European Review for Medical and Pharmacological Sciences 2018; 22: 2519-2526

MiR-133a inhibits fracture healing via targeting RUNX2/BMP2

H. PENG, S.-L. LU, Y. BAI, X. FANG, H. HUANG, X.-Q. ZHUANG

Department of Orthopedic, Affiliated Minzu Hospital of Guangxi Medical University, Nanning, China

Abstract. – OBJECTIVE: To analyze the mechanism of miR-133a in inhibiting fracture healing through regulating runt-related transcription factor 2 (RUNX2) signaling pathway.

PATIENTS AND METHODS: A total of 80 patients with fracture admitted to our hospital from January 2016 to January 2017 were divided into 2 groups according to fracture nonunion or fracture healing: fracture nonunion group (n = 40) and control group (n= 40). After admission, patients underwent the surgery, respectively, and the bone tissues were taken for stand-by application. The expression level of bone morphogenetic protein 2 (BMP2) was detected using immunohistochemical method, the expression level of RUNX2 protein was detected by Western blotting, and the expression level of micro ribonucleic acid (miR)-133a was detected by quantitative polymerase chain reaction (qPCR). Moreover, the bioinformatics method was used to predict the target gene of miR-133a, and the luciferase reporter gene was used to detect the binding of miR-133a to RUNX2.

RESULTS: Compared with those in control group, the expression of BMP2 and the relative expressions of RUNX2 protein and miR-133a were significantly decreased; the differences were statistically significant ($p < 0.05$). Pearson correlation analysis showed that miR-133a was negatively correlated with RUNX2. After overexpression of miR-133a, the expression level of RUNX2 was decreased, but it was increased significantly after interference in miR-133a. Besides, it was found in dual-luciferase reporter assay that miR-133a bound to RUNX2.

CONCLUSIONS: MiR-133a inhibits the bone formation through inhibiting the RUNX2/BMP2 signaling pathway, thereby negatively regulating the fracture healing.

Key Words:
miR-133a, RUNX2, BMP2, Fracture healing.

Introduction

Fracture nonunion is a common disease after fractures, especially the fractures caused by severe open wounds and those accompanied by severe vascular injury and soft tissue injury. The incidence rate of fracture nonunion after fractures is as high as 46%[1]. Various treatments are applied for fracture nonunion in clinical, but the effects of those treatment are not satisfied. The pathological reaction mechanism of fracture nonunion is very complex, and it is currently believed that fracture nonunion is mainly related to local ischemia, fracture factors, infection, improper treatment, susceptibility of patients, nutritional status, smoking and medication. Micro ribonucleic acid (miRNA) is a kind of non-coding RNA, consisting of approximately 18-25 nucleotides, and it can bind to the 3'-untranslated region of target RNA to inhibit target RNA translation[2-5]. MiR-133a, a member in the miRNA family, plays a regulatory role in bone formation and an important role in bone morphogenesis and sclerostin formation after fractures[6,7]. Animal experiments have shown that miR-133a can act on runt-related transcription factor 2 (RUNX2) that plays an important role in osteoblast differentiation and sclerostin formation, thus inhibiting the osteoblast differentiation in mice. However, the mechanism of action of miR-133a in fracture nonunion has not been confirmed clinically. Therefore, the primary purpose of this study was to investigate the mechanism of miR-133a regulating RUNX2 signaling pathway in fracture healing, which has an important guiding significance for effective clinical treatment of fracture nonunion .

Patients and Methods

General Data

A total of 40 patients with fracture nonunion admitted into our hospital from January 2016 to January 2017 were enrolled as fracture nonunion group, while 40 patients with fracture healing receiving internal fixator removal were selected as control group. This investigation was approved

Corresponding Author: Xiaoqiang Zhuang, BM; e-mail: 593198192@qq.com

by the Ethics Committee of Affiliated Minzu Hospital of Guangxi Medical University. Signed written informed consents were obtained from all participants before the study.

