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Original Article

Depletion of *MEIS2* inhibits osteogenic differentiation potential of human dental stem cells

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Abstract: Dental mesenchymal stem cells (MSCs) are a reliable and promising cell source for the regeneration of tooth,bone and other tissues . However, the molecular mechanisms underlying their differentiation are still largely unknown, which restricts their further wide application. Here, we investigate regulatory function of homeobox gene *MEIS2* in the osteogenic differentiation potential of MSCs using stem cells from apical papilla (SCAPs) and dental pulp stem cells (DPSCs) by loss-of-function experiments. Our findings demonstrated that knockdown of *MEIS2* in SCAPs and DPSCs decreased alkaline phosphatase (ALP) activity and mineralization, and inhibited the mRNA expression of *ALP,* bone sialoprotein (*BSP*), and osteocalcin (*OCN*). Besides, depletion of *MEIS2* resulted in reduced expression of the key osteogenesis-related transcription factor, osterix (*OSX)* but not in the expression of runt-related transcription factor 2 (*RUNX2*). Furthermore, *MEIS2* expression significantly increased during osteogenic induction and was strongly upregulated by BMP4 stimulation. Taken together, these results indicated that *MEIS2* played an essential role in maintaining osteogenic differentiation potential of dental tissue- derived MSCs. These findings will provide new insights into the mechanisms underlying directed differentiation of MSCs, and identify a potential target gene in dental tissues derived MSCs for promoting the tissue regeneration.

Keywords: Mesenchymal stem cell, osteogenic differentiation, MEIS2, homeobox gene, tooth

Introduction

The discovery of mesenchymal stem cells (MSCs), originally isolated from bone marrow, was a giant breakthrough in medicine and opened a new door for alternative therapies of various diseases for their potencies of self-renewal and multi-lineage differentiation. The successful isolation and identification of stem cells from the dental tissues, such as DPSCs [1], stem cells from exfoliated deciduous teeth (SHEDs) [2], periodontal ligament stem cells (PDLSCs) [3], dental follicle progenitor cells (DFPCs) [4] and SCAPs [5], has proven diverse populations of MSCs that exist in non-bone marrow tissues. Dental MSCs are not only easily accessible, but also possessing osteo/dentinogenic differentiation potentials as bone marrow mesenchymal stem cells. When delivered in vivo, dental MSCs could generate bone/dentin-like mineralized tissues and be capable of repairing bone or tooth defects [6, 7]. These make them promising and powerful candidates for therapy including bone and tooth regeneration. However, the mechanisms in osteo/dentinogenic differentiation of dental MSCs remain elusive, which has largely restricted their further potential application.

Homeobox genes, originally identified in drosophila but rapidly found in all animal species as well as in fungi and plants, are homologous genes highly conserved during evolution across lineages. Homeobox genes are characterized by a conserved 180 bp DNA sequence coding for a 60aa DNA-binding homeodomain. Home-

odomain proteins are transcription factors that can activate or inhibit transcription of downstream genes through the DNA binding. Recent studies show that homeobox genes play crucially regulatory roles in the process of maxillofacial and dental development. It's reported that prior to tooth development, homeobox genes had been expressed in the mesenchyme of the first branchial arch, and were subsequently expressed in different stages during odontogenesis, involving in both temporal and spatial control of dentition formation [8-10]. Distal-less homeobox5 (DLX5) was found as a requisite for mineralization of tooth. In *Dlx5-/-* mice, both maxillary and mandibular molars were malformed and had poorly mineralized crowns and both sets of incisors were shortened and misshapen [11]. In *Pbx1*-deficient mice, absence of pre-B-cell leukemia homeobox proteins 1 (*Pbx1*) caused precocious endochondral ossification and abnormal bone formation by perturbing chondrocyte proliferation and differentiation [12]. Homeobox a10 (Hoxa10) could mediate chromatin hyperacetylation and trimethyl histone K4 (H3K4) methylation, induce expression of osteogenic genes through activation of *Runx2*, or directly regulate other osteoblastic phenotypic genes, and contribute to the onset of osteogenesis and subsequent bone formation [13]. More importantly, it has been reported that homeobox gene msh-like 1 (*Msx1*) is essential for the proliferation and differentiation of dental mesenchymal cells at cap stage. Knockdown of *Msx1* resulted in decreased cell proliferation but enhanced odontoblast differentiation [14]. The DLX2 gene is highly expressed in dental tissue-derived MSCs. It's reported that DLX2 promotes the osteogenic differentiation potential of stem cells from apical papilla while knock-down of DLX2 in SCAPs decreased alkaline phosphatase (ALP) activity and mineralization [15]. Homeodomain protein HOXA10 and TALE-family protein PBX1 form coregulatory complexes. They are expressed in osteoprogenitors and mediated regulation of osteoblast commitment and the related gene expression. Overexpression of HOXA10 increased the expression of osteoblast-related genes, osteoblast differentiation and mineralization; expression of PBX1 impaired osteogenic commitment of pluripotent cells and the differentiation of osteoblasts. It's proposed that PBX1 probably attenuated the activity of HOXA10 as an activator of osteoblast-related genes, which functioned to establish the proper timing of gene expression during osteogenesis and resulted in proper matrix maturation and mineral deposition in differentiated osteoblasts [16]. Other recent studies also have showed that homeobox gene *DLX5 and HOXC6* function as key regulators in the lineage commitment of MSCs into osteoblasts [15, 17, 18], indicating that homeobox genes play essential roles in the development of hard tissue and the odonto/osteogenic differentiation of stem cells.

