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MicroRNA Expression Profile of Dexamethasone-Induced Human Bone Marrow-Derived Mesenchymal Stem Cells During Osteogenic Differentiation

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ABSTRACT

MiRNAs have been identified in various plants and animals where they function in post-transcriptional regulation. Although studies revealed that dexamethasone play a pivotal role in the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs), the identification of specific miRNAs and their regulatory roles in this process remain poorly defined. In this study, microarrays were used to analyze the miRNA expression profile of dexamethasone-induced hBMSCs derived from three donors, and RT-PCRs were used to confirm the microarray results. Nine upregulated miRNAs and seven downregulated miRNAs were identified. The putative target genes of these miRNAs were predicted using bioinformatics analysis. Subsequently, we focused our attention on the functional analysis of an upregulated miRNA, miR-23a. Overexpression of miR-23a inhibited osteogenic differentiation of hBMSCs at the cellular, mRNA, and protein levels. The results of our study provide an experimental basis for further research on miRNAs functions during osteogenic differentiation of dexamethasone-induced hBMSCs. J. Cell. Biochem. 115: 1683–1691, 2014.

KEY WORDS: DEXAMETHASONE; MARROW-DERIVED STEM CELLS; OSTEOGENIC DIFFERENTIATION; MicroRNA PROFILE

Human bone marrow-derived mesenchymal stem cells (hBMSCs) have the potential to differentiate into bone, cartilage, muscle, and adipose tissue [Pittenger et al., 1999], and thus have been applied to clinical field as cell-based therapeutics [Chen et al., 2004; Hare et al., 2012; Liang et al., 2012; Wang et al., 2012; Zhao et al., 2012]. Dexamethasone is a glucocorticoid drug that is widely used for the repression of allergic reactions and treatment of autoimmune diseases. A caveat of dexamethasone treatment is the induction of osteonecrosis of the femoral head via the possible pathway of inhibition of the osteogenic differentiation of hBMSCs [Cárcamo-Orive et al., 2010; Rauch et al., 2010]. Moreover, dexamethasone can induce the differentiation of MSCs into a large number of adipocytes, suppress the osteoblast proliferation and prolong the longevity of osteoclasts [Jia et al., 2006; Yin et al., 2006; Takano-Murakami et al., 2009]. It was also found that proliferation of osteoblastic MC3T3-E1 cells were significantly and directly inhibited by dexamethasone via aberrant glucocorticoid receptor activation and subsequently P53 activation [Li et al., 2012]. Although several theories have been postulated for the role of glucocorticoid-induced osteonecrosis of the femoral head [Miyanishi et al., 2002; Asano et al., 2004; Kogianni et al., 2004; Kitajima et al., 2007; Séguin et al., 2008], the underlying molecular mechanisms are not fully elucidated. Investigation of osteogenic differentiation of dexamethasone-induced hBMSCs can lead to a better understanding of the pathogenesis of femoral head necrosis and enhanced treatment options.

MicroRNAs (miRNAs) are small (17–25 nucleotides), single-stranded, non-coding RNAs that function in the negative regulation of gene expression by binding to complementary sequences in

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the 3-untranslated region (UTR) of target mRNAs, thereby leading to either mRNA degradation or translational repression [Bartel, 2004; Thomas et al., 2010; Bae et al., 2012]. MiRNAs have been shown to play roles in a broad spectrum of physiologic and pathological processes, such as cell proliferation and apoptosis, major signaling pathways, energy metabolism, tissue morphogenesis, and tumorigenesis [Cheng et al., 2005; Kloosterman and Plasterk, 2006; Raver-Shapira et al., 2007; Baltimore et al., 2008; Stefani and Slack, 2008]. Recently, mounting evidence has indicated that miRNAs play a vital role in osteogenic differentiation of hBMSCs [Goff et al., 2008; Mizuno et al., 2008; Gao et al., 2011; Baglìo et al., 2013]; however, there are no reports of osteogenic differentiation of dexamethasone-induced hBMSCs.

Herein, we sought to investigate the osteogenic differentiation of dexamethasone-induced hBMSCs which underlie the pathological basis of femoral head necrosis and osteoporosis. To further clarify the regulatory effects and underlying mechanisms of miRNAs in the osteogenic differentiation of dexamethasone-induced hBMSCs, we examined the differential expression of miRNAs in dexamethasone-induced hBMSCs compared to non-dexamethasone-induced hBMSCs during osteogenic differentiation from three individual human donors. Based on miRNA microarrays and confirmatory real-time reverse transcription-polymerase chain reactions (RT-PCR), nine overexpressed miRNAs and seven underexpressed miRNAs in dexamethasone-induced hBMSCs relative to non-dexamethasone-induced hBMSCs during osteogenic differentiation were identified. The putative target genes of these miRNAs were predicted using bioinformatics analysis. In addition, miR-23a upregulated in dexamethasone-induced hBMSCs during osteogenic differentiation was selected for further verification and function analysis.

MATERIALS AND METHODS

ISOLATION AND CULTURE OF hBMSCs

This study was approved by the Ethics Committee at Peking Union Medical College Hospital and written consent was obtained from the donors. The bone marrow tissue was obtained from three donors (A: a 30-year-old Chinese male; B: a 31-year-old Chinese female; and C: a 40-year-old Chinese female) who underwent total hip arthroplasty for congenital dislocation of hip joint or trauma-induced osteonecrosis of femoral head. hBMSCs were isolated from bone marrow using density gradient separation as previously described [Pittenger et al., 1999; Otsuru et al., 2013]. Bone marrow was collected after the osteotomy and placed in a sterile tube containing heparin (4,000 U/ml) for anticoagulation. Then the bone marrow diluted with an equal volume of PBS was layered over lymphocyte separate medium. The mononuclear cell layer was collected after centrifugation at 400*g* for 30 min at room temperature followed by washing with PBS. The cells were then resuspended at a density of 2×10^6 cells in 12 ml of regular growth medium and cultured in a 75 cm^2 flask. Growth medium contained 58% Dulbecco's modified Eagle's medium, Ham's F-12 medium (DF-12; Gibco, Grand Island, NY), 40% medium complete with trace elements-201 (MCDB; Gibco), 2% fetal bovine serum (FBS; Gibco), 1×10^9 mol/L insulin-trans-ferrin-selenium (ITS; Gibco), 1×10^{-4} mol/L ascorbic acid 2-phosphate, 20 ng/ml interleukin-6, 10 ng/ml epidermal growth factor, 10 ng/ml platelet derived growth factor BB (PDGF-BB) (all purchased from Sigma–Aldrich, St. Louis, MO), 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco) [Yang et al., 2011]. Cell cultures were maintained at 37°C in a humidified incubator with 5% CO_2. In some cultures 10^{-6} mol/L of dexamethasone was added to stimulate the hBMSCs during osteogenic differentiation.

