European Review for Medical and Pharmacological Sciences 2018; 22: 7633-7639

MiR-155 affects osteosarcoma cell proliferation and invasion through regulating NF-κB signaling pathway

S. LU[1], Q.-S. LIAO[2], L. TANG[1]

[1]College of Optoelectronic Engineering, Health Science Center Shenzhen University, Shenzhen, Guangdong, China
[2]Institute of Psychology of the Chinese Academy of Sciences, Shenzhen, Guangdong, China

Abstract. – OBJECTIVE: Osteosarcoma can form tumor osteoid tissue and bone tissue directly or indirectly through cartilage stage. It mainly occurs in children and adolescents with high mortality. MicroRNA (miRNA) possesses tissue sensitivity as tumor biomarker and plays a promoting or inhibitory role in tumor pathogenesis as oncogene or tumor suppressor gene. It was found that miR-155 was abnormally expressed in tumor and could be treated as a biomarker for cancer progression. However, miR-155 expression in osteosarcoma and related mechanism still remains unclear. This study aimed to explore the role of miR-155 in osteosarcoma occurrence and development.

MATERIALS AND METHODS: Osteosarcoma cell line MG-63 was cultured *in vitro* and transfected by miR-155 mimics or inhibitor. MiR-155 expression was examined by Real-time PCR (RT-PCR). Cell proliferation was evaluated by MTT assay. Caspase 3 activity was determined by caspase 3 activity detection kit. Cell invasion was measured by transwell assay. B-cell lymphoma-2 (Bcl-2) and nuclear factor κB (NF-κB) protein expressions were assessed by Western blot.

RESULTS: MiR-155 mimics significantly up-regulated miR-155 expression, promoted MG-63 cell proliferation and invasion, inhibited caspase 3 activity, and up-regulated expressions of NF-κB and Bcl-2 compared with control group ($p < 0.05$). However, miR-155 inhibitor significantly inhibited MG-63 cell proliferation and invasion, enhanced caspase 3 activity, and reduced expressions of NF-κB and Bcl-2 compared with control group ($p < 0.05$).

CONCLUSIONS: MiR-155 affected osteosarcoma cell proliferation and apoptosis through regulating NF-κB signaling pathway, indicating it might be a new biomarker for osteosarcoma diagnosis and treatment.

Key Words:
miR-155, NF-κB, Osteosarcoma, Proliferation, Invasion.

Introduction

Osteosarcoma is the most common type of bone malignant tumor with high malignant degree[1]. Osteosarcoma is derived from mesenchymal tissue in distal femur or proximal humeral bone and can form tumor osteoid tissue and bone tissue directly or indirectly through cartilage stage[2,3]. Osteosarcoma frequently occurs in children and adolescents, accounting for more than 30% of primary bone tumor[4]. Rapid tumor cell proliferation and early stage hematogenous metastasis of osteosarcoma are the causes for high mortality rate[5,6]. At present, the amputation combined with adjuvant chemotherapy, radiotherapy, and bone reconstruction are the main treatment methods for osteosarcoma[7]. However, fast growth, high malignancy, easy to invasion, metastasis, and recurrence lead to poor prognosis, bring huge mental and economic burdens to the patients and their families[8,9]. The pathogenesis of osteosarcoma is complicated and has not been fully elucidated. Therefore, identification of the effective molecular targets for the pathogenesis of osteosarcoma is helpful to improve the treatment effects[10]. MicroRNA (MiRNA) is a type of small non-coding RNA that can inhibit downstream target protein translation or degrade mRNA through negative complementary binding[11,12]. MiRNA accounts for about 1-5% of all predicted genes, while each miRNA can regulate more than 200 target genes, suggesting that about 1/3 coding genes were regulated by miRNA[13,14]. As a tumor biomarker, miRNA possesses the tissue sensitivity and regulates malignant tumor growth as oncogene or tumor suppressor gene[15]. It was showed that miR-155 was abnormally expressed in tumor, suggesting it might be a tumor biomarker[16]. MiR-155 was found up-regulated in multiple cancers, such as lung cancer and breast cancer[17,18]. However, miR-155 ex-

Corresponding Author: Li Tang, Ph.D; e-mail: f135155797999113@sina.com

pression and related mechanism in osteosarcoma have not been fully clarified. This study aimed to explore the role of miR-155 in the occurrence and development of osteosarcoma.

