European Review for Medical and Pharmacological Sciences 2019; 23: 10042-10048

Effects of lncRNA HOTAIR on proliferation and apoptosis of myeloma cells through NF-κB pathway

B.-Z. ZHU[1], L. LIN[2]

[1]Department of Laboratory Medicine, The Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, China
[2]Department of Neurobiology, School of Basic Medical Sciences, Shanxi Medical University, Taiyuan, Shanxi, China

Abstract. – OBJECTIVE: The aim of this study was to explore the influences of long non-coding ribonucleic acid (lncRNA) homeobox transcript antisense intergenic RNA (HOTAIR) on the proliferation and apoptosis of myeloma cells and its molecular mechanism.

MATERIALS AND METHODS: The myeloma cells were randomly divided into three groups, including: group A (myeloma cell group), group B [HOTAIR-small-interfering RNA (siRNA) group], and group C (HOTAIR negative control group). The Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was performed to detect the expression of lncRNA HOTAIR in myeloma cells. The flow cytometry was adopted to determine the apoptosis of myeloma cells. Meanwhile, the protein expression of nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-κB) was detected via Western blotting. In addition, the activity of the myeloma cells was measured using methyl thiazolyl tetrazolium (MTT) assay.

RESULTS: The expression of HOTAIR in group A, group B, and group C was (2.19 ± 0.33), (1.37 ± 0.25), and (2.51 ± 0.27), respectively. Compared with group A and group C, the expression of HOTAIR was significantly downregulated in group B, in which the cells interfered with siRNAs. The expression of HOTAIR was significantly higher in group C than group A and group B ($p<0.05$). HOTAIR expression reached the highest level in group C, followed by group A and group B, respectively. The results of MTT indicated that the activity of the myeloma cells significantly increased at 24 h, 48 h, and 72 h in group C when compared with group A and group B. However, the activity of the myeloma cells was relatively low in group B, showing a slow rising trend. The activity of myeloma cells in group A remarkably increased when compared with group B, but it was lower than group C. Furthermore, the activity of the myeloma cells was significantly higher in group C than group B ($p<0.05$). Western blotting indicated that the protein expression of NF-κB in group A, group B, and group C was (0.79 ± 0.22), (0.51 ± 0.17), and (0.95 ± 0.31), respectively. Compared with group A and group C, the protein expression of NF-κB was significantly downregulated in group B (the cells interfered with siRNAs) ($p<0.05$). Meanwhile, the protein expression of NF-κB was markedly higher in group C than in group A and group B ($p<0.05$). The protein expression of NF-κB was the highest in the cells of group C. Flow cytometry demonstrated that the apoptosis rate in group A, group B, and group C was (9.57 ± 1.71), (20.33 ± 1.63), and (8.74 ± 1.23), respectively. Compared with group A and group C, the apoptosis was significantly elevated in group B, in which the cells interfered with siRNAs ($p<0.05$). Compared with group A, the number of apoptotic myeloma cells significantly decreased in group C ($p<0.05$).

CONCLUSIONS: LncRNA HOTAIR activates the expression of NF-κB in myeloma cells and promotes the proliferation of myeloma cells.

Key Words:
LncRNA HOTAIR, NF-κB, Myeloma cells, Proliferation and apoptosis.

Introduction

Multiple myeloma (MM) is one of the most common malignant tumors in the blood system[1,2]. The decreased activity of osteoma cells and the changes of the hematopoietic microenvironment are the primary causes of myeloma. Current studies have found that the major components in hematopoietic micro-environment of bone marrow are bone marrow mesenchymal stem cells (BMMSCs)[3,4].