Myeloid ecotropic insertion site 2 (*MEIS2*), also known as *MRG1*, is a homeobox gene belonging to the three amino-acid loop extension (TALE) superclass. It contains a conserved homothorax (Hth) domain, which mediates interaction with PBX and allows for efficient DNA binding [19]. As an evolutionary conserved transcription factor, MEIS2 has been shown to participate in some developmental processes during embryogenesis, including development of proximal-distal limb patterning [20], heart [21], brain [22-24] and so on. For instance, MEIS2 is the only known transcriptional regulator so far that is capable to direct tectal fate specification and whose expression specifically marks the tectal anlage at mid to late somite stages [25]. MEIS2 is also showed to play an essential role in maintaining proliferation and regulating fate specification of retinal progenitor cells in chick and mouse embryos [26]. However, at present, functional role of *MEIS2* in differentiation of MSCs, especially, the ddental tissue- derived MSCs, hasn't been reported. Here, by loss-of-function study, we used SCAPs and DPSCs to investigate the function of *MEIS2* in osteogenic differentiation potential of MSCs. Our results showed that depletion of *MEIS2* inhibited the osteogenic differentiation potential in SCAPs and DPSCs, indicated that *MEIS2* played an essential role in maintaining osteogenic differentiation potential of dental tissue-derived MSCs.

Material and methods

Cell cultures and viral infection

Human tooth tissues from impact third molars were obtained under approved guidelines set by the Beijing Stomatological Hospital, Capital Medical University with informed patient consent. The isolation, culture and identification of

Table 1. Primers used in the Real-time RT-PCR

Gene Symbol	Primer Sequences (5'-3')
GAPDH-F	CGGACCAATACGACCAAATCCG
GAPDH-R	AGCCACATCGCTCAGACACC
MEIS2-F	CGGATGCCTAGATCACTTTCTTATCCG
MEIS2-R	TCTGCGCTCCAATAAACTCCTGGCT
BSP-F	CAGGCCACGATATTATCTTTACA
BSP-R	CTCCTCTTCTTCCTCCTCCTC
OSX-F	CCTCCTCAGCTCACCTTCTC
OSX-R	GTTGGGAGCCCAAATAGAAA
ALP-F	GACCTCCTCGGAAGACACTC
ALP-R	TGAAGGGCTTCTTGTCTGTG
OCN-F	AGCAAAGGTGCAGCCTTTGT
OCN-R	GCGCCTGGGTCTCTTCACT
SMAD4-F	GGTTGCACATAGGCAAAGGT
SMAD4-R	TGACCCAAACATCACCTTCA

SCAPs and DPSCs were performed as previously reported [27, 28]. Briefly, the third molars were first disinfected with 75% ethanol and then washed with phosphate buffered saline (PBS). SCAPs were gently separated from the apical papilla of the root, while DPSCs were separated from crown pulp. MSCs then respectively digested in a solution of 3 mg/ml collagenase type I (Worthington Biochemical Corp., Lakewood, NJ, USA) and 4 mg/ml Dispase (Roche Diagnostics Corp., Indianapolis, IN, USA) for 1 hour at 37°C. Single-cell suspensions were obtained by passing the cells through a 70 μm strainer (BD Biosciences, San Jose, CA). Then they were grown in a humidified 5% CO_2 incubator at 37°C in DMEM alpha modified Eagle's medium (Invitrogen) supplemented with 15% fetal bovine serum (FBS; Invitrogen), 2 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). The culture medium was changed every 3 days. Cells at passage 3-5 were used in subsequent experiments.