FLOW CYTOMETRIC ANALYSIS

hBMSCs were detached from the culture plate using 0.25% trypsin/EDTA (Gibco) and collected by centrifugation at 194*g* for 10 min. hBMSCs were suspended in PBS at a concentration of about 10^6 cells/ml and washed twice with PBS. About 5×10^5 cells/500 μl were incubated and stained with 5 ml of mouse anti-human CD29-fluorescein isothiocyanate (FITC), CD31-FITC, CD44-FITC, CD73-FITC, CD90-FITC, CD105-FITC, and HLA-DR-FITC antibodies for 20 min at room temperature. The cells were rinsed twice with PBS, resuspended in 500 ml of PBS, and the expression of surface antigens were analyzed using a flow cytometer.

OSTEOGENIC DIFFERENTIATION IN VITRO

hBMSCs were plated at a cell density of 2×10^5 cells in 6-well plates (for RNA and protein isolation) and 24-well plates (for staining) in growth medium. At 70–80% confluence, growth medium was replaced with normal osteogenic differentiation medium (high glucose Dulbecco's modified Eagle's medium supplemented with 10% FBS, 10 mM β-glycerophosphate, 0.2 mM L-ascorbic acid, and 10^{-9} M dexamethasone) [Zeng et al., 2012] or osteogenic differentiation medium with high dexamethasone concentration (high glucose Dulbecco's modified Eagle's medium supplemented with 10% FBS, 10 mM β-glycerophosphate, 0.2 mM L-ascorbic acid, and 10^{-6} M dexamethasone). The osteogenic differentiation medium was changed every 3 days for 12 days.

CYTOCHEMICAL STAINING

The osteoblast phenotype was determined based on alkaline phosphatase (ALP) activity, which is an early marker of osteogenic differentiation. ALP staining was performed on days 3 and 6 after the initiation of the differentiation cultures using the ALP staining kit (Blood Institute, Chinese Academy of Medical Sciences). Alizarin Red staining was performed to detect matrix mineralization deposition which occurs at the later stage of bone formation. In brief, cells were washed with PBS, fixed in 95% ethanol for 10 min, washed with distilled water, and stained using Alizarin Red solution (1 g Tris and 0.1 g Alizarin Red (Sigma–Aldrich) in 100 ml ultrapure water; regulating the pH to 8.3 with HCl) at 37°C for 30 min.

RNA EXTRACTION AND miRNA MICROARRAY ANALYSIS

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. Total RNA was isolated from non-dexamethasone-induced hBMSCs, dexamethasone-induced hBMSCs on day 3 of culture, and miR-23a and miR-NC transfected hBMSCs on days 3, 6, and 9 of cultures. The integrity of the RNA was confirmed by agarose gel electrophoresis and its concentration was determined by spectrophotometry. An aliquot of 1 μg total RNA was biotinylated using FlashTag™ Biotin

HSR RNA Labeling Kit and hybridized to a GeneChip miRNA 2.0 Array (Affymetrix, Inc., Santa Clara, CA) containing 15,644 mature miRNAs based on Sanger miRBase Release 15.0. Hybridization was performed at 45°C with rotation for 16 h with constant rotation at the speed of 60 rpm at hybridization oven 640. After washing and staining automatically on an Affymetrix fluidics station 450, the GeneChip arrays were scanned on Affymetrix GeneChip scanner 3000 7G. All miRNA microarrays were performed at the Beijing CapitalBio Corporation. Raw data were normalized to mean array intensity for inter-array comparison and analyzed using the Significant Analysis of Microarray software (SAM, version 3.02) to identify significantly differentially expressed genes between dexamethasone-induced hBMSCs and non-dexamethasone-induced hBMSCs groups.

RT-PCR ANALYSIS OF miRNA EXPRESSION

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen), and reverse transcription were performed as previously described [Cao et al., 2005]. RT-PCR analysis for the osteogenic genes were carried out in a total reaction volume of 25 μl containing 0.5 μl of template cDNA, 12.5 μl of SYBR Green I Master Mix (Takara, Otsu, Japan), and 200 nmol/L each of the sense and antisense primers. The expression of miR-23a was detected by RT-PCR using miScript SYBR Green PCR Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. The RT-PCRs were conducted in triplicates according to procedures reported previously [Cao et al., 2005]. Relative expression of mRNA or miRNA was evaluated using the $2^{-\Delta\Delta Ct}$ method and normalized to the expression of GAPDH or U6, respectively. The primers for the mRNAs and related osteogenic genes are listed in Table I.

TARGET PREDICTION

Target gene prediction of the miRNAs was performed using the online miRNA target predicting software (www.targetscan.org).

TRANSFECTION OF miRNA INTO hBMSCs

The synthetic miR-23a mimics and miR-negative control (miR-NC) were purchased from Invitrogen. The miRNAs were transfected into hBMSCs at the final concentration of 200 nM using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The transfection process was performed in Opti-MEM (a non-serum medium) (Gibco) at 37°C in 5% CO_2. After 6 h, the Opti-MEM medium was replaced with induction medium for differentiation.

WESTERN BLOT ANALYSIS

Cells were lysed in ice-cold Radio Immunoprecipitation Assay (RIPA) lysis buffer (Beyotime, Nanjing, China) for 15 min and manually scraped from culture plates. Whole cell lysates were quantified using the BCA assay. Protein samples (20 mg) were loaded and separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, and then transferred onto polyvinylidene difluoride membranes for immunoblotting. The membranes were incubated with the following primary antibodies overnight at 4°C: anti-ALP antibody (1/5,000; Abcam, USA), anti-OPN antibody (1/1,000; Abcam), anti-RUNX2 antibody (1/500; Cell Signaling Technology, USA), anti-IBSP antibody (1/500; Cell Signaling Technology), or anti-GAPDH antibody (1/2,000; Santa Cruz Biotechnology, USA). After washing, membranes were incubated with secondary anti-rabbit or anti-mouse horseradish peroxidase-conjugated antibodies (Zhongshan, Beijing, China) for 1 h at room temperature. Antibody and antigen complexes were detected using chemiluminescent ECL reagent (Millipore, USA).