Nuclear factor-kappa-light-chain-enhancer of activated B (NF-κB) is one of the most important

Corresponding Author: Lin Li, MD; e-mail: zbz5686@126.com

factors in nuclear transcription. NF-κB has been found involved in the apoptosis of many cancer cells and inflammatory responses[5,6]. Previous studies have demonstrated that the continuous activation of NF-κB participates in the progression of myeloma. Meanwhile, its importance in the pathogenesis of the myeloma patients has been discovered. These findings may provide a reasonable development basis for MM treatment by inhibiting the activity of NF-κB pathway[7,8]. In recent years, significant progress has been achieved in the research of molecular biology. Moreover, studies on myeloma[9,10] have begun to develop towards cytogenetics, molecular biological detection, and etc..

As a long non-coding ribonucleic acid (lncRNA), the homeobox transcript antisense intergenic RNA (HOTAIR) plays a significant role in regulating the expression of normal and cancer cells. Meanwhile, it can inhibit or promote the proliferation of tumor cells[11,12]. LncRNA HOTAIR has gradually become a new marker for assessing malignant tumors. HOTAIR is the first trans-acting lncRNA that has been first discovered in human fibroblasts[13,14]. Several authors have investigated the potential role of HOTAIR in malignancies. For example, the overexpression of HOTAIR is presented in numerous tumor cells or tissues. The upregulated expression of HOTAIR may increase the resistance of cancer cells to drugs[15,16]. In the present study, the myeloma cells were transfected with lncRNA HOTAIR-small-interfering RNA (siRNA) interference vectors, and different groups were established *in vitro*. The aim of this study was to explore the correlation between the expression of lncRNA HOTAIR and NF-κB in myeloma cells.

Materials and Methods

Cell Processing and Culture

Myeloma cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were sub-cultured in Roswell Park Memorial Institute-1640 (RPMI-1640, Hyclone, South Logan, UT, USA) medium containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% cyano-streptomycin in an incubator with 5% CO_2 at 37°C. When the confluence of cells reached 80-90%, they were centrifuged and passaged.

Main Reagents and Instruments

The main reagents and instruments used were: TRIzol (Invitrogen, Carlsbad, CA, USA) reagent and reverse transcription kit (Jiangsu Kejing Company, Nanjing, China), quantitative-Polymerase Chain Reaction (PCR) instrument (Beijing Keyu Technology Company, Beijing, China), FACS Calibur flow cytometer (Beijing DongXun Company, Beijing, China), HOTAIR-siRNA, and negative control (Shanghai Gemma Company, Shanghai, China).

Cell Transfection

The cells were impacted with 2 mg/mL DDP every 2 weeks during the routine culture. The cells in the logarithmic growth phase were inoculated into 6-well plates, with 1×10^6 cells per well. When the confluence reached 60-70%, HOTAIR-siRNA and the negative control sequences were transfected into the myeloma cells according to the instructions of Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). The primer sequences were shown in Table I. 48 h after successful transfection, the cells were collected and divided into 3 groups, including: group A (myeloma cell group), group B (HOTAIR-siRNA group), and group C (HOTAIR negative control group).

Detection of Expression of LncRNA HOTAIR in Myeloma Cells Via Reverse Transcription (RT)-PCR

The total RNA in the cells was extracted using the TRIzol (Invitrogen, Carlsbad, CA, USA) one-step method. Subsequently, extracted RNA was reverse transcribed into complementary deoxyribonucleic acids (cDNAs) according to the instructions. The expression level of HOTAIR was detected with DNA fluorochrome SYBR Green I. β-actin was used as an internal reference. The

Table I. Primer sequences.

Gene	Sequence	
HOTAIR	SiRNA	5'-UUAAGUCUAG-GAAUGAGCACGAAGC-3'
	Negative control	5'-UUCUCCGAACGUGUCACGUTT-3'

Table II. Primer sequences.

