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Original Article

miR-133b in predicting prognosis for patients with osteosarcoma, and its effect on apoptosis of osteosarcoma cells

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Abstract: Objective: To explore the expression of miR-133b in patients with osteosarcoma and its effect on apoptosis of osteosarcoma cells. Methods: The cancer tissues and corresponding adjacent tissues of 56 patients with osteosarcoma were collected in our hospital. The expression level of miR-133b in tissues was detected by qRT-PCR, and the relationship between miR-133b and prognosis was analyzed by Kaplan-Meier survival curve. The miR-133b overexpression vector (miR-133b-mimic) and the miR blank vector (miR-control) were designed and synthesized, and transfected into human osteosarcoma cell line MG-63. MTT and flow cytometry were used to detect the proliferation and apoptosis of MG-63 cells. The levels of p-p38, p-ERK and p-JNK were detected by Western blot. The correlation between miR-133b and p-p38, p-ERK, p-JNK was analyzed by Pearson analysis. Results: The expression level of miR-133b in cancer tissues was significantly lower than that in adjacent normal tissues ($P < 0.05$). The 5-year survival rate was 78.57% and 53.57% in the miR-133b high and low expression groups, respectively ($P = 0.038$). The expression level of miR-133b and cell apoptosis rate in miR-133b-mimic group were higher than those of miR-control group and control group ($P < 0.001$). Compared with miR-control group and control group, the cell proliferation, levels of p-p38, p-ERK and p-JNK in miR-133b-mimic group were lower ($P < 0.05$). Pearson analysis showed that miR-133b was negatively correlated with p-p38 ($r = -0.828$, $P < 0.001$), p-ERK ($r = -0.786$, $P < 0.001$) and p-JNK ($r = -0.928$, $P < 0.001$) expression. Conclusion: miR-133b expression is downregulated in osteosarcoma tissues. The 5-year survival rate of patients with low expression of miR-133b was decreased, and up-regulation of miR-133b could inhibit MAPKs signaling pathway and promote apoptosis of osteosarcoma cells.

Keywords: miR-133b, osteosarcoma, prognosis analysis, apoptosis

Introduction

Osteosarcoma is a rare malignant bone tumor that is prone to metastasis, especially lung tissue metastasis, which has a high degree of malignancy and a low prognosis [1, 2]. 2-3 of every one million people have osteosarcoma every year and most of them are minors and males [3]. Since the 1970s, the treatment of osteosarcoma has progressed more and more, and the prognosis of patients has been significantly improved. Cisplatin, doxorubicin, ifosfamide, and methotrexate were used to treat osteosarcoma, and the 5-year survival rate was increased by about 50-80%, but some patients still died of treatment failure [4, 5]. More researchers are needed to explore the ideal therapeutic target, as well as prognostic markers.

In recent years, many scholars have reported single-stranded non-coding microRNAs of about 19-24 nucleotides in length. MicroRNAs are 19-24 nucleotides endogenous non-coding RNAs that interact with the 3' untranslated region (UTR) of the mRNA target region and regulate the biological behavior of cells by regulating the transcription and translation of downstream RNAs. Similarly, abnormal changes in miRNA biosynthesis are involved in a variety of pathophysiological processes [6, 7]. Many studies have reported that miRNAs are closely relat-

ed to the development of tumors and the proliferation and invasion of tumor cells [8, 9]. Only a small fraction of the miRNAs currently associated with osteosarcoma are reported. In recent years, studies have reported that miR-133b is involved in the growth and invasion of osteosarcoma cells. Ying et al. [10] reported that miR-133b regulated the growth of osteosarcoma cells through the miR-133b/Sirt1 axis, and the overexpression of miR-133b inhibited the expression of Sirt1 and weakened cell proliferation and invasion. Deng et al. [11] also found that down-regulation of miR-133b expression can promote the expression of FOXC 1, thus promoting the proliferation and migration of osteosarcoma cells. Additional studies [12] reported that miR-133b overexpression inhibited proliferation and invasion of ovarian cancer cells by targeting MAPKs signaling pathway. This mode of action of miR-133b has not been reported in osteosarcoma.

