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Original Article

miR-508-5p suppresses osteogenic differentiation of human periodontal ligament stem cells via targeting sex-determining region Y-related HMG-box 11

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KEYWORDS
Periodontitis;
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Abstract *Background/Purpose:* The local inflammatory microenvironment created by periodontitis negatively impacts periodontal tissue regeneration, necessitating the development of methods to enhance the regenerative capacity of stem cells. This study explored the regulatory role and underlying mechanism of miR-508-5p in the osteogenic differentiation of human periodontal ligament stem cells (hPDLSCs).
Materials and methods: The regulatory roles of miR-508–5p in osteogenic differentiation of hPDLSCs were investigated through its inhibition or overexpression. Expression of the sex-determining region Y-related HMG-box 11 (SOX11) and osteogenic markers was analyzed using Western blot and real-time PCR. Osteogenesis was measured using alizarin red S (ARS) staining and alkaline phosphatase (ALP) staining. A dual luciferase reporter assay was performed to confirm SOX11 as a target of miR-508-5p.
Results: During the osteogenic differentiation of hPDLSCs, miR-508-5p expression level gradually decreased, while that of SOX11 increased. miR-508-5p inhibition significantly promoted osteogenesis in hPDLSCs, while overexpression inhibited the process. SOX11 overexpression reversed the suppressive effects of miR-508-5p on the osteogenic differentiation of hPDLSCs. miR-508–5p downregulation significantly increased SOX11; a dual luciferase reporter assay provided evidence for their direct targeting.
Conclusion: miR-508-5p downregulation promotes the osteogenic differentiation of hPDLSCs by targeting SOX11.

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Introduction

Periodontitis is a chronic inflammatory disease characterised by the destruction of periodontal supporting tissues, leading to alveolar bone resorption and attachment loss.[1] Regenerative therapy aims to regenerate these tissues and improve tooth prognosis,[2] with bone regeneration being a critical component. Periodontal ligament stem cells (PDLSCs) are ideal seed cells for periodontal regeneration that play a crucial role in this process.[3,4]

The inflammatory microenvironment in periodontitis impairs the regenerative capacity of stem cells, negatively affecting the therapeutic efficacy of periodontal regeneration.[5,6] Studies comparing PDLSCs from healthy individuals (H-PDLSCs) with those from periodontitis patients (P-PDLSCs) have reported reduced multi-lineage differentiation potential in P-PDLSCs, highlighting the detrimental impact of inflammation.[7] Pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α), further hinder the proliferation and differentiation of PDLSCs; for example, TNF-α suppresses osteogenic differentiation via the nuclear factor kappa-B (NF-κB) signalling pathway.[8–12] Therefore, mitigating inflammatory interferences and enhancing the differentiation capacity of PDLSCs are essential for improving periodontal regenerative therapy outcomes.

Epigenetic regulation, particularly that facilitated by microRNAs (miRNAs), offers a promising avenue for promoting tissue regeneration.[13] miRNAs are small noncoding RNAs that regulate gene expression in various biological processes[14–16] and can enhance the osteogenic potential of stem cells by modulating downstream genes, including transcription factors and signalling molecules.[17–19] Despite the identification of approximately 35 miRNAs related to PDLSC osteogenic differentiation, current research on miRNAs remains limited.[20,21] One study that analyzed the potential competing endogenous RNA (ceRNA) interplay during the osteogenic differentiation of human periodontal ligament stem cells (hPDLSCs) identified differentially expressed miRNAs.[22] The result suggests that miR-508-5p may be related to this biological process; however, experimental data to confirm this are lacking, necessitating further investigations.[22]

To address this knowledge gap, this study aimed to explore the regulatory role and mechanism of action of miR-508-5p in the osteogenic differentiation of hPDLSCs.

Materials and methods

Isolation and cultivation of hPDLSCs

hPDLSCs were isolated from the PDL of healthy premolars collected during extractions at the Peking University Stomatology Hospital. The procedure involved patients aged 18–25 years. Extracted premolars were rinsed with phosphate-buffered saline (PBS) (Servicebio, Wuhan, China) containing 5% penicillin-streptomycin, and PDL tissue was scraped from the middle third of the root surface. The PDL tissue was sectioned into small blocks (approximately 1–5 mm). After centrifugation at 1000 rpm for 5 min, the PDL tissue blocks were seeded into inverted 25 cm^2 cell culture flasks (NEST, Wuxi, China) for adherent growth.[23] The hPDLSCs were cultivated in α-minimum essential medium (α-MEM) (Gibco, Grand Island, NY, USA) supplemented with 1% penicillin-streptomycin (Pen Strep) (Gibco) and 20% foetal bovine serum (FBS) (Analysis Quiz, Beijing, China) at 37 °C in a 5% CO_2 incubator. After overnight culture, the inverted flasks were turned upright. The medium was replaced every 2 days and the hPDLSCs were observed under an inverted microscope (Olympus, Tokyo, Japan) approximately 10 days after seeding. Subsequently, hPDLSCs were cultured using a limited dilution method. Cells between passages 3 (P3) and 6 were used in all experiments. The Ethics Committee of Peking University Hospital of Stomatology approved this study (approval code PKUSSIRB-202497033).

Flow cytometry analysis

Cells were harvested using 0.25% trypsin–EDTA (Gibco) and resuspended in PBS containing 0.1% bovine serum albumin (BSA) (Solarbio, Beijing, China) and incubated with antibodies against CD29, CD31, CD34, CD45, CD73, CD90, CD105, and CD146 (BioLegend, San Diego, CA, USA) for 30 min at 4 °C in the dark. An isotype antibody served as a control. Characterisation of hPDLSCs (P3) was performed using flow cytometry (NovoCyte, San Diego, CA, USA) and analysed using NovoExpress 1.5.0 software.