Materials and Methods

Main Reagents and Instruments

Osteosarcoma cell line MG-63 (ATCC® CRL-1427™) was provided by American Type Culture Collection Cell Bank (ATCC, Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM) medium, fetal bovine serum (FBS), ethylene diamine tetraacetic acid (EDTA), and penicillin-streptomycin were purchased from HyClone (South Logan, UT, USA). Dimethylsulfoxide (DMSO) and 3-(4,5)- dimethylthiazol-(-z-yl)-3,5-di-phenytetrazoliumromide (MTT) were obtained from Gibco (Grand Island, NY, USA). Trypsin-EDTA was got from Sigma-Aldrich (St. Louis, MO, USA). Polyvinylidene difluoride (PVDF) membrane was derived from Pall Life Sciences (Covina, CA, USA). Western blot related reagents were provided by Beyotime Biotech. (Shanghai, China). Enhanced chemiluminescence (ECL) reagent was obtained from Amersham Biosciences (Piscataway, NJ, USA). Rabbit anti-human nuclear factor *κB* (NF-κB) monoclonal antibody (1:3000), B-cell lymphoma-*2 (*Bcl-2) monoclonal antibody (1:3000), and mouse-anti rabbit horseradish peroxidase (HRP) labeled IgG secondary antibody were provided by Cell Signaling Technology (Danvers, MA, USA). Transwell chamber was obtained from Corning (Corning, NY, USA). RNA extraction kit, reverse transcription kit, and lipo2000 were purchased from Invitrogen/Life Technologies (Carlsbad, CA, USA). Taqman MiRNA reverse transcription kit was got from Pierce (Rockford, IL, USA). MiR-155 mimics, miR-155 inhibitor, and negative control were synthesized by Genepharma (Shanghai, China). Other reagents were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). Benchtop was purchased from Sutai High-tech Materials Co. Ltd. (Shanghai, China). Equipment Engineering Co. Ltd (Suzhou, Jiangsu, China). Thermo Scientific Forma incubator was provided by Pierce (Rockford, IL, USA). ABI 7700 Fast PCR amplifier was derived from ABI (Foster City, CA, USA).

Methods

MG-63 Cell Culture and Grouping

MG-63 cells were unfrozen at 37°C and centrifuged at 1000 r/min for 3 min. Then, the cells were re-suspended in 1 ml Dulbecco's Modified Eagle Medium (DMEM) medium and cultured at 37°C with 5% CO_2 for 24-48 h. Next, the cells were seeded in dish at a density of $1\times10^6/cm^2$ in high-glucose DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells in logarithmic phase were used for the following experiments. MG-63 cells were randomly divided into four groups, miR-155 mimics, mimics normal control (NC), inhibitor NC, and miR-155 inhibitor group. This study was approved by the Ethical Committee of College of Optoelectronic Engineering, Health Science center Shenzheng University (Shenzhen, Guangdong, China).

MiR-155 Mimics and Inhibitor Transfection

MiR-155 mimics and inhibitors were transfected to MG-63 cells. miR-155 mimics, 5'-GAUAGUUCGGUGUGCACA-3'. miR-155 inhibitor, 5'-CGGAUAUGUGCAGUGCUA-3'. miR-155 mimicsNC, 5'-AUGGUCGUUAAGCCAGUG-3'. miR-155 inhibitor NC, 5'-AGGCAGUGUCGUCAAUUG-3'. MG-63 cells in logarithmic phase were seeded in 6-well plate. A total of 5 μl lipo2000, miR-155 mimics, or miR-155 inhibitor were added to 200 μl serum-free medium at room temperature for 15 min, respectively. Next, they were mixed and incubated at room temperature for 30 min. When the cell fusion reached 70-80%, they were used to transfect cells at 37°C with 5% CO_2 for 6 h. The medium was changed for further cultivation.