Reagents and Equipment

Primary antibodies: anti-bone morphogenetic protein 2 (BMP2) antibody (Abcam, Cambridge, MA, USA) and anti-RUNX2 antibody (Abcam, Cambridge, MA, USA). Immunohistochemical kits (Maxim, Fuzhou, China), AceQ quantitative polymerase chain reaction (qPCR) SYBR Green Master Mix kits (Vazyme, Nanjing, China), HiScript II Q RT perMix for qPCR (+gDNA wiper) kits (Vazyme, Nanjing, China), light microscope (Leica DMI 4000B/DFC425C, München, Germany), fluorescence quantitative PCR instrument (ABI 7500, Waltham, MA, USA), Image-lab image analysis system and Image-Pro image analysis system (Bio-Rad, Hercules, CA, USA).

Diagnostic Criteria

The diagnostic criteria of fracture nonunion are shown in Table I, and the criteria of fracture healing are shown in Table II.

Inclusion Criteria

The inclusion criteria in this study are as follows: (1) patients who met the above diagnostic criteria of fracture nonunion and met the above criteria of fracture healing, required internal fixator removal, and could tolerate the surgery; (2) patients aged 18-65 years old; (3) patients who agreed to participate in this research and signed the informed consent; (4) patients who did not take other drugs and receive other related treatments within 3 weeks; (5) patients who strictly cooperated with the treatment.

Exclusion Criteria

The exclusion criteria in this study are as follows: (1) patients who did not meet the above inclusion criteria; (2) pregnant or lactating female patients; (3) patients complicated with major internal medicine diseases, such as hypertension, diabetes mellitus or heart disease; (4) patients with a history of serious diseases, such as severe primary disease or mental illness; (5) patients with a past history of tumor.

Treatment Method in Each Group

After patients meeting the inclusion criteria, they were admitted into the hospital and immediately received routine preoperative examination. They underwent the surgery immediately after meeting the surgical conditions. In control group, the internal fixator was removed, and the excess bone blocks attached around the internal fixator were taken, marked and stored at -20°C for stand-by application. In fracture nonunion group, the surgery was performed to expose the fractured end, remove the sequestrum and break through the myelin sheath; the iliac bone was fixed by an appropriate internal fixation method after bone grafting, and the sequestrum removed was marked and stored at -20°C for stand-by application. Half of the bone blocks removed were decalcified in ethylenediaminetetraacetic acid (EDTA) decalcifying solution for 2 months, and then embedded into paraffin to be prepared into paraffin sections for immunohistochemical assay. The other half of the bone blocks were stored at -20°C for Western blotting and qPCR.

Immunohistochemistry

5 μm-thick paraffin tissue sections were conventionally dewaxed, placed into water, added with citric acid buffer solution and heated in a microwave oven for antigen retrieval. After sections were rinsed with phosphate-buffered saline (PBS), the endogenous peroxidase blocking agent was added for incubation for 10 min. After sections were rinsed with PBS, they were sealed using goat serum for 20 min; next, the serum blocking solution was thrown off and anti-BMP2 primary antibody (1:200) was added at 4°C over-

Table I. Diagnostic criteria of fracture nonunion .

No.	Symptom description
1	Pseudoarticulation formation, and abnormal activity and pain of fractured end
2	X-ray shows separation, obvious gap, osteosclerosis and closure of medullary cavity in fractured end
3	At 9 months after fracture, it still does not meet the requirement of bone healing, and there is no obvious porosis at 3 months after treatment
4	Hypertrophy, a large number of calluses or atrophy, thinning and no callus in fractured end

Table II. Criteria of bone healing.