This study analyzed the effect of miR-133b on osteosarcoma cell proliferation and apoptosis, as well as its effect on MAPKs signaling pathway, and explored the mechanism of action of miR-133b in osteosarcoma. In addition, this study analyzed the relationship between miR-133b and prognosis of patients with osteosarcoma.

Materials and methods

Research subjects

The cancer tissues and their corresponding adjacent tissues of 56 patients with osteosarcoma from July 5, 2010 to April 9, 2012 were collected in our hospital, including 35 males and 21 females, aged 15-30 years. Inclusion criteria: All patients were diagnosed with osteosarcoma by the pathology department of our hospital. Exclusion criteria: Pregnant and lactating women were excluded; patients who received any anti-tumor treatment before surgery were excluded; patients with excessive masses were excluded; patients with previous history of other tumors, or family genetic disease or autoimmune diseases history were excluded; patients with dysfunction of the heart, liver, kidneys, etc. were excluded; patients with abnormal bleeding or coagulopathy were excluded; patients with alcohol and nitroglycerin history were excluded. The study was approved by the hospital ethics committee, and the patients or their family signed an informed consent form.

The human osteosarcoma cell line MG-63 was purchased from ATCC, USA (No.: BNCC 338584). The medium composition contained DMEM (purchased from Xiamen Research Biotechnology Co., Ltd., Lot No. 10567014) + 10% fetal bovine serum (purchased from Beijing Zhijie Fangyuan Technology Co., Ltd., item number 10099141), L-glutamine (2 mM) (Invitrogen, Carl), 100 U/ml penicillin (Invitrogen, Carl) and 100 μg/ml streptomycin (Invitrogen, Carl). Cells were cultured in a 5% CO_2 humidified incubator at 37°C.

Cell passage

After the recovery of MG-63 cells, 0.25% trypsin was used to digest the cells when the cell adherent growth density reached 90%. When the cells became round, the intercellular space increased, and some of the cells were detached from the bottle wall under microscope observation. The cells were cultured in a 37°C, 5% CO_2 incubator.

Construction and transfection of miR-133b expression vector

The miR-133b overexpression vector (miR-133b-mimic), the p38 overexpression vector (sh-p38) and the miR blank vector (miR-control) were designed and synthesized by Thermo Fisher Scientific. The miR-control group, the miR-133b-mimic group, and the miR-133b-mimic + sh-p38 co-transfection group (referred to as the co-transfection group) were set. The MG-63 cells were digested with trypsin 24 h before transfection, and the expression vector was transfected when the cells reached 80% confluence, according to the kit instructions for specific procedures. The cells were cultured in a 37°C 5% CO_2 incubator for 48 hours, and the medium was changed every 6 hours. Transfection results, cell migration and apoptosis levels were detected by qRT-PCR. Lipofectamine TM 2000 transfection kit was purchased from Chengdu Dongsheng Kechuang Technology Co., Ltd., and the number was 11668019. Untreated MG-63 cells served as a control group.

qRT-PCR

After the cancer tissue or adjacent tissues were ground and pulverized, 1 ml of TRIzol lysate was added to extract total RNA from the tissue. The concentration of MG-63 cell suspension was adjusted to 1×10^7/ml, and the suspension

Table 1. Primer sequence

	Upstream primer	Downstream primer
miR-133b	5'-GAACCAAGCCGCCCGAGA-3'	5'-CCGCCCTGCTGTGCTGGT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

Table 2. General information

	Patients (n = 56)
Gender [n (%)]	
male	35 (62.50)
female	21 (37.50)
Age (years)	20.4±7.1
Tumor diameter [n (%)]	
≥ 8 cm	25 (44.64)
< 8 cm	31 (55.36)
Tumor type [n (%)]	
Osteoblast type	24 (42.86)
chondroblastic type	16 (26.79)
Fibroblast type	14 (25.00)
mixed type	2 (3.57)
Tumor site [n (%)]	
Femur	39 (69.64)
tibiofibula	9 (16.07)
other	8 (14.29)
Clinical stage [n (%)]	
I-IIA	23 (41.07)
IIB-III	33 (58.93)
Metastasis [n (%)]	
Yes	22 (39.29)
No	34 (60.71)

