miR-192-5p Inhibits Osteo-/odontogenic Differentiation in Dental Pulp Stem Cells by Targeting COL5A1

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Objective: *To investigate the biological function of miR-192-5p in osteogenic and odontogenic differentiation of dental pulp stem cells (DPSCs).*

Methods: *Alkaline phosphatase (ALP) activity assay, Alizarin Red S (ARS) staining and western blot analysis were applied to investigate the osteogenic differentiation potential of DPSCs in vitro. The osteogenic capacity was estimated by subcutaneous transplantation in nude mice in vivo. Carboxyfluorescein diacetate, succinimidyl ester (CFSE) assay was used to examine cell proliferation. Bioinformatics analyses, dual-luciferase reporter assays and real-time reverse transcriptase-polymerase chain reactions (RT-PCR) were applied to explore the regulatory mechanism of miR-192-5p.*

Results: *miR-192-5p decreased the ALP activity, nodule mineralisation and expression of BSP and OCN in DPSCs, and inhibited cell proliferation. Conversely, the miR-192-5p inhibitor motivated the osteogenic and odontogenic differentiation of DPSCs both in vitro and in vivo, along with promoting cell proliferation. COL5A1 was recognised as the target gene of miR-192-5p through bioinformatics analysis. Furthermore, dual luciferase reporter assays and RT-PCR experiments confirmed this interaction. Subsequent research verified that COL5A1 knockdown negatively affects the osteo-/odontogenic differentiation of DPSCs, with the PI3K/AKT signalling pathway involved in this process. COL5A1 knockdown promoted the proliferation of DPSCs.*

Conclusion: *miR-192-5p suppressed osteo/odontogenic differentiation by targeting COL5A1 in DPSCs and negatively regulated their proliferation. Conversely, COL5A1 knockdown promoted proliferation.*

Keywords: cell proliferation, COL5A1, DPSCs, miR-192-5p, osteo-/odontogenic differentiation

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Caries, trauma and other causes can cause pulp infections, necrosis or periapical lesions, ultimately destroying the dentine and alveolar bone.[1] Typically, root canal therapy is the most common treatment. Previous studies have shown that affected teeth are extremely susceptible to complications, such as tooth or bone loss,

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due to the lack of nutritional support and protection.[2-4] Consequently, tissue regeneration therapy has garnered significant attention. Mesenchymal stem cells (MSCs) show positive potential in tissue engineering because of their multipotency, which includes self-renewal and differentiation into various cell lineages.[5,6] Dental pulp stem cells (DPSCs), which are extracted and cultivated from the dental pulp, attract great interest in regenerative medicine because of their outstanding features, including pluripotency, elevated growth potential and the fewer ethical concerns surrounding their use.[7,8] Although DPSCs show the capability to generate dentine and bone-like structures, the molecular mechanism underlying the differentiation of DPSCs into the dentinogenic lineage remains unclear, and their current application is limited.[9]

MicroRNAs (miRNAs) are short, non-coding RNA molecules that typically range in length from 17 to 25 nucleotides. These molecules suppress the expression of specific mRNAs by binding to their complementary sequences, which results in the degradation of mRNA or the suppression of its translation. In general, miRNAs recognise the 3'-untranslated region (3'-UTR) of the target transcripts.[10] miRNAs are essential for regulating multiple physiological processes in organisms, covering cell differentiation, cell proliferation, cell division, cell senescence and apoptosis.[11] Furthermore, miRNAs have been reported to regulate osteo-/dentinogenic differentiation.[12] A previous study indicated that hypoxia could influence the osteo-/odontogenic differentiation capability, as well as the proliferative capacity of DPSCs. Researchers in this study conducted miRNA target gene network analysis, identifying 13 miRNAs that may play a role in DPSCs under hypoxic conditions. Notably, miR-192-5p was specifically expressed at a higher level in DPSCs under hypoxia in comparison with those under normoxia.[13] This indicates that miR-192-5p could act as a negative regulator for the osteo-/odontogenic differentiation of DPSCs. miR-192 is located on human chromosome 11.[14] Existing research indicated that miR-192-5p restrains the growth of non-small cell lung cancer cells while enhancing apoptosis in such cells.[15] miR-192-5p in adipose tissue–derived mesenchymal stem cells exosomes can attenuate fibrosis in hypertrophic scar-derived fibroblasts.[16] miR-192-5p is involved in hepatic trans-differentiation of human umbilical cord Wharton's jelly derived mesenchymal stem cells.[17] Until now, the ways in which miR-192-5p influences the differentiation and cell proliferation of DPSCs remain poorly understood. Thus, studies on the role and mechanism of miR-192-5p in DPSCs are necessary.

The present research investigated the biological regulatory function of miR-192-5p on the osteo-/odontogenic differentiation and proliferation of DPSCs and identified its target gene collagen type V alpha 1 (COL5A1). The findings revealed the underlying mechanisms of how miR-192-5p/COL5A1 affects DPSCs differentiation and identified potential targets for future clinical applications.