No.	Symptom description
1	No local tenderness, and no axial percussion pain
2	No abnormal local activity
3	X-ray shows blurred fracture line and continuous callus passing through the fracture line
4	In the absence of external fixation, the upper limbs can lift 1 kg weight for 1 min, and the lower limbs can walk for 3 consecutive minutes, not less than 30 steps
5	X-ray shows bone trabecula passing through the fracture line

night. Sections were rinsed again with PBS, added with secondary antibody for incubation for 10 min, rinsed with PBS, and added with streptavidin-peroxidase solution for incubation for 10 min, followed by color development via diaminobenzidine (DAB), hematoxylin re-staining, sealing using neutral gum, observation and photography under the microscope.

Western Blotting

Bone tissues stored at -20°C were added into lysis solution for ice bath for 60 min and centrifuged at 14,000 g for 10 min. The protein was quantified using bicinchoninic acid (BCA) method. The standard curve and optical density were obtained using a microplate reader and the protein concentration was calculated. After protein denaturation, the sample was separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in corresponding concentration. When the marker protein ran to the bottom of the glass plate and the sample protein sunk to the bottom almost in a straight line, the gel running was terminated. After that, the protein was transferred onto a polyvinylidene fluoride (PVDF) membrane, sealed and washed with Tris-buffered saline and Tween-20 (TBST-20) for 3 times. The protein was sealed using the blocking solution for 1.5 h, added with anti-RUNX2 primary antibody (1:1,000) and secondary antibody (1:1,000). The protein was rinsed with TBST between every two steps. After the protein was washed with TBST to remove the secondary antibody, the color began to be developed. The membrane was placed into the chemiluminescence reagent for reaction for 1 min, followed by development in a dark place, and analysis using the gel scanning imaging system.

qPCR Test

The total RNA was extracted from the bone block stored at -20°C using the RNA extraction kit. The total RNA was reversely transcribed into complementary DNA (cDNA) using the reverse transcription kit, and the reaction system was 20 μL. The reaction conditions are as follows: reaction at 51°C for 2 min, pre-denaturation at 96°C for 10 min, denaturation at 96°C for 10 s, annealing at 60°C for 30 s, for a total of 40 cycles. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal reference. The relative expression level of miR-133a was calculated. Primer sequences are shown in Table III.

Observation Indexes

The expression of BMP2 in bone tissues was detected using immunohistochemical method, the RUNX2 protein expression in bone tissues was detected *via* Western blotting, and the expression of miR-133a in bone tissues was detected *via* qPCR.

Cell Culture

MC3T3-E1 osteoblasts were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1000 U/mL penicillin/streptomycin in a thermostatic incubator with 5% CO_2 at 37°C, and the culture solution was replaced every 2-3 d. After more

Table III. Primer sequences.

Name	Primer sequences
miR-133a	Forward primer: 5'-TTTGGTCCCCTTCAAC-3' Reverse primer: 5'-TAGCTATCCTTTGCT-3'
GADPH	Forward primer: 5'-ACGGCAAGTTCAACGGCACAG-3' Reverse primer: 5'-GAAGACGCCAGTAGACTCCACGAC-3'

than 80% cells were fused, they were digested using trypsin, and inoculated into the plate for culture or passage. Cells were inoculated into a 6-well plate (2×10^5/well) and cultured for 24 h, and after the cell aggregation rate reached more than 70%, the transfection was performed in accordance with the instructions.

Dual-Luciferase Reporter Gene

Cells were cultured in a 24-well plate, and transfected with TOP flash-Luciferase plasmid (200 ng/3 wells), Renilla plasmid (50 ng/3 wells) and target gene to be detected (20-600 ng/3 wells based on the expression of plasmid). At 6 h after transfection, the medium was replaced, and Wnt3a or LiCl was added for stimulation. After 24-48 h, cells were collected, the medium was discarded, and cells were washed once with PBS. Cells were lysed using 120 μL passive lysis buffer (PLB) per well. After cell lysis, the 24-well plate was centrifuged at 2,000 rpm for 3 min to centrifuge the cell debris on the wall of culture dish. 20 μL cell lysate supernatant were taken to detect the Luciferase and Renilla fluorescence values, respectively.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Enumeration data were presented as mean ± standard deviation. *t*-test was used for data meeting the normal distribution and homogeneity of variance, corrected *t*-test was used for data meeting the normal distribution but with heterogeneity of variance, and non-parametric test was used for data without meeting the normal distribution and homogeneity of variance. Rank-sum test was used for ranked data, and x^2-test was used for enumeration data.

