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miR-375 Attenuates The Migration and Invasion of Osteosarcoma Cells by Targeting MMP13*

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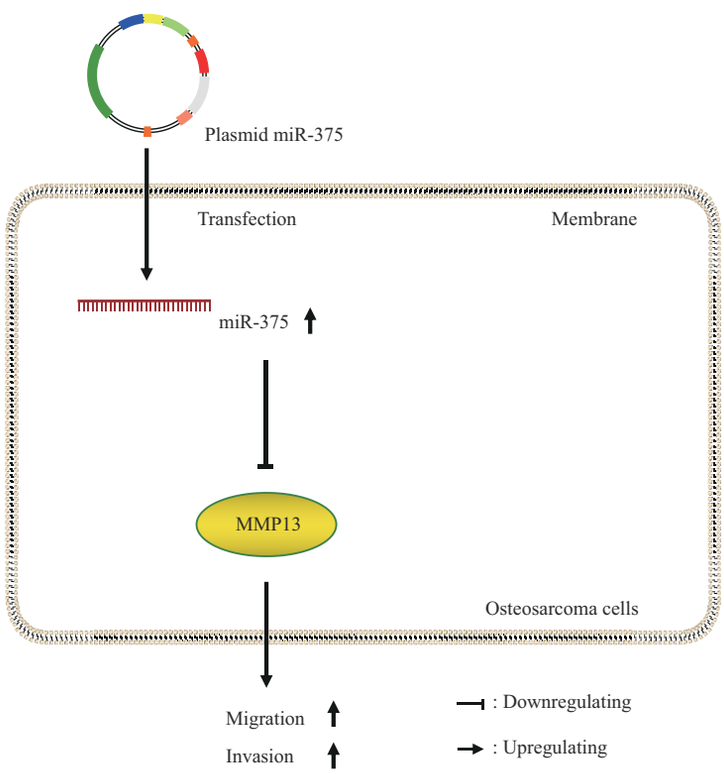
Graphical abstract



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Abstract **Objective** To explore whether miR-375 regulates the malignant characteristics of osteosarcoma (OS) by influencing the expression of MMP13. **Methods** Plasmid DNAs and miRNAs were transfected into OS cells and HEK293 cells using Lipofectamine 3000 reagent. Real-time quantitative polymerase chain reaction was performed to measure the expression of miR-375 and MMP13 in OS patients and OS cells. Western blot was performed to analyze the MMP13 protein in the patients with OS and OS cells. The targeting relationship between miR-375 and MMP13 was analyzed by luciferase assay. Migration and invasion were analysed by heal wound and transwell assays, respectively. **Results** miR-375 expression in OS tissues was lower than that in normal tissues. The expression of MMP13 was upregulated in OS tissues. MMP13 expression was negatively correlated with miR-375 expression in patients with OS. Migration and invasion were significantly inhibited in OS cells with the miR-375 mimic compared with OS cells with the miRNA control. MMP13 partially reversed the inhibition of migration and invasion induced by miR-375 in the OS cells. **Conclusion** miR-375 attenuates migration and invasion by downregulating the expression of MMP13 in OS cells.

Key words osteosarcoma, miR-375, MMP13, migration, invasion
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Osteosarcoma (OS) is one of the most common primary malignant bone tumors occurring in adolescents[1]. Although patients with OS have a 70% survival rate after surgery and chemotherapy[2], the 5-year survival rate is still less than 20%, which is attributed to the fact that 30% of patients develop lung metastases in the early stages of OS[3]. OS metastasis has become a bottleneck to further improve the survival rate of patients with OS. To improve the clinical efficacy and survival rate of patients, it is urgent to explore the metastasis mechanism of OS.

Matrix metalloproteinase (MMP) 13, a member of the MMP family, has been shown to be highly expressed in OS tissues and is associated with poor prognosis of patients with OS[4]. miRNAs are a class of single-stranded noncoding RNA molecules with a length of 20−24 nucleotides that usually bind to 3'-noncoding regions (3'UTRs) of potentially targeted mRNAs and participate in gene transcription or posttranscriptional regulation. Among miRNAs, the expression of miR-375 has attracted much attention in OS patients. It was reported that the expression level of serum miR-375 in patients with OS was significantly lower than that in healthy people, and OS patients with low expression of miR-375 often presented with clinically advanced tumours, large tumour volume, distant metastasis and poor chemotherapy efficacy[5]. It was concluded that miR-375 could increase the sensitivity of OS cells to chemotherapy drugs by regulating the expression of Mcl-1[6] and ATG2B[7]. A study showed that miR-375 alleviated osteoarthritis-related pain, inflammation and apoptosis by targeting MMP13[8]. It was confirmed that miR-375 markedly inhibits migration and invasion by regulating MMP13 in esophageal squamous cell carcinoma[9]. However, whether miR-375 accelerates the occurrence and development of OS by regulating the expression of MMP13 still needs to be explored.

In this study, we evaluated the relationship between miR-375 and the expression of MMP13 in the patients with OS. Meanwhile, we used a cell model to explore whether miR-375 regulates the migration and invasion of OS cells by inhibiting MMP13.