Materials and methods

Isolation, cultivation and multilineage differentiation of DPSCs

Healthy human teeth were sourced from patients (n = 3) who had given prior informed consent and were extracted for clinical reasons. All experiments followed the medical ethics and research guidelines of the Capital Medical University School of Stomatology. All experimental protocols received approval from the Ethics Committee of Capital Medical University School of Stomatology (ethics review no. 2011-02). After extraction, the dental pulp tissues were sliced into smaller segments and maintained in 6-well plates. The DPSCs were isolated, cultured and characterised following previously established methods, and the medium was refreshed every 72 hours.[18] All experiments were performed using 3 to 5 passages of cells. The multilineage differentiation assays of DPSCs were conducted in line with the approach outlined in a previous article.[19]

Viral transfection of miR-192-5p mimic/inhibitor

The miR-192-5p mimic, the miR-192-5p inhibitor, COL5A1 shRNA (COL5A1sh), and control shRNA (Consh) were purchased from GenePharma (Suzhou, China). The miR-192-5p mimic sequence was 5'-CTGACCTATGAATTGACAGCC-3', the miR-192-5p inhibitor sequence was 5'-GGCTGTCAATTCATAGGTCAG-3', the Consh sequence was 5'-TTCTCCGAACGTGTCACGT-3', the COL5A1sh sequence was 5'-GGGCATCAACCTGTCAGATGG-3' and the Consh sequence was 5-TTCTCCGAACGTGTCACGT-3'. Lentiviruses were transfected into DPSCs with polybrene (6 mg/ml, GenePharma) lasting 12 hours. Forty-eight hours later, 1 μg/ml puromycin was used to select the transfected DPSCs for 3 days.

Reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time RT-PCR

All RNA from DPSCs was extracted with TRIzol reagent (Invitrogen, Waltham, MA, USA) and subsequently ana-

lysed by quantitative RT-PCR. The expression ability of miRNAs was calculated using TaqMan MicroRNA Assays (Thermo Fisher Scientific, Waltham, MA, USA). RNA (1 ug) was converted into cDNA through reverse transcription and amplified with a QuantiTect SYBR Green PCR kit (Qiagen, Venlo, The Netherlands). The amplification process was monitored via the Icycler iQ Multi-color Real-time RT-PCR Detection System (Bio-Rad, Hercules, CA, USA). Primer sequences are shown in Table S1 (provided on request). These sequences were designed using Primer3 software. To normalise gene expression, U6 was used at the miRNA level, while glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used at the mRNA level.

ALP activity assay and Alizarin Red S staining (ARS)

Mineralisation-inducing medium was compounded by using α-MEM enriched with 15% foetal bovine serum (FBS), 100 U/ml penicillin-streptomycin, 100 μM ascorbic acid, 1.8 mM KH2PO4, 10 mM sodium β-glycerophosphate, 2 mM glutamine and 10 nM dexamethasone. The cells were induced for 5 days, after which the ALP activity assay was evaluated using the described research method.[18] ARS staining was performed after 14 days of stimulation of DPSCs, as well as quantitative calcium analysis, as previously described.[20]

Western blot analysis

DPSCs were induced in the osteogenic medium for 10 days and were lysed with RIPA buffer plus protease inhibitors. After total protein was collected, the present authors followed the procedures summarised in a previous study.[21] The primary antibodies applied were as follows: bone sialoprotein (BSP), GAPDH, COL5A1 (all from Bioss, Beijing, China), PI3K, phospho-PI3K, AKT and phospho-AKT (all from Cell Signaling Technology, Danvers, MA, USA), osteocalcin (OCN) (Abcam, Cambridge, UK) and β-actin (Abclonal, Wuhan, China).

Carboxyfluorescein succinimidyl ester (CFSE) assay

Carboxyfluorescein succinimidyl ester (CFSE) assays were carried out in accordance with the CellTrace CFSE Cell Proliferation Kit instructions (Invitrogen). DPSCs were stained and incubated at a proper concentration for 20 minutes, followed by washing with culture medium for 5 minutes, then the cells were cultured in six-well plates for an additional 3 days. After fixing the cells with 4% paraformaldehyde, a BD LSRII flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was used to analyse them, which featured excitation and emission filters at 635 nm optimised for far-red detection. Modfit LT software was employed to quantify the cell proliferation index (Verity Software House, Los Angeles, CA USA).

Nude mice subcutaneous transplantation

Experimental procedures were described in detail in a previous study, with 4.0×106 miR-192-5p inhibitor-treated DPSCs and 4.0×10^6 Consh-treated DPSCs with hydroxyapatite-tricalcium phosphate (HA/TCP) transplanted separately into 8-week-old nude mice (nu/nu, $n = 5$) by dorsal subcutaneous transplantation.[22] The transplant tissues were obtained after 12 weeks. All animal experimental steps were conducted under the guidelines approved by the Capital Medical University School of Stomatology.

Histological analysis

Transplanted tissue specimens were placed in 10% formalin for 24 hours, demineralised in 10% ethylenediaminetetraacetic acid (EDTA) for a month. Next, the transplants were embedded in paraffin, sectioned into 5-μm slices and stained with Masson's trichrome staining and haematoxylin-eosin (H&E) staining. Areas of positive staining were obtained using a microscope (DP71; Olympus, Shinjuku, Japan). After that, ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to calculate the area of dentine/bone tissue.

Dual luciferase gene reporter assay

The 293T cells were placed in a 12-well plate. miR-192-5p mimic or negative control (Consh) (GenePharma) were co-transfected with COL5A1-wt or COL5A1-mut constructs into 293T cells using Lipofectamine 3000 (Invitrogen), then the cells were incubated for an additional 48 hours. At day 3, the changes in luciferase activity were measured using the system provided in the product specification (E1910; Promega, Madison, WI, USA).

Statistical analysis

SPSS software (version 16.0; IBM, Armonk, NY, USA) was used for statistical analysis. Significance was assessed through one-way analysis of variance (ANOVA) or a Student *t* test. $P < 0.05$ was considered to indicate a statistically significant difference in all tests.

