Enriched Trimethylation of Lysine 4 of Histone H3 of WDR63 Enhanced Osteogenic Differentiation Potentials of Stem Cells from Apical Papilla

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Abstract

Introduction: Dental tissue–derived mesenchymal stem cells (MSCs) are a reliable cell source for dental tissue regeneration. However, the molecular mechanisms underlying their directed differentiation remain unclear, thus limiting their use. Trimethylation of lysine 4 of histone H3 (H3K4Me3) correlates with gene activation and osteogenic differentiation. We used stem cells from apical papilla (SCAPs) to investigate the effects of genomic changes in H3K4Me3 modification at gene promoter regions on MSC osteogenic differentiation. **Methods:** ChIP-on-chip assays were applied to compare the H3K4Me3 profiles at gene promoter regions of undifferentiated and differentiated SCAPs. Alkaline phosphatase activity assay, alizarin red staining, quantitative analysis of calcium, the expressions of osteogenesis-related genes, and transplantation in nude mice were used to investigate the osteogenic differentiation potentials of SCAPs. **Results:** In differentiated SCAPs, 119 gene promoters exhibited >2-fold increases of H3K4Me3; in contrast, the promoter regions of 21 genes exhibited >2-fold decreases of H3K4Me3. On the basis of enriched H3K4Me3 and up-regulated gene expression on the osteogenic differentiation of SCAPs, WDR63 may be a potential regulator for mediating SCAP osteogenic differentiation. Through gain-of-function and loss-of-function studies, we discovered that *WDR63* enhances alkaline phosphatase activity, mineralization, and the expression of *BSP*, *OSX*, and *RUNX2 in vitro*. In addition, transplant experiments in nude mice confirmed that SCAP osteogenesis is triggered by activated *WDR63*. **Conclusions:** These results indicate that *WDR63* is a positive enhancer for SCAP osteogenic differentiation and suggest that activation of *WDR63* signaling might improve tissue regeneration mediated by MSCs of dental origin. *(J Endod 2015;41:205–211)*

Key Words

Demethylation, osteogenic differentiation, stem cells from apical papilla (SCAPs), WDR63

Mesenchymal stem cells (MSCs) were originally isolated from bone marrow. They are multipotent cells that differentiate into a variety of cell types, including osteoblasts, chondrocytes, myocytes, and adipocytes. Increasing evidence indicates that MSCs are also present in non–bone marrow tissues (1, 2). Recently, a new population of MSCs has been isolated from dental and craniofacial tissues on the basis of their stem cell properties, including but not limited to periodontal ligament stem cells, dental pulp stem cells, and stem cells from apical papilla (SCAPs) (3–8). They are multipotent, destined for osteogenic lineages, dentinogenic lineages, and other lineages such as melanocytes, endothelial cells, and functionally active neurons, and are capable of self-renewal (3–13). When transplanted into mice or miniature pigs, they generate bone/dentin-like mineralized tissue and are capable of repairing tooth defects (4–16). Although MSCs are a reliable resource for tissue regeneration, the molecular mechanism underlying directed differentiation remains unclear; this restricts their potential applications.

The establishment of specific gene expression patterns during stem cell differentiation is a result of the subtly elaborated control of activation/silencing of large numbers of genes (17–20). Covalent histone modifications play an important role in regulating chromatin dynamics and functions (21). One type of histone modification, methylation, occurs on both lysine and arginine residues. This modification is involved in a diverse range of biological processes, including heterochromatin formation, X-chromosome inactivation, and transcriptional regulation (22–24). To date, histone modification profiles have not been extensively studied during the stem cell differentiation process, although they may be tightly associated with gene expression patterns. A technical method for genomic mapping of histone modifications *in vivo* was developed recently, allowing researchers to obtain a broader view of the distributions of various histone modifications. This method, known as ChIP-on-chip, is based on the chromatin immunoprecipitation (ChIP) assay and identifies enriched DNA fragments by hybridizing to microarrays with probes corresponding to genomic regions of interest (25–27).

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.09.027

Researchers recently discovered that trimethylation of lysine 4 of histone H3 (H3K4Me3) correlates with gene activation and function in MSCs (28–32), especially regarding osteogenic differentiation (28–30). The aim of this study was to investigate the effects of genomic changes in H3K4Me3 modifications at gene promoter regions on MSC osteogenic differentiation by using strategies that are based on ChIP-on-chip. In this study, SCAPs are used to investigate the function of H3K4Me3 with respect to osteogenic differentiation potentials by comparing H3K4Me3 profiles at gene promoter regions in undifferentiated and differentiated SCAPs. In addition, enriched H3K4Me3 and up-regulated gene expression on osteogenic differentiation combined with the results of gain-of-function and loss-of-function studies indicate that *WDR63* is a positive enhancer for SCAP osteogenic differentiation and suggest that activation of *WDR63* signaling might improve tissue regeneration mediated by MSCs of dental origin.