Real-Time PCR

Total RNA was extracted from hippocampus tissue using TRIzol reagent and reversely transcribed to complementary DNA (cDNA). The primers were designed using PrimerPremier 6.0 software and synthesized by Invitrogen/Life Technologies (Carlsbad, CA, USA). The primer sequences were listed in Table I. Real-time PCR was performed at 55°C for 1 min, followed by 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. GAPDH was selected as internal reference. The relative expression of mRNA was calculated by $2^{-\Delta Ct}$ method.

MTT assay

MG-63 cells in logarithmic phase were seeded in 96-well plate at a density of 5×10^3/well for 24 h. 20 μl MTT was then added into the plate for 4 h every 24 h. After that, 150 μl dimethylsulfoxide (DMSO) was

Table I. Primer sequences.

Gene	Forward 5′-3′	Reverse 5′-3′
GAPDH	AGTACCAGTCTGTTGCTGG	TAATAGACCCGGATGTCTGGT
mir-155	CCCCACAGTCTACTGTAAG	GCATTGCCGATGGTACTGATT

added into the plate for 10 min followed by measuring the OD value at a wavelength of 570 nm. Each experiment was repeated for at least three times.

Transwell Assay

The cells were further cultured for 24 h at 48 h after transfection. 50 mg/l Matrigel was used to coat the bottom of transwell chamber at 1:5. Next, 50 µl serum free medium containing 10 g/l BSA were added to the upper chamber at 37°C for 30 min. The chamber was put into the 24-well plate. A total of 500 µl DMEM medium containing 10% FBS was added to the lower chamber, while 100 µl tumor cell suspension were added to the upper chamber with serum free medium. After 48 h, the chamber was washed by phosphate-buffered saline (PBS) and fixed by absolute alcohol. At last, the membrane was stained by crystal violet for 30 min and observed under the microscope. Each experiment was repeated for three times.

Western Blot

The MG-63 cells were added with RIPA and cracked on ice for 15-30 min. Next, the tissues were treated by ultrasound at 5 s for 4 times and centrifuged at 10000 × g for 15 min. The protein was transferred to new Eppendorf (EP) tube and stored at -20°C. The protein was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane at 100 mA for 1.5 h. After blocked by 5% skim milk for 2 h, the membrane was incubated in Bcl-2 monoclonal antibody (dilution at 1:1000) and NF-κB monoclonal antibody (1:200) at 4°C overnight. Then the membrane was incubated in goat anti rabbit secondary antibody (1:2000) at room temperature for 30 min. Next, the membrane was treated by developer for 1 min and exposed to observe the result. The film was scanned by Quantity One software and analyzed by protein image processing system. Each experiment was repeated for four times.

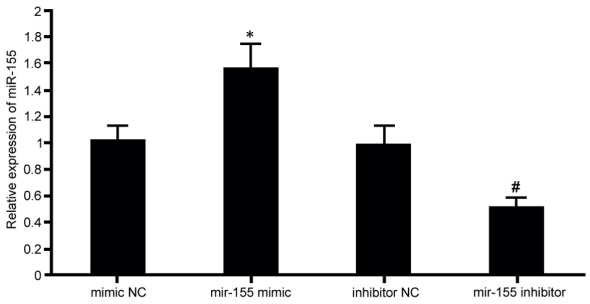

Figure 1. The impact of miR-155 transfection on miR-155 expression in osteosarcoma cells. $^{*}p < 0.05$, compared with mimic NC; $^{\#}p < 0.05$, compared with inhibitor NC.

Caspase 3 Activity Detection

Caspase 3 activity was tested according to the manual. The cells were digested by trypsin and centrifuged at 600×g and 4°C for 5 min. Next, the cells were added with 2 mM Ac-DEVD-pNA and detected at 405 nm to calculate caspase 3 activity.