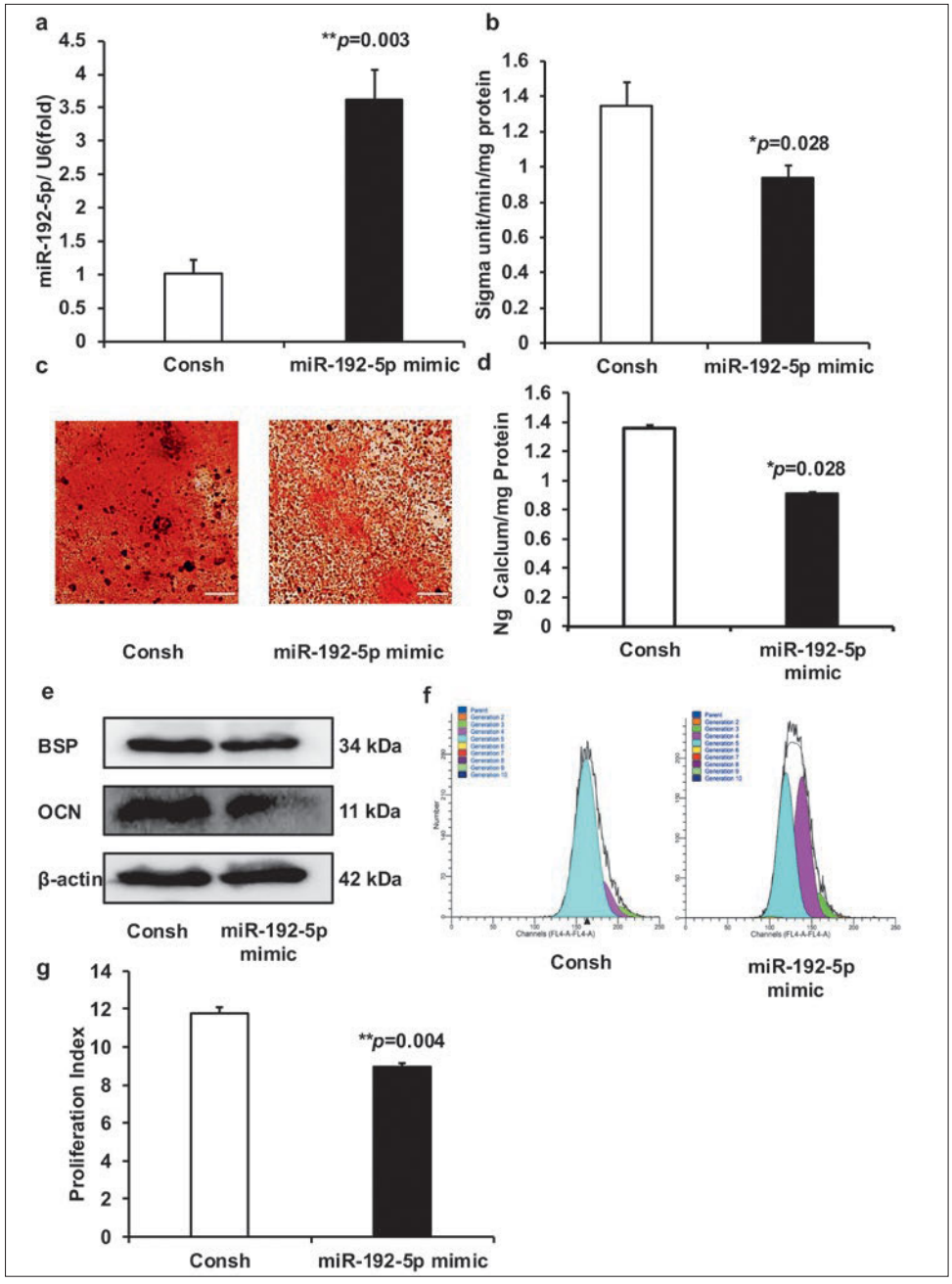

Fig 1a to g Effects of miR-192-5p on the osteo-/odontogenic differentiation and cell proliferation potential of DPSCs in vitro. Confirming the effectiveness of overexpressed miR-192-5p at the mRNA level in DPSCs **(a)**. The control group and miR-192-5p-treated DPSCs were under osteogenic induction for 5, 10 and 14 days **(b to e)**. ALP activity assay was performed at day 5 **(b)**. At day 14, ARS staining **(c)** and calcium quantification **(d)** were performed. Expression variation at the protein level of BSP and OCN was analysed at day 10 **(e)**. The control group and miR-192-5p treated DPSCs were stained with CFSE **(f and g)**. The CFSE assay **(f)** and its quantitative analysis **(g)** were performed at day 3. Student *t* tests were performed to determine statistical significance. Scale bar 200 μm. All error bars signify the standard deviation (n = 3). $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

Results

Effects of miR-192-5p on osteo-/odontogenic differentiation and proliferation of DPSCs in vitro

All DPSCs used in this experiment were validated by flow cytometry in a previously published study.[23] DPSCs showed multilineage differentiation potential (Fig S1, provided on request). miR-192-5p mimic was transfected into DPSCs using lentivirus infection and confirmed its overexpression via real-time RT-PCR (Fig 1a). The transduced DPSCs were kept in culture dishes and maintained with osteogenic induction medium to evaluate the effect of miR-192-5p on the osteo-/odontogenic differentiation of DPSCs for 5, 10 and 14 days. At day 5, distinctly reduced ALP activity was examined in miR-192-5p-treated DPSCs (Fig 1b). After 14 days of osteogenic induction, the miR-192-5p group showed minor mineralisation compared to the control group, as evidenced by ARS staining. Quantitative calcium measurements also proved the staining