Multiple differentiation potential of the hPDLSCs

The multipotent differentiation potential of hPDLSCs was assessed by inducing osteogenic and adipogenic differentiation. Cells were seeded into 6-well plates (Servicebio) and cultured in basal medium (α-MEM supplemented with 1% penicillin-streptomycin and 10% FBS) until 80% confluence. For osteogenic differentiation, cells were exposed to osteogenic medium (α-MEM supplemented with 50 mg/L dexamethasone, 10 mmol/L β-glycerophosphate, 50 mg/L vitamin D3, and 10% FBS). After seven days of culture, alkaline phosphatase (ALP) staining was performed using a BCIP/NBT Alkaline Phosphatase Color Development Kit (Beyotime, Guangzhou, China) according to the manufacturer's instructions. Alizarin red S (ARS) staining was used to evaluate extracellular calcium deposits after 21 days of culture in osteogenic medium. Stained cells were washed

with double-distilled water and imaged using an inverted light microscope (Olympus).

For adipogenic differentiation, hPDLSCs were plated in 12-well plates and cultured in the basal medium until 80% confluence. The medium was then replaced with adipogenic inductive medium (ProCell, Wuhan, China). After 21 days, cells were fixed in 4% paraformaldehyde for 30 min and stained with Oil red O (Solarbio) for an additional 30 min. Finally, the cells were washed with double-distilled water three times and observed under a microscope.

Alkaline phosphatase activity analysis

ALP activity was measured in hPDLSCs after 7 days of growth in osteogenic medium using an ALP Assay Kit (Jiancheng, Nanjing, China) according to the manufacturer's protocol. Absorbance was measured at 520 nm using a microplate reader.

Alizarin red S staining

For ARS, hPDLSCs cultivated in osteogenic medium for 21 days were washed with PBS and fixed with 4% paraformaldehyde for 30 min. After rinsing three times with deionised water, 1% alizarin red S staining (pH 4.2; Solarbio) was added to the cells for 15 min at 20—25 °C. After three rinses with deionised water, orange-red calcium nodules were observed using an inverted light microscope (Olympus).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. An ABScript II cDNA First Strand Synthesis Kit (Abclonal, Wuhan, China) was used to reverse transcribe the total RNA. The reaction included incubation at 37 °C for 2 min, 55 °C for 15 min, 85 °C for 5 min to inactivate the enzyme, and then maintenance at 4 °C. qRT-PCR was conducted using qPCR SYBR Green Master Mix (Abclonal) as previously described. Primer sequences for all probed genes are listed in Table 1. The relative expression of the target gene was calculated using the $2^{-\Delta\Delta CT}$ method after normalisation to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the standard.

Western blot analysis

Cells were harvested and rinsed with ice-cold PBS. Proteins were collected on ice using RIPA lysis buffer (Solarbio) according to manufacturer's instructions. Protein concentrations were determined using a BCA protein assay kit (EpiZyme, Shanghai, China) according to manufacturer's instructions. Protein samples (25 μg/well) were loaded onto 12.5% SDS-PAGE gels (EpiZyme) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% skim milk at 20—25 °C for 1.5 h and incubated with primary antibodies overnight at 4 °C using the following concentrations: anti-SOX11 (Proteintech, Wuhan, China) at 1:500, anti-Runx2 (Proteintech) and anti-β-actin (Proteintech) at 1:2000, and anti-COL1A1 (ABclonal) and anti-OCN (ABclonal) at 1:1000. Membranes were then rinsed with Tris-buffered saline containing Tween (TBST) (Solarbio) and incubated with a 1:2000 dilution of anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Proteintech) for 1.5 h. Subsequently, the membranes were immersed in enhanced chemiluminescence (ECL) Western Blotting Substrate (Solarbio) and analysed using a Touch Imager Contact Chemiluminescence Imaging System (e-BLOT, Shanghai, China).

Table 1 Sequences of primers used for qRT-PCR.

Gene	Primer sequences (5′—3′)
GAPDH	Forward: TCATTTCCTGGTATGACAACGA
	Reverse: GTCTTACTCCTTGGAGGCC
SOX11	Forward: CCTGTCGCTGGTGGATAAGG
	Reverse: GTGCAGTAGTCGGGGAACTC
COL1A1	Forward: GAGGGCCAAGACGAAGACATC
	Reverse: CAGATCACGTCATCGCACAAC
Runx2	Forward: CACTGGCGCTGCAACAAGA
	Reverse: CATTCCGGAGCTCAGCAGAATAA
OCN	Forward: GGCGCTACCTGTATCAATGG
	Reverse: GTGGTCAGCCAACTCGTCA
miR-508—5p	Forward: AACCGCTACTCCAGAGGGC
	Reverse: CAGAGCAGGGTCCGAGGTA
U6	Forward: CGCTTCGGCAGCACATATACTA
	Reverse: CGCTTCACGAATTTGCGTGTCA

GAPDH, glyceraldehyde-3-phosphate Dehydrogenase; SOX11, sex-determining region Y-related HMG-box 11; COL1A1, alpha-1 type 1 collagen; Runx2, runt-related transcription factor 2; OCN, osteocalcin.

miRNA target prediction

Target genes of miRNAs were predicted using the TargetScan (http://www.targetscan.org/) and starBase (https://rnasysu.com/encori/) databases.

Oligonucleotides and cell transfection

To assess the effects of miR-508-5p on the expression of SOX11 and osteogenic markers, including Runx2, OCN, and COL1A1, in hPDLSCs, the cells were transfected with several oligonucleotides. These included negative controls for miR-508-5p mimics (mimics NC), negative controls for miR-508-5p inhibitors (inhibitor NC), miR-508-5p mimics, and miR-508-5p inhibitors. All primers were purchased from GenePharma (Gemma Biotechnology, Shanghai, China). Transfection was carried out using HighGene Plus Transfection Reagent (Abclonal) according to the manufacturer's instructions. The cells were harvested after 48 h of incubation for SOX11 expression analysis. To determine the influence of miR-508-5p on osteogenic differentiation, cells were harvested at 0, 1, 4, and 7 d following exposure to the osteogenic medium.