TABLE I. The Primers of miRNAs and the Related Osteogenic Genes

Gene	Primer sequence 5′–3′
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT
hsa-miR-23a	ATCACATTGCCAGGGATTTCC
hsa-miR-23a-RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGGAAAT
hsa-miR-23a-AS	GATATCACATTGCCAGGGATT
hsa-miR-155	TTAATGCTAATCGTGATAGGGGT
hsa-miR-155-RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGCCCCT
hsa-miR-155-AS	GCGCTTAATGCTAATCGTGATAG
hsa-miR-744	TGCGGGGCTAGGGCtAACAGCA
hsa-miR-744-RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTGCTGT
hsa-miR-744-AS	TGCGGGGCTAGGGCTAAC
hsa-miR-140–3p	TACCACAGGGTAGAACCACGG
hsa-miR-140–3p-RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACCCGTGG
hsa-miR-140–3p-AS	ACCGTACCACAGGGTAGAACCA
GAPDH-F	GGTCACCAGGGCTGCTTTTA
GAPDH-R	GGATCTCGCTCCTGGAAGATG
ALP-F	CCACGTCTTCACATTTGGTG
ALP-R	AGACTGCGCCTGGTAGTTGT
OPN-F	ACTCGAACGACTCTGATGATGT
OPN-R	GTCAGGTCTGCGAAACTTCTTA
RUNX2-F	TGTCATGGCGGGTAACGAT
RUNX2-R	AAGACGGTTATGGTCAAGGTGAA
IBSP-F	TGGATGAAAACGAACAAGGCA
IBSP-R	AAACCCACCATTTGGAGAGGT

STATISTICAL ANALYSIS

Data presented were the mean ± standard deviation. Statistical analysis was conducted using two-tailed *t*-test (SPSS Statistics 17.0). Differences were considered significant when the *P*-value was less than or equal to 0.05.

RESULTS

CHARACTERISTICS AND PHENOTYPES OF hBMSCs

After 4 days of primary culture, the adherent cells are spindle shaped and flat (Supplemental Fig. S1A). The cells grew rapidly and reached almost 80% confluences after 14 days of culture. During subsequent cell passages the cell density was very high and the cells were swirling or radial arranged (Supplemental Fig. S1B).

To further confirm that the isolated cells were hBMSCs, seven cell surface antigens associated with hBMSCs were characterized by flow cytometric analysis. hBMSCs from passage 3 were positive for CD29, CD44, CD73, CD90, and CD105 (97.9%, 99.9%, 100%, 99.8%, and 99.7%, respectively), and negative for CD31 and HLA-DR (1.1% and 0.8%, respectively), showed in Supplemental Figure S2.

IDENTIFICATION OF OSTEOGENIC DIFFERENTIATION

hBMSCs at passage 4 were cultured in osteogenic medium for 12 days. In order to detect osteogenesis the cells were subjected to ALP

staining at day 6 and Alizarin Red staining at day 12. The positive ALP staining indicated that ALP, an osteogenic specific gene, was active (Supplemental Fig. S1C). The positive Alizarin Red staining indicated mineral deposition and nodule formation (Supplemental Fig. S1D).

miRNA MICROARRAY ANALYSIS

miRNA microarray chips were used to determine miRNA expression levels in hBMSCs during osteogenic differentiation. Four indicators were adopted to evaluate the differential expression between dexamethasone and non-dexamethasone-induced hBMSCs during osteogenic differentiation: T-statistic value, numerator of the T-statistic, denominator of the T-statistic, and fold change. Sixteen differentially expressed miRNAs were identified, of which nine miRNAs were up-regulated (hsa-miR-155, hsa-miR-196a, hsa-miR-199a-5p, hsa-miR-130a, hsa-miR-26a, hsa-miR-221, hsa-miR-23a, hsa-miR-22, and hsa-miR-27a) and 7 miRNAs were down-regulated (hsa-miR-21, hsa-miR-140-3p, hsa-miR-214, hsa-miR-744, hsa-miR-320a, hsa-miR-320b, and hsa-miR-320c). The differential expression of the 16 miRNAs is summarized in Table II. The heat map vividly reflects the differential expression of miRNAs between dexamethasone and non-dexamethasone-induced hBMSCs during osteogenic differentiation (Fig. 1).

Fig. 1. A heat map based on the differential expression of miRNAs between dexamethasone and non-dexamethasone-induced hBMSCs during osteogenic differentiation.

RT-PCR VALIDATION OF THE MICROARRAY RESULTS

To confirm the microarray results, RT-PCR was conducted to detect the expression levels of the differentially expressed miRNAs. RT-PCR showed that the some of the up-regulated miRNAs (miR-155 and miR-23a) and down-regulated miRNAs (miR-744 and miR-140-3p) were differentially expressed between dexamethasone and non-dexamethasone-induced hBMSCs during osteogenic differentiation, which was in line with the microarray results. Figure 2 shows that the fold-changes of miR-155, miR-23a, miR-744, and miR-140-3p by the RT-PCR agreed with the microarray results.

TARGET PREDICTION

Potential targets of the 16 differentially expressed miRNAs were predicted using the online software TargetScan. Predicted targets of each miRNA included genes involved in cell cycle regulation, cell differentiation, and metabolism. Table III lists the target genes related to osteogenic differentiation and bone formation. The predicted target genes of the nine up-regulated miRNAs include RUNX2, STAT5B, SMAD1, SMAD5, and BMPR2. The seven down-regulated miRNAs were predicted to target genes which inhibit osteogenic differentiation, such as such as Sox4, BMP3, Acvr2b, HDAC4, and TGF-β1.

FUNCTIONAL ANALYSIS AND FURTHER VERIFICATION OF miR-23a

miR-23a that was up-regulated in dexamethasone-induced hBMSCs during osteogenic differentiation was selected for functional analysis and further verification of our findings. Synthetic miR-

TABLE II. Differentially Expressed miRNAs in Dexamethasone-Induced hBMSCs During Osteogenic Differentiation

Gene name	Score (d)	Numerator (r)	Denominator (s + s0)	Fold change
Nine miRNAs overexpressed in dexamethasone-induced hBMSCs during osteogenic differentiation				
hsa-miR-155	−2.27	−302.68	133.55	3.34
hsa-miR-196a	−6.47	−52.82	8.16	2.59
hsa-miR-199a-5p	−11.55	−261.94	22.69	2.13
hsa-miR-130a	−6.22	−70.48	11.33	2.03
hsa-miR-26a	−6.06	−846.99	139.80	2.37
hsa-miR-221	−7.84	−3,278.34	418.32	2.07
hsa-miR-23a	−11.23	−2,375.33	211.49	3.66
hsa-miR-22	−12.97	−914.60	70.49	2.24
hsa-miR-27a	−5.68	−360.38	63.45	2.66
Seven miRNAs underexpressed in dexamethasone-induced hBMSCs during osteogenic differentiation				
hsa-miR-21	4.85	241.55	49.77	0.30
hsa-miR-140-3p	14.18	252.99	17.85	0.36
hsa-miR-214	22.39	4,276.91	191.03	0.17
hsa-miR-744	25.55	714.14	27.96	0.17
hsa-miR-320a	12.95	3,917.58	302.53	0.28
hsa-miR-320b	15.56	3,372.77	216.78	0.30
hsa-miR-320c	5.92	1,320.60	223.17	0.47

SAM score (d), T-statistic value; Numerator, numerator of the T-statistic; Denominator (s + s0), denominator of the T-statistic.