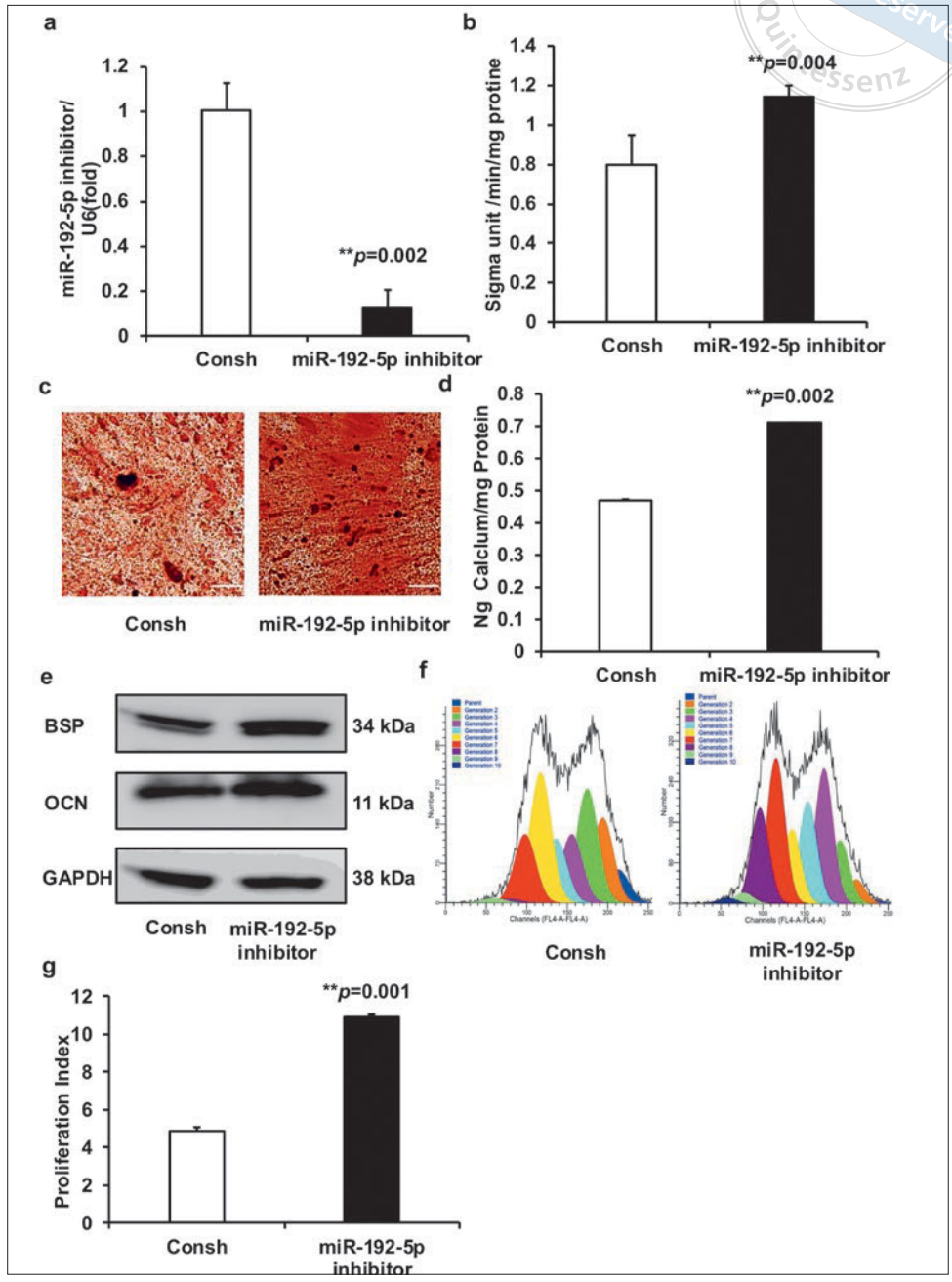

Fig 2a to g Effects of miR-192-5p inhibitor on osteo-/odontogenic differentiation and cell proliferation potential of DPSCs in vitro. Confirming the effectiveness of the miR-192-5p inhibitor at mRNA level in DPSCs **(a)**. The control group and miR-192-5p inhibitor treated-DPSCs were under osteogenic induction for 5, 10 and 14 days **(b to e)**. ALP activity assay was performed at day 5 **(b)**. At day 14, ARS staining **(c)** and calcium quantification **(d)** were performed. Expression variation at protein level of BSP and OCN at day 10 **(e)**. The control group and miR-192-5p inhibitor treated DPSCs were stained by CFSE **(f and g)**. The CFSE assay **(f)** and its quantitative analysis **(g)** were performed at day 3. Scale bar 200 μm. All error bars signify the standard deviation (n = 3). *$P \leq 0.05$, **$P \leq 0.01$.

results (Fig 1c and d). After 10 days of osteogenic induction, the osteogenesis-related proteins BSP and OCN showed lower expression levels in the miR-192-5p mimic group compared to those in the control group (Fig 1e).

The transduced cells were labelled with CFSE and incubated for 3 days. DPSC proliferation was markedly decreased in the miR-192-5p mimic group compared to the control group by day 3 (Fig 1f and g).

