01) (Fig. 5 and Table 3). In the Notch2 knockdown group, protein expression of these markers was also substantially lower than that observed in the control groups (0.04±0.01, 0.06±0.02, 0.04±0.01, 0.16±0.03, 0.10±0.02, P<0.01). Furthermore, the miR-146a-5p inhibition group exhibited markedly elevated protein expression of Notch2, CAP, BSP, OCN, and OPN compared with the overexpression group (P < 0.01) (Fig. 5 and Table 3).

Validation Results of miR-146a-5p Targeting Notch2

Bioinformatic predictions identified a possible interaction site through which miR-146a-5p may bind to Notch2 (Fig.6). For cells transfected with the wild-type pmirGLO-Notch2 construct, co-transfection of miR-146a-5p mimics led to an approximate 60percent reduction in relative luciferase intensity versus the miR-NC group (P<0.01), while the inhibitor produced the opposite effect (P<0.01). In contrast, when the mutant pmirGLO-Notch2 construct was introduced, the relative luciferase activity showed no statistically significant variation among the miR-NC, miR-146a-5p mimic, and inhibitor groups (P > 0.05) (Fig.7).

DISCUSSION

Periodontal disease, typically initiated by local factors like plaque and calculus that trigger gingival inflammation and alveolar bone loss, continues to pose a major clinical challenge (Jiang et al. 2020). Guided tissue regeneration is a key treatment strategy, with cementum regeneration being a critical step in this process (Raju et al. 2020). The results confirmed that human dental pulp stem cells

Fig. 5. Protein expression of Notch2, CAP, BSP, OCN and OPN in cells of each group

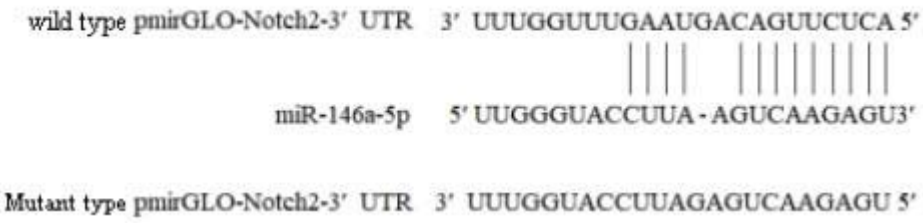


Fig. 6. MiR-146a-5p and Notch2 binding sites

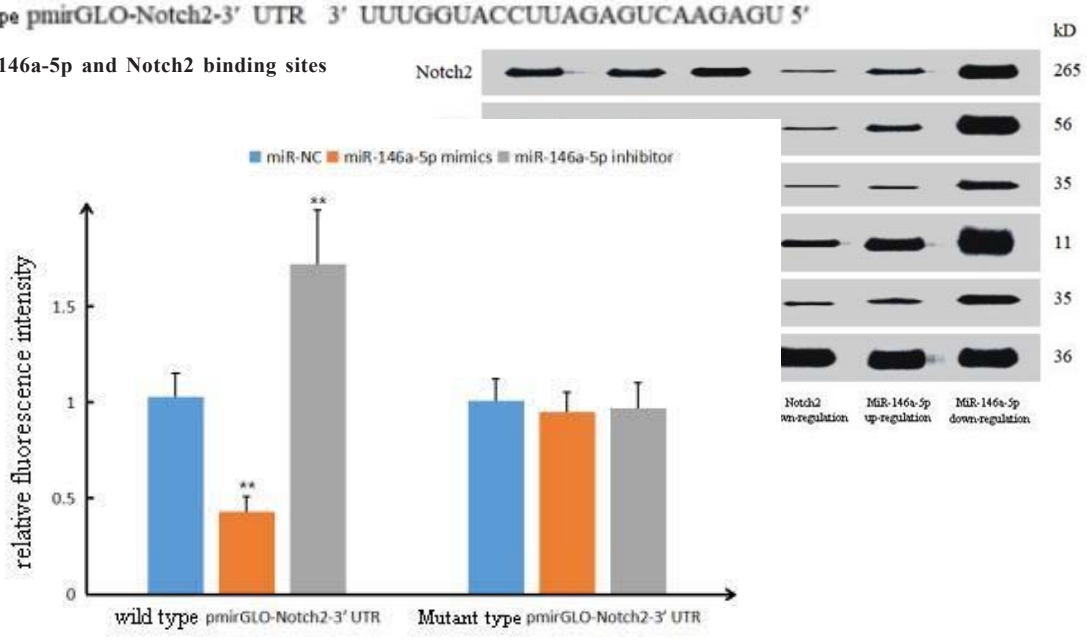


Fig. 7. Validation results of Dual Luciferase Reporter Gene
Note: Comparing to miR-NC group, $^{*}P<0.05$, $^{**}P<0.01$

were effectively isolated and expanded in culture, and that all experimental groups achieved successful transfection, ensuring their suitability for further analyses. Our results indicated that reduced miR-146a-5p expression upregulated ALP, increased its activity, facilitated mineralised nodule formation, and accelerated cementoblastic differentiation in dental pulp stem cells.