Statistical Analysis

All data were presented as mean ± standard deviation (SD) and compared by Student's *t*-test or one-way analysis of variance (ANOVA) with Newman-Keuls multiple comparison post-hoc analysis. All data analyses were performed on SPSS11.5 software (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was depicted as statistical significance.

Results

Effects of miR-155 Transfection on miR-155 Expression in Osteosarcoma Cells

Real-time PCR was applied to test miR-155 expression in osteosarcoma cells transfected with miR-155 mimics and inhibitor. MiR-155 mimics transfection significantly upregulated miR-155 expression in osteosarcoma cells compared with control ($p < 0.05$). Whereas, miR-155 inhibitor transfection effectively suppressed miR-155 level compared with control ($p < 0.05$, Figure 1).

Effects of miR-155 on Osteosarcoma Cell Proliferation

MTT assay was adopted to test the impact of miR-155 on osteosarcoma cell proliferation. MiR-155 mimics transfection significantly pro-

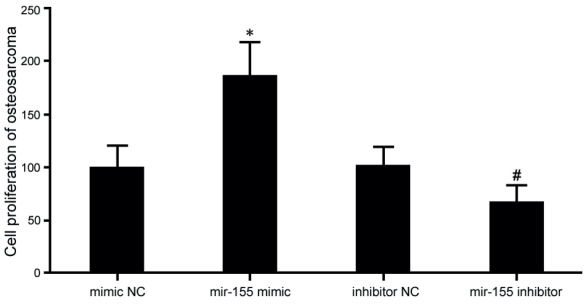

Figure 2. The impact of miR-155 on osteosarcoma cell proliferation. $^{*}p < 0.05$, compared with mimic NC; $^{\#}p < 0.05$, compared with inhibitor NC.

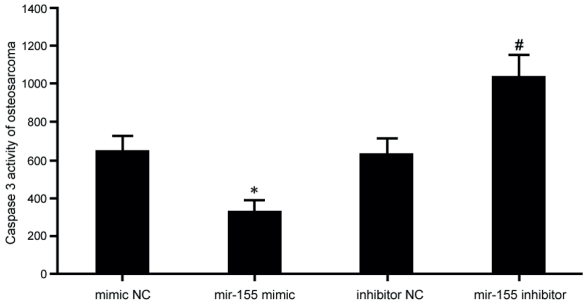

Figure 3. The influence of miR-155 on Caspase 3 activity in osteosarcoma cells. $^{*}p < 0.05$, compared with mimic NC; $^{\#}p < 0.05$, compared with inhibitor NC.

moted osteosarcoma cell proliferation compared with control ($p < 0.05$). MiR-155 inhibitor transfection significantly inhibited osteosarcoma cell proliferation compared with control ($p < 0.05$, Figure 2).

Effects of miR-155 on caspase 3 Activity in Osteosarcoma Cells

MiR-155 mimics transfection significantly decreased caspase 3 activity in osteosarcoma cells compared with control ($p < 0.05$). MiR-155 inhibitor transfection significantly increased caspase 3 activity in osteosarcoma cells compared with control ($p < 0.05$, Figure 3).

Effects of miR-155 on Osteosarcoma Cell Invasion

Transwell assay was selected to analyze the effect of miR-155 on osteosarcoma cell invasion. MiR-155 mimics transfection significantly accelerated osteosarcoma cell invasion compared with control ($p < 0.05$). MiR-155 inhibitor transfection attenuated osteosarcoma cell invasion compared with control ($p < 0.05$, Figure 4).