Effects of miR-192-5p inhibitor on osteo-/odontogenic differentiation and proliferation of DPSCs in vitro

To further validate the speculation about the effect of miR-192-5p in osteo-/odontogenic differentiation, the present authors induced miR-192-5p inhibitor into DPSCs using lentivirus transduction and confirmed the inhibition efficiency through real-time RT-PCR (Fig 2a). Subsequently, the miR-192-5p inhibitor treated DPSCs were cultured with osteogenic induction medium for

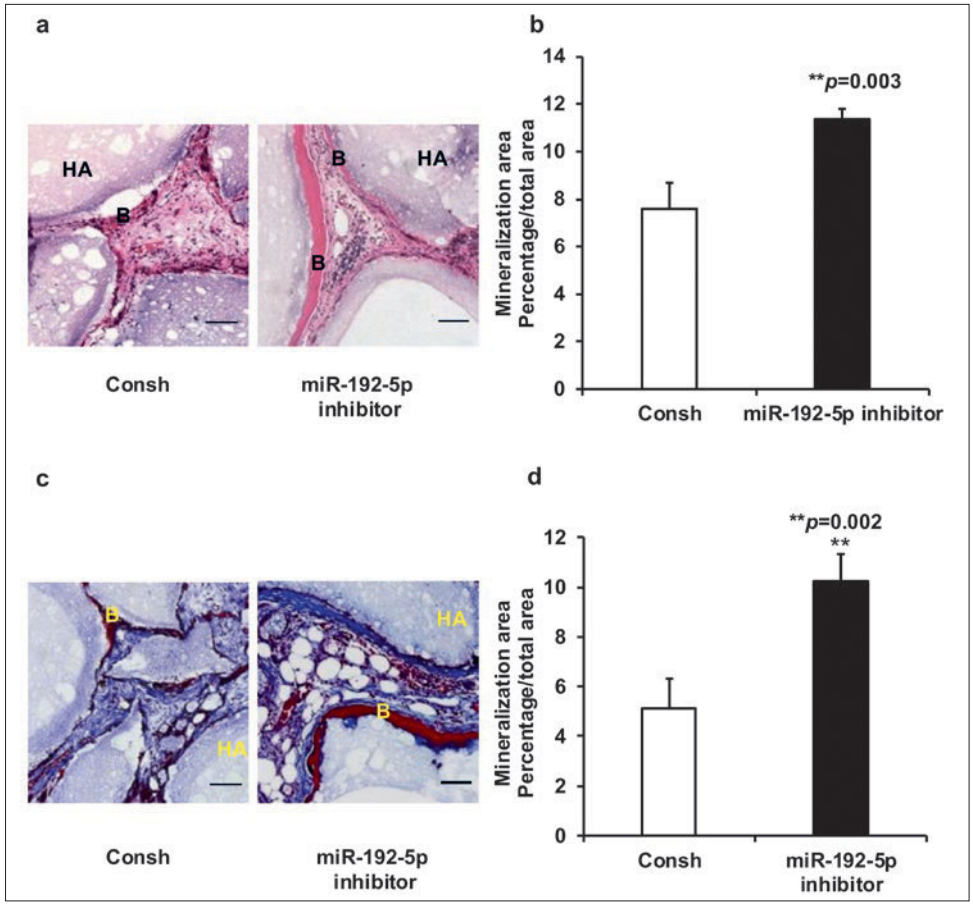

Fig 3a to d Effects of miR-192-5p inhibitor on osteo-/odontogenic differentiation potential of DPSCs in vivo. All slices were separately dyed with H&E staining and Masson trichrome staining. H&E staining and quantitative measurement of mineralized tissue **(a and b)**. Masson trichrome staining and quantitative measurement of mineralised tissue **(c and d)**. Scale bar 200 μm, B, bone-/dentine-like tissues; HA, hydroxyapatite tricalcium carrier. A Student *t* test was performed to determine statistical significance. All error bars signify the standard deviation (n = 5). *$P \leq 0.05$, **$P \leq 0.01$.

5, 10 and 14 days. At day 5, DPSCs treated with the miR-192-5p inhibitor showed a notable increase in ALP activity (Fig 2b). The miR-192-5p inhibitor group showed a notable enhancement in mineralised regions when compared to the control group after 14 days of induction, as evidenced by ARS. Quantitative analysis of calcium demonstrated that the group treated with the miR-192-5p inhibitor showed a greater degree of mineralisation than the control group (Fig 2c and d). BSP and OCN showed higher expression levels in the miR-192-5p inhibitor group than in the control group at day 10 (Fig 2e).

The result of CFSE assays showed markedly promoted proliferation of DPSCs treated with miR-192-5p inhibitor compared to the control group at day 3 (Fig 2f and g).

Effects of miR-192-5p inhibitor on osteo-/odontogenic differentiation of DPSCs in vivo

To validate the aforementioned in vitro findings, DPSCs treated with Consh and miR-192-5p inhibitor were transplanted into nude mice along with HA/TCP. The regenerated tissue was harvested 12 weeks later, and then processed and stained for analysis. Widespread areas of bone/dentine-like tissue were observed in the miR-192-5p inhibitor group rather than the control group in H&E staining images (Fig 3a). Not surprisingly, the quantitative measurements revealed that the miR-192-5p inhibitor group had greater osteo-/odontogenic formation capacity than the control group (Fig 3b). Furthermore, similar results were observed from Masson trichrome staining. The miR-192-5p inhibitor group prominently enhanced the osteo/odontogenic formation capacity in vivo (Fig 3c and d).