1 Materials and methods

1.1 Samples and ethics approval

Thirty-seven patients who had been diagnosed with OS at department of orthopedics in the First Affiliated Hospital of Shaoyang University were enrolled from January 2021 to September 2022. The OS and para-carcinoma normal tissues were collected at the time of surgery. The collected tissues were immediately extracted for total RNAs and proteins, or stored in a −80℃ refrigerator for further study. This research was approved by Medical Research Ethics Committee of the First Affiliated Hospital of Shaoyang University (approval number: 2020053) and abided by principles of Declaration of Helsinki. All patients had signed informed consent prior to samples collection.

1.2 Cell culture

The normal human osteoblast cells (HOB-C) and OS cells (MG63, U2OS, 143B) were respectively purchased from sigma-aldrich and American Type Culture Collection (ATCC), and cultured with Eagle’s Minimum Essential Medium (30-2003, ATCC, USA). Human embryonic kidney cells (HEK293) were

purchased from ATCC and cultured with DMEM (SH30022.01, Hyclone, USA). All medium was supplemented with 10% fetal bovine serum (FBS, F0193, Sigma, USA) and 1% penicillin-streptomycin (V900929, Sigma, USA). Cells were incubated in a 5% CO_2 humidified atmosphere at 37℃.

1.3 Plasmids generation, miRNAs acquisition and transfection

The homo species MMP13 cDNA from OS cells was ligated into *Eco*R I and *Xba* I sites of pcDNA3.1 vector to construct pcDNA3.1-MMP13 plasmids. The MMP13 cDNA was synthesized by polymerase chain reaction (PCR) using specific primers (forward primer: 5'-ATCGGAATTCATGCATCCAGGGGTCC-TG-3' and reverse primer: 5'-ATCGTCTAGATTAA-CACCACCAAAATGG-3'). The 3'UTR of MMP13 (including wild type and mutant) bound to miR-375 was synthesized by Sangon Biotech (China) and inserted into pMIR-REPORT luciferase vectors to generate the pMIR-REPORT-MMP13 plasmids. The plasmids were amplified in *Escherichia coli* and purified using TIANprep midi Plasmid Kit (DP106, TIANGEN, China) in according to manufacturer's protocol. All plasmids were validated by sequencing. The miRNA control (miRNA NC, 5'-UUCU-CCGAACGUGUCACG-3') and miR-375 mimic (5'-UUUGUUCGUUCGGCUCGCGUGA-3') were synthesized by GenPharma (Shanghai, China). The plasmids were transfected into cells (143B cells or HEK293 cells) according to the protocol of Lipofectamine 3000 reagent (L3000015, Thermo, USA).

1.4 Luciferase assay

The pMIR-REPORT-MMP13 plasmids were co-transfected with miRNAs (miRNA NC or miR-375 mimic) into HEK293 cells. Transfected cells were cultured continuously for 48 h. Based on activity of renilla luciferase, luciferase activity was evaluated with dual luciferase reporter gene assay kit (RG027, Beyotime, China) at the wavelength of 560 nm.

1.5 Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNAs were extracted from samples and cells in according to the protocol of TRNzol reagent (DP424, TIANGEN, China). The concentration of RNA was quantified using spectrophotometer (NanoDrop 2000, Thermo, USA). The RNA was reverse-transcribed into cDNA in according to the protocol of All-One RT MasterMix Kit (G492, abm, Canada). The expression of miR-375 and MMP13 were analyzed in according to the protocol of FastKing One Step RT-qPCR Kit (FP313, TIANGEN, China) with primers of target genes (miR-375 forward: 5'-CACAAAATTTGTTCGTTC-GGCT-3', reverse: 5'-GTGCAGGGTCCGAGGT-3'; MMP13 forward: 5'-GCCATTACCAGTCTCCGA-GG-3', reverse: 5'-TACGGTTGGGAAGTTCTGGC-3'; U6 forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-AACGCTTCACGAATTTGCGT-3'; 18S forward: 5'-AGAAACGGCTACCACATCCA-3', reverse: 5'-CA-CCAGACTTGCCCTCCA-3'). The RT-qPCR parameters were as follows: pre-denaturation at 95℃ for 3 min; 40 cycles at 95℃ for 15 s, 60℃ for 30 s. The U6 and 18S were used as internal control to standardize the expression of miR-375 and MMP13 respectively. The expression of miR-375 and MMP13 were analyzed with $2^{-\Delta\Delta Ct}$ method.

1.6 Western blot

The samples and cells were lysed in the pre-cooled RIPA buffer (P0013B, Beyotime, China) with cocktail protein inhibitor (P1005, Beyotime, China) using ultrasonic cell disruptor (JY92-IIN, Scientz, China). The lysates were centrifuged at 4℃ under 13 300*g* for 30 min. Supernatants of lysates were collected in the clean 1.5 ml EP tubes. Concentration of total protein was quantified by a spectrophotometer (NanoDrop 2000, Thermo, USA). Supernatants were mixed with 2×SDS-polyacrylamide gel electrophoresis (PAGE) sample loading buffer (P0015B, Beyotime, China) and boiled for 10 min to denature proteins. The proteins were separated by SDS-PAGE. The proteins were transferred on PVDF membranes, then, blocked with 5% bovine serum albumin (BSA, SRE0096, Sigma, USA) at room time (RT) for 2 h. The membranes were respectively incubated with MMP13 rabbit polyclonal antibody (1∶1 000, AF7479, Beyotime, China) and β-actin rabbit monoclonal antibody (1∶2 000, AF5003, Beyotime, China) at 4℃ overnight, then, incubated with horseradish peroxidase labeled goat anti-rabbit IgG (1∶2 000, A0208, Beyotime, China) at RT for 1 h. Finally, the protein on the membrane was visualized by chemiluminescence. The results were photographed and analyzed under the gel imaging system (Chemidoc MP; Bio-Rad, USA).