Plasmid construction and viral infection

The plasmids were constructed with standard methods; all structures were verified by appropriate restriction digest and/or sequencing. Short hairpin RNAs (shRNA) with the complementary sequences of the target genes were subcloned into the the pSIREN retroviral vector (Clontech Labtoratories, Mountain View, CA, USA) or pLKO.1 lentiviral vector (Addgene, Cambridge, MA, USA). Viral packaging was prepared according to the manufacturer's protocol using 293T cells (BD Clontech). For viral infections, MSCs were plated overnight, and then infected with lentiviruses or retroviruses in the presence of polybrene (6 μg/ml, Sigma-Aldrich, St. Louis, MO, USA) for 6 h. After 48 h, infected cells were selected with 2 μg/ml puromycin (Sigma-Aldrich, St. Louis, MO, USA). A Luciferase shRNA (*Lucsh*) was used as control. The target sequences for the shRNAs were: Luciferase shRNA, 5'-gtgcgttgctagtaccaac-3'; *MEIS2* shRNA1, 5'-ggaaccacactggagatca-3'; *MEIS2* shRNA2, 5'-gcttctgccaccgatacat-3'; sterile alpha motif domain containing 4 (*SMAD4*) shRNA (*SMAD4sh*), 5'-cattggatgggaggcttca-3'; A scramble shRNA (*Scramsh*) was purchased from Addgene.

Alkaline phosphatase and alizarin red detection

Cells were grown in osteogenic-inducing medium using the STEMPRO Osteogenesis Differentiation Kit (Invitrogen). ALP activity was assayed with an ALP activity kit according to the manufacturer's protocol (Sigma-Aldrich). Signals were normalized based on protein concentrations. For detecting mineralization, cells were induced for 3 weeks, fixed with 70% ethanol, and stained with 2% Alizarin red (Sigma-Aldrich). To quantitatively determine calcium, Alizarin Red was destained with 10% cetylpyridinium chloride in 10 mM sodium phosphate for 30 minutes at room temperature. The concentration was determined by measuring the absorbance at 562 nm on a microplate reader and comparing to a standard calcium curve with calcium dilutions in the same solution. The final calcium level in each group was normalized to the total protein concentration detected in a duplicate plate [27].

Reverse transcriptase-PCR (RT-PCR) and real-time RT-PCR

Total RNA was isolated from SCAP cells with Trizol reagents (Invitrogen). We synthesized cDNA from 2 μg aliquots of RNA, random hexamers or oligo (dT), and reverse transcriptase, according to the manufacturer's protocol (Invitrogen). Real-time PCR reactions were performed with the QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany) and an IcycleriQ Multi-color Real-time PCR Detection System (Bio-Rad). The expression of genes was calcu-