Dual luciferase reporter assay

The reporter vector was synthesised by GenePharma (Gemma Biotechnology, Suzhou, China). Either wild-type

SOX11 or its mutant fragment, termed SOX11 3′- untranslated region (3′-UTR)-wt and SOX11 3′-UTR-mut, respectively, was inserted into the vectors. The sequences of the miR-508-5p binding site and the mutant site are as follows: SOX11 3′-UTR-wt (5'-AGGUAGUUGCAUACCUAGUCUGGAGUU-3') and SOX11 3′-UTR-mut (5'-AGGUAGUUGCAUACCUAGUGACCUCAU-3'). To determine whether miR-508-5p binds to SOX11 3′-UTR, 293 T cells were seeded in 96-well plates (Corning Costar, Corning, NY, USA) for dual luciferase reporter gene assays. The cells were co-transfected with either the SOX11 3′-UTR-wt or the SOX11 3′-UTR-mut reporter plasmid along with mimics NC or miR-508-5p mimics using GP-transfect-Mate (Gemma Biotechnology) according to the manufacturer's instructions. After 48 h, luciferase activity was measured using a Dual-luciferase Reporter Assay Kit (Gemma Biotechnology) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were conducted using SPSS software (version 27.0) (IBM, Armonk, NY, USA). Differences between normally distributed values were analyzed using a two-tailed *t*-test. If the values were nonnormally distributed, a Mann—Whitney non-parametric test was performed. Differences between multiple groups were analysed using one-way analysis of variance with Bonferroni post-hoc tests. *P*-value <0.05 indicates statistical significance.

Results

Identification of hPDLSCs

After a 10-day isolation and cultivation period, hPDLSCs displayed irregular or elongated fusiform shapes arranged radially, resembling known periodontal ligament (PDL) cells (Fig. 1A). Flow cytometry analysis of third-generation hPDLSCs revealed negative expression of haematopoietic markers CD31, CD34, and CD45 (Fig. 1B) and positive expression of mesenchymal stem cell markers CD29, CD73, CD90, CD105, and CD146 (Fig. 1C).

Osteogenic and adipogenic differentiation assays were performed to assess the multilineage differentiation capacity of hPDLSCs. After 7 days of osteogenic induction, hPDLSCs exhibited increased ALP staining compared with the control group (Fig. 2A). The presence of irregular red-calcified nodules, as revealed by ARS staining, indicated osteoblastic differentiation potential of hPDLSCs (Fig. 2B). Additionally, Oil red O staining confirmed lipid droplet formation in hPDLSCs, indicating adipogenic differentiation (Fig. 2C). These results confirm the mesenchymal stem cell (MSC) properties of hPDLSCs.

miR-508-5p was downregulated during the osteogenic differentiation of hPDLSCs

We conducted qRT-PCR to observe the dynamic expression of miR-508-5p during osteogenic differentiation and observed a significant reduction in miR-508-5p levels at various time points during osteogenesis (Fig. 3A).

miR-508-5p inhibited osteogenic differentiation of hPDLSCs

Considering the correlation between miR-508-5p expression and osteogenic differentiation, we determined whether miR-508-5p influenced the osteogenic differentiation of hPDLSCs. After 7 days of osteogenesis induction, miR-508-5p overexpression significantly downregulated the expression of osteogenic markers COL1A1, Runx2, and OCN, and reduced ALP activity, compared with the mimic NC group (Fig. 3B—F). Similarly, decreased matrix mineralisation was observed with the overexpression of miR-508-5p compared with the mimic NC group via ARS staining after 21 days of induction (Fig. 3C). Conversely, miR-508-5p inhibition led to significant increases in osteogenic marker gene expression, ALP activity, and matrix mineralisation compared with the inhibitor NC group (Fig. 3B—F). These findings indicate that miR-508-5p negatively regulates the osteogenic differentiation of hPDLSCs.

SOX11 is a potential miR-508-5p target

Bioinformatic analysis was performed using the TargetScan and starBase databases to identify potential targets of miR-508-5p. Partial complementary sequences were identified between miR-508-5p and the 3′-UTR of sex-determining region Y-related HMG-box 11 (SOX11), suggesting that SOX11 is a potential miR-508-5p target (Fig. 4A).

We then examined the dynamic expression patterns of SOX11 during the osteogenic differentiation of hPDLSCs. Contrary to the expression of miR-508-5p, a significant increase was observed in the expression of SOX11 at both the mRNA and protein levels in cells undergoing osteogenic induction compared with controls (Fig. 4B—D).

miR-508-5p suppressed the expression of SOX11

To investigate the relationship between SOX11 and miR-508-5p in hPDLSC osteogenesis, we manipulated miR-508-5p levels using mimics or inhibitors. Overexpression of miR-508-5p significantly downregulated SOX11 at both the mRNA and protein levels compared with controls (Fig. 5A—C). Conversely, inhibiting miR-508-5p increased SOX11 expression at both levels compared with controls (Fig. 5A—C). These data imply that SOX11 is a target of miR-508-5p.

miR-508-5p suppressed the osteogenic differentiation of hPDLSCs by targeting SOX11

We hypothesised that miR-508-5p interacts with SOX11 during osteogenic differentiation. To test this, hPDLSCs were co-transfected with either miR-508-5p mimics or mimics NC, along with a SOX11 plasmid containing either a mutant (mut) 3′-UTR or a wild-type (wt) 3′-UTR (Fig. 6A). Co-transfection of miR-508-5p mimics with SOX11 3′-UTR-mut resulted in significantly higher expression of osteogenic genes compared with the SOX11 3′-UTR-wt group (Fig. 6B—D). Consistently, increased ALP activity and ARS staining were observed (Fig. 6E and F). However, co-transfection of miR-508-5p mimics with SOX11 3′-UTR-wt