Primer	RT	Sequence
HOTAIR	R	5'-CATGGATCCACATTCTGCCCTGATTTCCGGAACC-3'
	F	5'-ACTCTCGAGCCACACACACACACACACCTCAC-3'
β-actin	R	5'-GAG AGG GAA ATC GTG CGT GAC-3'
	F	5'-GACGTAGCACAGCTTCTCCTTAATG-3'

specific PCR conditions were: 60°C for 10 min, 95°C, and 72°C for 30 s, respectively, and 95°C for 5 min, for a total of 40 cycles. The relative expression level of the target gene was expressed by the $2^{-\Delta\Delta Ct}$ method. The experiment was performed for at least 3 times. The relative gene expression was finally calculated. The primer sequences used in this study were shown in Table II.

Apoptosis of Myeloma Cells Via Flow Cytometry

Changes in the apoptosis of the myeloma cells were detected via flow cytometry. At 2 d after transfection, the cells were washed with Phosphate-Buffered Saline (PBS) for 2 times. After centrifugation for 3-5 min, the cells were re-suspended with 100 μL of 1× binding buffer. Annexin V was labeled with 5 μL of fluorescein isothiocyanate (FITC) and mixed with 5 μL of propidium iodide (PI) for staining. After incubation in the dark for 15 min, the cells were added with 400 μL of 1× binding buffer, followed by washing with PBS for 3 times. Finally, the apoptosis of the myeloma cells was determined.

Changes in the Protein Expression of NF-κB in Myeloma Via Western Blotting

The total protein in the tissues was extracted, and its concentration was determined by the Bradford method. Subsequently, the protein samples were separated by 100 V dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After sealing with 10% goat serum for 0.5 h, the membranes were incubated with primary antibodies at 4°C overnight. On the next day, the membranes were incubated with the corresponding secondary antibody at room temperature for 1 h. Then, the membranes were washed with TBST at room temperature for 3 times. The Quantity One 4.0 software (Bio-Rad, Hercules, CA, USA) was adopted to analyze the protein expression of NF-κB. β-actin was used as an internal reference.

Activity of Myeloma Cells Via Methyl Thiazolyl Tetrazolium (MTT) Assay

The myeloma cells were first centrifuged for 3-5 min after transfection. Then, the cells were inoculated into 96-well plates (200 μL/well) and cultured in an incubator at 37°C with 5% CO_2. The duration of the culture was not less than 24 h and not more than 72 h. Subsequently, the cells were continuously cultured with methyl thiazolyl tetrazolium (MTT; Sigma-Aldrich, St. Louis, MO, USA) for not less than 3 h and not more than 4 h. Next, they were mixed with 150 μL of dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA). Optical density (OD_{490}) was determined using a microplate reader, and the activity of the cells was analyzed.

Statistics Analysis

The Statistical Product and Service Solutions (SPSS) 18.0 software (SPSS Inc., Chicago, IL, USA) was employed for all statistical analysis. The *t*-test was adopted for the comparisons of HOTAIR expression, the activity of myeloma cells, the changes in protein expression of NF-κB, and apoptosis of myeloma cells. The experimental results were presented as mean ± standard deviation. The single factor analysis was performed for the comparison between the two groups. $p<0.05$ was considered statistically significant.

Results

The Messenger RNA (mRNA) Expression of HOTAIR in Myeloma Cells of Different Groups

RT-PCR results indicated that the mRNA expression of HOTAIR in group A, group B, and group C was (2.19 ± 0.33), (1.37 ± 0.25), and (2.51 ± 0.27), respectively. In comparison with group A and group C, the expression of HOTAIR was

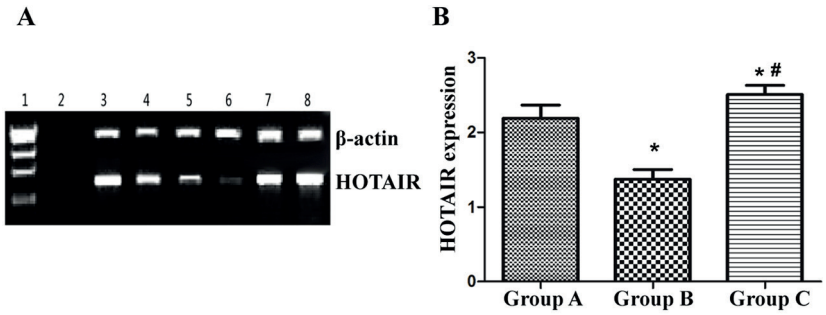

Figure 1. HOTAIR expression in myeloma cells. **A,** HOTAIR expression, **B,** HOTAIR expression in myeloma cells of different groups, *p<0.05 *vs.* group A, #p<0.05 *vs.* group B.

