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Original Article

MiR-203a-5p decreases the expression of Runx2 and inhibits the differentiation of BMSCs to osteoblasts

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Abstract: Objective: To study the expression of miR-203a-5p and Runx2 in differentiation of bone marrow mesenchymal stem cells to osteoblasts. Methods: The qRT-PCR was adopted to determine the expression of miR-203a-5p and Runx2 by inducing human bone marrow mesenchymal stem cells (BMSCs) to osteoblasts in vitro. miR-203a-5p mimic-transfected BMSCs were used to determine the effect of miR-203a-5p expression on differentiation of BMSCs. Western blot was applied to determine the expression of Runx2 protein. Results: Compared with the blank group and the unrelated sequence group, the expression of miR-203a-5p in the overexpression group increased significantly, and the expression of alkaline phosphatase (ALP) decreased significantly ($P < 0.05$). Compared with the overexpression group, the relative expressions of miR-203a-5p at different time points in the blank group and the unrelated sequence group were lower, and the relative expressions of Runx2 mRNA at different time points were higher ($P < 0.05$). The relative expressions of Runx2 mRNA in the blank group and the unrelated sequence group at day 7, 14 and 21 decreased significantly than those at day 1, and the expression increased gradually with time ($P < 0.05$). In the overexpression group, the expressions of Runx2 mRNA and ALP decreased gradually with the increase of miR-203a-5p, but the expressions increased gradually with the decrease of miR-203a-5p in the unrelated sequence group. Conclusions: miR-203a-5p can decrease the expression of Runx2 and inhibit the differentiation of BMSCs to osteoblasts.

Keywords: miR-203a-5p, Runx2, bone marrow mesenchymal stem cells

Introduction

As a health problem in the world, bone defect is mainly caused by trauma or related pathological diseases [1]. Currently, fibula graft, Papineau technique, Ilizarov technique, etc. are mainly used in the treatment of bone defect [2-4]. However, these techniques have the disadvantages of long treatment period, higher clinical technique and experience requirements for doctors, and many complications during treatment. Thus, the efficacy and quality of life of patients will be affected [5]. In recent years, the research of bone marrow mesenchymal stem cells has become a hot field [6]. Bone marrow mesenchymal stem cells (BMSCs) have the characteristics of multi-directional differentiation and proliferation, and lower immunogenicity relative to organ transplantation. Therefore, BMSCs are an ideal tissue engineering material, and are known as "Regenerative medicine seed cells" [7]. In recent years, many clinical studies have demonstrated the effectiveness of osteoarthritis cartilage repair in the treatment of osteoarthritis [8]. However, the mechanism of BMSCs in cartilage repair of osteoarthritis is still unknown.

Runx2 (Runt-related transcription factor 2) is also known as core-binding factor subunit alpha-1 (CBF-α-1). It is an important osteoblast differentiation related protein [9]. Study has shown that [10] the overexpression of Runx2 could induce the transformation of mouse primary skeletal muscle cells and 3T3-L1 pre-adipocytes to osteoblasts. Another study has demonstrated that [11] the overexpression of Runx2 gene in mice caused the osteoporosis, indicat-

Table 1. Primer sequence

Gene	Upstream sequence	Downstream sequence
miR-203a-5p	5'-GTGCAGGGTCCGAGGTATT-3'	5'-GCCGCGTGAAATGTTTAGGACCAC-3'
Runx2	5'-CAACATCCCCTACAAGATCGAG-3'	5'-CACGAAGAACAGAAGCACAAAG-3'
miR-203a-5p overexpression sequence	5'-GUGAAAUGUUUAGGACCACUAG-3'	5'-AGUGGUCCUAAACAUUUCACUU-3'
Blank sequence	5'-UUCUCCGAACGUGUCACGUTT-3'	5'-ACGUGACACGUUCGGAGAATT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
β-actin	5'-GCTGTCCCTGTATGCCTC-3'	5'-GATGTCACGCACGAT-3'