COL5A1 is targeted by miR-192-5p

Since miRNAs can bind to specific mRNAs and negatively regulate their expression, the present authors conducted bioinformatics analysis using the TargetScan and PicTar databases to explore the potential target of miR-192-5p. The 3'UTRs of COL5A1 were predicted to be targeted by miR-192-5p (Fig 4a). Next, a luciferase reporter was constructed that included both COL5A1-wt and COL5A1-mut to investigate the interaction between

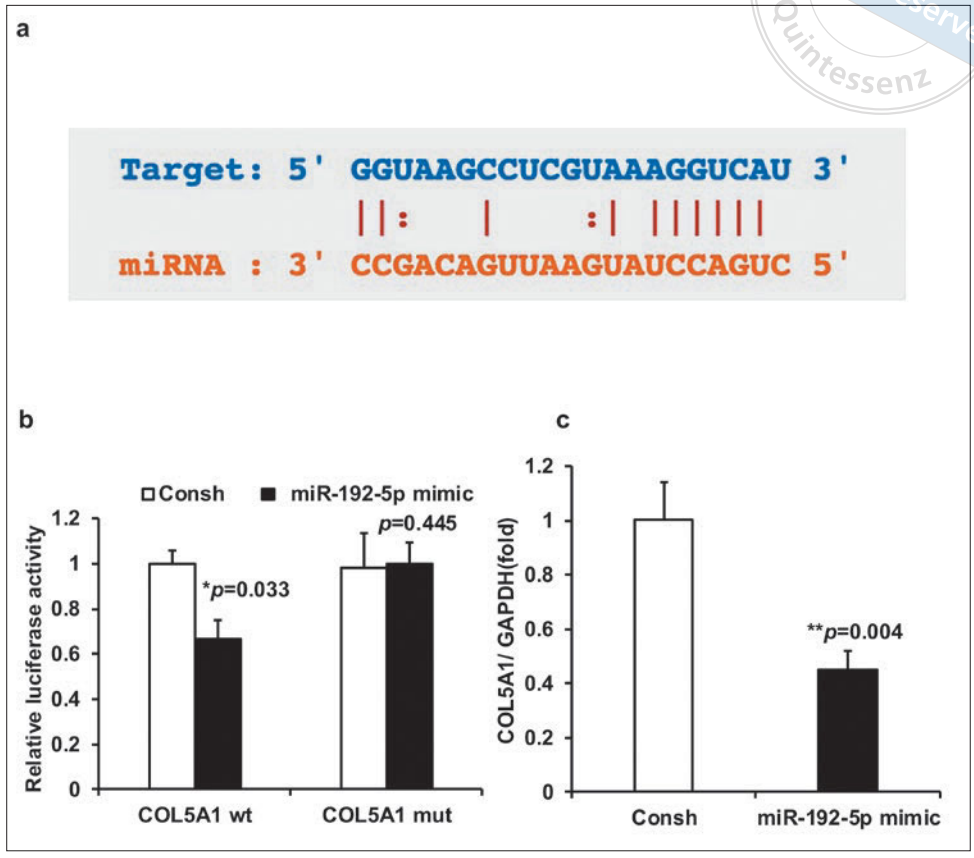

Fig 4a to c COL5A1 is targeted by miR-192-5p. The 3'-UTR of COL5A1 mRNA exhibits partial complementarity to the sequence of miR-192-5p **(a)**. Dual luciferase reporter gene assay was carried out following co-transfection **(b)**. miR-192-5p transfection induced significant downregulation of COL5A1 expression at mRNA level **(c)**. *$P \leq 0.05$, **$P \leq 0.01$.

miR-192-5p and COL5A1. Co-transfection with either the miR-192-5p mimic or negative control in 293T cells resulted in no change in luciferase activity variation in the COL5A1-mut group. When the miR-192-5p mimic was transfected into the 293T cells, luciferase activity was notably decreased in COL5A1-wt group. This indicated that miR-192-5p has specific binding affinity for COL5A1 (Fig 4b). Subsequent RT-PCR analysis revealed that the miR-192-5p mimic prominently downregulated mRNA expression of COL5A1 (Fig 4c). These results indicate that COL5A1 is targeted by miR-192-5p.

Effects of COL5A1sh on osteo-/odontogenic differentiation and proliferation of DPSCs

shRNA was employed to inhibit COL5A1 expression and the impact of COL5A1 on osteo/odontogenic differentiation of DPSCs was investigated. Western blot analysis confirmed the knockdown efficiency (Fig 5a). Next, DPSCs treated with COL5A1sh were induced for 5, 10 and 14 days with osteogenic induction medium. At day 5, ALP activity declined due to COL5A1 knockdown in DPSCs (Fig 5b). ARS illustrated that knockdown of COL5A1 hindered mineralisation in DPSCs compared to the control DPSCs, and the quantitative calcium measurements present the identical results at day 14 (Fig 5c and d). The depletion of COL5A1 resulted in lower levels of BSP and OCN expression compared to the control group at day 10 (Fig 5e).

CFSE results showed that, in contrast to the control group, the COL5A1sh group promoted cell proliferation on day 3 (Fig 5f and g). Further mechanistic investigations revealed that COL5A1sh markedly decreased the phosphorylation levels of PI3K and AKT in DPSCs, whereas the overall protein levels of PI3K and AKT did not change (Fig 5h).