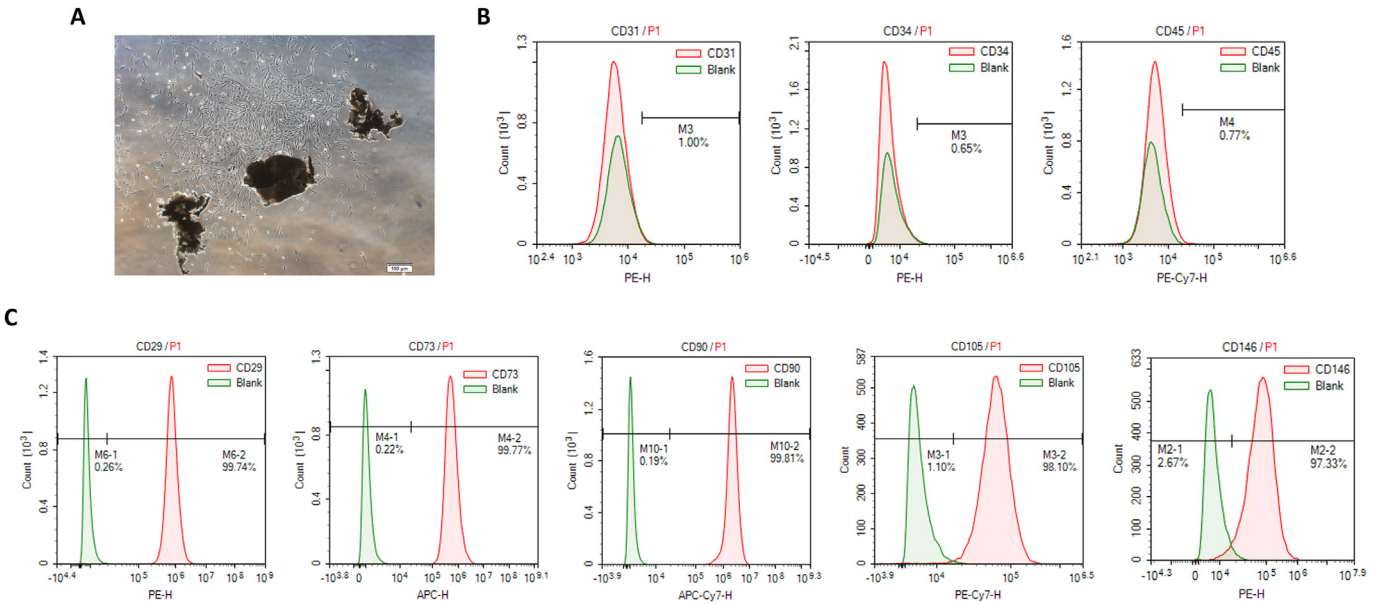

Figure 1 Culture and identification of hPDLSCs. (A) Morphology of cultured hPDLSCs. (B) Flow cytometry results showing negative expression of CD31, CD34, and CD45. (C) Flow cytometry results showing positive expression of CD29, CD73, CD90, CD105, and CD146. hPDLSCs, human periodontal ligament stem cells.