evidently downregulated in group B, in which the cells interfered with siRNAs (p<0.05). Meanwhile, the mRNA expression of HOTAIR was significantly higher in group C than group A and group B (p<0.05) (Figure 1).

Changes in the Activity of Myeloma Cells Detected Via MTT Assay

MTT assay demonstrated that the activity of the myeloma cells significantly increased at 24 h, 48 h, and 72 h in group C when compared with group A and group B (p<0.05). The activity of the cells slowly increased in group B. Meanwhile, it was evidently elevated in group A when compared with group B (p<0.05). The activity of the cells was evidently higher in group C than group B, which remained highest in group C (p<0.05). These results suggested that with upregulated expression of HOTAIR increased the activity of myeloma cells (Figure 2).

The Protein Expression of NF-κB in Myeloma Cells Determined via Western Blotting

The protein expression of NF-κB was detected via Western blotting. The results showed that NF-κB protein expression in group A, group B, and group C was (0.79 ± 0.22), (0.51 ± 0.17), and (0.95 ± 0.31), respectively. Compared with group A and group C, the protein expression of NF-κB decreased significantly in group B, in which the cells interfered with siRNAs (p<0.05). Furthermore, the expression of NF-κB protein was markedly higher in group C than group A and group B (p<0.05) (Figure 3).

Changes in the Apoptosis of Myeloma Cells Detected Via Flow Cytometry

The apoptosis rate in group A, group B, and group C was (9.57 ± 1.71), (20.33 ± 1.63), and (8.74 ± 1.23), respectively. In comparison with group A and group C, the apoptosis of myeloma cells was evidently suppressed in group B (p<0.05). Compared with group B, the apoptosis of myeloma cells remarkably decreased in group C (p<0.05) (Figure 4).

Discussion

Multiple myeloma has always been a big challenge in hematological oncology. Although the effects of stem cell transplantation and targeted

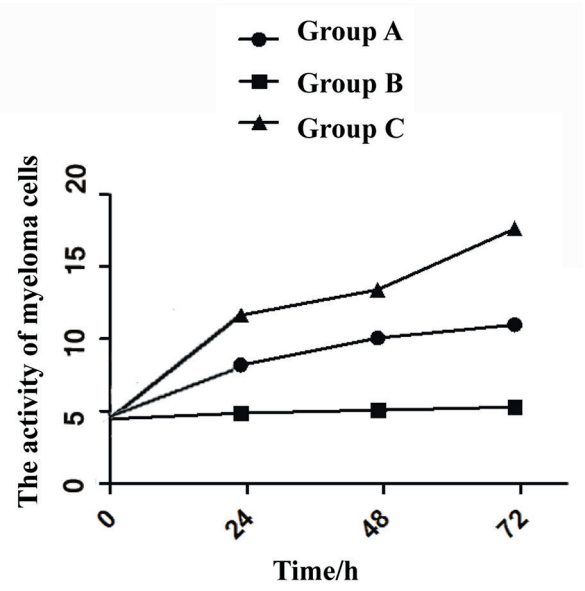

Figure 2. Changes in the activity of myeloma cells. No significant differences were observed in the activity of myeloma cells at 0 h among the three groups. The activity of the myeloma cells was significantly higher in group C than group A and group B at 24-48 h (p<0.05). Meanwhile, the cell activity tended to be stable. The differences in the activity of the myeloma cells at 72 h among the three groups were the most significant (p<0.05).