Results

Comparisons of General Conditions

A total of 40 patients with fracture nonunion meeting the diagnosis criteria of fracture nonunion were enrolled as fracture nonunion group, and 40 patients meeting the criteria of bone healing and receiving internal fixator removal were enrolled as control group. Gender and age of each group are shown in Table IV. Comparisons showed that there were no differences in gender and age between the two groups ($p > 0.05$), and they were comparable.

Detection of Relative Expression Level of miR-133a in Bone Tissues in Each Group Via qPCR

The relative expression level of miR-133a in control group was lower, while that was higher in fracture nonunion group. The relative expression level of miR-133a in fracture nonunion group was significantly higher than that in control group, and the difference was statistically significant ($p < 0.01$).

RUNX2 Expression in Fracture nonunion Group Was Significantly Decreased

qPCR showed that the relative expression level of RUNX2 mRNA was higher in control group, but lower in fracture nonunion group (Figure 1B). Similarly, Western blotting revealed that the relative expression level of RUNX2 protein in control group was higher, but lower in fracture nonunion group. It could be seen that the relative expression level of RUNX2 protein in fracture nonunion group was significantly lower than that in control group, and the difference was statistically significant (Figure 1C) ($p < 0.05$).

Immunohistochemical Detection of Relative Expression Level of BMP2 in Bone Tissues in Each Group

Immunohistochemical results showed that the positive-expression BMP2 was tan and mainly distributed in the cytoplasm. The positive-expression BMP2 in control group showed a darker color, and the number of it was large. In fracture nonunion group, the positive-expression BMP2 showed a lighter color, and the number of it was small. Statistical analysis of the positive-expression BMP2 in each group (Figure 1B) showed that the relative expression level of BMP2 in control group was higher, but that was lower in

Table IV. General conditions in each group.

Group	Male	Female	Average age
Control group	22	18	(45.7 ± 10.67) years old
Fracture nonunion group	24	16	(39.1 ± 12.27) years old