ing that Runx2 plays an very important role in osteoblast formation and bone development. As one of the hot subjects, microRNA has drawn more and more attention from scholars in recent years. As a non-coding short-chain RNA about 22 nt in length, microRNA (mir) inhibits the translation and transcription by binding to the untranslated regions (UTR) of downstream mRNA3'. Thus, the expression of target gene is changed [12]. Studies have shown that miRNAs play the regulatory role in many diseases, such as tumor, cardiovascular disease and bone defect [13-15]. miR-203a-5p is located on 32 and 33 of human chromosome 14. After analyzing miRNA gene chip in the tissue of esophageal cancer patients, the study of Feber et al. [16] found that the expression of miR-203 was 2~10 times higher than that in normal esophageal tissues. The study of Li et al. [17] has shown that the overexpression of miR-203 could inhibit the expression of downstream target gene ZNF217. Thus, apoptosis is accelerated, and cell proliferation and invasion are inhibited. However, the effect of miR-203a-5p on the expression of Runx in BMSCs by regulating other target genes has not been studied.

Therefore, the relevant mechanism was studied by investigating the expression of miR-203a-5p in BMSCs to provide reference for clinical practice.

Materials and methods

Cell source

The BMSCs were obtained from 10 patients with open fracture and ilium transplantation in our hospital. The study was approved by the Medical Ethics Committee of the hospital. The patients and their family members were aware of the purpose of this study, and signed the informed consent form. Tumor, osteoporosis, blood or immune diseases were excluded.

Main reagents and instruments

The main reagents included total RNA extraction kit EasyPure RNA Kit, PCR kit TransScript miRNA First-Strand cDNA Synthesis SuperMix, TransScript II Green Two-Step qRT-PCR SuperMix (Beijing Transgen Biotech Co., Ltd., ER101-01, AT351-01, AQ301-01), anti-RUNX2 antibody, β-Actin, HRP labelled goat anti-mouse IgG secondary antibody (ab76956, ab124964, ab20272), ALP kit (Shanghai Beyotime Biotechnology Co., Ltd., P0321), fetal bovine serum, MEMα medium (low glucose), DMEM medium, Opti-MEM medium, ECL chemiluminescence detection kit, LipofectAMINE 2000 kit, RIPA and BCA protein kit (Shanghai Thermo Fisher Scientific, 10099141, 12561072, 10569044, 11058021, 35050, 11668019, 23225, 15224041). MiR-203a-5p primer and relevant sequences were designed and synthesized by Shanghai Jemma Biological Co., Ltd. The main instruments included PCR instrument (U.S. ABI Company, 7500) (**Table 1**).

Tissue collection and cell culture

10 mL of fresh bone marrow was collected from patient during surgery and placed in the EDTA anticoagulated tube. The bone marrow (10 mL) was added to the serum-free MEMα medium, and mixed well. The resulting substance was centrifuged for 10 min at 1337 rpm, and the fat layer was removed. MEMα medium was used for resuspension. Separation liquid was added by adherence. Afterwards, gradient centrifugation was performed for 30 min, and the mononuclear cell layer was collected. After washing, resuspension (10% fetal serum, MEMα meidum) was conducted again. Cell density was adjusted (2×10^6/mL), and the cells were inoculated into culture flask (37°C, 5% CO_2, constant temperature incubator). After 24 hours, the culture solution was changed, and the non-adherent growth cells were cleaned up, once daily.

After 3~5 days, 0.25% trypsin was used for digestion and collection of passage BMSC cells.

BMSCs osteoblast culture

The third-generation BMSCs in logarithmic phase were collected. After digestion with 0.25% trypsin, they were inoculated to a 24-well plate with 2×10^4 cells in each well. The medium was replaced with osteoblast induction medium (10 mM β-sodium glycero-phosphate, 10 nM dexamethasone, 0.2 mM ascorbic acid, 10% FBS DMEM medium) when the cells grew with adherence to 80%. Induction culture lasted for 21 days.