was added to the TRIzol lysate for total RNA extraction at a ratio of 3:1. RNA integrity was analyzed by 1.5% agarose gel electrophoresis after extraction. The purity of the extracted RNA was measured by a micronucleic acid analyzer, and the A260/A280 value was considered to satisfy the experimental requirements between 1.8 and 2.1. After the RNA extraction is completed, the reverse transcription reaction is carried out, and the first strand cDNA is synthesized and then subjected to PCR amplification. PCR amplification system: 2 μL of cDNA template, 1 μL of cDNA dilution, 5 μL of 2×SYBR Green mixture, 1 μL of upstream primer and downstream primer, and added to 10 μL with distilled water. It was denatured at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s for 40 cycles. The dissolution curve analysis was performed after the end of the experiment. U6 was used as the reaction internal reference. All samples were repeated in 10 wells and the results were analyzed by $2^{-\Delta\Delta Ct}$ method. TRIzolTM Reagent was purchased from Chengdu Dongsheng Kechuang Technology Co., Ltd., and the item number was 15596026. The SYBER GREEN real-time PCR kit was purchased from Nanjing Kehao Biotechnology Co., Ltd., and the number is 4310251. The primer sequence was designed and synthesized by Herzen (Shanghai) Biotechnology Co., Ltd. (**Table 1**).

MTT in vitro proliferation experiment

The MG-63 cells after 48 hours of chemotherapy were prepared into 4×10^6 cells/mL of single array cell suspension, and the cells were routinely inoculated and cultured in 96-well cell culture plates. The four collection time points were set at 24 h, 48 h, 72 h, and 96 h, and three parallel holes were set at each time point. When the cells were cultured to various times, a 20 μL LTTT (5 mg/mL) solution was added, and incubation was continued at 37°C for 4 h. The supernatant containing the impurities was removed, and a dimethyl sulfoxide preparation was added and placed on a horizontal shaking table for 10 min. Finally, the absorbance at 570 nm was measured on a light absorption microplate reader VersaMax (Meigu Molecular Instruments (Shanghai) Co., Ltd.). The MTT assay kit was purchased from Sigma-Aldrich, USA.

Apoptosis detected by flow cytometry

After being treated with trypsin (0.25%)-EDTA, the cells were centrifuged at 1000 rpm at room temperature for 5 min. Cells were labeled with Annexin V-FITC and 7AAD for 20 min. Fluorescence (DNA content) was measured using a standard flow cytometer. AnnexinV-FITC(+) and 7AAD(-) cells are considered to be early apoptotic cells, and all negative are considered to be late apoptotic cells. Three sets of parallel experiments were set and performed simultaneously.

Western blot

The protein in MG-63 cells was extracted by repeated freeze-thaw method. The protein was

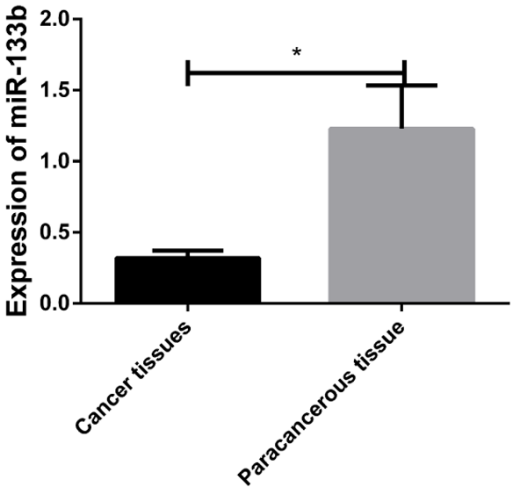

Figure 1. Expression levels of miR-133b in tissues of patients with osteosarcoma. * indicated P < 0.05.