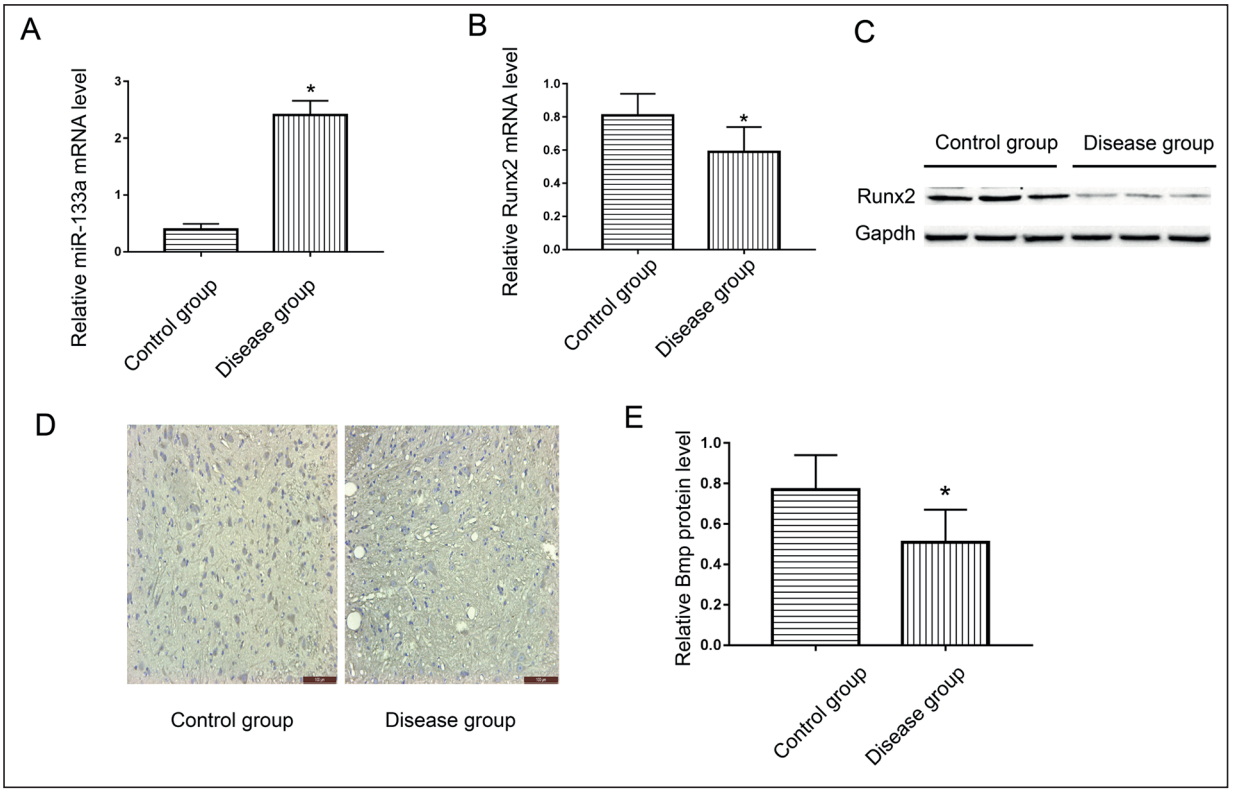

Figure 1. The miR-133a expression is significantly increased in patients with fracture nonunion . ***A,*** The miR-133a expression in patients with fracture nonunion is significantly increased. ***B-C,*** RUNX2 mRNA and protein expressions are significantly reduced in patients with fracture nonunion . ***D,*** The BMP expression is decreased significantly in patients with fracture nonunion (×40). ***E,*** The BMP protein expression is decreased significantly in patients with fracture nonunion (*$p < 0.05$).

fracture nonunion group; the difference was statistically significant between the two groups (Figure 1D-E) ($p < 0.05$).

MiR-133a Inhibited Osteoblast Proliferation Via Decreasing RUNX2 Expression

The target genes of miR-133a were predicted via the websites (DIANA, miRanda and PicTar), and RUNX2 was found, followed by further study on binding site (Figure 2C). Correlation analysis showed that miR-133a was negatively correlated with RUNX2 (Figure 2A). Results of qPCR showed that the expression of RUNX2 was significantly decreased after overexpression of miR-133a, but the expression of RUNX2 was significantly increased after interference in miR-133a (Figure 2B). MiR-133a could reduce the relative luciferase activity of RUNX2 (Figure 2C), suggesting that RUNX2 was a target gene of miR-133a. Results of Western blotting also showed that the expression of RUNX2 was significantly decreased after overexpression of miR-133a, but that was significantly increased after interference in miR-133a (Figure 2E).

Discussion

Fracture nonunion includes atrophic fracture nonunion and hypertrophic fracture nonunion . Atrophic fracture nonunion is mainly caused by the poor blood supply in fractured end, resulting in lack of nutrition and difficult fracture healing. Hypertrophic fracture nonunion is mainly due to the unstable fractured end, which is not conducive to fracture healing. Fracture healing is a series of complex biological repair process, involving a variety of molecular substances and molecular repair mechanisms. BMP2, as an important member in transforming growth factor-β (TGF-β) superfamily, plays an important role in cartilage formation and periosteal osteogenesis[8]. In addition, studies[9] have further confirmed that BMP2 can promote fracture healing though inducing the differentiation of bone marrow mesenchymal stem cells into osteoblasts via the Smad-dependent BMP signaling pathway under the regulation of RUNX2[10,11]. Moreover, researches[12] have shown that BMP2 can also promote the osteoclast activity and the remod-