Transfection and grouping

The third-generation BMSCs were collected. After digestion with 0.25% trypsin, they were inoculated to a 24-well plate with 2×10^4 cells in each well. Transfection was performed when cells grew with adherence to 85%. Three groups were set, including blank group, unrelated sequence group, and overexpression group. No sequence was added to the blank group. Negative control-miRNA was added to the unrelated sequence group (**Table 1**). miR-203a-5p-mimics was added to the overexpression group (**Table 1**). The transfection was performed following the instructions of LipofectAMINE ^{2000} kit. After transfection for 48 h, osteoblast induction medium was used for culture for 21 days.

Alkaline phosphatase activity detection

All cells were determined for alkaline phosphatase (ALP) activity at day 7 of culture. The specific scheme was as follows: The cells in culture medium were collected, and rinsed by PBS reagent. Afterwards, cell lysis solution was added. After full lysis, the supernatant was collected by centrifugation, and buffer solution was added. Then, incubation was performed at 37°C for 30 min. Finally, sodium hydroxide was added to stop the reaction. The OD value was determined by ELIASA at 405 mm. This experiment was repeated for 3 times and the mean was finally analyzed.

WB detection

The total protein of the third-generation BMSCs was extracted by RIPA lysis method. The concentrations of proteins were determined using BCA method. The protein concentration was adjusted to 4 μg/μL, and 12% SDS-PAGE was used for electrophoretic separation. Then, the resulting was transferred to a PVDF membrane. Ponceau working solution was adopted for staining. PBST was applied for soaking for 5 min and washing. 5% skim milk powder was used for sealing for 2 h. Afterwards, primary antibody (1:1000) was added, and the resulting substance was sealed at 4°C overnight. The primary antibody was removed by washing the membrane. Horseradish peroxidase labelled goat anti-mouse secondary antibody (1:5000) was added. After incubation at 37°C for 1 h, PBS was used for rinsing 3 times, 5 min each time. Imaging development was performed in a dark room. Surplus liquid on the membrane was dried with a filter paper. ECL luminescence was used for imaging development. Protein band was scanned. The gray value was analyzed with Quantity One software. The relative expression level of protein was equal to the target protein band gray value/β-Actin protein band gray value.

PCR detection

The total RNA of the third-generation BMSCs was extracted by EasyPure miRNA Kit. The purity, concentration and integrity were determined by UV spectrophotometer and agarose gel electrophoresis. The method for determination of miR was as follows: TransScript® miRNA RT Enzyme Mix and 2×TS miRNA Reaction Mix were used for reverse transcription of total RNA. The operating steps were followed strictly in accordance with the instructions of manufacturer's kit. Afterwards, PCR amplification was performed. PCR reaction system consisted of 1 μL of cDNA, 0.4 μL of upstream primer, 0.4 μL of downstream primer, 10 μL of 2×TransTaq® Tip Green qPCR SuperMix, and 0.4 μL of Passive Reference Dye (50X). Finally, ddH_2O was added to 20 μL. PCR reaction condition was as follows: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing and extending at 60°C for 30 s, 40 cycles in total. 3 repeated wells were set for each sample, and the experiment was repeated 3 times. In this study, U6 was used for the internal reference, and the data were analyzed with $2^{-\Delta ct}$. The detection scheme of Runx2 was as follows: 5X TransScript® II All-in-One SpuperMix for qPCR and gDNA Remover kit were used for inversion

Table 2. Relative expression of miR-203a-5p in three groups of cells

Group	MiR-203a-5p relative expression	F value	*P* value
Blank group	1.054±0.084***	81.011	< 0.001
Unrelated sequence group	1.022±0.071***		
Overexpression group	2.174±0.189		

Note: ***implied the difference compared with the overexpression group (P < 0.001).

transcription. The operating steps were strictly followed in accordance with the instructions of manufacturer's kit. Afterwards, PCR amplification was performed. PCR reaction system consisted of 1 μL of cDNA, 0.4 μL of upstream primer, 0.4 μL of downstream primer, 10 μL of 2X TransScript® Tip Green qPCR SuperMix, and Passive Reference Dye (50X). Finally, Nuclease-free Water was added to 20 μL. The PCR reaction condition was as follows: predenaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing and extending at 60°C for 30 s, 40 cycles in total. 3 repeated wells were set for each sample, and the experiment was repeated 3 times. In this study, β-actin was used for the internal reference (Prime sequence was in **Table 1**), and the data were analyzed with $2^{-\Delta\Delta ct}$.