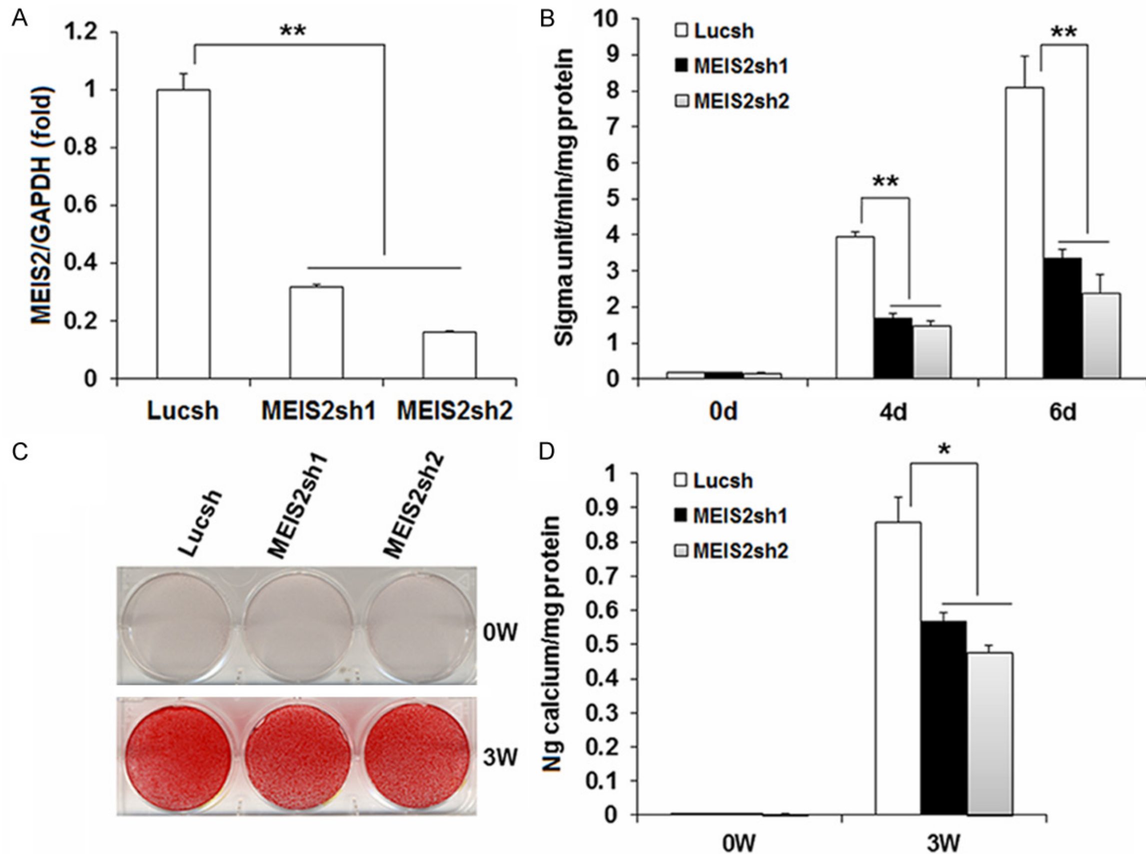

Figure 1. Knockdown of *MEIS2* inhibited osteogenic potential in SCAPs. SCAPs were infected with short hairpin RNAs (shRNA) that silenced *MEIS2* with different knockdown efficiency or Luciferase shRNA (*Lucsh*). Real-time RT-PCR showed *MEIS2* expression (30% left in *MEIS2sh1* and 15% left in *MEIS2sh2*). GAPDH was used as an internal control (A). The knock-down of *MEIS2* reduced alkaline phosphatase activity (B), Alizarin red staining (C) and calcium quantitative analysis (D) in SCAPs. Analysis of variance was performed to determine statistical significance. All error bars represent s.d. (n=3). *P ≤ 0.05. **P ≤ 0.01.

lated by the method of $2^{-\Delta\Delta CT}$ as described previously [29]. The primers for specific genes were shown in **Table 1**.

Statistics

All statistical calculations were performed with SPSS13.0 statistical software. The student's t test or Analysis of variance (ANOVA) test were performed to determine statistical significance. A *P*-value ≤ 0.05 was considered significant.

Results

Depletion of MEIS2 inhibited osteogenic differentiation potential in SCAPs

In order to investigate the function of *MEIS2* in SCAPs, we designed two short hairpin RNAs to target *MEIS2* and introduced them into SCAPs with retroviral infection (*MEIS2sh1* and *MEIS2-sh2*, respectively). After selection, the knockdown efficiency (70% in *MEIS2sh1* and 85% in *MEIS2sh2*) was verified by real-time RT-PCR (**Figure 1A**). Next, we examined whether *MEIS2* intrinsically affected the osteogenic differentiation potential of SCAPs. Transduced SCAPs were cultured in osteogenic-inducing medium, examined ALP activity on 0, 4, 6 days, stained Alizarin Red and detected calcium in 0 and 3 weeks. The results indicated that the depletion of *MEIS2* markedly reduced ALP activity, an early marker for osteogenic differentiation in SCAPs (**Figure 1B**), and significantly deceased mineralization determined by Alizarin Red staining and quantitative calcium measurements (**Figure 1C**, **1D**). Consistent with that, real-time RT-PCR results showed that the osteogenic marker gene *ALP* was strongly reduced when *MEIS2* were knocked down on 3, 7, 10 days

