



Regulatory role of microRNA184 in osteosarcoma cells

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ABSTRACT. Osteosarcoma is a highly malignant cancer that often appears in teenagers. It is the most frequently occurring primary bone tumor, and can easily metastasize, resulting in high mortality. MicroRNAs express abnormally in osteosarcoma, and may function as oncogenes or tumor suppressors. Recent studies showed that microRNA184 (miR-184) is abnormally expressed in multiple tumors, and is involved in tumor cell growth, differentiation, invasion, and metastasis. Nevertheless, the role of miR-184 in osteosarcoma cells remains unknown. We evaluated the expression and function of microRNA184 in osteosarcoma cells. SOSP-M osteosarcoma cells were divided into normal control, miR-184 mimic, and miR-184 inhibitor groups. Real-time PCR was applied to detect miR-184 expression. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to evaluate cell proliferation. Transwell assays were performed to detect changes in cell invasion ability. Compared with the control group, miR-184 expression was significantly increased in the miR-184 mimic group ($P < 0.05$). After miR-184 inhibitor transfection, miR-

184 expression was obviously reduced ($P < 0.05$). Tumor cell proliferation was enhanced in the miR-184 mimic group ($P < 0.05$), whereas miR-184 inhibition suppressed cell proliferation ($P < 0.05$). Furthermore, tumor cell invasion increased after miR-184 mimic transfection ($P < 0.05$), and decreased after inhibiting miR-184 ($P < 0.05$). MiR-184 promotes tumor cell proliferation and invasion, and may represent a new biological target for osteosarcoma.

Key words: miR-184; Osteosarcoma; Proliferation; Invasion

INTRODUCTION

Osteosarcoma mainly occurs during the bone growth stage. Thus, it is common in children and adolescents. It is highly malignant, and is the most common cause of primary bone tumors. Pulmonary metastasis occurs in early stages of osteosarcoma, causing high mortality. Osteosarcoma originates from the mesenchymal tissue, and is characterized by spindle stromal cells and osteoid tissue (Vijayakumar et al., 2014; Trosman and Krakovitz, 2015). No obvious clinical symptoms occur in the early phase of osteosarcoma. Rather, it is usually misdiagnosed as growing pains or trauma, since it occurs during bone growth. Osteosarcoma progresses and metastasizes quickly, and is resistant to chemotherapy. Due to its high mortality rate, it can cause great pain and heavy economic burden to the patient and family (Ebrahimzadeh et al., 2013; Sun et al., 2015).

Current research suggests that osteosarcoma is associated with abnormal gene expression. The pathogenesis of osteosarcoma, including development and metastasis, involves multiple genes (Garajova et al., 2014; Phuah and Nagoor, 2014). MicroRNAs (miRNAs), which widely exist in animals and plants, are non-coding, small, regulatory RNAs (Orang and Barzegari, 2014; Saadatian et al., 2014) that can negatively regulate gene expression. They function via complete or incomplete pairing with target genes, or by inhibiting the downstream transcription of target proteins, mRNA degradation, and protein translation (Gandhi et al., 2014). MiRNAs have numerous functions, affecting growth, metabolism, the endocrine system, hormone secretion, and embryonic stem cells, thereby regulating adaption to the environment. MiRNAs are closely associated with tumor occurrence and development. Some miRNAs can promote tumor proliferation and metastasis, whereas others inhibit tumorigenesis (Gallach et al., 2014; Lindner et al., 2015). Previous research showed that miRNAs are abnormally expressed in osteosarcoma, and may function as oncogenes or tumor suppressors. Recent studies suggest that microRNA184 (miR-184) is abnormally expressed in various tumors, and can regulate tumor cell growth, differentiation, invasion, and metastasis (Wong et al., 2009; Cui et al., 2014; Gao et al., 2014). Nevertheless, the role of miR-184 in osteosarcoma cells remains unclear. We evaluated the expression and function of miR-184 in osteosarcoma cells to identify new osteosarcoma markers and therapeutic targets.