1.7 Wound healing analysis

The migration was analyzed by wound healing analysis. Briefly, the 143B cells were seeded into 12-well plates. When cells reached 100% confluent, the scratch was drawled evenly from top to bottom in the plates using a tip, which was defined as 0 h. Floating cells were washed away with PBS. Then, the cells were cultured continuely for 48 h in a 5% CO_2 incubator at 37℃. Wound healing area was visualized under microscope at 0 h and 48 h, and analyzed by ImageJ software (NIH, USA). The migration was evaluated using the following formula: rate of migration= (area of wound healing at 0 h−area of wound healing at 48 h)/area of wound healing at 0 h× 100%.

1.8 Transwell analysis

The invasion was analyzed by transwell analysis. Transwell chambers (MCEP24H48, Millipore, USA) were pre-treated with BD Matrigel (356234, BD, USA) for 3 h in a 5% CO_2 incubator at 37℃. The chambers were inserted into the 24-well plate containing the medium without FBS. 1×10^4 cells were implanted into each transwell chamber. Then, the cells of plate were cultured in an incubator with 5% CO_2 at 37℃ for 48 h. The cells in chamber were fixed with 5% paraformaldehyde. After washing with PBS, cells were stained with 0.1% crystal violet (BL802A, Biosharp, China). Swab was used to scrub the cells in the upper chamber and the cells in bottom chamber were randomly photographed and quantified.

1.9 Statistical analysis

All data from three independent experiments were presented as mean±standard deviation (SD). The SPSS23.0 and GraphPad Prism 9.0 software were used to analyze the data in this study. The difference between two groups was analyzed using student's *t* test. The correlation between miR-375 and MMP13 in the patients with OS was analyzed using Spearman correlation analysis. It was considered as statistically significant when $P<0.05$.

2 Results

2.1 The expression of miR−375 was decreased in patients with OS

To explore the expression of miR-375 in patients with OS, 37 OS tissues and 37 normal tissues adjacent to OS were collected. Then, the expression of miR-375 was measured by RT-qPCR assays. As shown in Figure 1, the miR-375 expression in OS tissues was significantly downregulated compared with that in normal tissues ($P<0.000\ 1$).

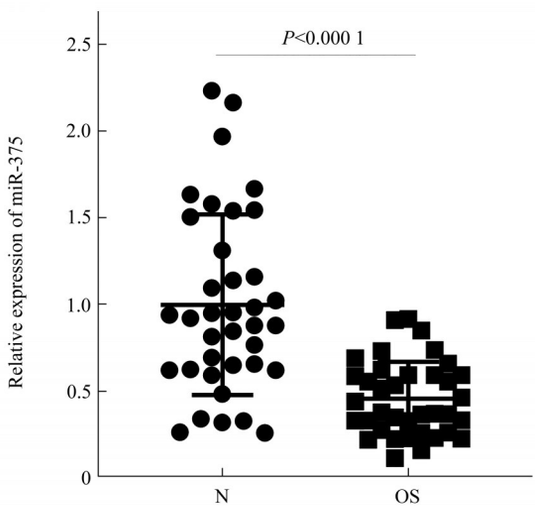

Fig. 1 The expression of miR−375 was analyzed in patients with OS

Thirty-seven patients with OS were enrolled and the expression of miR-375 was detected by RT-qPCR in the normal (N) and OS tissues.

2.2 The expression of MMP13 was increased and negatively correlated with the expression of miR−375 in patients with OS

To investigate the correlation between miR-375 expression and MMP13 expression in patients with OS, the expression of MMP13 was analysed. The results of the database analysis (https://tnmplot.com) showed that the expression of MMP13 in OS tissues was higher than that in normal tissues ($P=1.73\times10^{-101}$) (Figure 2a). Then, the expression of MMP13 was analysed in patients with OS. As expected, the expression of MMP13 mRNA was significantly increased in OS tissues compared with normal tissues ($P=0.025\ 6$) (Figure 2b). Five OS patients were randomly selected for analysis of MMP13 protein expression. Consistent with the MMP13 mRNA results, MMP13 protein expression in OS tissues was upregulated compared with that in normal tissues ($P<0.000\ 1$) (Figure 2c, d). The correlation between miR-375 and MMP13 was analysed. As shown in Figure 2e, the expression of miR-375 was negatively correlated with the expression of MMP13 in OS patients ($P=0.042\ 2$).