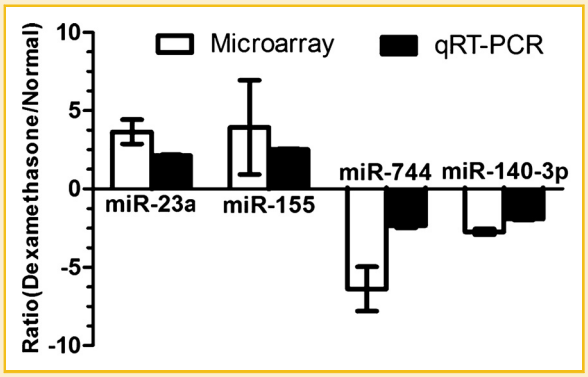

Fig. 2. RT-PCR confirmation of related upregulated miRNAs (miR-155 and miR-23a) and downregulated miRNAs (miR-744 and miR-140-3p) in dexamethasone-induced hBMSCs.

23a mimics and miR-NC were transfected into hBMSCs and osteogenesis was examined by evaluating stainings, as well as mRNA and protein expression of osteo-specific proteins.

At days 6 and 12 of osteogenic differentiation, ALP staining and Alizarin Red staining revealed significantly decreased ALP activity and depressed matrix mineralization in the miR-23a group compared to the miR-NC group (Fig. 3A,B). Moreover, lower mRNA expression levels of osteo-specific markers, such as ALP, OPN, RUNX2, and IBSP, were detected in hBMSCs that overexpressed miR-23a (Fig. 3C). In addition, we also found lower protein expression of ALP, OPN, RUNX2, and IBSP in the miR-23a group (Fig. 3D), which were compatible with RT-PCR results and staining. Therefore, overexpression of miR-23a inhibited the osteogenesis of hBMSCs.

DISCUSSION

miRNAs are important negative regulators of post-transcriptional gene expression that have emerged as key players in various cellular processes. Recently, the regulatory role of miRNAs on mesenchymal stem cells activities, such as self-renewal and differentiation into osteoblasts, chondrocytes, or adipocytes, have garnered increasing interest and attention of scientific researchers [Lakshmipathy and Hart, 2008; Kim et al., 2009b; Schoolmeesters et al., 2009; Han et al., 2010; Huang et al., 2010 ; Li et al., 2013]. Osteogenic differentiation is a complex sequence of events that involves the contributions of numerous signaling pathways and transcription factors. Baglìo et al. [2013] evaluated the miRNA expression profile of hBMSCs during osteogenic differentiation and found 17 up-regulated and 12 down-regulated miRNAs. Moreover, Oskowitz et al. [2008] identified 19 miRNAs that were up-regulated during osteogenic differentiation. A major limitation of these studies was that simple osteogenic differentiation is a very ideal state that does not typically occur in the normal adult environment. We therefore sought to examine osteogenic differentiation in dexamethasone-induced hBMSCs. Dexamethasone treatment underlies the pathological basis of the femoral head necrosis and osteoporosis which are widely detected in systemic lupus erythematosus patients who are treated with dexamethasone. To the best of knowledge, this current report is the first one that describes the role of miRNAs in dexamethasone-induced hBMSCs during osteogenic differentiation and it provides a new insight into the pathology and treatment of the femoral head necrosis and osteoporosis.

In this study, we utilized miRNA microarrays to investigate the miRNAs involved in dexamethasone-induced hBMSCs during osteogenic differentiation. Nine up-regulated miRNAs and seven down-regulated miRNAs were identified in this process. To further validate the reliability of the microarray results, the expression fold changes of some of the up-regulated miRNAs (miR-23a and miR-155) and down-regulated miRNAs (miR-744 and miR-140-3p) were analyzed by RT-PCR. The expression level of miR-155 and miR-23a increased and the level of miR-744 and miR-140-3p decreased in dexamethasone-induced hBMSCs during osteogenic differentiation, which was in accordance with previous microarray results.

Herein, we identified nine miRNAs that were up-regulated in dexamethasone-induced hBMSCs during osteogenic differentiation incidating that they inhibit the osteogenic differentiation of hBMSCs. Our findings were in line with previous reports of differential regulation of miRNAs during osteogenesis. Hassan

TABLE III. Predicted Target Genes of Differentially Expressed miRNAs

miRNA	Target gene	Functions
miRNAs overexpressed in dexamethasone-induced hBMSCs during osteogenic differentiation		
hsa-miR-155	SATB2	Osteogenesis
hsa-miR-196a	COL1A1, COL1A2, COL3A1, FOXO1	Osteogenesis and inhibition of adipogenesis
hsa-miR-199a-5p	TMEM135, Sox4	Osteogenesis
hsa-miR-130a	SMAD5, SMAD4, BMPR1B, BMPR2	Osteogenesis
hsa-miR-26a	SMAD1, Wnt5α, SMAD4, TMEM135	Osteogenesis
hsa-miR-221	Wnt1, Sox4	Osteogenesis
hsa-miR-23a	SATB1,STAT5B, TMEM135, FGF2,NFIB, SMAD5, IHH, RUNX2	Osteogenesis
hsa-miR-22	SMAD4, SATB2, HDAC6	Osteogenesis and inhibition of adipogenesis
hsa-miR-27a	RUNX1, SMAD5, SATB2, LRP6, FOXO1	Osteogenesis and inhibition of adipogenesis
miRNAs underexpressed in dexamethasone-induced hBMSCs during osteogenic differentiation		
hsa-miR-21	SMAD7, Sox2, TGFBR2	Inhibit of osteogenesis
hsa-miR-140-3p	Acvr2b, CBL, HDAC4	Inhibit of osteogenesis
hsa-miR-214	CBL, GSK3β	Inhibit of osteogenesis and adipogenesis
hsa-miR-744	TGFBβ1	Inhibit of osteogenesis
hsa-miR-320a	CBL, BMP3, HDAC4, Sox4, Acvr2b, TGFBR2	Inhibit of osteogenesis
hsa-miR-320b	CBL, BMP3, HDAC4, Sox4, Acvr2b, TGFBR2	Inhibit of osteogenesis
hsa-miR-320c	CBL, BMP3, HDAC4, Sox4, Acvr2b, TGFBR2	Inhibit of osteogenesis