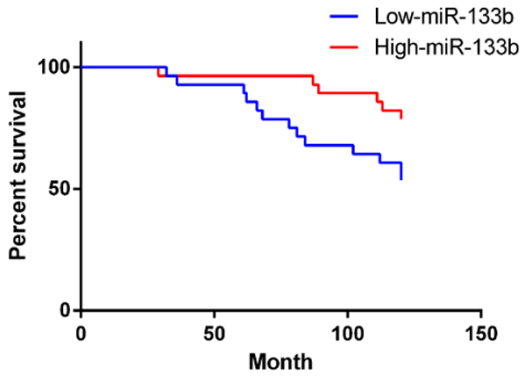

Figure 2. Relationship between miR-133b and prognosis in patients with osteosarcoma. * indicated P < 0.05.

separated by polyacrylamide gel electrophoresis at an initial voltage of 90 V, after which the voltage was increased to 120 V to move the sample to the appropriate position of the separation gel. After electrophoresis was completed, the membrane was transferred, at a constant pressure of 100 V for 100 min, and blocked at 37°C for 60 min. The transfer membrane was then placed in 5% skim milk for blocking and then subjected to an immune response. The membrane was incubated with primary antibody (1:1000) overnight at 4°C, and was washed three times with PBS for 5 min each time the next day. Then, it was incubated with the secondary antibody (1:1000) for 1 h at room temperature. After completion, the ECL luminescence reagent was developed and fixed. Statistical analysis of the strips after film scanning was performed using Quantity One software. Protein relative expression level = band gray value/internal parameter gray value. The Western blot kit was purchased from Shanghai Youyu Biotechnology Co., Ltd., with lot number JC-445. RhoA antibody (batch number: RS-1180R) was purchased from Abcam, UK. The p-p38, p-ERK, and p-JNK monoclonal antibodies were purchased from Shenyang Wanji Biotechnology Co., Ltd., and the lot numbers were WL02983, WLP1512, and WL01813, respectively. A goat anti-mouse IgG secondary antibody (1:1000) was purchased from Santa Cruz Biotechnology, CA, USA.

Follow-up

The survival duration of the patients after discharge was recorded. The final status of the patient regarding survival was gathered via phone calls every three months. After 5 years of follow up, 5-year survival rate of patients with high and low expression of miR-133b was compared.

Statistical methods

SPSS19.0 (Asia Analytics Formerly SPSS China) was used for statistics. The count data was expressed as [n (%)], and the ratio was compared using the χ^2 test. The measurement data was expressed as $\bar{x} \pm sd$. An independent sample t test was used for comparison between the two groups. One-way analysis of variance was used for multigroup comparison. Repeated variance measurement was used for comparison at different time point in the group, and LSD test was used for post hoc test. Kaplan-Meier survival curve was used to analyze the relationship between miR-133b and patient prognosis. The correlation between miR-133b and p-p38, p-ERK, p-JNK was analyzed by Pearson correlation analysis. P < 0.05 indicated statistical significance.

Results

General information

There were 56 patients with osteosarcoma, aged 15-30 years, with an average age of

Table 3. Assignment table

Independent variable	Assignment
Gender	Male = 0, Female = 1
Age	Continuous variable
Diameter of tumor	≥ 8 cm = 0, < 8 cm = 1
Type of tumor	Osteoblast type = 0, chondroblast type = 1, fibroblast type = 2, mixed type = 3
Location of tumor	Femur = 0, tibiofibula= 1, other = 2
Clinical state	I-IIA = 0, IIB-III = 1
metastasis	Yes = 0, no = 1
miR-133b	Contimuous variable
Prognosis	Survival = 0, death = 1

Table 4. Analysis of COX

Factors	Single factor		Muti-factor	
	HR (95% CI)	P	HR (95% CI)	P
Gender	0.643 (0.566-1.778)	0.256		
Age	0.616 (0.344-1.117)	0.165		
Diameter of tumor	0.435 (0.273-0.806)	0.023	0.659 (0.342-1.336)	0.162
Type of tumor	2.741 (1.589-6.832)	0.034	1.102 (2.358-12.248)	0.421
Location of tumor	4.562 (1.443-17.625)	0.091		
Clinical stage	6.172 (2.267-7.844)	0.002	3.529 (3.365-24.604)	0.009
Metastasis	6.574 (1.884-10.158)	0.003	3.757 (3.247-26.784)	0.013
miR-133b	1.465 (0.743-2.275)	0.006	1.212 (0.485-2.238)	0.011