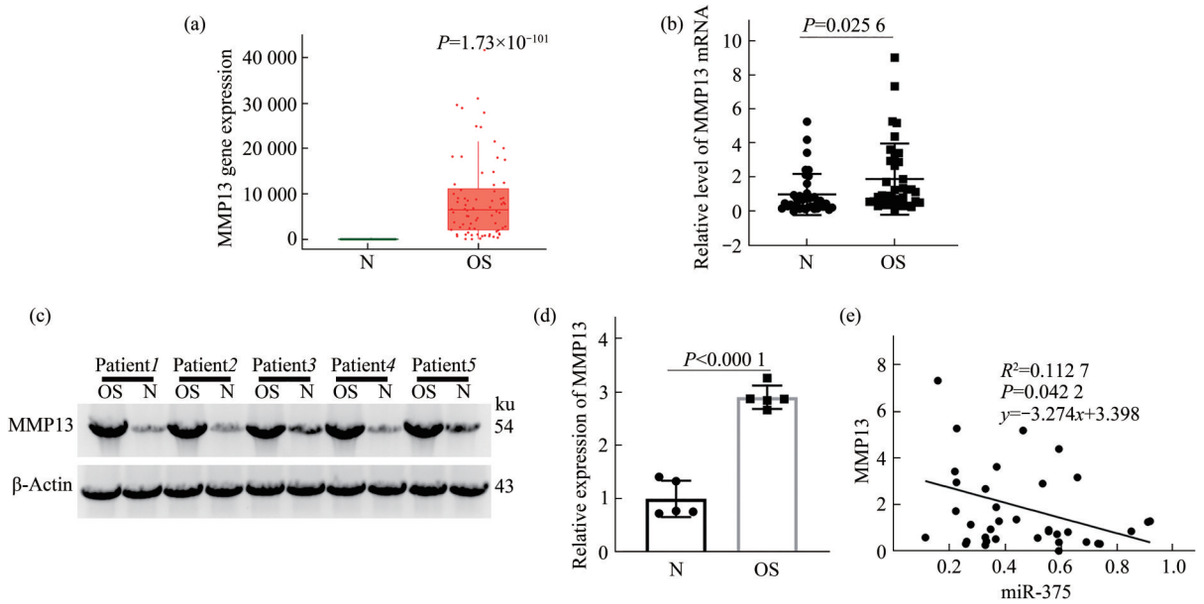

Fig. 2　MMP13 was upregulated and negatively correlated with miR−375 in patients with OS

(a) The expression of MMP13 was analyzed online (https://tnmplot.com). (b) The expression of MMP13 mRNA was measured by RT-qPCR in patients with OS. (c) The expression of MMP13 protein was represented in five randomized patients with OS. (d) The expression of MMP13 was analyzed statistically, which showed that MMP13 expression in OS tissues was higher than that in normal tissues. (e) The correlation analysis showed that MMP13 expression was negatively correlated with miR-375 expression in patients. N: normal tissue; OS: osteosarcoma.

2.3　Overexpression of miR−375 repressed the migration and invasion of OS cells

To select appropriate OS cells for investigating the effects of miR-375 on the biological characteristics in OS cells, the expression of miR-375 was measured in human osteoblasts and OS cells. As shown in Figure 3a, miR-375 expression in OS cells was significantly lower than that in HOB-C cells (MG63, U2OS, 143B *vs*. HOB-C, P<0.000 1 respectively). Based on the expression of miR-375, 143B cells were used for subsequent experiments. The 143B cells were transfected with miR-375 mimic, then the expression of miR-375 was analysed by RT-qPCR. The expression of miR-375 in the 143B cells transfected with the miR-375 mimic was significantly upregulated compared to that in control cells (P<0.000 1), which showed that miR-375 was successfully expressed in 143B cells transfected with the miR-375 mimic (Figure 3b).

The migration of 143B cells was analysed by wound healing assay. The wound area of cells with mimic was almost the same as that of NC cells at 0 h; however, it was significantly wider than that of NC cells at 48 h (Figure 3c). That was, the migration rate of cells with mimic was observably decreased compared with that of NC cells (P=0.012 8), which indicated that miR-375 inhibited the migration of 143B cells (Figure 3d). Invasion was analysed by transwell assay. Compared with the control group, the number of cells penetrating the membrane in the 143B cells with mimic was decreased (P<0.000 1), which revealed that miR-375 repressed the invasion of 143B cells (Figure 3e, f).

2.4　miR−375 could downregulate the expression of MMP13

To explore whether miR-375 directly regulates the expression of MMP13, a dual luciferase reporter assay was performed. The alignment of miR-375 and MMP13 3'UTR sequences showed that 6 consecutive bases at positions 100−113 of the MMP13 3'UTR were complementarily paired with miR-375 bases (Figure 4a). Then, positions 100−113 of the MMP13 3'UTR wild-type or mutant sequences were inserted into a luciferase expression plasmid (Figure 4b). HEK293 cells were cotransfected with plasmids and miR-375 mimic. Subsequently, luciferase activity was measured. As shown in Figure 4c, the activity of luciferase in the cells cotransfected with the wild-type