Target prediction

miR-203a-5p downstream target genes were predicted by http://www.targetscan.org/vert_72/ and miRwalk.

Target gene prediction and verification

MiR-203a was predicted by Targetscan online prediction software. The miR-203a-mimic and blank sequences were constructed. The recombinant Runx2-WT plasmid and the mutant Runx2-MUT plasmid were transfected into BMSCs for 48 h. And the collected cells were detected by double luciferase reporter gene detection kit. Three replicates were designed for each set of experiments, and each experiment was repeated for three times.

Outcome measures

The immediate expression of miR-203a-5p after transfection in three groups of cells was observed. The expression of ALP at day 7 was observed. The relative expressions of miR-203a-5p and Runx2mRNA at day 1, 7, 14 and 21 were observed. The expressions of Runx2 protein at day 1, 7, 14 and 21 were observed. In the unrelated sequence group and the overexpression group, Pearson test was applied to observe the relationship between miR-203a-5p and Runx2mRNA, ALP at day 7 (at day 7, the experiment was repeated 10 times in each group, and the data were collected for correlation analysis).

Statistical analysis

In this study, the collected data were statistically analyzed with SPSS20.0 (Cabit (Shanghai) Information Technology Co., Ltd.), and the figures were plotted with the GraphPad Prism 7 (Softhead (Shenzhen) Technology Co., Ltd.). The enumeration data utilization rate (%) was analyzed with Chi-square test, and expressed with χ^2. The distribution of data was analyzed with K-S test. The measurement data were expressed as mean ± standard deviation. The normally distributed data between two groups were compared with independent sample t test, and expressed with t. The data among groups were compared with analysis of variance, and expressed with F. The LSD-t test was used for posterior pairwise comparison. The intra-group comparison at each time point was performed with one-way ANOVA and LSD-t test was used for post hoc test analysis. Pearson correlation analysis was used to analyze the correlation between mir-203a and Runx2. P < 0.05 implied the statistical difference between two groups.

Results

Expression of miR-203a-5p in BMSCs after transfection

The expression of miR-203a-5p was detected by PCR. The results showed that the expression in overexpression group was significantly higher than that in the blank group and the unrelated sequence group (P < 0.05). However, the expression between the blank group and unrelated sequence group was not significantly different (P>0.05) (**Table 2** and **Figure 1**).

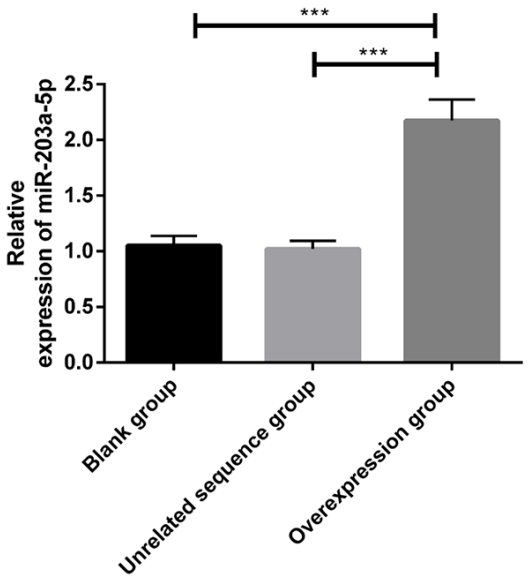

Figure 1. Expression of miR-203a-5p in BMSCs. ***implied the significant difference between two groups (P < 0.001).