Effects of miR-155 on Bcl-2 Expression in Osteosarcoma Cells

Western blot was adopted to analyze the impact of miR-155 on Bcl-2 protein expression in

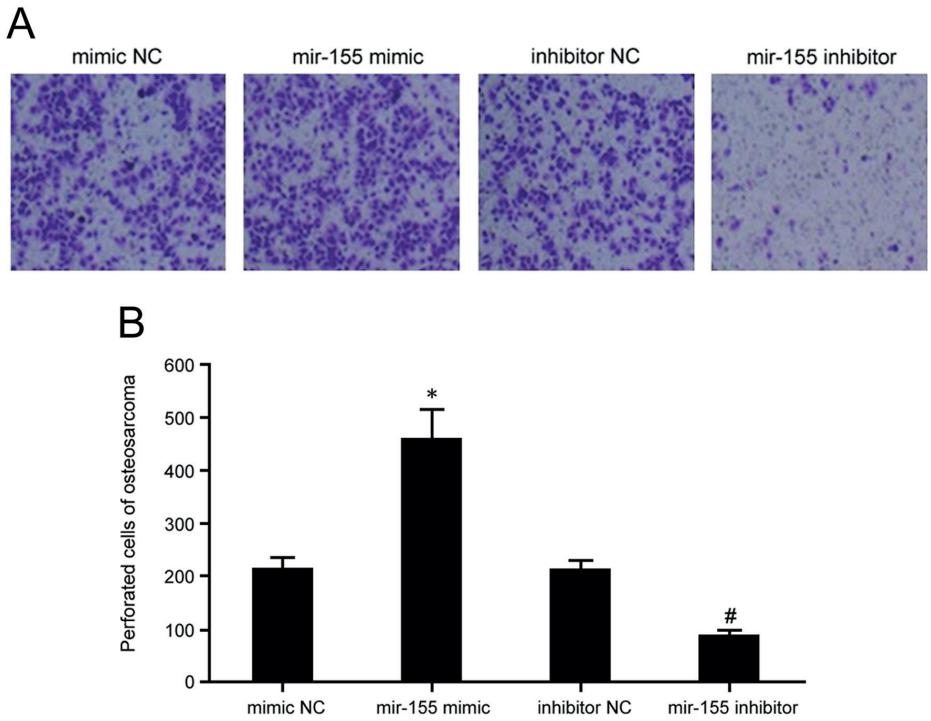

Figure 4. The effect of miR-155 on osteosarcoma cell invasion. ***A,*** Transwell assay detection of cell invasion. ***B,*** Cell invasive ability analysis. $^{*}p < 0.05$, compared with mimic NC; $^{\#}p < 0.05$, compared with inhibitor NC.

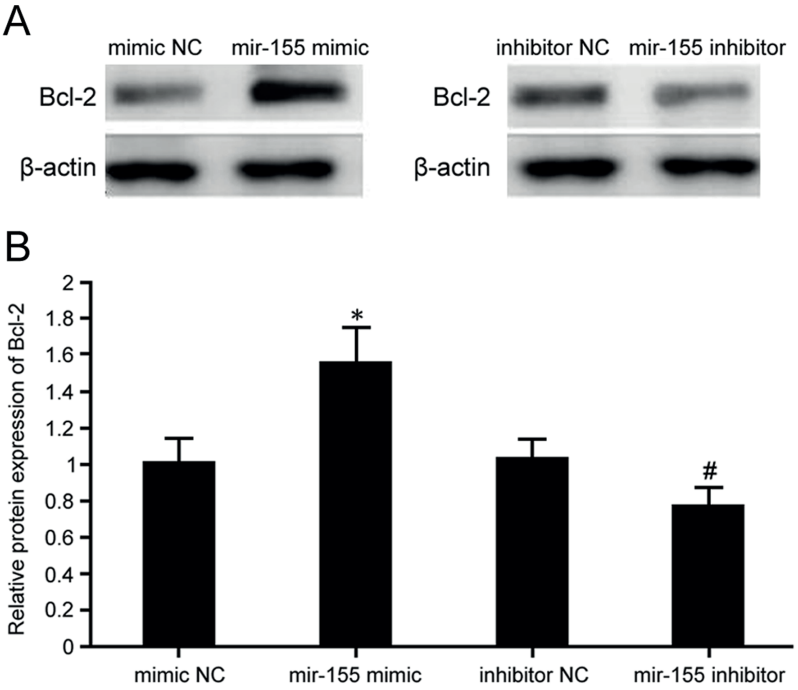

Figure 5. The impact of miR-155 on Bcl-2 protein expression in osteosarcoma cells. A, Western blot detection of Bcl-2 protein expression. B, Bcl-2 expression analysis. $^{*}p < 0.05$, compared with mimic NC; $^{\#}p < 0.05$, compared with inhibitor NC.