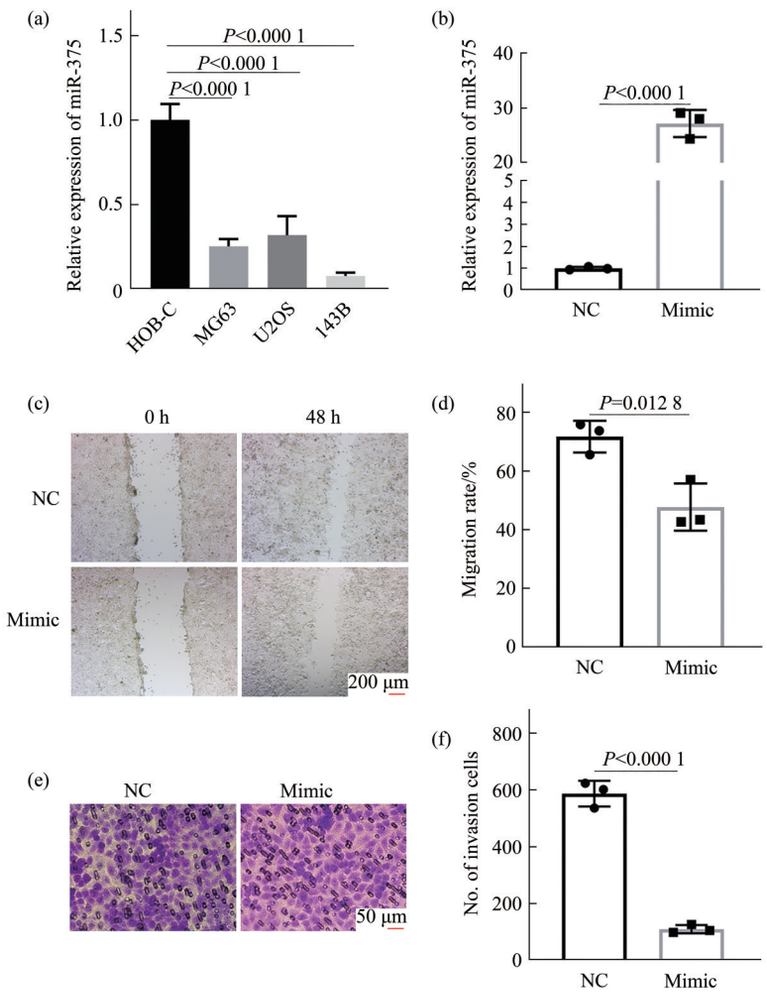

Fig. 3　miR−375 inhibited the migration and invasion in 143B cells

(a) The expression of miR-375 was detected by RT-qPCR in human osteoblasts and OS cells. (b) The expression of miR-375 was analyzed by RT-qPCR in 143B cells transfected with or without miR-375 mimic. (c, d) Migration was analyzed by wound healing assay in 143B cells transfected with miR-375 mimic, which showed that miR-375 could attenuate the migration of 143B cells. (e, f) Transwell assay was performed to evaluate the invasion of 143B cells transfected with miR-375 mimic, which revealed that miR-375 inhibited the invasion of 143B cells. NC: cells transfected with miRNA control; Mimic: cells transfected with miR-375 mimic.

of MMP13 3'UTR and miR-375 mimic was significantly lower than that in the cells cotransfected with the mutant MMP13 3'UTR and miRNA control (P<0.000 1); however, compared with cells transfected with the mutant MMP13 3'UTR and miRNA control, the activity of luciferase was not different in cells transfected with the mutant MMP13 3'UTR and miR-375 mimic (P=0.984 9), which showed that MMP13 may be one of the target genes of miR-375.

2.5　Overexpression of miR−375 abrogated migration and invasion by downregulating MMP13 expression in 143B cells

To verify whether miR-375 regulated migration and invasion by MMP13, 143B cells were cotransfected with miRNAs and plasmids. The expression of miR-375 in the cells transfected with miR-375 mimic and pcDNA3.1 vector was significantly increased compared with that in the cells transfected with miRNA control and pcDNA3.1