MATERIAL AND METHODS

Instruments and reagents

The osteosarcoma cell line, SOSP-M, was bought from ATCC cell bank (Manassas, VA,

USA). Transwell chambers, Dulbecco's modified Eagle medium (DMEM) and penicillin-streptomycin were purchased from Hyclone (Logan, UT, USA). Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Co., Ltd. (Zhejiang, China). Trypsin-EDTA, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethylsulfoxide (DMSO) were obtained from Sigma (St. Louis, MO, USA). The miR-184 mimetic and inhibitor were synthesized by Genepharma Co. Ltd., (Shanghai, China). The RNA extraction kit, reverse transcription kit, and Lipo2000 were from Invitrogen (Grand Island, NY, USA). DNA amplification was performed using the PE Gene Amp PCR System 2400 (Foster City, CA, USA).

Methods

SOSP-M cell culture

SOSP-M cells were revived in a 37°C water bath. After centrifugation at 1000 rpm for 3 min, the cells were resuspended in 1 mL medium and maintained at 37°C in 5% CO₂. Cells (1 x 10⁷) were cultured in high glucose DMEM with 10% FBS and penicillin-streptomycin. Cells in the logarithmic growth phase were used for all experiments. The cells were divided into three groups: normal control, miR-184 mimic, and miR-184 inhibitor.

Cell transfection

MiR-184 mimic sequence: 5'-GGCAUUCUGUAUACAUCGGAG-3'; miR-184 inhibitor sequence: 5'-GAUCGGAGGUGCAUUCUA-3'. The miR-184 mimic and inhibitor were mixed with 200 µL serum free medium. After incubation at room temperature for 15 min, the medium was incorporated with Lipo2000 at room temperature for 30 min. After washing with PBS, the cells were incubated with medium and maintained at 37°C and 5% CO₂ for 6 h.

Real-time PCR

Total RNA was extracted from the cells using Trizol. The cDNA was synthesized using the reverse transcription kit. The primers used were designed by PrimerPremier 6.0 and synthesized by Sangon (Shanghai, China). The primers were as follows: miR-184 sense: 5'-TACGACTATGTGACCTGCCCTG-3'; anti-sense: 5'-TGGTTCAACTCTCCTTTCCA-3'; GAPDH sense: 5'-ATCTGGAGTTTACCGCTGG-3'; Anti-sense: 5'-TACCGATGTCTGGTAGACGAT-3'.

The cycling conditions were: 40 cycles of 60 s at 55°C, 30 s at 92°C, 45 s at 58°C, and 35 s at 72°C. Gene expression levels were quantified using an optimized comparative Ct ($\Delta\Delta C_t$) value method.

MTT assay

SOSP-M cells (5 x 10³) were plated in 96-well plates. After 24 h incubation, the cells were divided into three groups. MTT (20 µl) was added at different time points. After 4 h, MTT solution was removed and DMSO was added. The absorbance at 570 nm was read on a microplate reader (Synergy™ 2 Multi-Mode Microplate Reader, BioTek, Winooski, VT, USA). The experiments were performed in triplicate, and the cell proliferation rate was calculated.

Transwell assay

After 48 h transfection, cells were seeded in Transwell chambers coated with Matrigel. After 48 h incubation, the chambers were fixed with ice-cold ethanol and stained with crystal violet for 30 min. Cells that migrated to the lower side of the membrane were counted in 10 fields. The experiment repeated three times.

Statistical analysis

All statistical analyses were performed using SPSS13.0 software (Chicago, IL, USA). Numerical data were presented as the mean and standard deviation (\pm SD). Differences between means were analyzed using one-way ANOVA or paired t-test. P values less than 0.05 were considered statistically significant.

RESULTS

MiR-184 expression in SOSP-M cells

The miR-184 mimic and inhibitor were transfected to SOSP-M cells using Lipo2000. Real-time PCR was used to detect miR-184. Compared to normal control cells, miR-184 expression was significantly increased in the miR-184 mimic group ($P < 0.05$). However, miR-184 inhibitor expression obviously reduced miR-184 expression ($P < 0.05$) (Figure 1).