osteosarcoma cells. MiR-155 mimics transfection significantly upregulated Bcl-2 protein expression in osteosarcoma cells compared with control ($p < 0.05$). MiR-155 inhibitor transfection significantly inhibited Bcl-2 protein expression in osteosarcoma cells compared with control ($p < 0.05$, Figure 5).

Effects of miR-155 on NF-κB Expression in Osteosarcoma Cells

Western blot was used to analyze the impact of miR-155 on NF-κB protein expression in osteosarcoma cells. MiR-155 mimics transfection markedly promoted NF-κB protein expression in osteosarcoma cells compared with control ($p < 0.05$). MiR-155 inhibitor transfection apparently suppressed NF-κB protein expression in osteosarcoma cells compared with control ($p < 0.05$, Figure 6).

Discussion

Osteosarcoma is featured as high morbidity and poor prognosis in bone malignant tumor. The mortality of osteosarcoma attracts much attention. In spite of improved treatment methods, the survival rate of osteosarcoma has not been substantially increased[19,20]. As small molecule nucleotides, miRNAs play important roles in cell proliferation, differentiation, apoptosis, immune response[21,22]. MiRNA expression is regulated by a variety of factors, including transcription level, pathological state, and environments. Therefore, miRNA expression and regulation are affected in different tissue cells even in the same tissue cells[23]. MiRNA is closely associated with disease type, thus can be treated as important targets for disease diagnosis and prognosis[24]. Our findings showed that miR-155 mimics transfection promo-

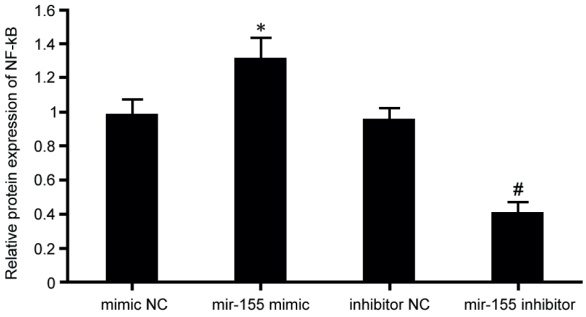

Figure 6. The influence of miR-155 on NF-κB expression in osteosarcoma cells. ***A,*** Western blot detection of NF-κB protein expression. ***B,*** NF-κB expression analysis. $^{*}p < 0.05$, compared with mimic NC; $^{\#}p < 0.05$, compared with inhibitor NC.

ted osteosarcoma cell proliferation and invasion, while miR-155 inhibitor transfection suppressed cell proliferation and invasion, which was consistent with previous studies on its role in breast cancer and lung cancer[17,18], further supporting a promoting role of miR-155 in osteosarcoma. Further mechanism analysis revealed that miR-155 mimics transfection inhibited caspase 3 activity and facilitated NF-κB and Bcl-2 expressions, whereas miR-155 inhibitor transfection enhanced caspase 3 activity and inhibited NF-κB and Bcl-2 expressions. As a nuclear transcriptional factor, NF-κB participates in multiple physiological processes, such as inflammation and immune response. NF-κB signaling pathway plays a critical role in tumor occurrence and development[25]. MiR-155 affects tumor cell apoptosis by regulating NF-κB. Bcl-2 is an anti-apoptotic protein that regulates caspase 3 activity. As a member of Caspase family, caspase 3 is the executor of apoptosis that induces tumor cell apoptosis[26,27]. Thus, this study confirmed that down-regulation of miR-155 inhibited osteosarcoma cell proliferation and induced cell apoptosis through NF-κB signaling pathway, leading to reduced Bcl-2 expression and decreased caspase 3 activity.