19.3±6.8 years old. There were 35 male patients (62.50%) and 21 females (37.50%). There were 25 patients (44.64%) with tumor diameter over 8 cm and 31 patients with tumor diameter less than 8 cm. The location of osteosarcoma was concentrated in the femur. There were 3 patients with osteosarcoma (41.07%) in phase II-IIA and 33 patients (58.93%) in phase IIB-III. Tumor metastasis occurred in 22 patients (39.29%) (**Table 2**).

miR-133b expression level in osteosarcoma patients

The expression level of miR-133b in cancer tissues was significantly lower than that in adjacent tissues (P < 0.05) (**Figure 1**).

Relationship between miR-133b and prognosis of osteosarcoma patients

The median relative expression level of miR-133b was 0.357 based on the relative expression level of miR-133b in tumor tissues. With the median as the critical value, the two groups were divided into high (> 0.357) and low (≤ 0.357), and Kaplan-Meier survival analysis was performed. The analysis showed that the 5-year survival rate of patients with high expression of miR-133b was 78.57% (22 cases), and the 5-year survival rate of patients with low expression of miR-133b was 53.57% (15 cases). The 5-year survival rate was higher in the miR-373 low-expression group than in the miR-373 high-expression group (P = 0.038) (**Figure 2**).

Analysis of prognostic risk factors

COX multivariate analysis showed that miR-133b, clinical stage, and metastasis were independent risk factors for prognosis of patients with osteosarcoma (**Tables 3**, **4**).

Analysis of transfection results of osteosarcoma MG-63 cells

The results of qRT-PCR showed that the relative expression level of miR-133b in miR-133b-mimic was 1.461±0.219 after transfection, and the relative expression level of miR-133b in miR-control group and control group was 0.405±0.054 and 0.416±0.033. There was a significant difference between miR-133b-mimic and miR-control group and control group (P < 0.001). There was no significant difference between miR-control group and control group (P > 0.05) (**Figure 3**).

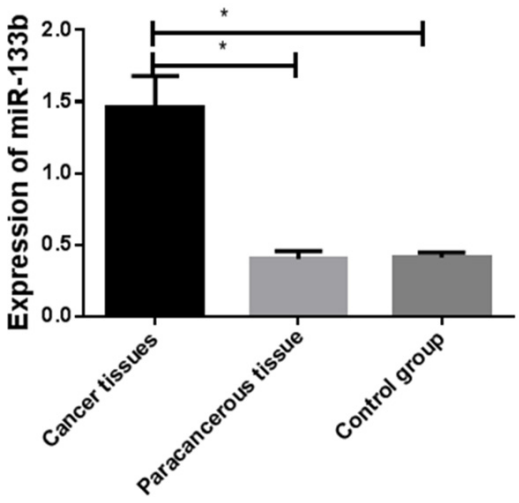

Figure 3. Analysis of transfection results of osteosarcoma MG-63 cells. * indicated P < 0.05.

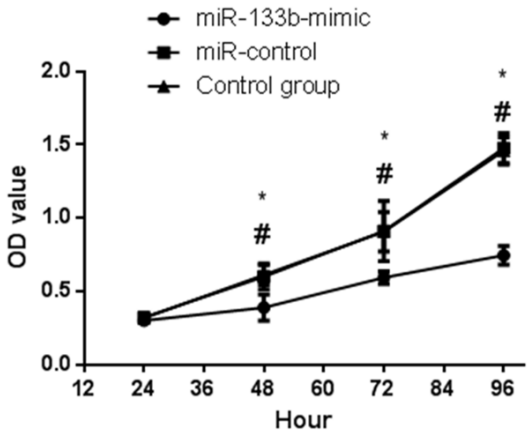

Figure 4. Analysis of proliferation of osteosarcoma MG-63 cells. * indicated P < 0.05 compared to the miR-control group. # indicated P < 0.05 compared to the control group.

Analysis of proliferation of osteosarcoma MG-63 cells

Compared with the miR-control group, the OD values of the miR-133b-mimic group were lower at 48 h, 72 h, and 96 h (both P < 0.05), and there was no significant difference between miR-control group and control group (P > 0.05) (**Figure 4**).