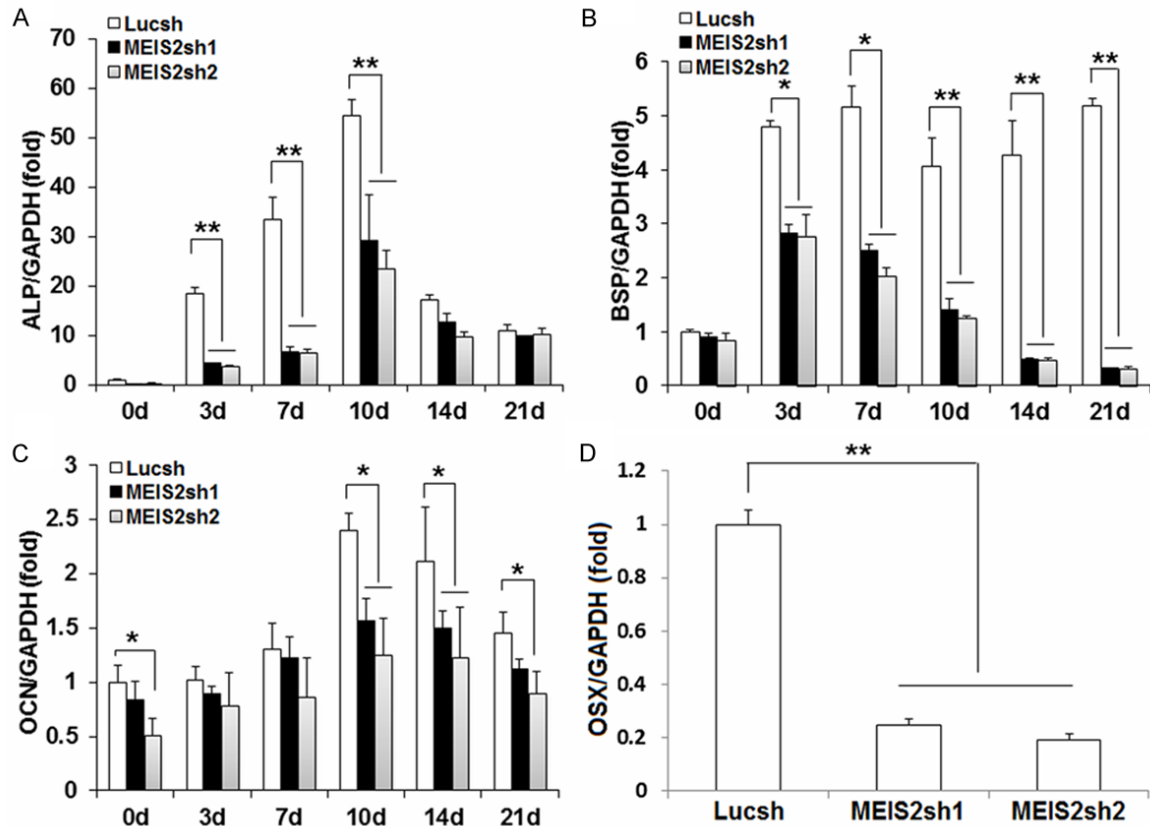

Figure 2. Knockdown of *MEIS2* reduced the expression of osteogenic related genes in SCAPs. Real-time RT-PCR results showed depletion of *MEIS2* reduced the expression of *ALP* (A), *BSP* (B), *OCN* (C), and *OSX* (D). GAPDH was used as an internal control. Analysis of variance was performed to determine statistical significance. All error bars represent s.d. (n=3). $*P \leq 0.05$. $**P \leq 0.01$.

after induction (**Figure 2A**). Other osteogenic marker genes, *BSP* and *OCN*, which encode extracellular matrix proteins of bone, were also significantly reduced after induction (**Figure 2B**, **2C**). On the third day BSP was decreased by about 40% while on the 21th day it dropped by approximately 90% after induction. Changes of OCN after induction took place earlier but slightly than BSP. At the beginning day of induction, OCN quickly declined by 20% in *MEIS2sh1* and 50% in *MEIS2sh2* and on the day 21, it reduced by 26% in *MEIS2sh1* and 40% in *MEIS2sh2.* Next, we examined the expression of *OSX* and *RUNX2* which are important key transcription factors for regulating osteogenic differentiation. It found that the mRNA level of *OSX* was decreased by about 80% when *MEIS2* were knocked down (**Figure 2D**). However, the mRNA level of *RUNX2* was not obviously changed (data not show). Notably, inhibition of *MEIS2sh* to osteogenic differentiation of SCAPs seemed dose-dependent because almost all the osteogenic differentiation markers described above were decreased more in *the MEIS2sh2 group* than those in *the MEIS2sh1 group*.

BMP signaling induced MEIS2 expression

To explore the possible mechanisms of *MEIS2* in osteogenic differentiation potential in SCAPs, we first examined the expression of *MEIS2* in SCAPs during osteogenisis process and found that *MEIS2* was significantly up-regulated (more than 1.6 times) in SCAPs when cultured with osteogenic-inducing medium (**Figure 3A**). We further loaded three different concentrations of BMP4 (0, 50, 100 ng/ml) in basic culture medium. We found that *MEIS2* was strongly induced in SCAPs after BMP4 treatment (**Figure 3B**). For example, at the eighth hour, expression of *MEIS2* was increased by 1.7 times in medium with 50 ng/ml BMP4 and increased by 1.9 times in medium with 100 ng/