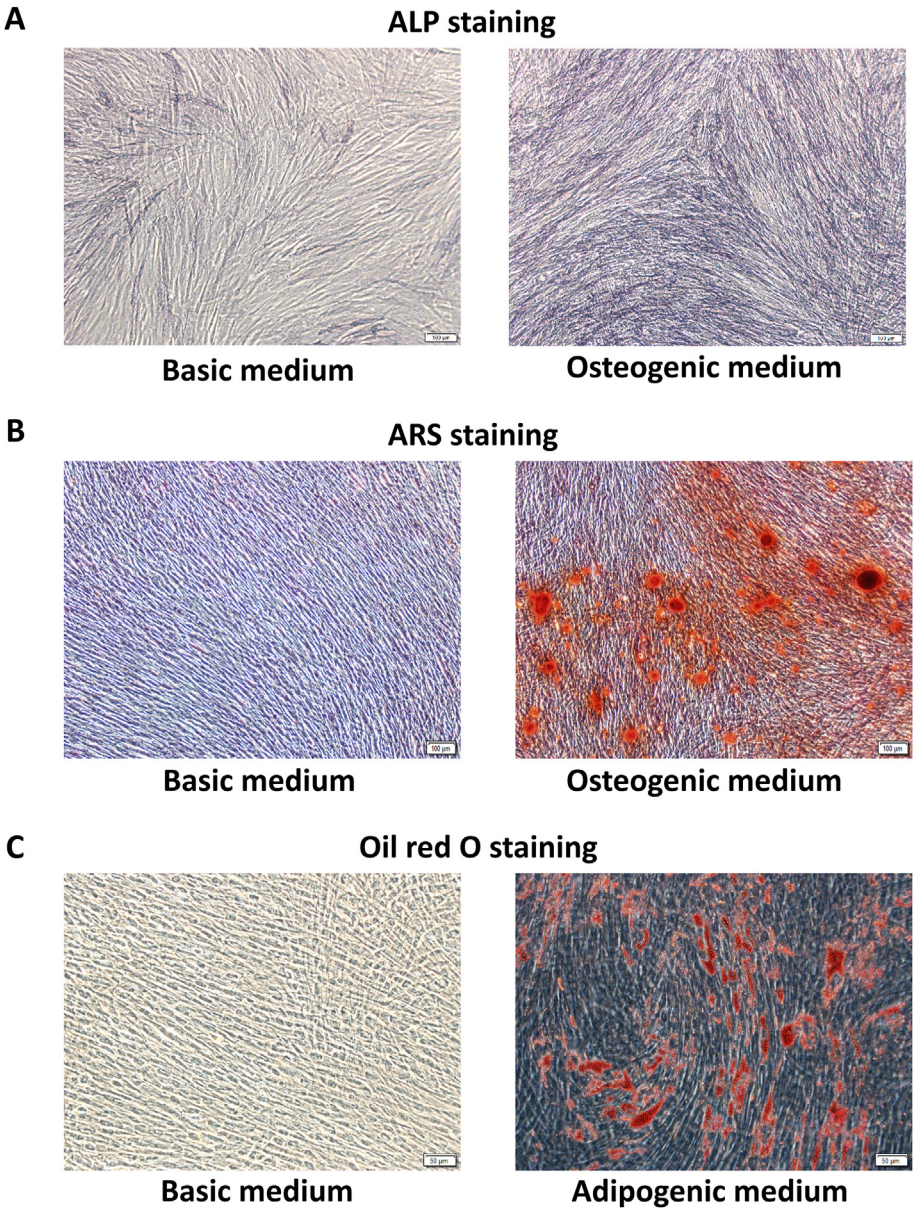

Figure 2 Identification of hPDLSC differentiation. (A) ALP staining after 7 d of osteogenic induction. (B) ARS staining after 21 d of osteogenic induction. (C) Oil red O staining confirming adipogenic differentiation after 21 d hPDLSCs, human periodontal ligament stem cells; ALP, alkaline phosphatase; ARS, alizarin red S.(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

did not reverse the suppressive effects of miR-508-5p (Fig. 6B—F).

To confirm the direct suppression of SOX11 by miR-508-5p, fragments of wt and mut 3′-UTR of SOX11 were inserted into a luciferase reporter vector. Overexpression of miR-508-5p inhibited luciferase activity in the presence of wild-type 3′-UTR, but not with the mutated 3′-UTR (Fig. 6G). These results indicate that miR-508-5p suppresses the osteogenic differentiation of hPDLSCs by directly targeting SOX11.

Discussion

Periodontitis and its associated inflammatory microenvironment not only cause tissue destruction but also hinder the regenerative process by influencing the behaviour of PDLSCs, which are crucial for periodontal regeneration.[9] Therefore, strategies to enhance the osteogenic differentiation of PDLSCs are warranted.[24—26] Our study demonstrated that miR-508-5p regulates the osteogenic differentiation of hPDLSCs by modulating SOX11 expression. Specifically, the downregulation of miR-508-5p promotes osteogenic differentiation, suggesting that hPDLSCs could have increased clinical utility in periodontal regeneration.

To the best of our knowledge, this is the first study to identify miR-508-5p as a regulator of osteogenic differentiation in hPDLSCs. Our experiments demonstrated that inhibiting miR-508-5p enhances osteogenesis, as evidenced by the increased expression of osteogenic markers and