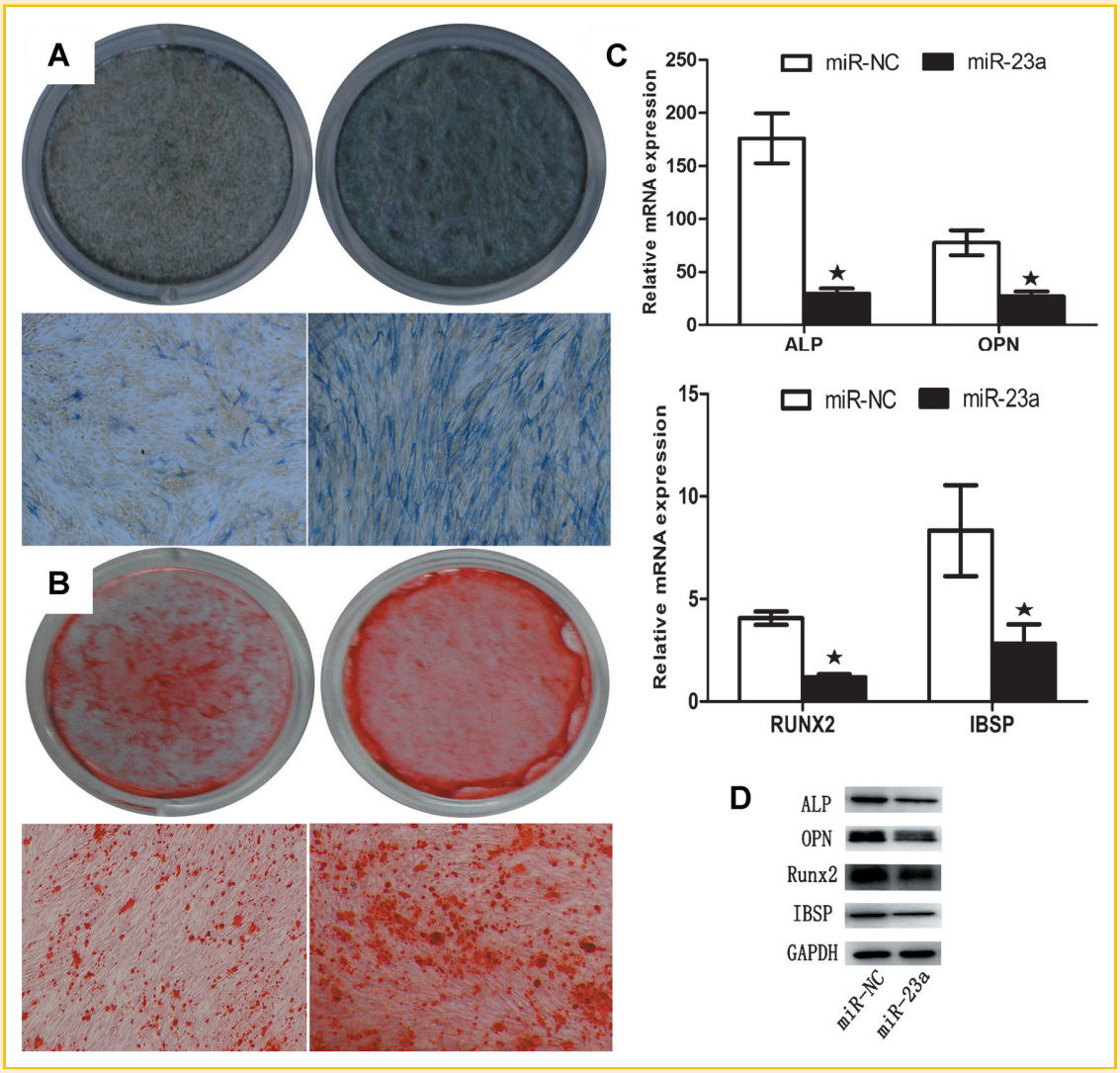

Fig. 3. Functional analysis of miR-23a. Synthetic miR-23a mimics and miR-NC were transfected into hBMSCs and osteogenesis was examined by evaluating stainings, as well as mRNA and protein expression of osteo-specific proteins. A: ALP staining at day 6 of osteogenic differentiation. The upper are gross appearances and the lower are the corresponding microscopic appearances. B: Alizarin Red staining at day 12 of osteogenic differentiation. The upper are gross appearances and the lower are the corresponding microscopic appearances. C: mRNA expression levels of ALP, OPN, RUNX2, and IBSP. D: protein expression of ALP, OPN, RUNX2, and IBSP. $^*P < 0.05$.

et al. [2010] showed that miR-23a was a potent inhibitor of osteoblastogenesis and miR-27a delayed osteoblast differentiation. According to Luzi et al. [2008], inhibition of miR-26a in treated osteoblasts by 2′-*O*-methyl-antisense RNA increased protein levels of its predicted target, SMAD1 transcription factor, which upregulated bone marker genes and enhanced osteoblast differentiation. Wu et al. [2012] found that knockdown of miR-155 relieved the inhibition of TNF-α on BMP-2-induced osteogenic differentiation. However, our findings were different from that of at least two other studies. First, Kim et al. [2009a] found that overexpression of miR-196a decreased the proliferation of human adipose tissue-derived mesenchymal stem cells (hAMSCs) and enhanced osteogenic differentiation. Second, according to Huang et al. [2012], the upregulation of miR-22 promoted osteogenic differentiation and inhibited adipogenic differentiation of hAMSCs via repression of HDAC6 protein expression. The distinct research findings were likely due to differences in the variability among MSCs from different tissues or the cultured microenvironment of the MSCs.