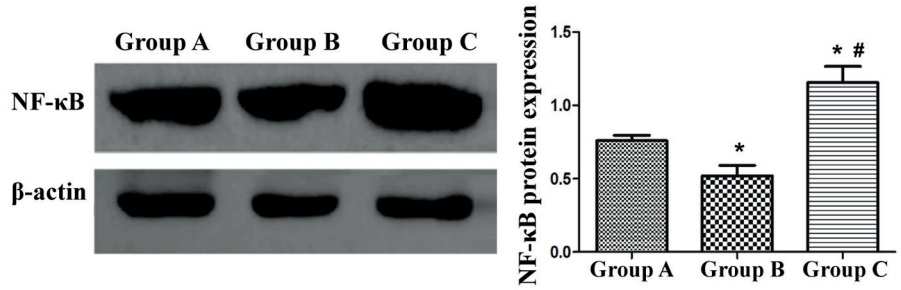

Figure 3. NF-κB protein expression in myeloma cells. **A,** NF-κB protein expression, **B,** NF-κB protein expression in myeloma cells of different groups, *p<0.05 *vs.* group A, #p<0.05 *vs.* group B.

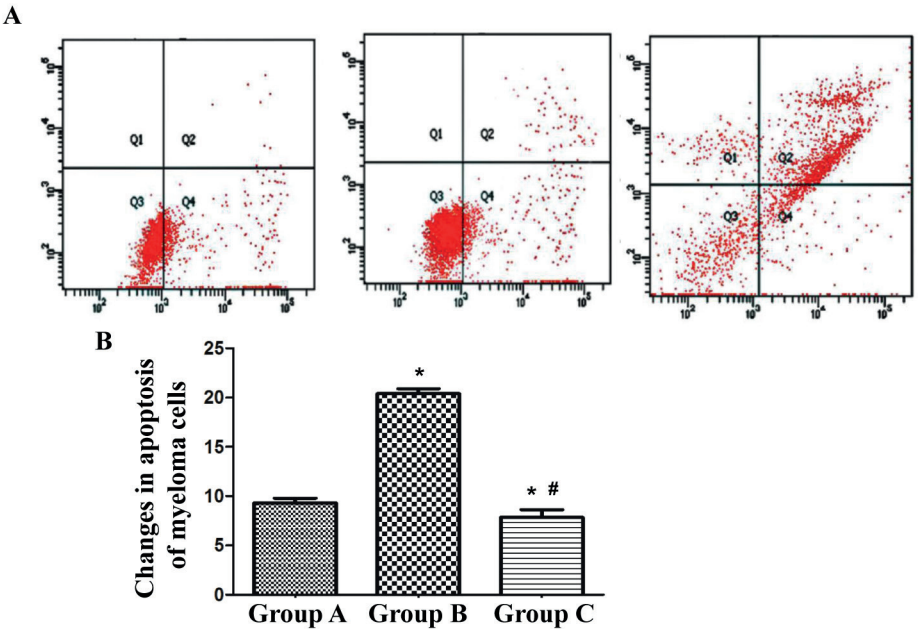

Figure 4. Changes in the apoptosis of myeloma cells. **A,** Changes in the apoptosis of myeloma cells detected via flow cytometry, **B,** Comparison of apoptosis of myeloma cells among different groups, *p<0.05 *vs.* group A, #p<0.05 *vs.* group B.