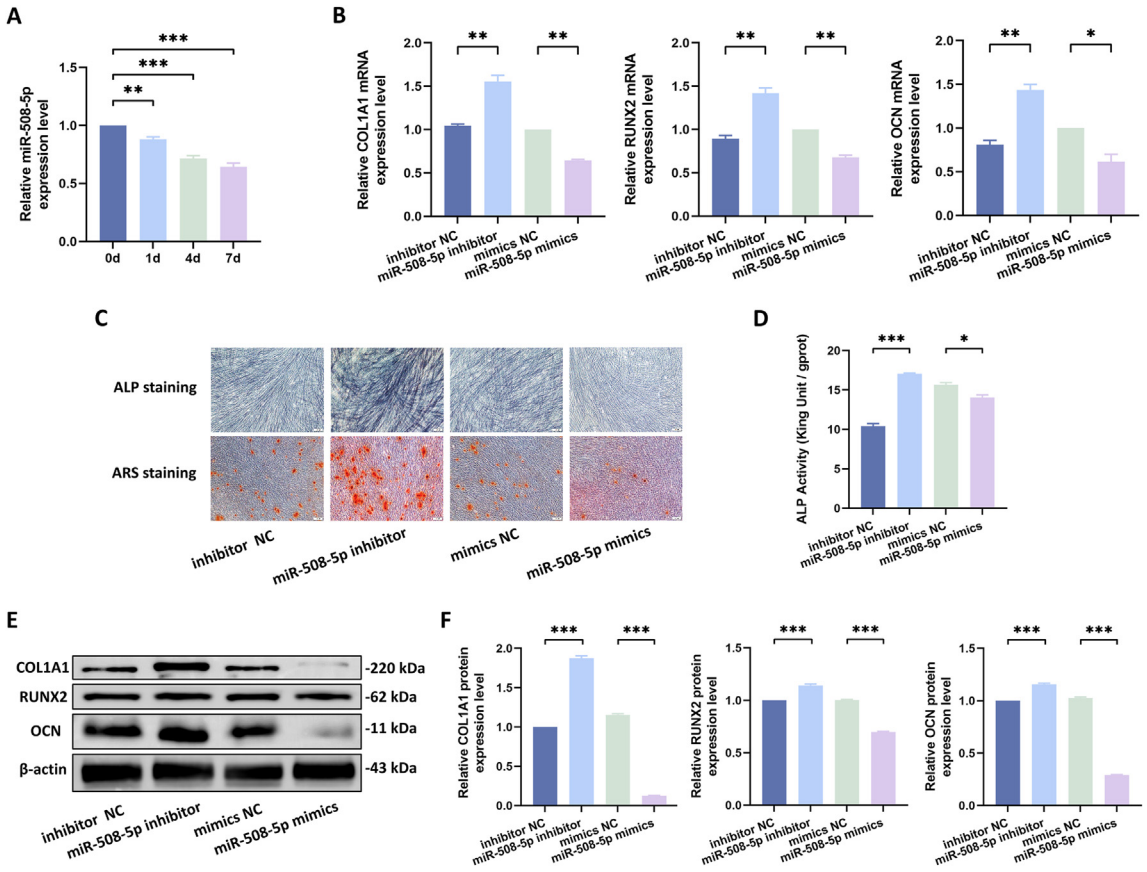

Figure 3 miR-508—5p is downregulated during osteogenic differentiation and inhibits this process. (A) miR-508—5p expression levels detected using qRT-PCR during osteogenic differentiation. (B) mRNA expression levels of COL1A1, Runx2, and OCN on day 7 of osteogenic differentiation. (C) ALP and ARS staining. (D) ALP activity changes in miR-508—5p mimics and inhibitor groups. (E) Protein expression levels of COL1A1, Runx2, and OCN detected using Western blot. (F) The quantified Western blot results. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Mimics NC, negative controls for miR-508—5p mimics; Inhibitor NC, negative controls for miR-508—5p inhibitors; COL1A1, alpha-1 type 1 collagen; Runx2, runt-related transcription factor 2; OCN, osteocalcin; ALP, alkaline phosphatase; ARS, alizarin red S.(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

greater mineralisation. Conversely, upregulating miR-508-5p reduces osteogenic differentiation. This finding is consistent with that of recent research showing that the downregulation of miR-508-5p facilitates odontogenic differentiation of human dental pulp stem cells (hDPCs) by targeting glycoprotein non-metastatic melanomal protein B (GPNMB),[27] thereby supporting the regulatory role of miR-508-5p in stem cell differentiation. However, our study specifically reports the role of miR-508-5p in the osteogenic differentiation of hPDLSCs, highlighting a novel aspect of its influence on stem cell behaviour.

Moreover, the influence of miR-508-5p on the osteogenic differentiation of hPDLSCs in this study was consistent throughout the process. During osteogenic differentiation and extracellular matrix mineralisation, ALP activity and the expression of COL1A1, Runx2, and OCN, as well as calcified nodules identified by ARS, serve as markers of osteoblast activity.[28—30] These markers were used to assess osteogenic capability. ALP, expressed during the matrix deposition and maturation phases, is widely utilised as an early indicator of bone formation,[31] while OCN is typically expressed during the terminal stage of osteogenic differentiation.[32] Changes in the expression of genes affected by miR-508-5p indicate the potential for the development of a new method for enhancing the osteogenic differentiation capacity of hPDLSCs.

In the regulatory pathway of miR-508-5p, we, for the first time, identified SOX11 as a target molecule regulated by miR-508-5p in hPDLSCs, playing a pivotal role in their osteogenic differentiation. Bioinformatics analysis showed that SOX11 was a downstream target gene of miR-508-5p. SOX11 is a transcription factor involved in various biological processes.[33,34] Our results showed that SOX11 levels significantly increased during the osteogenic differentiation of hPDLSCs. Co-transfection experiments revealed that SOX11 overexpression reversed the suppressive effects of miR-508-5p on the osteogenic differentiation of hPDLSCs. Furthermore, we introduced mutations into the 3′-UTR region of wild-type SOX11 to construct a mutated version. Subsequent luciferase reporter assays indicated that miR-508-5p directly bound to the 3′-UTR of wild-type SOX11 but not the mutated version, thereby negatively regulating

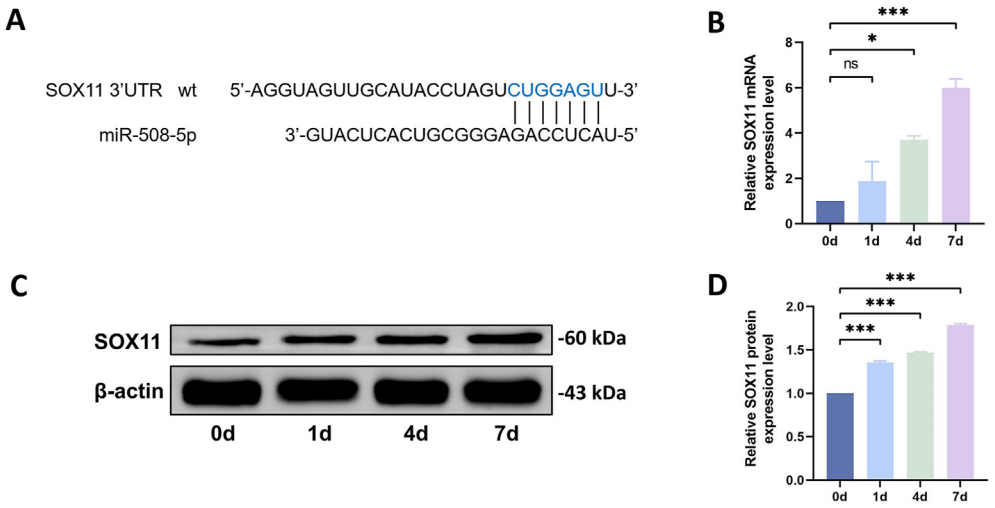

Figure 4 SOX11 is a potential miR-508—5p target. (A) Schematic illustration of the complementary sequence between miR-508—5p and the SOX11 3′-UTR. (B) SOX11 mRNA expression levels detected using qRT-PCR during osteogenic differentiation of hPDLSCs at indicated time points. (C) SOX11 protein levels detected using Western blot during osteogenic differentiation of hPDLSCs at the indicated time points. (D) Quantitative analyses of Western blot showing increased SOX11 levels during the osteogenic differentiation of hPDLSCs at the indicated time points. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ns, not significant. SOX11, sex-determining region Y-related HMG-box 11; 3′-UTR, 3′-untranslated region; Wt, wild-type.