Materials and Methods

Cell Cultures

All research involving human stem cells is in compliance with the ISSCR "Guidelines for the Conduct of Human Embryonic Stem Cell Research." Tooth tissues were obtained under approved guidelines set by the Beijing Stomatological Hospital, Capital Medical University, with informed patient consent. Wisdom teeth were first disinfected with 75% ethanol and then washed with phosphate-buffered saline. SCAPs were isolated, cultured, and identified as previously described (6). Briefly, the tissues were gently separated from the apical papilla of the root and then digested in a solution of 3 mg/mL collagenase type I (Worthington Biochemical Corp, Lakewood, NJ) and 4 mg/mL dispase (Roche Diagnostics Corp, Indianapolis, IN) for 1 hour at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-μm strainer (Falcon, BD Labware, Franklin Lakes, NJ). SCAPs were grown in a humidified, 5% CO_2 incubator at 37°C in Dulbecco modified Eagle medium (Invitrogen, Carlsbad, CA) supplemented with 15% fetal bovine serum (Invitrogen), 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). The culture medium was changed every 3 days. Cells from passages 2–4 were used in further experiments.

ChIP-on-Chip Data Analysis

Because of both experimental and technical variability, the signal must be standardized for proper comparison among experiments. This can be done by using the stochastic part of log IP/Input distributions corresponding to the regions in which there is no binding (33). The distribution of log IP/Input may be asymmetric, with a heavy tail on the right corresponding to enrichment (33). The frequencies of loci with different IP signal intensities were modeled by using an EM algorithm to separate loci into enriched and non-enriched groups (33). The LOWESS program was used to measure the variability between different ChIP-on-chip arrays (34, 35). To identify H3K4Me3 targets, which are differentially methylated in differentiating versus undifferentiating conditions of SCAPs, a 2-fold change was defined as the threshold for significant change of H3K4Me3. Similarly, the nominal *P* value threshold of .05 was also used to distinguish the changed genes. Briefly, two-channel image data ratios were normalized by using the LOWESS method, and the *P* value calculation was implemented by using the same method as in the expression analysis (Supplemental Tables S1 and S2 and Supplemental Materials and Methods).

Transplantation in Nude Mice

The present study was approved by the Animal Care and Use Committee of Beijing Stomatological Hospital, Capital Medical University. The care and use of animals followed the guidelines of the Experimental Animal Management Ordinance. Animals were purchased from the Institute of Animal Science of the Vital River Co, Ltd; no drugs or previous procedures were used. Approximately 4.0×10^6 cells were mixed with 40 mg hydroxyapatite/tricalcium phosphate ceramic particles (Engineering Research Center for Biomaterials, Sichuan University, Chengdu, China) and then transplanted subcutaneously into the dorsal surface of five 10-week-old immunocompromised beige mice (nu/nu nude mice), as previously described (28, 29). In each animal, the SCAP-Vector cells were transplanted subcutaneously into the left side of the dorsal surface, and the SCAP-WDR63 cells were transplanted subcutaneously into the right side of the dorsal surface. These procedures were performed in accordance with the specifications of an approved animal protocol. Eight weeks after transplantation, the transplanted cells were harvested, fixed with 10% formalin, decalcified with buffered 10% EDTA (pH 8.0), and embedded in paraffin. Sections were deparaffinized, hydrated, and stained with hematoxylin–eosin (H&E). Qualitative measurement of tissue mineralization was calculated by using the Image-Pro Plus 6.0 program (Media Cybernetics, Rockville, MD).

Statistics

All statistical calculations were performed by using SPSS10 (SPSS Inc, Chicago, IL) statistical software. The Student *t* test was performed to determine statistical significance. A *P* value $\leq .05$ was considered statistically significant.

Results

Use of ChIP-on-Chip Assays to Generate Histone Modification Profiles at Gene Promoter Regions

We wanted to examine the genome-wide distributions of H3K4Me3 at gene promoter regions on SCAP osteogenic differentiation. SCAPs were cultured with osteogenesis-inducing medium and with normal culture medium for 7 days, and ChIP-on-chip assays were performed with anti-H3K4Me3 antibody by co-hybridizing differentially labeled ChIP-enriched and total input DNAs to human promoter arrays. The ChIP-on-chip data revealed that the gene promoters were enriched with H3K4Me3 after osteogenic induction (Supplemental Table S3). Moreover, when we compared the H3K4Me3 profiles at gene promoter regions from undifferentiated versus differentiated SCAPs, we found that 119 gene promoters exhibited >2-fold increases in H3K4Me3 and the promoter regions of 21 genes exhibited >2-fold decreases in H3K4Me3 (Supplemental Table S3).

To confirm the ChIP-on-chip data, *WDR63*, *TREX1*, *FOXO24*, *ARNTL*, *FOXP4*, and *TMEM106 B* were selected for ChIP assay validation. The results showed that osteogenesis-inducing medium increased H3K4Me3 in the *WDR63*, *TREX1*, *FOXO24*, and *ARNTL* promoters and decreased H3K4Me3 in the *FOXP4* and *TMEM106 B* promoters compared with normal medium (Fig. 1*A–F*). Moreover, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) demonstrated that *WDR63* and *ARNTL* exhibited >2-fold up-regulation on differentiation, and *FOXP4* was down-regulated during SCAP differentiation (Fig. 1*G–I*). However, the expression of *TREX1*, *FOXO24*, and *TMEM106 B* did not differ in undifferentiated versus differentiated SCAPs (data not shown). Overall, these data led us to speculate that *WDR63* and *ARNTL* might play an activating role in SCAP osteogenic differentiation.

WDR63 Overexpression Enhanced the Osteogenic Differentiation Potential of SCAPs

To further confirm the function of *WDR63* in SCAPs, we inserted the *WDR63* sequence into a retroviral vector. This construct overexpressed