therapy are significant, there is still a chance of relapse[17,18]. Currently, the NF-κB signaling pathway has become a focus of the targeted therapy for myeloma[19,20]. As a kind of lncRNAs, HOTAIR plays a significant role in regulating the expression of normal cells and cancer cells. It can inhibit or promote the proliferation of tumor cells, as well[21,22]. In our study, RT-PCR results indicated that the mRNA expression of HOTAIR in group A, group B, and group C was (2.19 ± 0.33), (1.37 ± 0.25), and (2.51 ± 0.27), respectively. Compared with group A and group C, the mRNA expression of HOTAIR was evidently downregulated in group B, in which the cells interfered with siRNAs (p<0.05). Meanwhile, the mRNA expression of HOTAIR was significantly higher in group C than group A and group B (p<0.05). Scholars[23,24] have shown that HOTAIR is the first trans-acting lncRNA that has been first discovered. The role of HOTAIR in human fibroblasts has been widely investigated. Overexpression of HOTAIR is presented in numerous tumor cells or tissues. Meanwhile, this will increase the risk of tumor recurrence and even severe resistance to drugs[23]. Liu et al[24] have found that lncRNA HOTAIR plays a significant role in the occurrence of malignant tumors. Furthermore, it is abnormally expressed in multiple tumor cells, which is consistent with our results.

In the present study, the apoptosis of myeloma cells was determined using flow cytometry. The

results indicated that the apoptosis rate in group A, group B, and group C was (9.57 ± 1.71), (20.33 ± 1.63), and (8.74 ± 1.23), respectively. In comparison with group A and group C, the proliferation of myeloma cells was evidently inhibited in group B, with significant apoptosis of myeloma cells. Compared with group B, the apoptosis of myeloma cells significantly decreased in group C (p<0.05). The results of MTT indicated that the activity of the myeloma cells was significantly elevated at 24 h, 48 h, and 72 h in group C when compared with group A and group B (p<0.05). The activity of cells slowly increased in group B. Moreover, it was significantly elevated in group A when compared with group B (p<0.05). In addition, the activity of the cells was evidently higher in group C than group B, which remained higher in group C (p<0.05). Tenshin et al[25] have discovered that lncRNA HOTAIR plays a significant role in the occurrence of malignant tumors. Meanwhile, it is abnormally expressed in multiple tumor cells. The upregulated expression of HOTAIR in the cells increases the activity of myeloma cells. These findings indicate that HOTAIR is involved in the regulation of many malignant phenotypes of tumors, including cell proliferation, apoptosis, invasion, and metastasis. Additionally, it can also increase the resistance of tumor cells to drugs.

The protein expression of NF-κB was detected via Western blotting. The results showed that NF-κB protein expression in group A, group B, and group C was (0.79 ± 0.22), (0.51 ± 0.17), and (0.95 ± 0.31), respectively. Compared with group A and group C, the expression of NF-κB protein significantly decreased in group B (p<0.05). The protein expression of NF-κB was the highest in the cells of group C. Meanwhile, it was markedly higher in group C than group A and group B (p<0.05). Sun et al[26] have found that the number of myeloma cells will increase by stimulating the activity of NF-κB in the cell signal transduction pathways. Some investigations have discovered that the continuous activation of NF-κB is involved in the attack of myeloma. Furthermore, it takes part in the apoptosis of many cancer cells and inflammatory responses. NF-κB plays a significant role in the myeloma cells, causing the proliferation of internal myeloma cells. Finally, this may negatively affect the curing process of the disease[27]. Park et al[28] have reported that the activity of the NF-κB pathway can be influenced by the expression of lncRNA HOTAIR. This promotes its expression in myeloma cells, thus accelerating the growth of myeloma cells.