ALP activity detection at day 7

After detection, the results showed that the expression of ALP in the overexpression group was significantly lower than that in the blank group and the unrelated sequence group (P < 0.05). However, the expression between the blank group and the unrelated sequence group was not significantly different (**Table 3** and **Figure 2**).

Expressions of miR-203a-5p and Runx2 mRNA at different time points

The expressions of miR-203a-5p and Runx2 mRNA at day 1, 7, 14 and 21 were detected. The results showed that the relative expressions of miR-203a-5p at each time point in the blank group and unrelated sequence group were lower than those in the overexpression group (P < 0.05). In the blank group and the unrelated sequence group, the relative expression at day 7, 14 and 21 decreased significantly than that at day 1, and the expression declined gradually with time (P < 0.05). The expression of Runx2 mRNA was detected. The results showed that the expressions at each time point in the blank group and the unrelated sequence group were higher than those in the overexpression group. In the blank group and unrelated sequence group, the relative expression at day 7, 14 and 21 increased significantly compared with that at day 1, and the expression increased gradually with time (P < 0.05) (**Tables 4** and **5**).

Expression of Runx2 protein

The expression of Runx2 protein at day 1, 7, 14 and 21 in three groups of cells was detected by WB. The results showed that the expression in the blank group and unrelated sequence group was significantly higher than that in the overexpression group (P < 0.05). In the blank group and the overexpression group, the expression was significantly higher than that at day 1, and increased gradually with time (**Table 6**, **Figure 3**).

Target prediction

We predicated the downstream target genes by targetscan and miRwalk online software, and found that the Runx2 had the binding site to miR-203a-5p, and might be the potential target gene. Dual luciferase reporter assay displayed that after transfection with Runx2-WT, luciferase activity in miR-148a-mimic group was significantly lower than that in the unrelated sequence group (P < 0.05), while after transfection with Runx2-MUT, luciferase activity in miR-148a-mimic group and the unrelated sequence group showed no significant difference (P>0.05) (**Figure 4**).

Relationship between miR-203a-5p, and Runx-2mRNA, ALP in the overexpression group and unrelated sequence group

The data of the two groups were collected at day 7 (**Table 7**), and then Pearson correlation analysis was performed. The results showed that the expression of Runx2mRNA and ALP in the overexpression group decreased gradually with the increase of miR-203a-5p. However, the expression in the unrelated sequence group increased gradually with the decrease of miR-203a-5 (**Figure 5**).

Discussion

Mesenchymal stem cells have the ability of self-renewal and multiple differentiation. Relevant studies have shown that mesenchymal stem cells can be differentiated into chondrocytes, osteoblasts, adipocytes, myocytes, and many

Table 3. Expression of ALP activity (total protein of OD/) at day 7 in three groups of cells

Group	ALP expression on the 7th day	F value	*P* value
Blank group	2.154±0.325***	41.696	< 0.001
Unrelated sequence group	2.284±0.344***		
Overexpression group	0.428±0.088		

Note: ***implied the difference compared with the overexpression group (P < 0.001).

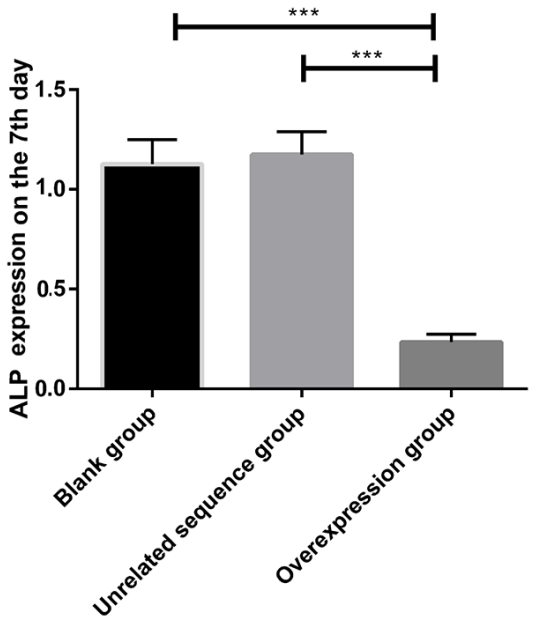

Figure 2. ALP activity detection at day 7. ***implied the significant difference between two groups (P < 0.001).