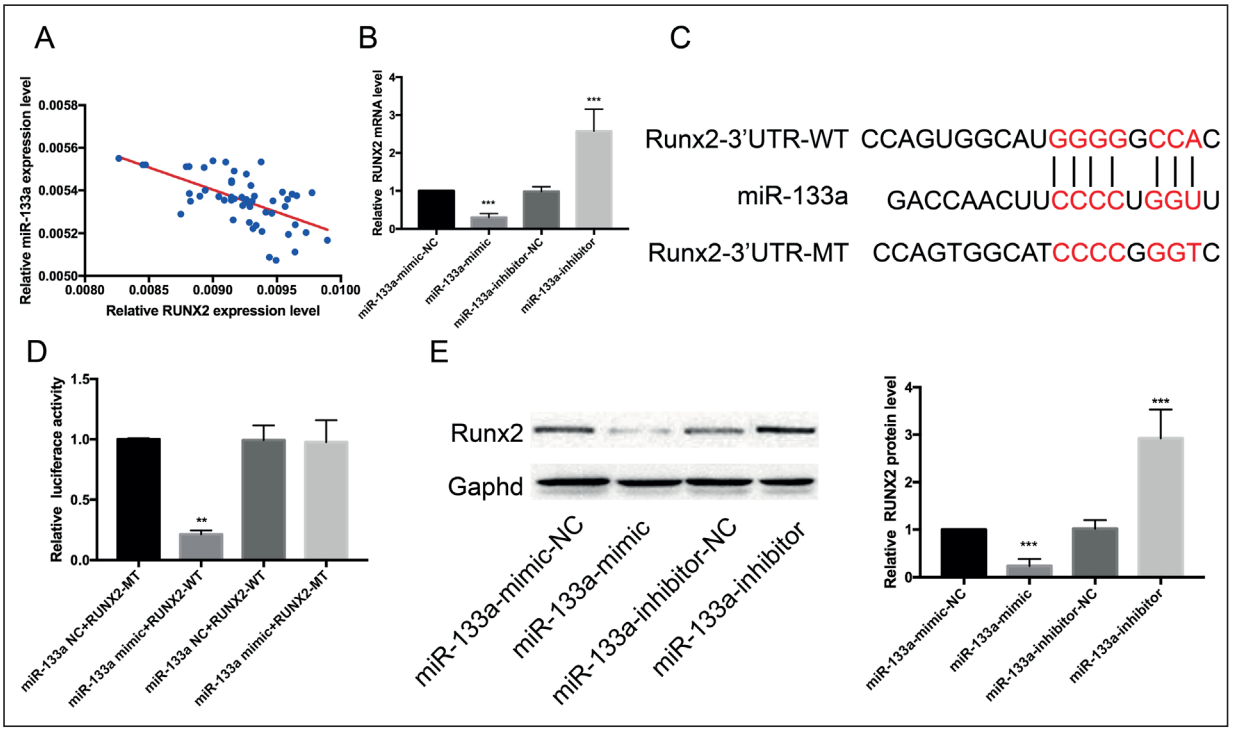

Figure 2. MiR-133a inhibits osteoblast proliferation by degrading RUNX2. ***A,*** The expression levels of miR-133a and RUNX2 are inversely proportional. ***B,*** After interference in miR-133a, the RUNX2 expression is significantly increased, but it is significantly reduced after overexpression of miR-133a. ***C,*** Binding sequences of miR-133a and RUNX2. ***D***, MiR-133a can reduce the RUNX2 luciferase activity. ***E,*** After interference in miR-133a, the RUNX2 protein expression is significantly increased, but it is significantly reduced after overexpression of miR-133a.