Conclusions

To sum up, lncRNA HOTAIR activates the expression of NF-κB in myeloma cells and promotes the proliferation of myeloma cells, thereby worsening the progression of MM.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Xiang S, Zou P, Wu J, Zheng F, Tang Q, Zhou J, Hann SS. Crosstalk of NF-kappaB/P65 and lncRNA HOTAIR-mediated repression of MUC1 expression contribute to synergistic inhibition of castration-resistant prostate cancer by polyphyllin 1-enzalutamide combination treatment. Cell Physiol Biochem 2018; 47: 759-773.
2) Xiao Z, Qu Z, Chen Z, Fang Z, Zhou K, Huang Z, Guo X, Zhang Y. LncRNA HOTAIR is a prognostic biomarker for the proliferation and chemoresistance of colorectal cancer via miR-203a-3p-mediated Wnt/ss-catenin signaling pathway. Cell Physiol Biochem 2018; 46: 1275-1285.
3) Yang Q, Jia L, Li X, Guo R, Huang Y, Zheng Y, Li W. Long noncoding RNAs: new players in the osteogenic differentiation of bone marrow-and adipose-derived mesenchymal stem cells. Stem Cell Rev 2018; 14: 297-308.
4) Shi B, Wang Y, Zhao R, Long X, Deng W, Wang Z. Bone marrow mesenchymal stem cell-derived exosomal miR-21 protects C-kit+ cardiac stem cells from oxidative injury through the PTEN/PI3K/Akt axis. PLoS One 2018; 13: e0191616.
5) Zhao W, Geng D, Li S, Chen Z, Sun M. LncRNA HOTAIR influences cell growth, migration, invasion, and apoptosis via the miR-20a-5p/HMGA2 axis in breast cancer. Cancer Med 2018; 7: 842-855.
6) Wu B, Liu J, Wang B, Liao X, Cui Z, Ding N. Association on polymorphisms in lncRNA HOTAIR and susceptibility to HNSCC in Chinese population. Eur Rev Med Pharmacol Sci 2018; 22: 702-706.
7) Pietrovito L, Leo A, Gori V, Lulli M, Parri M, Becherucci V, Piccini L, Bambi F, Taddei ML, Chiarugi P. Bone marrow-derived mesenchymal stem cells promote invasiveness and transendothelial migration of osteosarcoma cells via a mesenchymal to amoeboid transition. Mol Oncol 2018; 12: 659-676.
8) Matsuda Y, Sasaki M, Kataoka-Sasaki Y, Takayanagi A, Kobayashi K, Oka S, Nakazaki M, Masumori N, Kocsis JD, Honmou O. Intravenous infusion of bone marrow-derived mesenchymal stem cells reduc-

es erectile dysfunction following cavernous nerve injury in rats. Sex Med 2018; 6: 49-57.

9) Ri M. Endoplasmic-reticulum stress pathway-associated mechanisms of action of proteasome inhibitors in multiple myeloma. Int J Hematol 2016; 104: 273-280.

10) Sun MY, Zhu JY, Zhang CY, Zhang M, Song YN, Rahman K, Zhang LJ, Zhang H. Autophagy regulated by lncRNA HOTAIR contributes to the cisplatin-induced resistance in endometrial cancer cells. Biotechnol Lett 2017; 39: 1477-1484.

11) Zhang ZS, Wang J, Zhu BQ, Ge L. Long noncoding RNA UCA1 promotes multiple myeloma cell growth by targeting TGF-β. Eur Rev Med Pharmacol Sci 2018; 22: 1374-1379.

12) Edwards SK, Han Y, Liu Y, Kreider BZ, Liu Y, Grewal S, Desai A, Baron J, Moore CR, Luo C, Xie P. Signaling mechanisms of bortezomib in TRAF3-deficient mouse B lymphoma and human multiple myeloma cells. Leuk Res 2016; 41: 85-95.

13) Li H, Nie B, Du Z, Zhang S, Long T, Yue B. Bacitracin promotes osteogenic differentiation of human bone marrow mesenchymal stem cells by stimulating the bone morphogenetic protein-2/Smad axis. Biomed Pharmacother 2018; 103: 588-597.

14) Li H, Jia Y, Cheng J, Liu G, Song F. LncRNA NCK1-AS1 promotes proliferation and induces cell cycle progression by crosstalk NCK1-AS1/miR-6857/CDK1 pathway. Cell Death Dis 2018; 9: 198.

15) Nuermaimaiti N, Liu J, Liang X, Jiao Y, Zhang D, Liu L, Meng X, Guan Y. Effect of lncRNA HOXA11-AS1 on adipocyte differentiation in human adipose-derived stem cells. Biochem Biophys Res Commun 2018; 495: 1878-1884.