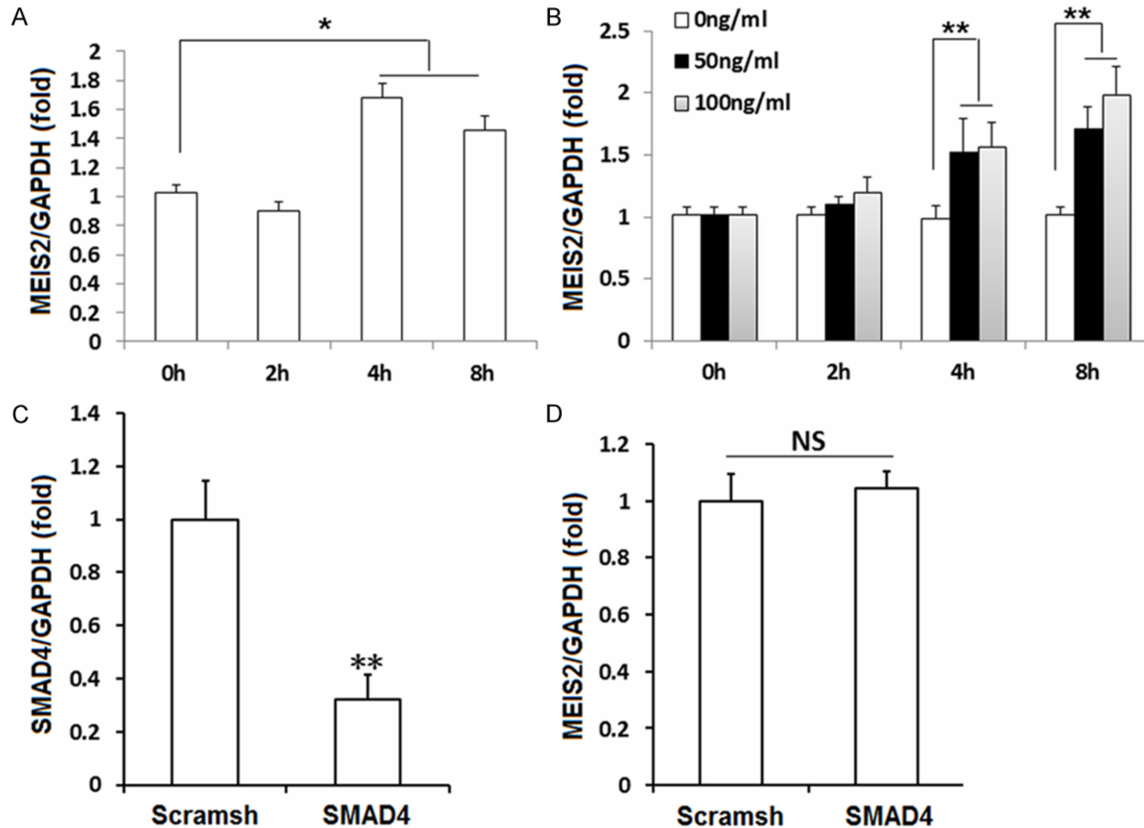

Figure 3. BMP signaling induced *MEIS2* expression. *MEIS2* was significantly up-regulated in SCAPs when cultured with osteogenic-inducing medium (A). *MEIS2* was strongly induced in SCAPs immediately after treated with different concentrations of BMP4 (0, 50, 100 ng/ml respectively.) in basic culture medium (B). Real-time RT-PCR results showed that knockdown of *SMAD4* had no obvious effect on *MEIS2* expression (C, D). GAPDH was used as an internal control. Analysis of variance (A, B) or student's t test (C, D) was performed to determine statistical significance. All error bars represent s.d. (n=3). $*P \leq 0.05$. $**P \leq 0.01$.

ml BMP4. Last, we examined the effects of *SMAD4* on *MEIS2* expression. It's demonstrated that knockdown of *SMAD4* had no obvious effect on *MEIS2* transcription (**Figure 3C**, **3D**). Taken together, these findings suggested that *MEIS2* act as a downstream DNA-binding protein in BMP signaling cascade but its transcriptional activity may be independent of the regulation of *SMAD4*.

Depletion of MEIS2 inhibited osteogenic differentiation potential in DPSCs

To confirm that *MEIS2* is indeed crucial in maintaining osteogenic differentiation potential of dental tissue- derived MSCs, we further depleted *MEIS2* in DPSCs and investigated the changes of their osteogenic differentiation potential. Similar effects with those in SCAPs were observed in DPSCs. Transduced DPSCs with 60% knockdown efficiency (**Figure 4A**) had a decreased ALP activity (**Figure 4B**), mineralization also determined by Alizarin Red staining (**Figure 4C**) and quantitative calcium measurements (**Figure 4D**), compared to the control group (*Lucsh*). Besides, the expression of the key transcription factor, *OSX*, was down-regulated by 40% when *MEIS2* depleted (**Figure 4E**). *MEIS2* was also significantly induced in DPSCs immediately following BMP4 (50 ng/ml) loaded in basic culture medium (**Figure 4F**). Collectively, these observations further confirmed our hypothesis that *MEIS2* was important in maintaining osteogenic differentiation potential of dental tissue derived MSCs.

Discussion

MEIS2 is a critical member of homeobox genes and plays a key role in regulating cell fate. Evidence for the potential function of *MEIS2* in controlling differentiation of stem cells involved

