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Research Article

Depletion of histone demethylase KDM2A enhanced the adipogenic and chondrogenic differentiation potentials of stem cells from apical papilla

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ABSTRACT

Mesenchymal stem cells (MSCs) are a reliable resource for tissue regeneration, but the molecular mechanism underlying directed differentiation remains unclear; this has restricted potential MSC applications. The histone demethylase, lysine (K)-specific demethylase 2A (KDM2A), is evolutionarily conserved and ubiquitously expressed members of the JmjC-domain-containing histone demethylase family. A previous study determined that KDM2A can regulate the cell proliferation and osteo/dentinogenic differentiation of MSCs. It is not known whether KDM2A is involved in the other cell lineages differentiation of MSCs. Here, we show that depletion of KDM2A by short hairpin RNAs can enhance adipogenic and chondrogenic differentiation potentials in human stem cells from apical papilla (SCAPs). We found that the stemness-related genes, *SOX2*, and the embryonic stem cell master transcription factor, *NANOG* were significantly increased after silence of KDM2A in SCAPs. Moreover, we found that knock-down of the KDM2A co-factor, BCOR also up-regulated the mRNA levels of *SOX2* and *NANOG*. Furthermore, Chromatin immunoprecipitation assays demonstrate that silence of KDM2A increased the histone H3 Lysine 4 (H3K4) trimethylation in the *SOX2* and *NANOG* locus and regulates its expression. In conclusion, our results suggested that depletion of KDM2A enhanced the adipogenic and chondrogenic differentiation potentials of SCAPs by up-regulated *SOX2* and *NANOG*, BCOR also involved in this regulation as co-factor, and provided useful information to understand the molecular mechanism underlying directed differentiation in MSCs.

Introduction

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

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tissues on the basis of their stem cell properties, including stem cells from the periodontal ligament (PDLSCs), from dental pulp (DPSCs), from apical papilla (SCAPs) [3–8]. They are multipotent, destined for osteo/dentinogenic lineages and other lineages such as melanocytes, endothelial cells, and functionally active neurons, and capable of self-renewal [3–13]. MSCs are a reliable resource for tissue regeneration, but the molecular mechanism underlying directed differentiation remains unclear; this restricts their potential applications.

Covalent histone modifications play an important role in regulating chromatin dynamics and functions [14]. 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 [15–17]. The steady-state level of covalent histone methylation is controlled by histone methyltransferases and demethylases. Lysine (K)-specific demethylase 2A (KDM2A) is an uncharacterized protein that was originally identified through bioinformatic searches for F-box-containing proteins [14,16]. In addition to an F-box domain, KDM2A also contains a JmjC domain, a CxxC zinc finger, a PHD domain, and three leucine-rich repeats. The molecular function of KDM2A uniquely parallels that of the human paralogue KDM2B, which contributes to gene activation by binding to and demethylating the gene promoters. Recently, KDM2A/2B were found to be key effectors of somatic cell reprogramming downstream of vitamin C. And it was found that KDM2A had a specific requirement for recognition of nucleosome-free linker DNA in vitro, and the binding mode was required for specific mucleation of KDM2A at CpG islands in vivo [18–20]. The previous studies found that KDM2A can regulate MSCs osteo/dentinogenic differentiation and cell proliferation [21,22]. Thus, we wondered whether KDM2A can also regulate other cell lineage differentiation in MSCs.

In this study, we used MSCs derived from dental apical papilla to investigate the function of KDM2A on adipogenic and chondrogenic differentiation potentials. Our results showed that depletion of KDM2A enhanced the adipogenic and chondrogenic differentiation potentials of SCAPs.

Materials and methods

Cell cultures

Five human impacted third molar with immature roots were collected from 3 healthy patients (16–20 years old) under approved guidelines set by Beijing Stomatological Hospital, Capital Medical University (Ethical committee agreement is Beijing Stomatological Hospital Ethics Review No. 2011-02), with informed patient consent. Wisdom teeth were first disinfected with 75% ethanol and then washed with phosphate buffered saline (PBS). SCAPs were isolated, cultured, and identified, as previously described [6]. Briefly, SCAPs were gently separated from the apical papilla of the root and then digested in a solution of 3 mg/mL collagenase type I (Worthington Biochem, USA) and 4 mg/mL dispase (Roche, Germany) for 1 h at 37 °C. Single-cell suspensions were obtained by passing the cells through a 70 μm strainer (Falcon, BD Labware, USA). MSCs from human bone marrow (BMSCs) were obtained from ScienCell Research Laboratories (USA). MSCs were grown in a humidified, 5% CO_2 incubator at 37 °C in DMEM alpha modified Eagle's medium (Invitrogen, USA), 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. MSCs at passages 3–5 were used in subsequent experiments. Human embryonic kidney 293T cells were maintained in complete DMEM medium with 10% fetal bovine serum (FBS; Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen).

Plasmid construction and viral infection

Plasmids were constructed by standard methods; all structures were verified by appropriate restriction digest and/or sequencing. The short hairpin RNAs (shRNA) of target genes were subcloned into the pLKO.1 lentiviral vector (Addgene, USA). Viral packaging was prepared according to the manufacturer's protocol by using 293T cells (Addgene). For viral infection, SCAPs were plated overnight and then infected with lentiviruses in the presence of polybrene (6 μg/mL, Sigma-Aldrich, USA) for 6 h, and then after 48 h, selected by antibiotics. A scramble shRNA (Scramsh) was purchased from Addgene (USA). The target sequences for shRNAs were KDM2A shRNA1 (KDM2Ash1), 5′-gcttgagatcctctgattt-3′; KDM2A shRNA2 (KDM2Ash2), 5′-tttccaagccaatggtttc-3′; BCOR shRNA (BCORsh), 5′-gatggcttcagtgctatat-3′

Oil Red O Staining

Adipogenic differentiation was induced by using the STEMPRO Adipogenesis differentiation Kit (Invitrogen). SCAPs were grown in the adipose-inducing medium for three weeks. For Oil Red O staining, cells were fixed with 10% formalin for at least 1 h at room temperature. Next, cells were stained with the 60% Oil Red O in isopropanol as working solution for 10 min. The proportion of Oil Red O-positive cells was determined by counting stained cells under a light microscope. Oil Red O dye was eluted with 100% isopropanol for 10 min, and the OD was measured at 500 nm, using 100% isopropanol as blank. The final OD value in each group was normalized with the total protein concentrations prepared from a duplicate plate.

Alcian Blue stain analysis

Chondrogenic differentiation was induced by using the STEMPRO Chondrogenesis Differentiation Kit (Invitrogen). SCAPs were grown in the chondrogenic medium for 3 weeks. For Alcian Blue staining, Cells were rinsed once with DPBS, and fixed with 4% formaldehyde solution for 30 min. After fixation, rinsed wells with DPBS and stained cells with 1% Alcian Blue solution prepared in 0.1 N HCL for 30 min. And then rinsed wells 3 times with 0.1 N HCl, added distilled water to neutralize the acidity, visualized under light microscope, and captured images for analysis. Blue staining indicated synthesis of proteoglycans by chondrocytes. To quantify proteoglycans synthesis, Alcian Blue was extracted by 4 M guanidine-HCl overnight at 4 °C. Absorbance values were read at 600 nm after temperature equilibration. The final OD value in each group was normalized with the total protein concentrations prepared from a duplicate plate.