Downregulation of miRNAs in the dexamethasone-induced hBMSCs during osteogenic differentiation would indicate that these miRNAs enhance the osteogenic differentiation of hBMSCs. Our findings were supported by previous studies. MiR-21 was confirmed to promote the osteoblast differentiation of MSCs by repressing Spry1, which negatively regulates osteogenic differentiation of MSCs [Yang et al., 2013]. Palmieri et al. [2010] found that miR-214 and miR-320 were up-regulated in an osteoblast-like cell line (MG63) exposed to Bio-Oss, a biomaterial that enhances osteoblast activity to promote bone formation. On the other hand, Kim et al. [2009b] found that lentiviral overexpression of miR-21 enhanced adipogenic differentiation. In addition, it was reported that miR-140

is associated with chemoresistance in human osteosarcoma and osteoarthritis [Song et al., 2009; Miyaki et al., 2010]. Taken together these data highlight a putative role for miRNAs during osteogenesis and the promoting or inhibitory effects varied depending on the specific tissue or the microenvironment of cells.

To further understand the regulatory mechanism of the differentially expressed miRNAs in dexamethasone-induced hBMSCs during osteogenic differentiation, the putative target genes for the candidate miRNAs were predicted using TargetScan 5.1. Several of the putative target genes for the upregulated miRNAs were associated with bone formation, including RUNX2, STAT5B, SMAD1, SMAD5, and BMPR2. Runx2 directs the formation of immature bone by inducing the differentiation of multipotent mesenchymal cells into immature osteoblasts [Komori, 2010]. Studies demonstrated that osteoblastic differentiation was associated primarily with increased Runx2 activity in bone marrow stromal cells [Wojtowicz et al., 2010]. Activation of STAT5B, which mediates the signal transduction triggered by various ligands, such as IL-2, IL-4, and CSF-1, promotes osteogenic differentiation of MSCs and bone growth [Joung et al., 2012, 2013]. The BMP-Smad signaling pathway plays a vital role in osteogenic differentiation and bone-related disorders. Phosphorylated Smad1 or Smad5 forms a complex with Smad4, which is then translocated to the nucleus where it binds to the consensus DNA sequence to regulate the transcription of BMP target genes [Li, 2008].

The miRNAs that were down-regulated in the dexamethasone-induced hBMSCs during osteogenic differentiation were predicted to target genes, such as Sox4, BMP3, Acvr2b, HDAC4, and TGF-β1. The Sox4 gene belongs to a gene family (SOX and SRY) comprising transcription factors that bind to DNA through their high mobility group (HMG)-type binding domain. They regulate bone formation by acting upstream of Osx and independent of Runx2 [Reppe et al., 2000; Nissen-Meyer et al., 2007]. Kokabu et al. [2012] found that BMP3 inhibits Smad signaling via its interaction with Acvr2b, thereby, suppressing osteoblast differentiation of bone marrow stromal cells. Shimizu et al. [2010] demonstrated that histone deacetylase 4 (HDAC4) suppressed osteoblast differentiation by binding to Runx2 at the runt domain (RD) site of the MMP-13 promoter. Ochiai et al. [2012] showed that TGF-β1 inhibited osteoblast differentiation via suppression of IGF-1 expression and subsequent down-regulation of the PI3K/Akt pathway. Moreover, Zhou [2011] demonstrated that TGF-β1 inhibited osteoblast differentiation in hMSCs through the regulation of β-catenin signaling.

Functional studies and analysis of miRNAs can provide direct evidence to prove that miRNAs modulate osteogenic differentiation of hBMSCs. In previous studies, hsa-miR-23a was associated with B-cell development [Kong et al., 2010] and gastrointestinal cancer [Zheng et al., 2013]. Zhang et al. [2011] showed that miR-23a inhibits osteogenesis and downregulates RUNX2 protein expression in MC3T3-E1 cells. In our study, miR-23a upregulated in dexamethasone-induced hBMSCs during osteogenic differentiation was selected for the further function verification. Subsequently, miR-23a effects were assessed by the RT-PCR, western blot, ALP and Alizarin Red staining by transfecting miR-23a mimics in to hBMSCs. The results showed that miR-23a inhibit the osteogenic differentiation of hBMSCs. There are three highlights in our study compared with the study by Zhang et al. [2011]. Firstly, the cells we treated in our study are human BMSCs, whereas Zhang et al. used MC3T3-E1 osteoblasts. Secondly, we examed the osteoginic ability from mRNA (ALP, OPN, RUNX2, and IBSP), protein and stainings levels, which is more comprehensive than previous study.

To the best of our knowledge, this is the first report on the miRNA expression profile of dexamethasone-induced hBMSCs during osteogenic differentiation. We discovered nine upregulated miRNAs and seven downregulated miRNAs in dexamethasone-induced hBMSCs during osteogenic differentiation. Bioinformatic analyses were used to predict the putative target genes for these miRNAs. Functional analysis of hsa-miR-23a further validated the results and highlighted its putative role during osteogenesis. Further studies involving target verification for the differentially regulated miRNAs are required to more fully explain the underlying miRNAs regulatory mechanisms. Taken together, the results of our study provided an experimental basis for further research of miRNA functions during osteogenic differentiation of dexamethasone-induced hBMSCs, which plays a role in the pathogenesis and development of bone diseases, such as femoral head necrosis and osteoporosis.

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REFERENCES

Asano T, Takahashi KA, Fujioka M, Inoue S, Ueshima K, Hirata T, Okamoto M, Satomi Y, Nishino H, Tanaka T, Hirota Y, Kubo T. 2004. Relationship between postrenal transplant osteonecrosis of the femoral head and gene polymorphisms related to the coagulation and fibrinolytic systems in Japanese subjects. Transplantation 77:220–225.

Bae Y, Yang T, Zeng HC, Campeau PM, Chen Y, Bertin T, Dawson BC, Munivez E, Tao J, Lee BH. 2012. MiRNA-34c regulates Notch signaling during bone development. Hum Mol Genet 21:2991–3000.

Baglìo SR, Devescovi V, Granchi D, Baldini N. 2013. MicroRNA expression profiling of human bone marrow mesenchymal stem cells during osteogenic differentiation reveals Osterix regulation by miR-31. Gene 527: 321–331.

Baltimore D, Boldin MP, O′Connell RM, Rao DS, Taganov KD. 2008. MicroRNAs: New regulators of immune cell development and function. Nat. Immunol 9:839–845.

Bartel DP. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116:281–297.

Cao Y, Sun Z, Liao L, Meng Y, Han Q, Zhao RC. 2005. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. Biochem Biophys Res Commun 332:370–379.

Cárcamo-Orive I, Gaztelumendi A, Delgado J, Tejados N, Dorronsoro A, Fernández-Rueda J, Pennington DJ, Trigueros C. 2010. Regulation of human bone marrow stromal cell proliferation and differentiation capacity by glucocorticoid receptor and AP-1 cross-talk. J Bone Miner Res 25:2115–2125.

Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, Sun JP. 2004. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol 94: 92–95.