Conclusions

We showed that miR-155 affected osteosarcoma cell proliferation and apoptosis through regulating NF-κB signaling pathway, indicating it might be a new biomarker for osteosarcoma diagnosis and treatment.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) 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.
2) Rudnick-Glick S, Corem-Salkmon E, Grinberg I, Margel S. Targeted drug delivery of near ir fluorescent doxorubicin-conjugated poly (ethylene glycol) bisphosphonate nanoparticles for diagnosis and therapy of primary and metastatic bone cancer in a mouse model. J Nanobiotechnology 2016; 14: 80.
3) Wu CC, Huang YF, Hsieh CP, Chueh PJ, Chen YL. Combined use of zoledronic acid augments ursolic acid-induced apoptosis in human osteosarcoma cells through enhanced oxidative stress and autophagy. Molecules 2016; 21: pii: E1640.
4) Lopez C, Correa A, Vaporciyan A, Austin M, Rice D, Hayes-Jordan A. Outcomes of chest wall resections in pediatric sarcoma patients. J Pediatr Surg 2017; 52: 109-114.
5) Echchikhi Y, Loughlimi H, Touil A, Kebdani T, Benjaafar N. Radiation-induced osteosarcoma of the skull base after radiation therapy in a patient with nasopharyngeal carcinoma: a case report and review of the literature. J Med Case Rep 2016; 10: 334.
6) Dong CH, Du QY, Wang ZM, Wang Y, Wu SY, Wang AM. Microrna-665 suppressed the invasion and metastasis of osteosarcoma by directly inhibiting rab23. Am J Transl Res 2016; 8: 4975-4981.
7) Hlavaty J, Wolfesberger B, Hauck M, Obermayer-Pietsch B, Fuchs-Baumgartinger A, Miller I, Walter I. Ezrin and moesin expression in canine and feline osteosarcoma. Histol Histopathol 2017; 32: 805-816.
8) Zhan C, Li C, Zhang H, Tang H, Ji F, Qiao SC, Xu WD, Wang ZW. Microrna-150 upregulation reduces osteosarcoma cell invasion and metastasis by downregulating ezrin. Oncol Lett 2016; 12: 3457-3462.
9) Engel N, Adamus A, Schauer N, Kuhn J, Nebe B, Seitz G, Kraft K. Synergistic action of genistein and calcitriol in immature osteosarcoma mg-63 cells by sgpl1 up-regulation. PLoS One 2017; 12: E0169742.
10) Leichter AL, Sullivan MJ, Eccles MR, Chatterjee A. Microrna expression patterns and signalling pathways in the development and progression of childhood solid tumours. Mol Cancer 2017; 16: 15.
11) Chen JJ, Gao SM, Wang CJ, Wang ZG, Zhang HX, Huang KT, Zhou B, Li HY, Yu ZJ, Wu JB, Chen CS. Pathologically decreased expression of mir-193a contributes to metastasis by targeting wt1-e-cadherin axis in non-small cell lung cancers (vol 35, pg 173, 2016). J Exp Clin Cancer Res 2017; 36: 31.
12) Scano A, Ratto D, Occhinegro A, Pedroncelli A, Rossi P. Microrna-552 in colorectal cancer with poor prognosis. Its role as a novel molecular biomarker. Eur Rev Med Pharmacol Sci 2018; 22: 1171-1174.
13) Bi MJ, Chen W, Yu HM, Wang JX, Ding F, Tang DJ, Tang CY. Mir-543 is up-regulated in gefitinib-resistant non-small cell lung cancer and promotes cell proliferation and invasion via phosphatase and tensin homolog. Biochem Biophys Res Comm 2016; 480: 369-374.
14) Alipoor SD, Adcock IM, Garssen J, Mortaz E, Varahram M, Mirsaeidi M, Velayati A. The roles of mirnas as potential biomarkers in lung diseases. Eur J Pharmacol 2016; 791: 395-404.
15) Sun CC, Li SJ, Yuan ZP, Li DJ. Microrna-346 facilitates cell growth and metastasis, and suppresses cell apoptosis in human non-small cell lung cancer by regulation of xpc/erk/snail/e-cadherin pathway. Aging (Albany NY) 2016; 8: 2509-2524.
16) Han JG, Jiang YD, Zhang CH, Yang YM, Pang D, Song YN, Zhang GQ. A novel panel of serum mir-