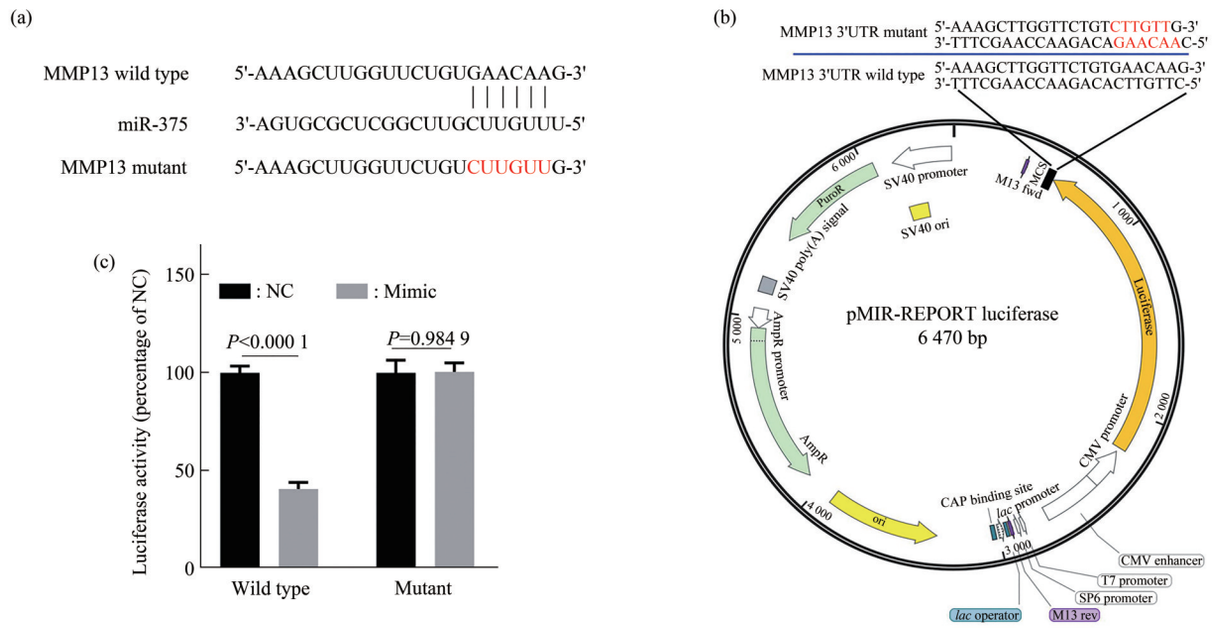

Fig. 4　The expression of MMP13 was directly regulated by miR−375

(a) The sequence positions 100 – 113 of the MMP13 3'UTR was bound by miR-375. Red bases represent the mutation of the MMP13 3'UTR. (b) The wild-type or mutant MMP13 3'UTR was inserted into pMIR-REPORT luciferase vectors. (c) A dual luciferase reporter assay analyzed in HEK293 cells cotransfected with miRNA control/mimic and wild-type/mutant MMP13 3'UTR. Renilla luciferase activity was normalized to firefly luciferase activity. NC: cells transfected with miRNA control; Mimic: cells transfected with miR-375 mimic.

vector (*P*<0.000 1). Meanwhile, the expression of miR-375 in the cells transfected with miR-375 mimic and pcDNA3.1-MMP13 plasmids was the same as that in the cells transfected with miR-375 mimic and pcDNA3.1 (*P*=0.838 6), which indicated that MMP13 had no effect on the expression of miR-375 in 143B cells (Figure 5a). Then, the expression of MMP13 protein was measured. As shown in Figure 5b, c, MMP13 expression in cells transfected with miR-375 mimic and pcDNA3.1 was dramatically decreased compared with that in miRNA control and pcDNA3.1 cells (*P*<0.000 1); however, MMP13 expression in cells cotransfected with miR-375 mimic and pcDNA3.1-MMP13 plasmids was markedly increased compared to that in cells cotransfected with miR-375 mimic and pcDNA3.1 (*P*<0.000 1), which suggested that MMP13 could partially eliminate the decrease of MMP13 expression caused by miR-375 in 143B cells. Migration was analyzed by wound healing assay. After 48 h of scratching, the wound area of cells with miR-375 mimic and pcDNA3.1 was wider than that of cells with miRNA control and pcDNA3.1 (*P*=0.001 2); however, the wound area of cells with miR-375 mimic and pcDNA3.1-MMP13 was less than that of cells with miR-375 and pcDNA3.1 (*P*=0.011 0) (Figure 5d), which suggested that MMP13 partially nullified the inhibition of migration induced by miR-375 in 143B cells (Figure 5e). The invasion of cells was analyzed by transwell assays. The number of cells penetrated the membrane in the 143B cells transfected with miR-375 mimic and pcDNA3.1 was less than that in the cells transfected with miRNA control and pcDNA3.1 (*P*<0.000 1); however, the number of cells in the cells transfected with miR-375 mimic and pcDNA3.1-MMP13 was increased compared with that in the cells transfected with miR-375 mimic and pcDNA3.1 (*P*<0.000 1) (Figure 5f), which indicated that MMP13 could partially reverse the inhibitory effect of miR-375 on invasion in 143B cells (Figure 5g).

Taken together, these results verified that overexpressed miR-375 suppressed osteosarcoma cell migration and invasion by downregulating MMP13 expression.