Discussion

DPSCs provide crucial biological support in maintaining the homeostasis of pulp tissue and present great potential for tissue regeneration. DPSCs possess the potential of multi-lineage differentiation to form dentine- and bone-like tissue.[24,25] It is acknowledged that improved tissue regeneration can be achieved by using cytokines, scaffold materials and cell aggregates/

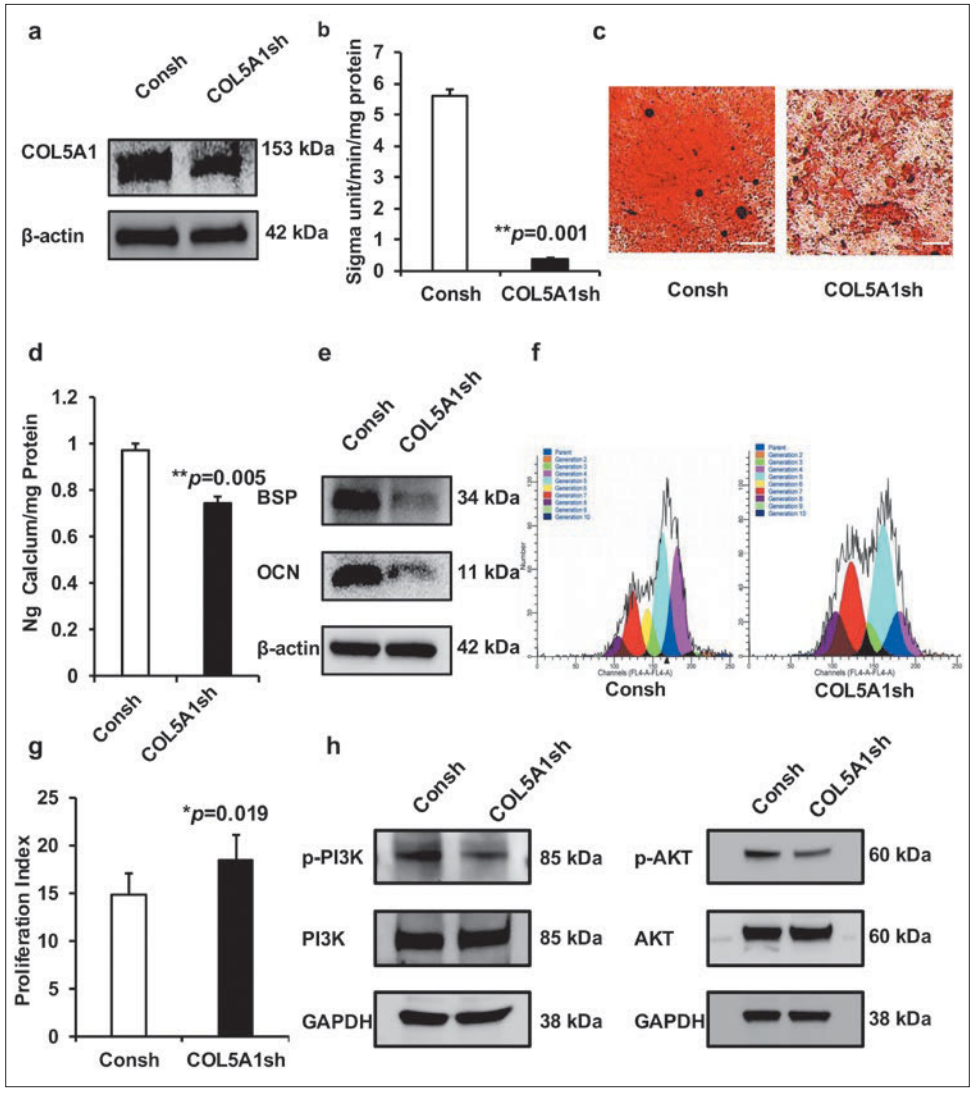

Fig 5a to h Effects of COL5A1sh on osteo-/odontogenic differentiation and cell proliferation of DPSCs. Western blot analysis confirming the effectiveness of knockdown of COL5A1 at protein level in DPSCs **(a)**. The control group and COL5A1sh-treated DPSCs were under osteogenic induction for 5, 10 and 14 days **(b to e)**. ALP activity assay was performed at day 5 **(b)**. At day 14, ARS staining **(c)** and calcium quantification **(d)** were performed. Expression variation at protein level of BSP and OCN at day 10 **(e)**. The control group and COL5A1sh treated DPSCs were stained with CFSE **(f and g)**. The CFSE assay **(f)** and its quantitative analysis **(g)** were performed at day 3. Western blot analysis demonstrated the activation status of the PI3K/AKT signalling pathway in COL5A1sh treated DPSCs **(h)**. Scale bar 200 μm. All error bars signify the standard deviation (n = 3). $*P \leq 0.05$, $**P \leq 0.01$.