other connective tissue cells under different induction conditions [18-20]. However, BMSCs have become the ideal seed cells in tissue engineering for its easy amplification in vitro and differentiation into osteoblasts under osteogenic induction conditions [21]. As the important marker of new bone formation, osteoblasts are differentiated into new bones, and osteoclasts absorb necrotic bones. Thus, normal bone metabolism is maintained [22]. However, it is easy to cause excessive bone resorption and deficient bone formation if the balance is broken, resulting in bone defect and osteoporosis [23].

More and more studies have shown that many genes were differentially expressed in the process of osteoporosis and osteopenia [24]. As an important transcription gene, Runx2 plays a regulatory role in bone development and osteoblast formation. Meanwhile, Runx2 can participate in bone metabolism in many ways [25]. However, the specific mechanism is still unclear. As a hot research in recent years, miR plays a key role in the cell differentiation, morphogenesis and tumorigenesis [25]. miR is the tumor-promoting or tumor suppressor gene in the occurrence and development of various malignancies. Thus, miR can be used as an effective molecular biological marker for tumor diagnosis, treatment and prognosis evaluation [26]. miR-203a-5p is one of the important members of miR family. In the study of Wang et al. [27], the gene chip screening showed that miR-203a-5p was significantly under expressed in hepatoma cells. However, the trend of miR-203a-5p expression with differentiation of BMSCs to osteoblasts is not clear.

In this study, the activity of ALP at day 7 was firstly detected. ALP exists extensively in tissues and organs, and half of ALP in human serum is synthesized by osteoblasts [27]. After detection, we found that the activity of ALP in the overexpression group was significantly lower than that in the blank group and unrelated sequence group at day 7. We speculated that the differentiation of BMSCs to osteoblasts was inhibited, and the activity of ALP decreased. Afterwards, we determined the expression of Runx2 mRNA, and the result showed that the expression increased gradually with time. The study of Li et al. [28] has shown that the expression of Runx2 increased gradually with the differentiation of BMSCs to osteoblasts. Hence, the result was similar to that in the study. Moreover, we detected the expression of miR-203a-5p in the blank group. The result showed that the expression decreased gradually with the differentiation of BMSCs to osteoblasts, indicating that miR-203a-5p participates in the differentiation of BMSCs to osteoblasts. Meanwhile, we predicated the downstream target genes of miR-203a-5p by miR predication software, and found that there were binding sites between Runx2 and miR-203a-5p. However, the target regulatory relationship is not clear. For this purpose, miR-203a-5p overexpression vector was transfected to BMSCs, and the effect of miR-203a-5p overexpression on expression of Runx2 was ob-

Table 4. Relative expression of miR-203a-5p at different time points in three groups of cells

Group	1 d	7 d	14 d	21 d	F value	*P* value
Blank group	1.046±0.068#	0.684±0.035*,#	0.568±0.068*,#	0.442±0.029*,#	155.551	0.001
Unrelated sequence group	1.001±0.015#	0.709±0.032*,#	0.629±0.033*,#	0.548±0.037*,#	138.463	0.001
Overexpression group	2.344±0.086	2.300±0.108	2.163±0.109	2.384±0.118	1.429	0.347
F value	427.580	554.520	417.833	665.070		
P value	< 0.001	< 0.001	< 0.001	< 0.001		

Note: *implied the difference compared with day 1 of culture (P < 0.05), and #implied the difference at each time point compared with the overexpression group (P < 0.05).