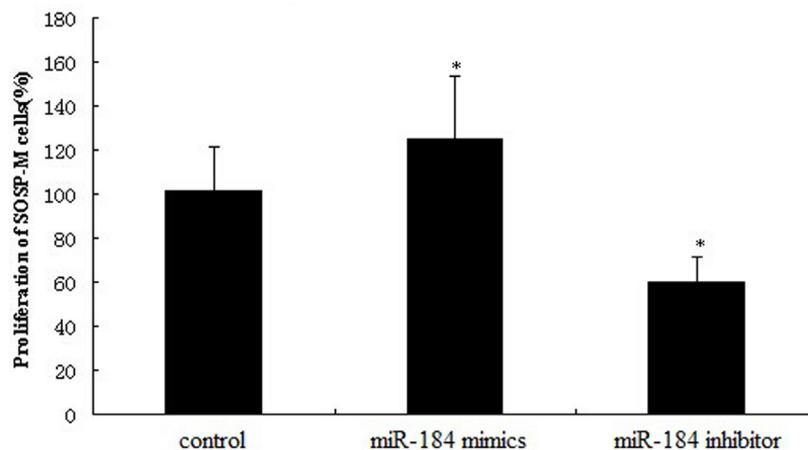


Figure 1. MiR-184 expression in SOSP-M cells. * $P < 0.05$ compared with control.

Effect of miR-184 on SOSP-M cell proliferation

The MTT assay was used to evaluate osteosarcoma cell proliferation. Tumor cell proliferation was enhanced in cells expressing the miR-184 mimic ($P < 0.05$), whereas miR-184 inhibition suppressed cell proliferation ($P < 0.05$) (Figure 2).

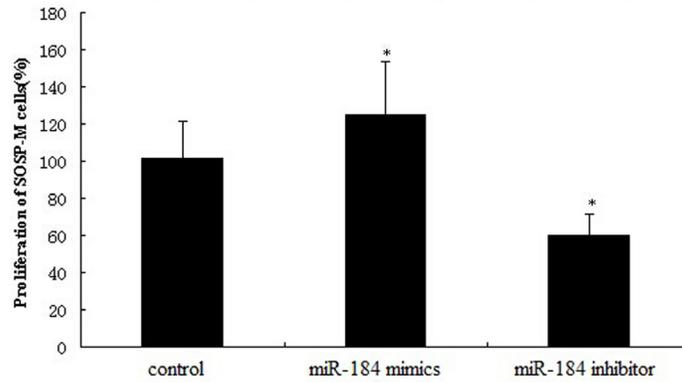


Figure 2. Effect of miR-184 on SOSP-M cell proliferation. *P < 0.05 compared with control.

Effect of miR-184 on SOSP-M cell invasion

A Transwell assay was performed to determine cell invasion. Tumor cell invasion increased after miR-184 mimic transfection (P < 0.05), and decreased after miR-184 was inhibited (P < 0.05) (Figures 3 and 4).

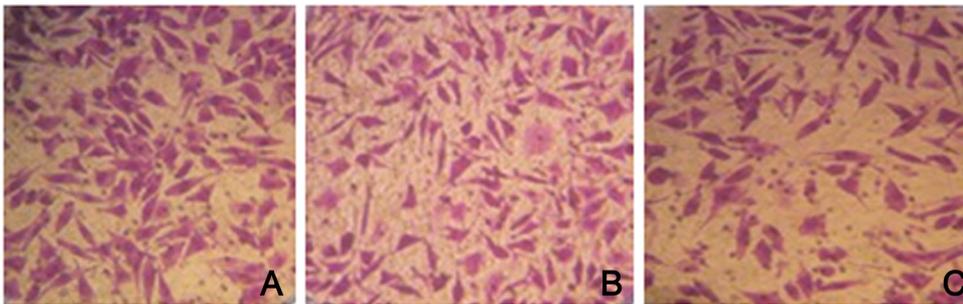


Figure 3. Effect of miR-184 on SOSP-M cell invasion.