eling and shaping of calluses in the middle and late stages of fracture healing, so that fractures can be healed and restored to original biological state. Therefore, BMP2 is a key molecule in fracture healing and plays an important role in the formation of primary callus and the remodeling and shaping of callus. miRNA can degrade the target gene or inhibit the translation of target gene via binding to its target gene, and regulate the transcription process[13-15]. Among them, miR-133a plays an important role in bone morphogenesis and fracture healing[16], and RUNX2 and its several downstream molecules involved in osteoblast differentiation and bone formation are the major targets of miR-133a. RUNX2, as a core-binding factor, is a specific transcription factor that regulates the differentiation of mesenchymal stem cells into osteoblasts[17,18]. Approximately 80% people with clavicle or skull defects are found to suffer from RUNX2 gene deletion to varying degrees[19]. Animal experiments have also demonstrated[20] that RUNX2-knockout mice have poor osteoblastic development, skeletal dysplasia or partial bone loss. The reason is that RUNX2 can up-regulate the expressions of a variety of osteogenesis-related genes, such as osteocalcin and type I collagen, and promote and induce the differentiation of bone marrow mesenchymal stem cells into osteoblasts, thus promoting the osteogenic capability[17,21]. Therefore, RUNX2 is a key factor that promotes osteogenesis and ostosis. MiR-133a can bind to RUNX2 to regulate the RUNX2 expression, inhibit the secretion and expression of bone formation-promoting substances, and inhibit the expressions of BMP2 and other molecules related to osteoblast differentiation, thereby inhibiting bone formation and delaying fracture healing[22].

Results of this study showed that the expression level of miR-133a was significantly increased in bone tissues of patients with fracture nonunion , suggesting that the miR-133a expression level is closely related to fracture healing. At the same time, the RUNX2 protein expression level in bone tissues of patients with fracture nonunion was significantly reduced, suggesting that the translation process of RUNX2 gene is inhibited.

RUNX2, as a transcription factor, binds to miR-133a to be degraded under the high expression of miR-133a, so its transcription process is blocked. Moreover, dual-luciferase reporter gene assay revealed that miR-133a could degrade the RUNX2 expression, which was consistent with the finding in previous research. BMP2 is a downstream molecule of RUNX2, and its expression level is decreased in the case that RUNX2 is inhibited. Therefore, we observed that the BMP2 expression level was decreased significantly in patients with fracture nonunion , so its effects of inducing osteoblast differentiation and sclerostin formation were weakened, which was not conducive to fracture healing.