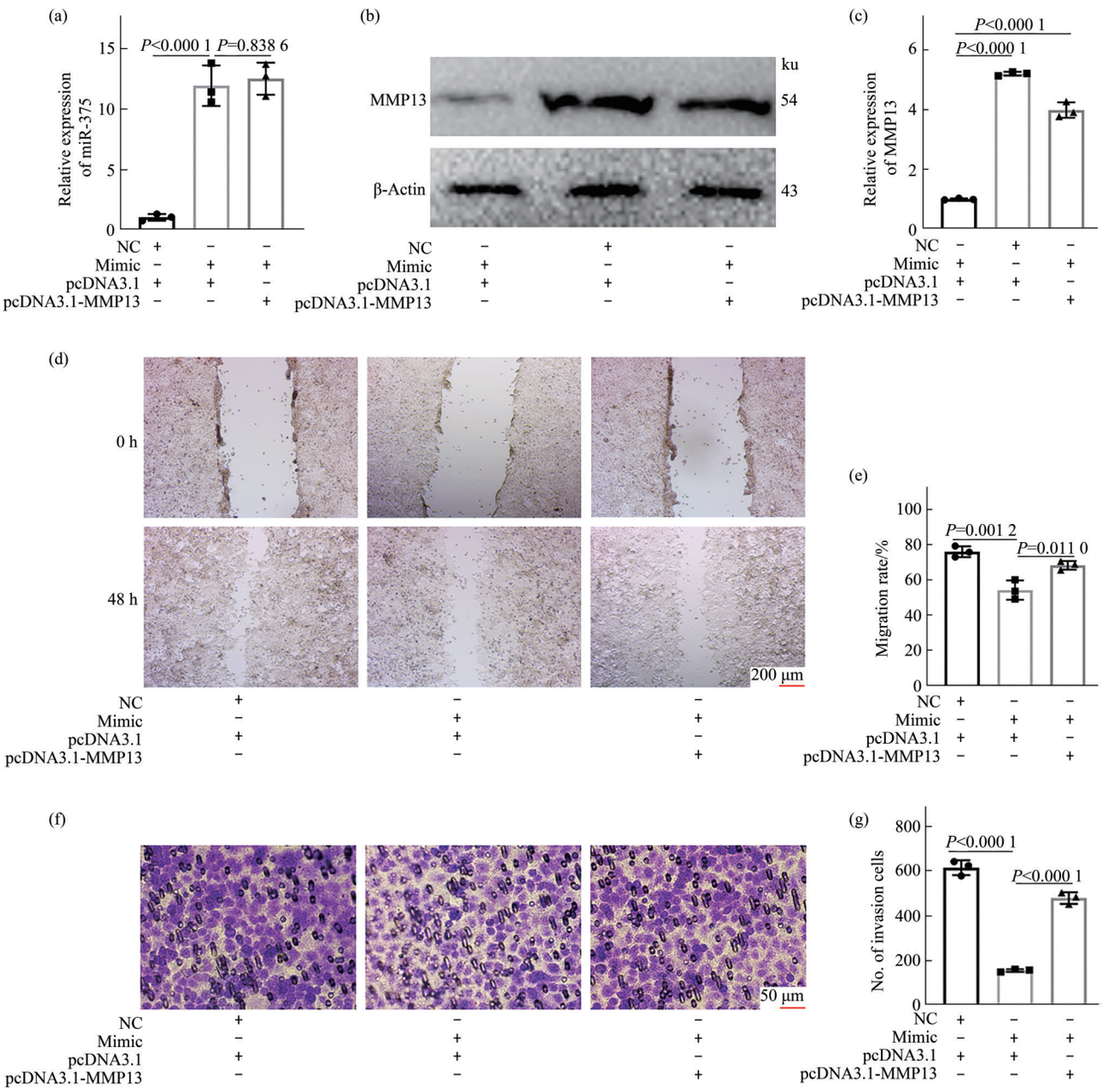

Fig. 5　Overexpression miR−375 inhibited the migration and invasion by sequestrating MMP13 in 143B cells

The 143B cells were transfected with miRNAs and plasmids. (a) The expression of miR-375 was analysed by RT-qPCR assays in the transfected cells. (b, c) The expression of MMP13 protein was analyzed by Western blot in the transfected 143B cells. (d, e) Migration was analysed by wound healing assays in the transfected 143B cells. (f, g) Invasion was analyzed by transwell assays in the transfected 143B cells.

3　Discussion

OS is one of the most common primary malignant bone tumours in adolescents. It develops from a mesenchymal cell line with a low survival rate and poor systemic condition. OS is prone to lung metastasis in the early stage due to its faster growth, leading to systemic deterioration. With the development of medical technology, the means of treating OS are diversified, such as classical surgical treatment, chemotherapy, immunotherapy, traditional Chinese medicine therapy and combination therapy. Even after amputation and chemotherapy, approximately 40% of patients still die from tumour

lung metastasis[3]. In 80%−90% of patients, systemic micrometastasis had already occurred before diagnosis[10]. Patients with OS who only have lung metastases can be surgically resected, but there is no effective treatment for multiple metastases. Therefore, metastasis has become a clue to further improve the survival rate of OS patients. Based on the characteristics of rapid OS growth and easy transfer, it is particularly important to investigate the occurrence and development of OS.

MMP13 plays a key role in the activation of MMPs, which are closely associated with the invasion, metastasis and prognosis of multiple tumours[11-12]. It has been reported that MMP13 can reduce or deactivate almost all extracellular matrix and basement membrane components except collagen[13]. Studies have shown that MMP13 is highly expressed in a variety of malignant tumour tissues, such as oral squamous cell carcinomas[14-15], breast cancer[12, 16-17], hepatocellular carcinoma[18], myeloma[19], and gastric cancer[20]. MMP13 can degrade extracellular matrix and promote tumour cell metastasis. In our study, we measured the expression of MMP13 in patients with OS. Not surprisingly, our results are consistent with those previously reported, which showed that the expression of MMP13 was upregulated in OS tissues[4, 21]. At the same time, a large number of studies have shown that traditional Chinese medicine, such as bisdemethoxycurcumin[22], fistein[23], ouabain[24] and shikonin[25-26], can downregulate the expression of MMP13 to inhibit the malignant characteristics of OS cells. Therefore, the specific molecular mechanism of MMP13 is conducive to slowing down the development process and prevention to improve the clinical prognosis of patients with OS.