Table 5. Relative expression of Runx2 mRNA at different time points in three groups of cells

Group	1 d	7 d	14 d	21 d	F value	*P* value
Blank group	1.033±0.054#	1.510±0.119*,#	1.697±0.121*,#	1.763±0.162*,#	166.179	0.001
Unrelated sequence group	1.058±0.076#	1.487±0.104*,#	1.709±0.119*,#	1.798±0.174*,#	89.673	0.007
Overexpression group	0.348±0.107	0.364±0.086	0.354±0.069	0.317±0.056	0.374	0.157
F value	69.084	119.311	162.671	107.755		
P value	< 0.001	< 0.001	< 0.001	< 0.001		

Note: *implied the difference compared with day 1 of culture (P < 0.05), and #implied the difference at each time point compared with the overexpression group (P < 0.05).

Table 6. Comparison of Runx2 protein expression at different time points in three groups of cells

Group	1 d	7 d	14 d	21 d	F value	*P* value
Blank group	0.434±0.084#	0.594±0.075*,#	0.884±0.092*,#	1.125±0.124*,#	329.550	0.002
Unrelated sequence group	0.415±0.075#	0.622±0.087*,#	0.902±0.101*,#	1.174±0.115*,#	172.797	0.004
Overexpression group	0.225±0.052	0.239±0.042*,#	0.244±0.051*,#	0.235±0.038*,#	3.564	0.171
F value	7.814	27.427	62.846	83.686		
P value	0.021	0.001	< 0.001	< 0.001		

Note: *implied the difference compared with day 1 of culture (P < 0.05), and #implied the difference at each time point compared with the overexpression group (P < 0.05).

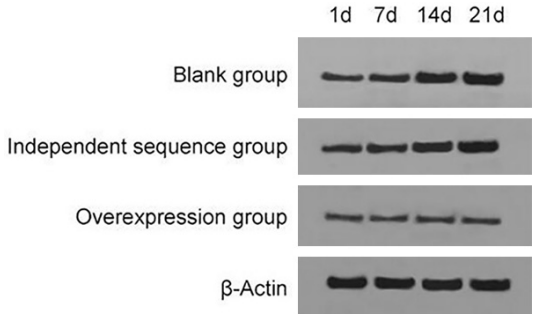

Figure 3. Western Blot.

served. We tested the relative expressions of miR-203a-5p, Runx2 mRNA and protein at different time points. The results showed that the expressions of miR-203a-5p at different time points in the overexpression group was not significantly different. However, the expression in unrelated sequence group decreased gradually with the increase of induction time. After observation, the relative expressions of Runx2 mRNA and protein at different time points in the overexpression group were not different. However, the relative expressions increased gradually with time in the unrelated sequence group. The comparison between groups showed that the relative expression in the unrelated sequence group was significantly higher than that in the overexpression group. However, the relative expression of miR-203a-5p in the unrelated sequence group was significantly lower than that in the overexpression group. It suggests that there may be a regulatory relationship between the overexpression of miR-203a-5p and the expression of Runx2. Therefore, we further analyzed the relationship between miR-203a-5p and Runx2 mRNA, ALP at day 7. The results showed that the expression of Runx2mRNA and ALP in the overexpression

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P_{CT}
Position 934-940 of RUNX2 3' UTR hsa-miR-203a-3p.1	5' ...AUUUGGACCCUUGAAACAUUUCU... 3' GAUCACCAGGAUUUGUAAAGU	7mer-m8	-0.10	87	-0.10	2.864	0.23
Position 2344-2351 of RUNX2 3' UTR hsa-miR-203a-3p.1	5' ...CCAGUGUCUGGUUAC--ACAUUUCA... 3' GAUCACCAGGAUUUGUAAAGU	8mer	-0.03	67	-0.03	3.256	0.23

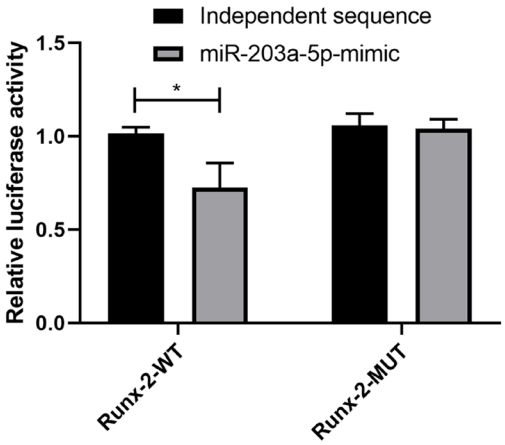

Figure 4. Double luciferase report.