Analysis of apoptosis in osteosarcoma MG-63 cells

The apoptosis rate of miR-133b-mimic group was higher than that of miR-control group and control group (P < 0.05), and there was no significant difference between miR-control group and control group (P > 0.05) (**Figure 5**).

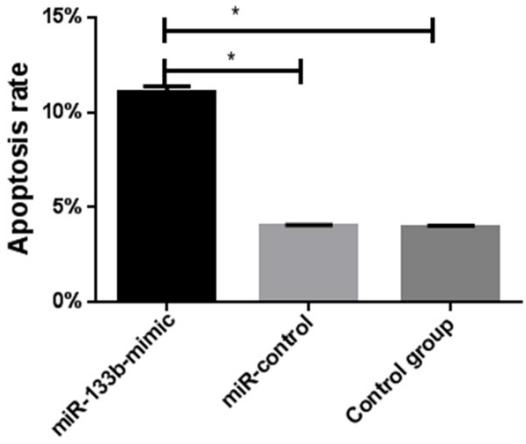

Figure 5. Analysis of apoptosis in osteosarcoma MG-63 cells. * indicated P < 0.05.

Results of p-p38, p-ERK, p-JNK

The protein levels of p-p38, p-ERK and p-JNK in the miR-133b-mimic group were lower than those in the miR-control group and control group (P < 0.05), and there was no significant difference between miR-control group and control group (P > 0.05) (**Table 5**).

Correlation between miR-133b and p-p38, p-ERK and p-JNK

Pearson analysis showed that miR-133b was negatively correlated with p-p38 (r = -0.828, P < 0.001), p-ERK (r = -0.786, P < 0.001), and p-JNK (r = -0.928, P < 0.001) (**Figure 6**).

Rescue experiment

Compared with transfection of miR-133b-mimic alone, the expression levels of p-p38, p-ERK, and p-JNK after co-transfection were significantly increased (P < 0.05), but there was no significant effect on miR-133b expression (P > 0.05). The cell proliferation of co-transfection group was significantly higher than that of miR-133b-mimic group (P < 0.05), and the apoptosis rate was lower than that of miR-133b-mimic group (P < 0.05) (**Figure 7**).

Discussion

Osteosarcoma is a highly heterogeneous malignant primary bone tumor with vascular invasion

Table 5. Results of p-p38, p-ERK and p-JNK

	miR-control	miR-133b-mimic	control group	F	P
p-p38	0.393±0.040	0.254±0.042*	0.394±0.038	12.308	0.008
p-ERK	0.456±0.027	0.365±0.032*	0.472±0.025	9.705	0.013
p-JNK	0.407±0.041	0.244±0.026*	0.405±0.019	28.286	0.001

Note: *indicates the significant difference comparing with miR-control and control group (P < 0.05).

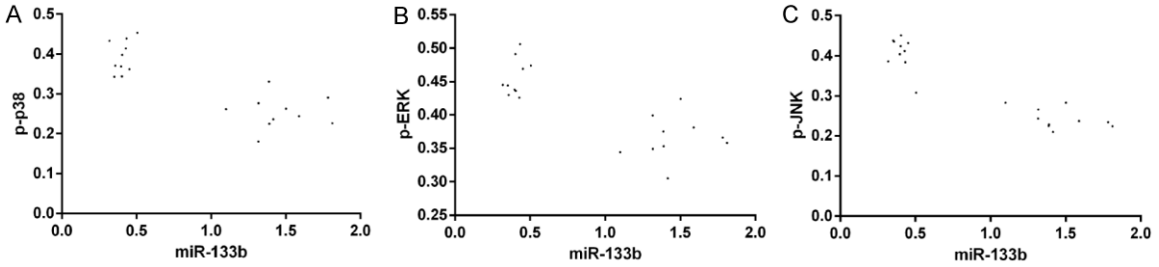

Figure 6. Correlation of miR-133b with p-p38, p-ERK, p-JNK. A: Correlation of miR-133b with p-p38. r = -0.828, P < 0.001. B: Correlation between miR-133b and p-ERK. r = -0.786, P < 0.001. C: Correlation between miR-133b and p-JNK. r = -0.928, P < 0.001.