Cheng AM, Byrom MW, Shelton J, Ford LP. 2005. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 33:1290–1297.

Gao J, Yang T, Han J, Yan K, Qiu X, Zhou Y, Fan Q, Ma B. 2011. MicroRNA expression during osteogenic differentiation of human multipotent mesenchymal stromal cells from bone marrow. J Cell Biochem 112:1844–1856.

Goff LA, Boucher S, Ricupero CL, Fenstermacher S, Swerdel M, Chase LG, Adams CC, Chesnut J, Lakshmipathy U, Hart RP. 2008. Differentiating human multipotent mesenchymal stromal cells regulate microRNAs: Prediction of microRNA regulation by PDGF during osteogenesis. Exp Hematol 36:1354–1369.

Han J, Yang T, Gao J, Wu J, Qiu X, Fan Q, Ma B. 2010. Specific microRNA expression during chondrogenesis of human mesenchymal stem cells. Int J Mol Med 25:377–384.

Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, Tracy M, Ghersin E, Johnston PV, Brinker JA, Breton E, Davis-Sproul J, Schulman IH, Byrnes J, Mendizabal AM, Lowery MH, Rouy D, Altman P, Wong Po Foo C, Ruiz P, Amador A, Da Silva J, McNiece IK, Heldman AW, George R, Lardo A. 2012. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: The POSEIDON randomized trial. JAMA 308:2369–2379.

Hassan MQ, Gordon JAR, Beloti MM, Croce CM, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2010. A network connecting Runx2, SATB2, and the miR-23a–27a–24-2 cluster regulates the osteoblast differentiation program. Proc Natl Acad Sci USA 107:19879–19884.

Huang J, Zhao L, Xing L, Chen D. 2010. MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. Stem Cells 28:357–364.

Huang S, Wang S, Bian C, Yang Z, Zhou H, Zeng Y, Li H, Han Q, Zhao RC. 2012. Upregulation of miR-22 promotes osteogenic differentiation and inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells by repressing HDAC6 protein expression. Stem Cells Dev 21:2531–2540.

Jia D, O'Brien CA, Stewart SA, Manolagas SC, Weinstein RS. 2006. Glucocorticoids act directly on osteoclasts to increase their life span and reduce bone density. Endocrinology 147:5592–5599.

Joung YH, Lim EJ, Darvin P, Chung SC, Jang JW, Do Park K, Lee HK, Kim HS, Park T, Yang YM. 2012. MSM enhances GH signaling via the Jak2/STAT5b pathway in osteoblast-like cells and osteoblast differentiation through the activation of STAT5b in MSCs. PLoS ONE 7:e47477.

Joung YH, Lim EJ, Darvin P, Jang JW, Park KD, Lee HK, Kim HS, Cho BW, Park T, Chung S, Park JH, Yang YM. 2013. Hwanggeumchal sorghum extract enhances BMP7 and GH signaling through the activation of Jak2/STAT5B in MC3T3–E1 osteoblastic cells. Mol Med Rep 8:891–896.

Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS. 2009a. MiR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. J Bone Miner Res 24:816–825.

Kim YJ, Hwang SJ, Bae YC, Jung JS. 2009b. MiR-21 regulates adipogenic differentiation through the modulation of TGF-beta signaling in mesenchymal stem cells derived from human adipose tissue. Stem Cells 27:3093–3102.

Kitajima M, Shigematsu M, Ogawa K, Sugihara H, Hotokebuchi T. 2007. Effects of glucocorticoid on adipocyte size in human bone marrow. Med Mol Morphol 40:150–156.

Kloosterman WP, Plasterk RH. 2006. The diverse functions of microRNAs in animal development and disease. Dev Cell 11:441–450.

Kogianni G, Mann V, Ebetino F, Nuttall M, Nijweide P, Simpson H, Noble B. 2004. Fas/CD95 is associated with glucocorticoid-induced osteocyte apoptosis. Life Sci 75:2879–2895.

Kokabu S, Gamer L, Cox K, Lowery J, Tsuji K, Raz R, Economides A, Katagiri T, Rosen V. 2012. BMP3 suppresses osteoblast differentiation of bone marrow stromal cells via interaction with Acvr2b. Mol Endocrinol 26:87–94.

Komori T. 2010. Regulation of osteoblast differentiation by Runx2. Adv Exp Med Biol 658:43–49.

Lakshmipathy U, Hart RP. 2008. Concise review: MicroRNA expression in multipotent mesenchymal stromal cells. Stem Cells 26:356–363.

Kong KY, Owens KS, Rogers JH, Mullenix J, Velu CS, Grimes HL, Dahl R. 2010. MIR-23A microRNA cluster inhibits B-cell development. Exp Hematol 38:629–640.

Li B. 2008. Bone morphogenetic protein-Smad pathway as drug targets for osteoporosis and cancer therapy. Endocr Metab Immune Disord Drug Targets 8:208–219.

Li H, Qian W, Weng X, Wu Z, Li H, Zhuang Q, Feng B, Bian Y. 2012. Glucocorticoid receptor and sequential P53 activation by dexamethasone mediates apoptosis and cell cycle arrest of osteoblastic MC3T3-E1 cells. PLoS ONE 7:e37030.

Li H, Li T, Wang S, Wei J, Fan J, Li J, Han Q, Liao L, Shao C, Zhao RC. 2013. MiR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res 10:313–324.

Liang J, Li X, Zhang H, Wang D, Feng X, Wang H, Hua B, Liu B, Sun L. 2012. Allogeneic mesenchymal stem cells transplantation in patients with refractory RA. Clin Rheumatol 31:157–161.

Luzi E, Marini F, Sala SC, Tognarini I, Galli G, Brandi ML. 2008. Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Miner Res 23:287–295.

Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, Takada S, Lotz MK, Ueno-Kudo H, Asahara H. 2010. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev 24:1173–1185.

Miyanishi K, Yamamoto T, Irisa T, Yamashita A, Jingushi S, Noguchi Y, Iwamoto Y. 2002. Bone marrow fat cell enlargement and a rise in intraosseous pressure in steroid-treated rabbits with osteonecrosis. Bone 30:185–190.

Mizuno Y, Yagi K, Tokuzawa Y, Kanesaki-Yatsuka Y, Suda T, Katagiri T, Fukuda T, Maruyama M, Okuda A, Amemiya T, Kondoh Y, Tashiro H, Okazaki Y. 2008. MiR-125b inhibits osteoblastic differentiation by down-regulation of cell proliferation. Biochem Biophys Res Commun 368:267–272.