ALP serves as a key indicator during the differentiation of cells into cementoblasts. Previous studies have reported that increased ALP expression and activity can drive human dental follicle cells toward cementoblastic differentiation (Liu et al. 2021). The present findings align with previous reports, indicating that reduced miR-146a-5p levels may facilitate the cementoblastic differentiation of human dental pulp stem cells through increased ALP expression and activity.

The researchers further revealed that low miR-146a-5p expression upregulates Notch2, CAP, BSP, OCN, and OPN at both mRNA and protein levels, thereby promoting cementoblast differentiation. Conversely, downregulating Notch2 inhibits this differentiation. miR-146a-5p directly targets Notch2, which is a key regulator of cementoblast differentiation and osteogenic capacity. Earlier research has demonstrated that elevated Notch2 levels enhance the osteogenic differentiation capability of dental pulp stem cells (Zhou et al. 2023). CAP is involved in guiding the differentiation of cementoblast precursor cells into mature cementoblast-like cells and can strengthen their osteogenic capacity. According to Hoz et al. (2021), CAP expression is elevated in the course of cementoblast regeneration. Cementoblasts are functional cells that form cementum. At the initial stage of cementum formation, BSP and OPN play important functions. BSP contributes to cementoblast adhesion and differentiation, whereas OPN regulates the pace of cementum mineralisation. Li et al. (2021) reported that suppression of ALP, BSP, and OPN expression hampers cementoblast differentiation and consequently impedes cementum formation. OCN serves as a marker of osteoblast differentiation, indicative of their osteogenic function, and is abundantly expressed in cementum. Fu et al. (2022) pointed out that inhibiting the expression of BSP and OCN in cementum can reduce the mineralisation ability of cementoblasts and inhibit the formation of cementum. Liu et al. (2022) pointed out that inhibiting OPN and OCN expression in cementoblasts could inhibit the formation of cementum. The Notch2 signaling pathway participates in controlling stem cell proliferation and the preservation of stemness, and is essential for tooth development and regenerative processes. Ma et al. (2018) pointed out that inhibiting tumour necrosis factor-á (TNF-á) can inhibit Notch2 pathway and ALP activity, and then inhibit periodontal ligament stem cells' osteogenic differentiation.

MiRNA acts importantly in the osteogenic differentiation of mesenchymal stem cells (Ma et al. 2018). Zheng et al. (2021) found that miR-146a-5p functions as an important inhibitor of osteoblast and bone formation, limiting the osteogenic differentiation capacity of bone marrow–derived mesenchymal stem cells. Miao et al. (2023) indicated that miR-146a-5p influences the osteogenic differentiation of periodontal ligament stem cells through its regulatory action on Notch2. Consistent with these previous findings, our study indicates that reduced miR-146a-5p expression facilitates cementoblastic differentiation of DPSCs through activation of the Notch2 pathway and upregulation of critical markers including Notch2, CAP, BSP, OCN, and OPN.

CONCLUSION

In conclusion, the study provides new insights into the molecular mechanisms of cementoblast differentiation and potential therapeutic targets for periodontal regeneration. The regulatory interaction between miR-146a-5p and Notch2 may serve as a valuable therapeutic target for future strategies aimed at enhancing periodontal tissue regeneration.

RECOMMENDATIONS

Chronic periodontal disease can progressively destroy periodontal tissues and ultimately result in tooth loss. To address periodontal damage, treatment approaches have ranged from conventional supportive therapies to the application of advanced biomaterials. In recent years, gene-based and small-molecule therapeutic strategies have emerged to promote the regeneration of compromised periodontal structures. Understanding the molecular mechanisms that

regulate cementoblast differentiation offers valuable insights and holds significant potential for improving periodontal disease management.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

COMPETING INTERESTS

No conflicts of interest exist in this manuscript.

CONSENT FOR PUBLICATION

Manuscript is approved by all authors for publication.

AVAILABILITY OF DATA AND MATERIALS

The data and materials of this experiment are available.

ACKNOWLEDGEMENTS

Not applicable.

AUTHOR CONTRIBUTIONS

Xiaoguang Tian was responsible for conception and design. YanHua Zhu and Xiaoguang Tian were responsible for manuscript writing. Zizhong Wu and Feifei Gong were responsible for collection and assembly of data. Jianwei Pan and YanHua Zhu were responsible for data analysis and interpretation. All authors were responsible for manuscript writing. All authors were responsible for the final approval of the manuscript.

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