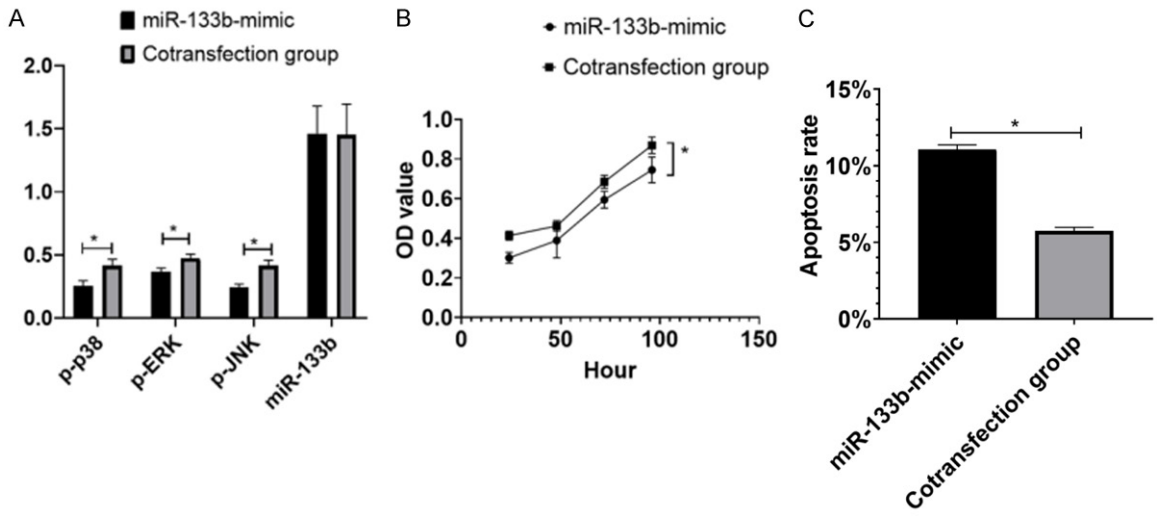

Figure 7. Rescue experiment. A: Effect of co-transfection on the expression of miR-133b with p-p38, p-ERK, p-JNK. B: Effect of co-transfection on proliferation of osteosarcoma cells. C: Effect of co-transfection on apoptosis of osteosarcoma cells.

and local soft tissue infiltration as the main clinical features [13, 14]. In recent years, many studies have reported that the development of osteosarcoma is closely related to the expression levels of many miRNAs. In recent years, there have been studies related to miR-133b and osteosarcoma. Among them, Zou et al. [15] reported that miR-133b was decreased in tissue and serum of osteosarcoma patients, and low expression of miR-133b was closely related to osteosarcoma's high differentiation, metastasis and recurrence. The overall survival rate and disease-free survival rate of patients with low expression of miR-133b were also lower than those with high expression of miR-133b. Multivariate analysis showed that miR-133b was an independent predictor of prognosis in patients with osteosarcoma. Ying et al. [16] reported that, by up-regulating miR-133b, procaine can inhibit tumor growth, reduce p/t-AKT

and p/t-ERK levels, and inhibit proliferation and migration of osteosarcoma cells, thereby promoting apoptosis. These studies confirmed the role of miR-133b as a tumor suppressor in osteosarcoma. The inhibitory mechanism of miR-133b in osteosarcoma was analyzed in this study.

56 patients with osteosarcoma were included in the study. We first validated the expression of miR-133b in tumor tissues of patients with osteosarcoma and its relationship with prognosis. The results of the analysis showed that the expression of miR-133b in osteosarcoma cancer tissues was significantly lower than that of adjuvant tissues. Kaplan-Meier survival curve analysis showed that the 5-year survival rate of patients with low expression of miR-133b was significantly lower than that of miR-133b high expression group, and miR-133b is an independent risk factor for prognosis in patients with osteosarcoma, which was consistent with previous studies. Gao et al. [17] reported that compared with normal bone tissue and normal osteoblasts, miR-133b was down-regulated in osteosarcoma tissue and osteosarcoma cell lines MG-63 and U2OS, and inhibited FGFR 1. This, in turn, inhibited the signaling cascade within RAS/MAPK and PI3K/Akt cells, and the migration and invasion of osteosarcoma cells, thus promoting their apoptosis. They also reported that low expression of miR-133b is associated with tumorigenesis, advanced clinical stage, and distant metastasis. Zhang et al. [18] also reported in the study that the expression of miR-133b in osteosarcoma tissue and patient serum was decreased and was associated with the prognosis of patients. The overall survival rate and disease-free survival rate of patients with low expression were lower than those of high expression group of miR-133b.