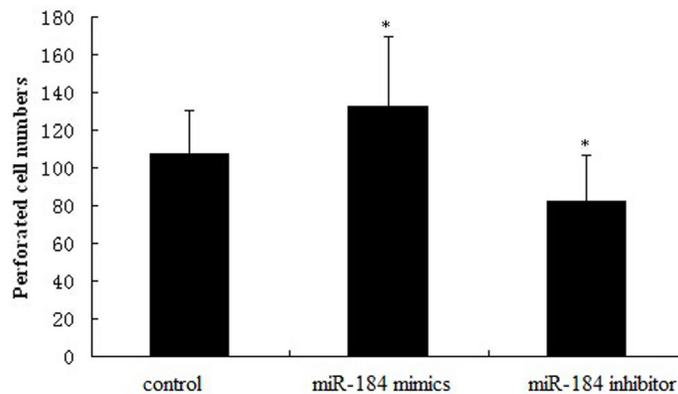


Figure 4. Effect of miR-184 on SOSP-M cell invasion. *P < 0.05 compared with control.

DISCUSSION

Osteosarcoma is a highly aggressive primary bone tumor that poses numerous problems to orthopedic and oncology doctors, due to its quick progression, frequent metastasis, lack of treatment efficacy, and poor clinical prognosis. Genetic abnormalities contribute to tumor development, including normal gene overexpression, mutation, or deficiency, or oncogene expression. Interactions between related genes and the immune system regulate tumor protein expression (Huang and Ouyang, 2015). MiRNAs were first discovered in *Caenorhabditis elegans*, as Lin-4 can regulate nematode sequential development. Subsequent studies identified miRNAs in rice, fruit flies, viruses, nematodes, mice, and humans. Currently, over 4500 miRNAs have been identified. MiRNAs can post-transcriptionally inhibit gene expression, based on sequence specificity. They regulate cell growth, proliferation, differentiation, metabolism, and apoptosis. In general, miRNAs are composed of 19-25 nucleotides. Science magazine considered the discovery of miRNA to be one of the top ten scientific advancements in 2002 and 2003 (Garajova et al., 2014; Luo et al., 2014). The relationship between miRNAs and tumors was first identified in chronic lymphocytic leukemia (CLL). MiRNA microarray detection suggested that miR-15a and miR-16-1 were decreased in CLL patients, as compared to healthy controls. MiRNA microarrays revealed that changes in miRNA expression depended on tumor type. MiR-155 and miR-21 may negatively regulate cancer-suppressor genes and the cell cycle to promote cancer pathogenesis, whereas let-7, miR-15/16, and miR-34 negatively regulate oncogenes to inhibit tumor development. MiRNA expression and biological function have been studied in various tumors. MiRNA microarrays have clarified their relationship with tumor occurrence, development, and differentiation, and in favor of tumor diagnosis and classification (Li et al., 2015; Pei et al., 2015).

In view of their important roles in tumors, miRNAs are widely considered a biomarker of hematological and tumor tissues. MiRNAs may participate in bone formation through Twist, Hedgehog, fibroblast growth factors (FGF), and bone morphogenetic proteins (BMP) signaling. MiRNAs are abnormally expressed during osteosarcoma pathogenesis. Multiple genes were upregulated or downregulated, as determined by miRNA microarray. MiRNA expression changes before and after tumor treatment can be used to detect chemotherapy sensitivity (Zhao et al., 2014; Xiao et al., 2015). MiR-184 is a novel miRNA, and its role in tumors remains unclear. Studies show that it is abnormally expressed in diverse tumors and may regulate tumorigenesis. MiR-184 was overexpressed in glioma, suggesting that it promotes glioma pathogenesis. Furthermore, miR-184 upregulation significantly promotes glioma invasion (Wong et al., 2009; Cui et al., 2014; Gao et al., 2014). However, its role in osteosarcoma remains unknown. This study suggested that miR-184 overexpression promotes SOSP-M cell proliferation, whereas miR-184 inhibition obviously impedes SOSP-M cell proliferation. Tumor cells invasiveness increased after microRNA184 mimic transfection and decreased after miR-184 inhibition. These data indicate that miR-184 is directly associated with osteosarcoma occurrence and development, and participates in osteosarcoma metastasis.

In conclusion, miR-184 participates in the regulation of osteosarcoma development and metastasis, and may represent a novel clinical marker for osteosarcoma diagnosis and therapeutic target for metastatic osteosarcoma. Further evaluation of its mechanism in osteosarcoma is required.

Conflicts of interest

The authors declare no conflict of interest.

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