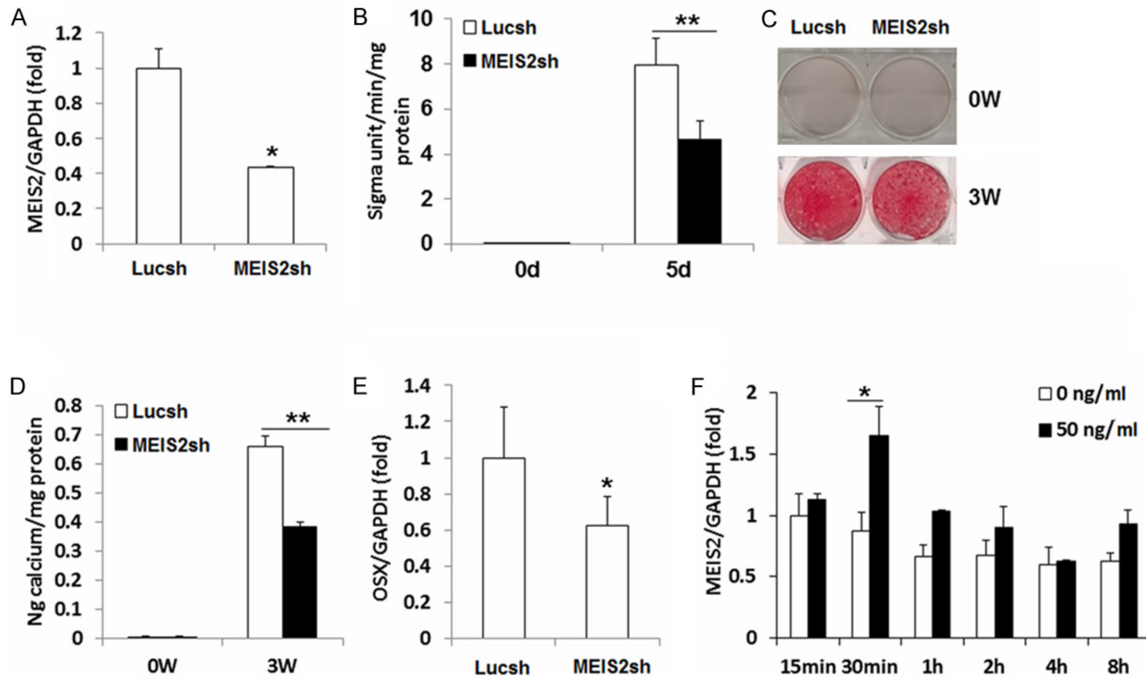

Figure 4. Knockdown of *MEIS2* inhibited osteogenic potential in DPSCs. DPSCs were infected with short hairpin RNAs (shRNA) that silenced *MEIS2* (*MEIS2sh*) or Luciferse shRNA (*Lucsh*). Real-time RT-PCR showed *MEIS2* expression. GAPDH was used as an internal control (A). The knock-down of *MEIS2* reduced alkaline phosphatase activity (B), Alizarin red staining (C) and calcium quantitative analysis (D) in DPSCs. Real-time RT-PCR showed decreased *OSX* expression after depletion of *MEIS2* in DPSCs. GAPDH was used as an internal control (E). Real-time RT-PCR showed *MEIS2* was strongly induced in DPSCs immediately after loading BMP4 (50 ng/ml) in basic culture medium (F). Student's t test was performed to determine statistical significance. All error bars represent s.d. (n=3). *P ≤ 0.05. **P ≤ 0.01.

in its critical roles in regulating the differentiation of embryonic stem cells into the cardiac, brain, and retinal cell lineage. Here, we present evidence to extend the functions of homeobox gene *MEIS2* to be a critical regulator in maintaining osteogenic differentiation of postnatal MSCs in vitro.

The commitment of MSCs into osteogenic lineages requires activation of multiple transcription factors [30, 31]. *Runx2* and *Osx* are two of the key transcription factors necessary for the osteogenic differentiation [32-34]. Genetic and molecular studies have shown that *Runx2* functions as an early transcriptional regulator of osteogenesis directing the differentiation of MSCs into an osteoblastic lineage [35]. After differentiating into preosteoblasts, *Runx2* and *Osx* can drive them into immature osteoblasts and produce bone matrix. Then, *Runx2* inhibits maturation of osteoblast and transition into osteocytes [34]. As a downstream gene of *Runx2*, *Osx* is anther transcription factor, which is essential at the early and late stages of osteogenesis [36-38] and, specifically expressed in all developing bones [39]. In the process of osteogenesis, ALP, OCN, BSP are the distinctive and key proteins of bone extracellular matrix. ALP is an enzyme mainly participating in hydrolysis of pyrophosphate during the early and late stage of osteogenesis. The activity of ALP indicates the extent of differentiation of preosteoblasts into osteoblasts; therefore, it's identified as an early marker of osteogenesis. OCN and BSP are both important markers in the later stage of osteogenic differentiation, suggesting mature of osteoblasts.

In the present study, by loss-of-function experiment, we found that targeted depletion of *MEIS2* by short hairpin RNA markedly reduced ALP activity in a dose-dependent manner in SCAPs, consistent with the following Alizarin Red staining and quantitative calcium measurements. These results indicated that *MEIS2* was important in maintaining normal osteogenic differentiation of dental MSCs. Next, by real-time RT-PCR, we identified the important osteo-

genic marker genes and found that loss of *MEIS2* largely decreased *ALP, BSP* and *OCN,* which encode extracellular matrix proteins during osteogenesis. We further examined the expressions of *OSX* and *RUNX2*, which are the important key transcription factors for regulating osteogenic differentiation. It revealed that depletion of *MEIS2* evoked the significant down-regulation of *OSX*, but didn't affect *RUNX2* expression, indicating that regulation of *MEIS2* on osteogenesis didn't mediate by *RUNX2*, but by its downstream transcription factor *OSX*. *MEIS2* evoked the activity of *OSX*, driving the preosteoblasts into osteoblasts and producing bone matrix like ALP, BSP and OCN. In parallel, results of additional experiments from DPSCs were consistent with those from SCAPs, further confirming our supposition that *MEIS2* was a critical factor for osteogenic differentiation potential of MSCs.