spheroids, which are currently the primary focus of research in this field.[26] However, the detailed biology of odontogenic MSC transplantation and regeneration remains unclear; hence, future studies should explore these mechanisms. MiRNAs have been investigated in various studies as regulatory molecules in the osteo-/odontogenic differentiation of DPSCs.[12] For instance, miR-140-5p inhibits odontogenic differentiation of DPSCs by targeting BMP2 and FGF9.[27] This indicates that there is substantial potential for further investigation into the molecular mechanisms of miRNAs in regulating the osteo-/odontogenic differentiation of DPSCs. In the previous study,[13] hypoxic environments affected the osteo-/odontogenic differentiation capabilities of DPSCs, as well as their proliferation capacity. According to the miRNA array analysis, it was identified that miR-192-5p exhibits differential expression in DPSCs under hypoxic circumstances when compared to normoxic settings,[13] which induced the present authors' significant interest. Furthermore, research showed that miR-192-5p is involved in the development of mandibular prognathism.[28] Combined with the above findings, miR-192-5p could be crucial for the osteo-/odontogenic differentiation of DPSCs. Thus, the present authors examined the distinct effects of miR-192-5p on DPSCs, with a specific emphasis on their osteo-/odontogenic differentiation. miR-192-5p exhibited reduced osteo-/odontogenic differentiation capacity, as evidenced by lower ALP activity and ARS intensity. Additionally, BSP and OCN were dramatically inhibited at the protein level. Furthermore, miR-192-5p restricted cell proliferation, which was consistent with previous research.[29,30] The present findings indicated that miR-192-5p is barely capable of forming dentine- or

bone-like tissue in vitro. On the other hand, the present authors sequentially demonstrated that the miR-192-5p inhibitor could markedly strengthen the mineralisation ability of DPSCs both in vitro and in vivo, while also exhibiting enhanced cell proliferation. Taken together, miR-192-5p suppresses the osteo-/odontogenic differentiation and cell proliferation of DPSCs.

It is widely acknowledged that the specific mRNAs serve as essential targets, enabling miRNAs to carry out their physiological roles. The present authors speculate that COL5A1 is regulated by miR-192-5p in DPSCs, as predicted by the TargetScan and PicTar databases. Collagens contribute largely to the composition of the extracellular matrix (ECM) as structural protein and are essential for connective tissue, including bone and dentine.[31,32] Type V collagen regulates fibril formation and incorporates into the fibrils of type I collagen and plays a role in bone formation.[33-35] Several investigations have indicated the significant roles of COL5A1 in cellular movement and proliferation.[36] More importantly, COL5A1 is involved in the odontogenic differentiation process.[37] To confirm that COL5A1 is regulated by miR-192-5p, the present authors conducted dual luciferase reporter assays and real-time RT-PCR experiments. The results suggest that miR-192-5p directly targets COL5A1.

The present authors analysed the role of COL5A1 through loss-of-function assays and observed that COL5A1sh suppressed osteo/odontogenic forming capacity of DPSCs. These data strongly support that miR-192-5p serves as a critical factor for bone or dentine formation through the target of COL5A1. miR-192-5p inhibited the expression of COL5A1 through complementary binding to the 3'UTR of COL5A1 mRNA. COL5A1 is essential for collagen formation, and the depletion of collagen disrupts the ECM structure, impairs integrin binding and activates various signalling pathways, such as the PI3K/AKT signalling pathway and the TGF-β/Smads pathway, thereby modulating cell adhesion, migration and differentiation.[37-39] Earlier studies have shown that the PI3K/AKT signalling pathway can influence stem cell proliferation and differentiation.[40,41] More importantly, it is involved in the osteogenic differentiation of DPSCs, as evidenced by recent research.[42,43] Therefore, the present authors examined the expression of PI3K/AKT in COL5A1 knockdown DPSCs, and western blot analysis demonstrated that the phosphorylation levels of both PI3K and AKT were notably reduced while their total expression remained unchanged. The experimental results showed that miR-192-5p interacts with COL5A1, and knockdown of COL5A1 represses the osteo-/odontogenic differentiation of DPSCs by inactivating the PI3K/AKT signalling pathway.

The present authors' results revealed that COL5A1sh promotes the proliferation of DPSCs, a finding that contrasts with the function of miR-192-5p in DPSCs. One miRNA can regulate various cellular processes by targeting numerous genes. In other words, the authors suggest that the reductionist paradigm of one molecule affecting one target to cause a given phenotype is not typically applied to miRNAs. Due to the characteristics of miRNAs, the specific binding between miR-192-5p and COL5A1 might only influence the differentiation of DPSCs, rather than their proliferation. TGF-β plays an important role in dentine formation and tissue repair.[44] Previous research has demonstrated that COL5A1 is regulated by TGF-β in osteoblasts.[34] The present results showed that miR-192-5p regulated the osteo-/odontogenic differentiation of DPSCs by targeting COL5A1, which indicated that miR-192-5p may be an important molecular mediator that regulates TGF-β signalling to affect odontoblast differentiation. Previous studies have shown that c-Myc, which is identified as a target of miR-192-5p, stimulates the proliferation of MSCs. In contrast, the present results suggest that miR-192-5p suppresses the cell proliferation of DPSCs.[45,46] Thus, the present authors speculate that miR-192-5p suppresses the proliferation of DPSCs through its interaction with c-Myc. Moreover, the precise mechanism through which miR-192-5p influences DPSC function requires further study.

Conclusion

In summary, miR-192-5p suppresses the osteo-/odontogenic differentiation of DPSCs by interacting with COL5A1. Cell proliferation reduction caused by miR-192-5p could potentially be ascribed to other pathways. miR-192-5p may be considered as one possible molecular target for modulating osteo-/odontogenic differentiation, offering promising therapeutic implications in dental medicine.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Zi Yan SUN and Yi Jie ZHANG contributed to the acquisition, analysis and interpretation of data and manuscript writing; Dr Hao Qing YANG contributed to

the data collection; Drs Hui Na LIU, Dong Mei YANG and Zhi Peng FAN were responsible for the conception and design and manuscript writing and revising. All authors read and approved the final version of the manuscript.

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