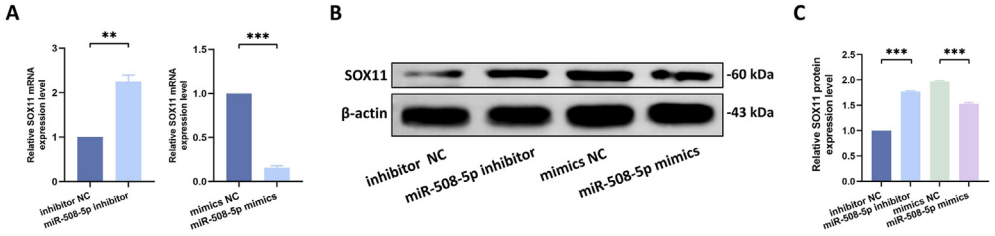

Figure 5 Expression of SOX11 is suppressed by miR-508—5p. (A) The SOX11 expression levels of hPDLSCs transfected with miR-508—5p inhibitors or mimics determined using qRT-PCR. (B) SOX11 expression levels detected using Western blot. (C) The quantified SOX11 Western blot results. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. hPDLSCs, human periodontal ligament stem cells; SOX11, sex-determining region Y-related HMG-box 11; Mimics NC, negative controls for miR-508—5p mimics; Inhibitor NC, negative controls for miR-508—5p inhibitors.

its expression and influencing osteogenic differentiation. This confirms the importance of the 3′-UTR region of wild-type SOX11 for the interaction of miR-508-5p with SOX11. Our study identified the direct binding sites between miR-508-5p and SOX11 and validated the regulatory interaction during the osteogenic differentiation of hPDLSCs.

Accumulating evidence suggests that SOX11 promotes stem cell osteogenic differentiation and bone formation.[35—38] Long noncoding RNAs (lncRNA) cytoskeleton regulator RNA (CYTOR), enhance osteogenic differentiation of PDLSCs by functioning as a ceRNA to increase SOX11 levels via sponging miR-6512—3p.[35] This finding coincides with our perspective on the positive role of SOX11 in osteogenic differentiation, corroborating its remarkable impact on hPDLSCs. As for the downstream target genes of SOX11, Runx2 and osterix (Osx) are directly regulated by SOX11.[36] Moreover, SOX11 mediates the Wnt7b-induced increase in bone formation by enhancing the self-renewal and osteogenic differentiation of BMSCs.[36] Additionally, allogeneic MSCs engineered with SOX11 have been shown to accelerate bone fracture healing.[37] Notably, most existing research on SOX11 in this field is based on animal studies, whereas our study focuses on the mechanisms underlying osteogenic differentiation at the cellular level, contributing to molecular function research.

This study highlights miR-508-5p's regulatory effect on osteogenic differentiation and the role of epigenetics in promoting tissue regeneration. While we have established SOX11 as a downstream target of miR-508-5p, the mechanisms governing its upstream regulation merit further investigation. Using miRanda with a maximum binding free energy of −20, seven lncRNAs were predicted to share at least two binding sites with miR-508-5p.[22] These lncRNAs include TCONS_00155742, TCONS_00187080, TCONS_00212984, TCONS_00068354, TCONS_00221188, TCONS_00212979, and TCONS_0008635, all of which are potential upstream regulators that may exert sponging effects on miR-508-5p.[22] Furthermore, the lncRNA known as metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) positively regulates osteogenic differentiation of