16) Zhao J, Li H, Deng H, Zhu L, Zhou B, Yang M, Liu Q, Luo G, Yang Y, Ma W. LncRNA gadd7, increased in varicocele patients, suppresses cell proliferation and promotes cell apoptosis. Oncotarget 2017; 9: 5105-5110.

17) Chen Z, Lin S, Li JL, Ni W, Guo R, Lu J, Kaye FJ, Wu L. CRTC1-MAML2 fusion-induced lncRNA LINC00473 expression maintains the growth and survival of human mucoepidermoid carcinoma cells. Oncogene 2018; 37: 1885-1895.

18) Ding D, Li C, Zhao T, Li D, Yang L, Zhang B. LncRNA H19/miR-29b-3p/PGRN axis promoted epithelial-mesenchymal transition of colorectal cancer cells by acting on Wnt signaling. Mol Cells 2018; 41: 423-435.

19) Lu S, Dong W, Zhao P, Liu Z. LncRNA FAM83H-AS1 is associated with the prognosis of colorectal carcinoma and promotes cell proliferation by targeting the Notch signaling pathway. Oncol Lett 2018; 15: 1861-1868.

20) Zheng C, Li X, Qian B, Feng N, Gao S, Zhao Y, Zhou B. The lncRNA myocardial infarction associated transcript-centric competing endogenous RNA network in non-small-cell lung cancer. Cancer Manag Res 2018; 10: 1155-1162.

21) Zhou W, Chen X, Hu Q, Chen X, Chen Y, Huang L. Galectin-3 activates TLR4/NF-κB signaling to promote lung adenocarcinoma cell proliferation through activating lncRNA-NEAT1 expression. BMC Cancer 2018; 18: 580.

22) Wang Y, Zhang XL, Sun CM. BAY-11-7082 induces apoptosis of multiple myeloma U266 cells through inhibiting NF-κB pathway. Eur Rev Med Pharmacol Sci 2018; 22: 2564-2571.

23) Balaji S, Ahmed M, Lorence E, Yan F, Nomie K, Wang M. NF-kappaB signaling and its relevance to the treatment of mantle cell lymphoma. J Hematol Oncol 2018; 11: 83.

24) Liu S, Zheng LL, Zhu YM, Shen HJ, Zhong Q, Huang J, Li C, Liu Z, Yao MD, Ou RM, Zhang Q. Knockdown of REGgamma inhibits the proliferation and migration and promotes the apoptosis of multiple myeloma cells by downregulating NF-κB signal pathway. Hematology 2018; 23: 277-283.

25) Tenshin H, Teramachi J, Oda A, Amachi R, Hiasa M, Bat-Erdene A, Watanabe K, Iwasa M, Harada T, Fujii S, Kagawa K, Sogabe K, Nakamura S, Miki H, Kurahashi K, Yoshida S, Aihara K, Endo I, Tanaka E, Matsumoto T, Abe M. TAK1 inhibition subverts the osteoclastogenic action of TRAIL while potentiating its antimyeloma effects. Blood Adv 2017; 1: 2124-2137.

26) Sun Y, Pan J, Zhang N, Wei W, Yu S, Ai L. Knockdown of long non-coding RNA H19 inhibits multiple myeloma cell growth via NF-κB pathway. Sci Rep 2017; 7: 18079.

27) Huang H, Sun Z, Wang X, Liu X, Na W, Xu R, Ding R, Liu H. The effect of marrow stromal cells on TRAF6 expression levels in myeloma cells. Oncol Lett 2017; 14: 1464-1470.

28) Park GB, Chung YH, Jeong JY, Kim D. A p110δ-specific inhibitor combined with bortezomib blocks drug resistance properties of EBV-related B cell origin cancer cells via regulation of NF-κB. Int J Oncol 2017; 50: 1711-1720.