The cell proliferation ability after miR-133b overexpression was significantly decreased, and the apoptosis level was significantly increased. This is also consistent with previous studies, which reported the role of miR-133b in tumor suppressor genes in osteosarcoma. Novello et al. [19] reported that the expression level of miR-133b was decreased in osteosarcoma cells, possibly through the expression of MET protein, blocking the G1 phase of osteosarcoma cells, prolonging the cell cycle, promoting apoptosis and inhibiting cell proliferation. Zhao et al. [20] also reported that in the osteosarcoma cell lines U2-OS and MG-63, stable and high expression of miR-133b inhibited cell proliferation, invasion and migration, and induced apoptosis. In addition, overexpression of miR-133b reduced expression of the predicted target genes BCL2L2, MCL-1, IGF1R and MET, and decreased expression of p-Akt and FAK. Then, the expression levels of MAPKs signaling pathway-related proteins p-p38, p-ERK, and p-JNK were detected in the two groups of cells. The results showed that the expression levels of p-p38, p-ERK and p-JNK were decreased in the miR-133b-mimic group, suggesting that up-regulation of miR-133b can inhibit the activation of MAPKs signaling pathway. Moreover, our Pearson correlation analysis also showed that miR-133b was negatively correlated with p-p38, p-ERK and p-JNK. Through rescue experiments, it was found that simultaneous activation of EAPKs signaling pathway can significantly interfere with the inhibition of miR-133b up-regulation on osteosarcoma cells, suggesting that EAPKs signaling pathway is a target for miR-133b to play a role in tumor suppression in osteosarcoma. Liu et al. [11] showed that compared with normal ovarian epithelial cells, the level of miR-133b was significantly decreased and the expression of EGFR protein was significantly increased in ovarian cancer cells. Up-regulation of miR-133b inhibited the proliferation and invasion of ovarian cancer cells. It was also found that overexpression of miR-133b inhibited phosphorylation of ERK 1/2 and Akt. Their results demonstrated that miR-133b overexpression inhibited proliferation and invasion of ovarian cancer cells by targeting EGFR to inhibit MAPK and PI3K/Akt signaling pathways. The results of Bandrés et al. [21] showed that miR-133b is down-regulated in colorectal cancer and may regulate tumor cell proliferation and apoptosis via the RAS/RAF/MEK/ERK pathway. These results all confirmed the regulation of miR-133b on MAPKs signaling pathway and verified our results to some extent. This may also be a molecular mechanism by which miR-133b inhibits osteosarcoma cell proliferation and promotes apoptosis.

There were some limitations in this study. The clinical research object of this study is the osteosarcoma tissue and its paracancerous tissue preserved in our hospital, which were not fresh tissue and might have a certain impact on the results. We will collect fresh tis-

sue for research in the future. This study confirmed the relationship between miR-133b and MAPKs signaling pathway through correlation analysis. However, since no target gene prediction and dual fluorescein report analysis were performed, we cannot confirm whether it was direct regulation or indirect regulation. In addition, in the basic experimental part of this study, only one osteosarcoma cell line was used, which may reduce the credibility of some results. We will include more osteosarcoma cell lines in future studies to further confirm these regulatory relationships.

In summary, miR-133b is low-expressed in osteosarcoma tissues. The 5-year survival rate of patients with low expression of miR-133b was decreased, and up-regulation of miR-133b could inhibit the proliferation of osteosarcoma cells and promote apoptosis by inhibiting MAPKs signaling pathway.

Disclosure of conflict of interest

None.

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