Table 7. Expression of miR-203a-5p, Runx2mRNA, ALP in two groups of cells

Group	miR-203a-5p	Runx2mRNA	ALP
Overexpression group	2.301±0.138	0.329±0.115	0.413±0.077
Unrelated sequence group	0.701±0.030	1.538±0.111	2.252±0.220
t Value	35.853	23.893	24.929
P Value	< 0.001	< 0.001	< 0.001

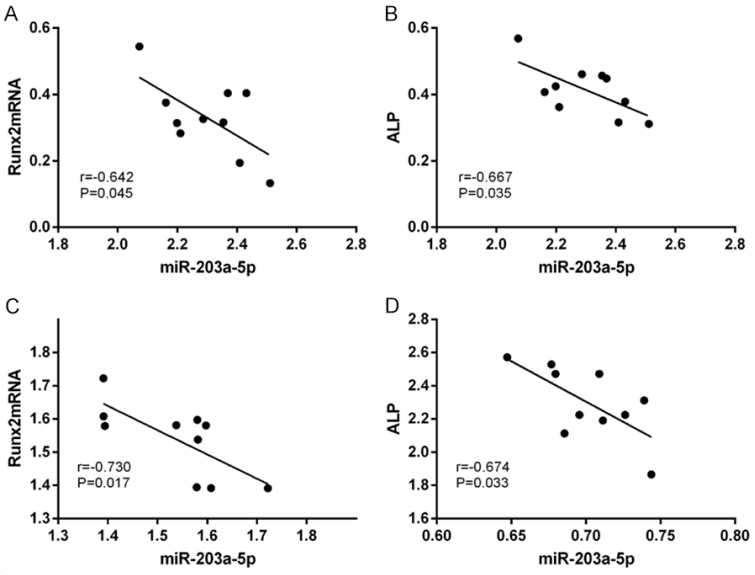

Figure 5. Relationship between miR-203a-5p and Runx2mRNA, ALP.

group decreased gradually with the increase of miR-203a-5p. However, the expression in the unrelated sequence group increased gradually with the decrease of miR-203a-5p. It suggests that the overexpression of miR-203a-5p can inhibit the expression of Runx2, and reduce the activity of ALP. In this study, we confirmed the relationship between miR-203a-5p and Runx2 by dual luciferase reports. The study of Khalid et al. [29] has shown that GATA4 can increase the expression of Runx2, and promote the differentiation of mesenchymal stem cells to osteoblasts. However, the study of Zhu et al. [30] has shown that miR-217 can inhibit the differentiation of rat mesenchymal stem cells to osteoblasts by binding to Runx2. Hence, the result was similar to that in this study. This study showed that the overexpression of miR-203a-5p decreased the expression of Runx2. Thus, the differentiation of BMSCs to osteoblasts was inhibited.

However, there are still some defects in this study. First, the effect of miR-203a-5p on other pathways is not clear. Second, miR-203a-5p inhibition group is not set in this study, and the effect of inhibiting the expression of miR-203a-

5p on osteoblast differentiation is not clear. Therefore, we hope that these study items are added in future study, and the relationship between miR-203a-5p and Runx2, osteoblast related pathways is further studied to make up the defects in this study.

In summary, miR-203a-5p can decrease the expression of Runx2, and inhibit the differentiation of BMSCs to osteoblasts.

Disclosure of conflict of interest

None.

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