Nissen-Meyer LS, Jemtland R, Gautvik VT, Pedersen ME, Paro R, Fortunati D, Pierroz DD, Stadelmann VA, Reppe S, Reinholt FP, Del Fattore A, Rucci N, Teti A, Ferrari S, Gautvik KM. 2007. Osteopenia, decreased bone formation and impaired osteoblast development in Sox4 heterozygous mice. J Cell Sci 120:2785–2795.

Ochiai H, Okada S, Saito A, Hoshi K, Yamashita H, Takato T, Azuma T. 2012. Inhibition of insulin-like growth factor-1 (IGF-1) expression by prolonged transforming growth factor-β1 (TGF-β1) administration suppresses osteoblast differentiation. J Biol Chem 287:22654–22661.

Oskowitz AZ, Lu J, Penfornis P, Ylostalo J, McBride J, Flemington EK, Prockop DJ, Pochampally R. 2008. Human multipotent stromal cells from bone marrow and microRNA: Regulation of differentiation and leukemia inhibitory factor expression. Proc Natl Acad Sci USA 105:18372–18377.

Otsuru S, Hofmann TJ, Olson TS, Dominici M, Horwitz EM. 2013. Improved isolation and expansion of bone marrow mesenchymal stromal cells using a novel marrow filter device. Cytotherapy 15:146–153.

Palmieri A, Pezzetti F, Brunelli G, Martinelli M, Lo Muzio L, Scarano A, Scapoli L, Arlotti M, Guerzoni L, Carinci F. 2010. Anorganic bovine bone (Bio-Oss) regulates miRNA of osteoblast-like cells. Int J Periodontics Restorative Dent 30:83–87.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147.

Rauch A, Seitz S, Baschant U, Schilling AF, Illing A, Stride B, Kirilov M, Mandic V, Takacz A, Schmidt-Ullrich R, Ostermay S, Schinke T, Spanbroek R,

Zaiss MM, Angel PE, Lerner UH, David JP, Reichardt HM, Amling M, Schütz G, Tuckermann JP. 2010. Glucocorticoids suppress bone formation by attenuating osteoblast differentiation via the monomeric glucocorticoid receptor. Cell Metab 11:517–531.

Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, Bentwich Z, Oren M. 2007. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell 26:731–743.

Reppe S, Rian E, Jemtland R, Olstad OK, Gautvik VT, Gautvik KM. 2000. Sox-4 messenger RNA is expressed in the embryonic growth plate and regulated via the parathyroid hormone/parathyroid hormone-related protein receptor in osteoblast-like cells. J Bone Miner Res 15:2402–2412.

Schoolmeesters A, Eklund T, Leake D, Vermeulen A, Smith Q, Force Aldred S, Fedorov Y. 2009. Functional profiling reveals critical role for miRNA in differentiation of human mesenchymal stem cells. PLoS ONE 4:e5605.

Séguin C, Kassis J, Busque L, Bestawros A, Theodoropoulos J, Alonso ML, Harvey EJ. 2008. Non-traumatic necrosis of bone (osteonecrosis) is associated with endothelial cell activation but not thrombophilia. Rheumatology (Oxford) 47:1151–1155.

Shimizu E, Selvamurugan N, Westendorf JJ, Olson EN, Partridge NC. 2010. HDAC4 represses matrix metalloproteinase-13 transcription in osteoblastic cells, and parathyroid hormone controls this repression. J Biol Chem 285:9616–9626.

Song B, Wang Y, Xi Y, Kudo K, Bruheim S, Botchkina GI, Gavin E, Wan Y, Formentini A, Kornmann M, Fodstad O, Ju J. 2009. Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. Oncogene 28:4065–4074.

Stefani G, Slack FJ. 2008. Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol 9:219–230.

Takano-Murakami R, Tokunaga K, Kondo N, Ito T, Kitahara H, Ito M, Endo N. 2009. Glucocorticoid inhibits bone regeneration after osteonecrosis of the femoral head in aged female rats. Tohoku J Exp Med 217:51–58.

Thomas M, Lieberman J, Lal A. 2010. Desperately seeking microRNA targets. Nat Struct Mol Biol 17:1169–1174.

Wang S, Qu X, Zhao RC. 2012. Clinical applications of mesenchymal stem cells. J Hematol Oncol 5:19.

Wojtowicz AM, Templeman KL, Hutmacher DW, Guldberg RE, Garcia AJ. 2010. Runx2 overexpression in bone marrow stromal cells accelerates bone formation in critical-sized femoral defects. Tissue Eng A 16:2795–2808.

Wu T, Xie M, Wang X, Jiang X, Li J, Huang H. 2012. MiR-155 modulates TNF-α-inhibited osteogenic differentiation by targeting SOCS1 expression. Bone 51:498–505.

Yang Z, Bian C, Zhou H, Huang S, Wang S, Liao L, Zhao RC. 2011. MicroRNA hsa-miR-138 inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells through adenovirus EID-1. Stem Cells Dev 20:259–267.

Yang N, Wang G, Hu C, Shi Y, Liao L, Shi S, Cai Y, Cheng S, Wang X, Liu Y, Tang L, Ding Y, Jin Y. 2013. Tumor necrosis factor α suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. J Bone Miner Res 28:559–573.

Yin L, Li YB, Wang YS. 2006. Dexamethasone-induced adipogenesis in primary marrow stromal cell cultures: Mechanism of steroid-induced osteonecrosis. Chin Med J (Engl) 119:581–588.

Zeng Y, Qu X, Li H, Huang S, Wang S, Xu Q, Lin R, Han Q, Li J, Zhao RC. 2012. MicroRNA-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting BMPR2. FEBS Lett 586:2375–2381.

Zhang Y, Xie RL, Croce CM, Stein JL, Lian JB, van Wijnen AJ, Stein GS. 2011. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. Proc Natl Acad Sci USA 108:9863–9868.

Zhao D, Cui D, Wang B, Tian F, Guo L, Yang L, Liu B, Yu X. 2012. Treatment of early stage osteonecrosis of the femoral head with autologous implantation of bone marrow-derived and cultured mesenchymal stem cells. Bone 50:325–330.

Zheng H, Li W, Wang Y, Xie T, Cai Y, Wang Z, Jiang B. 2013. MiR-23a inhibits E-cadherin expression and is regulated by AP-1 and NFAT4 complex during Fas-induced EMT in gastrointestinal cancer. Carcinogenesis 35:173–183.

Zhou S. 2011. TGF-β regulates β-catenin signaling and osteoblast differentiation in human mesenchymal stem cells. J Cell Biochem 112:1651–1660.

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