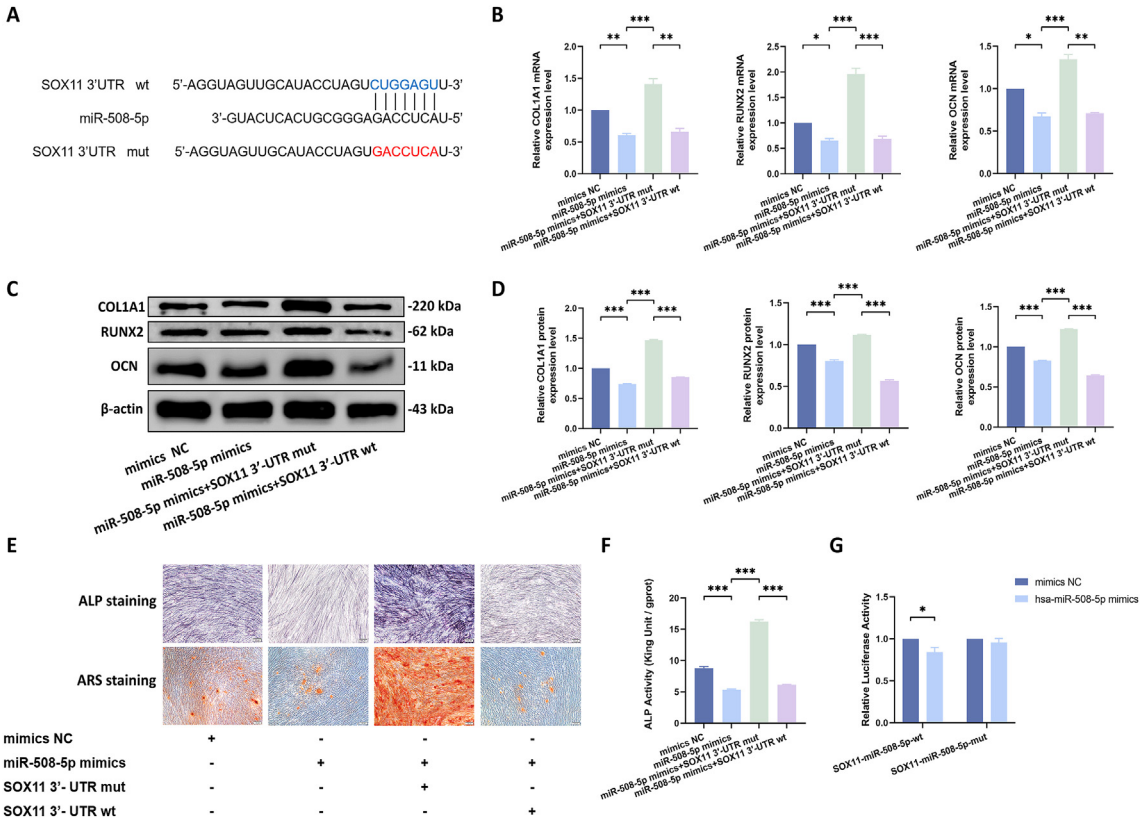

Figure 6 SOX11 3′-UTR-mut rescues the effect of miR-508–5p. (A) Schematic illustration of the complementary sequence between miR-508–5p and the SOX11 3′-UTR. Mutation sites on the 3′-UTR of SOX11 are highlighted in red. (B) mRNA expression levels of COL1A1, Runx2, and OCN detected using qRT-PCR. (C) Protein expression levels of COL1A1, Runx2, and OCN detected using Western blot. (D) Quantification of the Western blot results. (E) hPDLSCs fixed and stained for ALP and ARS, respectively. (F) ALP activity significantly increased on day seven after co-transfection of miR-508–5p mimics and SOX11 3′-UTR mut in hPDLSCs. (G) Luciferase reporter assay showing decreased luciferase activity in 293 T cells co-transfected with miR-508–5p and wild-type SOX11 3′-UTR, but not with the mutated 3′-UTR. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. hPDLSCs, human periodontal ligament stem cells; SOX11, sex-determining region Y-related HMG-box 11. 3′-UTR, 3′-untranslated region; Wt, wild-type; Mut, mutant; Mimics NC, negative controls for miR-508–5p mimics; COL1A1, alpha-1 type 1 collagen; Runx2, runt-related transcription factor 2; OCN, osteocalcin; ALP, alkaline phosphatase; ARS, alizarin red S.(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

stem cells.[38] Moreover, the direct targeting relationship between MALAT1 and miR-508-5p, confirmed in colorectal cancer progression,[39] suggests that MALAT1 may modulate osteogenic differentiation in hPDLSCs through the sequestration of miR-508-5p. Further exploration is needed to understand the potential sponging effects of lncRNA and circular RNAs (circRNAs) and to identify potential upstream regulators or signals of miR-508-5p.

Given these insights, further exploration of the role of miR-508-5p in an inflammatory microenvironment is warranted. miR-508-5p and miR-508-3p originate from different stem-loop arms of the same miRNA precursor, and there are variations in their nucleotide composition. While miR-508-3p is known to negatively regulate the progression of diabetic periodontitis,[40] the relationship between miR-508-5p and periodontitis, as well as its role in inflammatory contexts, remains unclear and merits further investigation.

This study provides new insights into enhancing the osteogenic differentiation capabilities of hPDLSCs, which could aid in promoting periodontal tissue regeneration. Future studies should explore the upstream regulators of miR-508-5p and its role in inflammatory conditions. Confirming these findings through *in vivo* experiments is essential to fully elucidating the therapeutic potential and applications in clinical settings.

In summary, our study demonstrated the regulatory role of miR-508–5p by targeting SOX11 in the osteogenic differentiation of hPDLSCs. Fig. 7 shows a schematic illustration of the role of miR-508-5p in the osteogenic differentiation of hPDLSCs. Our findings present a promising strategy for enhancing the differentiation capacity of

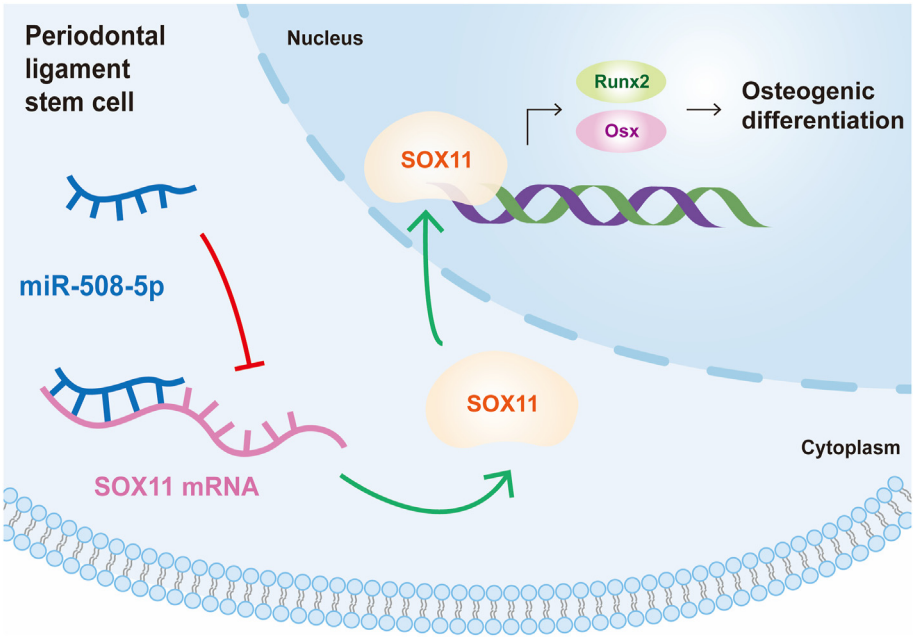

Figure 7 Schematic diagram of miR-508—5p in osteogenic differentiation of hPDLSCs. hPDLSCs, human periodontal ligament stem cells.

hPDLSCs, with potential application in periodontal regeneration therapy.

Declaration of competing interest

The authors have no conflicts of interest in this study.

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