21/mir-155/mir-365 as a potential diagnostic biomarker for breast cancer. Ann Surg Treat Res 2017; 92: 55-66.

17) Fernandez C, Bellosillo B, Ferraro M, Seoane A, Sanchez-Gonzalez B, Pairet S, Pons A, Barranco L, Vela MC, Gimeno E, Colomo L, Besses C, Navarro A, Salar A. Micrornas 142-3p, mir-155 and mir-203 are deregulated in gastric malt lymphomas compared to chronic gastritis. Cancer Genomics Proteomics 2017; 14: 75-82.

18) Cao SZ, Wang YZ, Li JQ, Lv ML, Niu HT, Tian Y. Tumor-suppressive function of long noncoding rna malat1 in glioma cells by suppressing mir-155 expression and activating fbxw7 function. Am J Cancer Res 2016; 6: 2561-2574.

19) Seiter M, Al Maaieh M, Rosenberg A, Conway S. Primary osteosarcoma of the bone with rhabdoid features: a rare, previously undescribed primary malignant tumor of bone. Case Rep Surg 2016; 2016: 5901769.

20) Ando T, Kudo Y, Iizuka S, Tsunematsu T, Umehara H, Shrestha M, Matsuo T, Kubo T, Shimose S, Arihiro K, Ogawa I, Ochi M, Takata T. Ameloblastin induces tumor suppressive phenotype and enhances chemosensitivity to doxorubicin via src-stat3 inactivation in osteosarcoma. Sci Rep 2017; 7: 40187.

21) Bilbao-Aldaiturriaga N, Patino-Garcia A, Martin-Guerrero I, Garcia-Orad A. Cytotoxic t lymphocyte-associated antigen 4 rs231775 polymorphism and osteosarcoma. Neoplasma 2017; 64: 299-304.

22) Li Y, Liu J, Liu ZZ, Wei WB. Microrna-145 inhibits tumour growth and metastasis in osteosarcoma by targeting cyclin-dependent kinase, cdk6. Eur Rev Med Pharmacol Sci 2016; 20: 5117-5125.

23) Li X, Sun X, Wu J, Li Z. Microrna-613 suppresses proliferation, migration and invasion of osteosarcoma by targeting c-met. Am J Cancer Res 2016; 6: 2869-2879.

24) Fei D, Zhao K, Yuan H, Xing J, Zhao D. Microrna-187 exerts tumor-suppressing functions in osteosarcoma by targeting zeb2. Am J Cancer Res 2016; 6: 2859-2868.

25) Cheng S, Zhang X, Huang N, Qiu Q, Jin Y, Jiang D. Down-regulation of s100a9 inhibits osteosarcoma cell growth through inactivating mapk and nf-kappab signaling pathways. BMC Cancer 2016; 16: 253.

26) Yi WR, Li ZH, Qi BW, Ernest MER, Hu X, Yu AX. Downregulation of idh2 exacerbates the malignant progression of osteosarcoma cells via increased nf-kappa b and mmp-9 activation. Oncol Rep 2016; 35: 2277-2285.

27) Wang Y, Zhao YR, Zhang AY, Ma J, Wang ZZ, Zhang X. Targeting of miR-20a against CFLAR to potentiate TRAIL-induced apoptotic sensitivity in HepG2 cells. Eur Rev Med Pharmacol Sci 2017; 21: 2980.