No exception, miRNAs have attracted increasing attention for regulating the malignant characteristics of OS tumour cells by promoting or inhibiting the expression of target genes. miRNA-151a-3p regulates the invasion and migration of OS by inhibiting the expression of RAB22A[27]. miRNA-221-3p derived from M2-polarized tumour-associated macrophage exosomes promotes the proliferation and metastasis of OS by the SOCS3/JAK2/STAT3 pathway[28]. miR-195-5p aggravates the progression and metastasis of OS by downregulating FGF2 expression[29]. It was reported that miRNA-218 was a therapeutic target for OS by targeting multiple oncogenes[30]. The study of Luo *et al*.[31] showed that miRNA-18b-5p facilitates the proliferation and metastasis of OS by attenuating PHF2. Extracellular vesicle-mediated delivery of miR-101 repressed lung metastasis in OS[32]. A study confirmed that miRNAs are potential targets for diagnosis, inhibiting metastasis and increasing chemosensitivity in patients with OS[31, 33]. Therefore, it can be concluded that abnormal miRNA expression is one of the crucial factors in the occurrence and development of OS. Among miRNAs, the expression level of serum miR-375 in patients with OS was significantly lower than that in healthy people, and OS patients with low miR-375 presented with clinically advanced tumours, large tumour volume, distant metastasis and poor chemotherapy efficacy[5]. Our research also showed that miR-375 expression in OS tissues was downregulated compared with that in normal bone tissues, and overexpressed miR-375 inhibited the migration and invasion of OS cells, which suggested that miR-375 may be a biomarker for the diagnosis and prognosis of OS. It is well known that miRNAs participate in gene transcription or posttranscriptional regulation by binding to the 3'UTR of potential targets. It was shown that miR-375 increased the sensitivity of OS cells to chemotherapy drugs by regulating the expression of Mcl-1[6]. miR-375 targeting ATG2B restrained autophagy and tumorigenesis in cisplatin-resistant OS cells[7]. miR-375 promoted proliferation by targeting the expression of RPBJ by sponging circular RNA circ_0060428 in OS cells[34]. Liu *et al*.[35] suggested that the circFAT1/miR-375/YAP1 axis regulated human OS cell growth, apoptosis, migration, invasion and tumorigenesis. In this study, we revealed that miR-375 expression was negatively correlated with MMP13 expression in patients with OS. However, whether miR-375 regulates migration and invasion by targeting the expression of MMP13 in OS cells has not been studied.

Based on the study by Osako *et al*.[9] showing that antitumour miR-375 markedly inhibited migration and invasion by regulating MMP13 expression in esophageal squamous cell carcinoma, we speculate that the regulation of migration and invasion by miR-375 is related to MMP13 expression. In this study, we found that miR-375 was downregulated in OS tissues, and low expression of miR-375 was associated with high expression of MMP13 in patients with OS. Meanwhile, it was confirmed that

overexpressed miR-375 weakened the malignant characteristics of OS cells, such as migration and invasion. To explore the specific underlying mechanism by which miR-375 regulates the biological behavior of OS cells, we demonstrated that miR-375 can regulate cell migration and invasion by targeting MMP13 through bioinformatics, dual luciferase assays and rescue experiments. These results will expand the mechanism of OS occurrence and development, and provide evidence for the treatment of OS by targeting miR-375.

4 Conclusion

miR-375 was downregulated in OS tissue, and overexpressed miR-375 attenuated the migration and invasion of OS cells by inhibiting MMP13 expression *in vitro*.

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miR-375靶向MMP13抑制骨肉瘤细胞的迁移和侵袭*

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摘要 **目的** 为了探究miR-375是否通过影响基质金属蛋白酶13（MMP13）的表达来调控骨肉瘤（osteosarcoma，OS）恶性特征。**方法** 用Lipofectamine 3000试剂盒将质粒、miRNA转染至骨肉瘤细胞和HEK293细胞中。实时定量聚合酶链反应（real-time quantitative PCR，RT-qPCR）检测OS患者和OS细胞中miR-375和MMP13的表达。蛋白质印迹法（Western blot）分析OS患者和OS细胞中MMP13蛋白的表达。双荧光素酶法分析miR-375与MMP13的靶向关系。伤口愈合和transwell实验分别分析OS细胞的迁移和侵袭。**结果** OS组织中miR-375的表达低于正常组织。MMP13在OS组织中表达上调。在OS患者中，MMP13的表达与miR-375呈负相关。与转染miRNA对照的OS细胞相比，转染miR-375模拟物OS细胞的迁移和侵袭明显被抑制。MMP13能部分逆转miR-375对OS细胞迁移和侵袭的抑制作用。**结论** 在OS细胞中，过表达miR-375通过调控MMP13的表达抑制细胞的迁移和侵袭。

关键词 骨肉瘤，miR-375，基质金属蛋白酶13，细胞迁移，细胞侵袭
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