The process that commitment of mesenchymal stem cells to osteoblast lineages in vivo is usually mediated by a variety of extracellular signals, including canonical WNT signals and bone morphogenetic proteins (BMPs) [31, 40, 41]. Homeodomain proteins have been identified as downstream targets or regulators of osteogenic BMP signaling [42-44]. Therefore, we further investigated the effects of BMP4 on the expression of *MEIS2* in SCAPs and DPSCs. We found that *MEIS2* was strongly stimulated immediately following BMP4 treatment, indicating that *MEIS2* enhancing the osteogenic differentiation of SCAPs and DPSCs may be mediated by BMP signaling. With similar mechanism, *Hoxa10* played a vital role in regulating formation and maintenance of bone tissues [45, 46]. Previous study showed that *Hoxa10* was markedly stimulated in osteoblasts after BMP2 treatment, coincident with the robust expression of *Runx2* [47]. However, in our findings, it was with no change in the expression of *RUNX2*, probably because that osteogenic differentiation of SCAPs and DPSCs mediated by *MEIS2* is independent of *RUNX2*. Furthermore, we examined the effects of *SMAD4*, a key mediator of BMP canonical signaling pathway, on *MEIS2* expression and found that knockdown of *SMAD4* didn't result in decrease of *MEIS2* transcription, suggesting that *SMAD4* was not required in BMP4-induced *MEIS2* expression. Evidence from previous studies showed that canonical SMAD signaling had different effects on the expression of homeobox genes during odontogenesis. It's demonstrated that canonical SMAD signaling played a positive role in regulating BMP4-induced *DLX2* activity, and depletion of *SMAD4* decreased the expressions of *DLX2* after *BMP4* stimulation [15]. On the contrary, all the 39 paralogous proteins in HOX family were BMPs downstream transcription factors but *Smads* oppose *Hox* transcriptional activities. For example, *Smad6* was found to inhibit *Hoxc8*- and *Hoxb7*-induced osteoprotegerin (*OPG*) transactivation [44]. However, consistent with our findings, the expression of homeobox gene *Msx1* in dental mesenchymal cells during odontogenesis was also independent of *Smad4*. In the absence of *Smad4*, BMPs were still able to induce *phospho-Smad1/5/8* nuclear translocation and direct binding to the *Msx1* promoter in dental mesenchymal cells [48]. Based on these findings, we could believe that *MEIS2* probably act as a downstream DNA-binding protein in BMP signaling cascade, and we could further speculate that an atypical canonical BMP signaling (*SMAD4*-independent) pathway may be regulate homeobox gene MEIS2 in the dental mesenchyme during odontogenesis. Yet future work will deserve to elucidate precise signaling pathways and regulation mechanisms of *MEIS2* in osteogenic differentiation of MSCs.

Dental tissues have been identified as easily accessible sources of multipotent postnatal stem cells. Dental tissue derived MSCs are using as a new and powerful tool in dental/bone tissue engineering [49]. DPSCs had the ability to differentiate into osteoblasts and endothelial cells, and form the woven bone [50]. When transplanted into immunocompromised rats, DPSCs generated bone tissue with an integral microcirculation, similar to that of mature bone [51]. This ability of DPSCs in osteogenesis as well as their high proliferation rate makes them good candidates for the study of bone formation [52]. SCAPs, combined with PDLSCs in hydroxyapatite/tricalcium phosphate (HA/TCP) scaffolds, when transplanted into the socket of swine, could regenerate a bio-root structure capable of supporting a porcelain crown and exhibited normal functions [53]. Therefore, SCAPs and DPSCs are believed to have broad prospects as novel seed cells for bone and dental regeneration [54]. However, the mechanisms of osteogenic differentiation

of dental MSCs are still largely unknown, which have largely restricted their further wide application in regeneration. Our studies provide in vitro evidence that *MEIS2* functions as a positive regulator in maintenance of the osteogenic differentiation of dental MSCs and implicate a promising gene target to improve the process of osteogenesis.

In conclusion, the foregoing observations collectively revealed that *MEIS2* was an important transcriptional factor regulator in osteogenic commitment of dental tissue derived MSCs, and BMP signaling might account for this ability of *MEIS2*. The present study sheds light on the mechanisms of osteogenic differentiation of MSCs, yet more detailed analysis deserves our further investigation.

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Disclosure of conflict of interest

None.

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