Conclusions

We showed that miR-133a is involved in the pathological reaction of fracture nonunion , and negatively regulates the fracture healing via RUNX2/BMP2 signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Tzioupis C, Giannoudis PV. Prevalence of long-bone non-unions. Injury 2007; 38: S3-S9.
2) Lee RC, Ambros V. An extensive class of small RNAs in caenorhabditis elegans. Science 2001; 294: 862-864.
3) Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001; 294: 853-858.
4) Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.
5) Xu M, Zhang YY, Wang HF, Yang GS. The expression and function of miRNA-106 in pediatric osteosarcoma. Eur Rev Med Pharmacol Sci 2017; 21: 715-722.
6) Liao XB, Zhang ZY, Yuan K, Liu Y, Feng X, Cui RR, Hu YR, Yuan ZS, Gu L, Li SJ, Mao DA, Lu Q, Zhou XM, de Jesus PV, Yuan LQ. MiR-133a modulates osteogenic differentiation of vascular smooth muscle cells. Endocrinology 2013; 154: 3344-3352.
7) Zhou PP, Li Y, Ma ZD, Li ZY, Chen FY, Jiang YX. Single nucleotide polymorphisms in the promoter region of mir-133a-1 and in pre-mir-152 rs1707 may contribute to the risk of asthma in a Chinese Han population. Eur Rev Med Pharmacol Sci 2016; 20: 2642-2649.
8) Cho TJ, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J Bone Miner Res 2002; 17: 513-520.
9) Liu DD, Zhang JC, Zhang Q, Wang SX, Yang MS. TGF-beta/BMP signaling pathway is involved in cerium-promoted osteogenic differentiation of mesenchymal stem cells. J Cell Biochem 2013; 114: 1105-1114.
10) Sawyer AA, Song SJ, Susanto E, Chuan P, Lam CX, Woodruff MA, Hutmacher DW, Cool SM. The stimulation of healing within a rat calvarial defect by mPCL-TCP/collagen scaffolds loaded with rhBMP-2. Biomaterials 2009; 30: 2479-2488.
11) Govender S, Csimma C, Genant HK, Valentin-Opran A, Amit Y, Arbel R, Aro H, Atar D, Bishay M, Borner MG, Chiron P, Choong P, Cinats J, Courtenay B, Feibel R, Geulette B, Gravel C, Haas N, Raschke M, Hammacher E, van der Velde D, Hardy P, Holt M, Josten C, Ketterl RL, Lindeque B, Lob G, Mathevon H, McCoy G, Marsh D, Miller R, Munting E, Oevre S, Nordsletten L, Patel A, Pohl A, Rennie W, Reynders P, Rommens PM, Rondia J, Rossouw WC, Daneel PJ, Ruff S, Ruter A, Santavirta S, Schildhauer TA, Gekle C, Schnettler R, Segal D, Seiler H, Snowdowne RB, Stapert J, Taglang G, Verdonk R, Vogels L, Weckbach A, Wentzensen A, Wisniewski T; BMP-2 Evaluation in Surgery for Tibial Trauma (BESTT) Study Group. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am 2002; 84-A: 2123-2134.
12) Kaneko H, Arakawa T, Mano H, Kaneda T, Ogasawara A, Nakagawa M, Toyama Y, Yabe Y, Kumegawa M, Hakeda Y. Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. Bone 2000; 27: 479-486.
13) Cai B, Pan Z, Lu Y. The roles of microRNAs in heart diseases: a novel important regulator. Curr Med Chem 2010; 17: 407-411.
14) Chiyomaru T, Enokida H, Tatarano S, Kawahara K, Uchida Y, Nishiyama K, Fujimura L, Kikkawa N, Seki N, Nakagawa M. MiR-145 and miR-133a function as tumour suppressors and directly regulate FSCN1 expression in bladder cancer. Br J Cancer 2010; 102: 883-891.
15) Small EM, Frost RJ, Olson EN. MicroRNAs add a new dimension to cardiovascular disease. Circulation 2010; 121: 1022-1032.
16) Koutsoulidou A, Mastroyiannopoulos NP, Furling D, Uney JB, Phylactou LA. Expression of miR-1, miR-133a, miR-133b and miR-206 increases during development of human skeletal muscle. BMC Dev Biol 2011; 11: 34.
17) Saito T, Ogawa M, Hata Y, Bessho K. Acceleration effect of human recombinant bone morphogenetic protein-2 on differentiation of human pulp cells into odontoblasts. J Endod 2004; 30: 205-208.

18) Okazaki K, Sandell LJ. Extracellular matrix gene regulation. Clin Orthop Relat Res 2004: S123-S128.

19) Tou L, Quibria N, Alexander JM. Transcriptional regulation of the human RUNX2/Cbfa1 gene promoter by bone morphogenetic protein-7. Mol Cell Endocrinol 2003; 205: 121-129.

20) Komori T. A fundamental transcription factor for bone and cartilage. Biochem Biophys Res Commun 2000; 276: 813-816.

21) Zhang X, Aubin JE, Inman RD. Molecular and cellular biology of new bone formation: Insights into the ankylosis of ankylosing spondylitis. Curr Opin Rheumatol 2003; 15: 387-393.

22) Li Z, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM, Lian JB, Stein GS. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A 2008; 105: 13906-13911.