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LncRNA NEAT1 regulates cervical carcinoma proliferation and invasion by targeting AKT/PI3K

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Abstract. – OBJECTIVE: Cervical cancer is a common tumor in gynecological malignancies. Recent studies showed that long non-coding RNAs (lncRNAs) play a key role in tumorigenesis and development. LncRNA nuclear-rich transcripts 1 (NEAT1) has been found to play a role in gynecological tumors, such as endometrial cancer. However, expression of lncRNA NEAT1 and mechanism in cervical cancer has not been elucidated.

MATERIALS AND METHODS: The tumor tissue and adjacent tissue of cervical cancer patients were collected. HeLa cells were cultured in vitro and lncRNA NEAT1 expression was interfered with small interfere RNA (siRNA). Cell proliferation was detected by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. Cell invasion ability was assessed by transwell assay. LncRNA NEAT1, Cyclin D1, and cyclin-dependent kinase 4 (CDK4) expressions were detected by Real-time PCR. Caspase 3 expression was detected by caspase 3 activity kit. Phosphorylated protein kinase B (p-AKT), phosphatidylinositol 3-kinase (p-PI3K), and matrix metalloproteinase-2 (MMP2) levels were evaluated by Western blot.

RESULTS: Compared with the adjacent tissue, lncRNA NEAT1 expression was significantly increased in cervical cancer ($p<0.05$). LncRNA NEAT1 level was decreased in HeLa cells transfected by siRNA, which inhibited the proliferation and invasion of tumor cells, reduced cyclin D1 and CDK4 expressions, enhanced caspase 3 activity, and declined the expressions of p-AKT, p-PI3K, and MMP2 ($p<0.05$).

CONCLUSIONS: LncRNA NEAT1 siRNA transfection can inhibit the proliferation of cervical cancer by regulating the AKT/PI3K signaling pathway, promote cell apoptosis, and restrain cell invasion. Therefore, the lncRNA NEAT1 may be used as a molecular potential for the diagnosis and treatment of cervical cancer through regulating AKT/PI3K signaling pathway, which would be confirmed in the following study.

Key Words:
lncRNA NEAT1, Cervical cancer, AKT/PI3K, Proliferation, Apoptosis, Invasion.

Introduction

Cervical cancer is the second most common gynecological malignant tumor with the incidence only after breast cancer, lung cancer, and colorectal cancer. As one of the women with gynecological cancers, cervical cancer is a serious threat to women's health[1,2]. The incidence and mortality of cervical cancer in developing countries and economically underdeveloped areas keeps high, thus becoming one of the malignant tumors that threaten the world, especially in developing countries. Limited by not universal cervical cancer screening, poor prevention, and imbalanced economic development, the incidence and mortality of cervical cancer in developing countries are higher than the developed countries[3,4]. As a developing country, China has a vast territory and a large population. Therefore, the incidence and mortality of cervical cancer account for a high proportion in gynecologic cancer[5,6]. As most cervical cancer patients are in the advanced stage when diagnosed, the cervical cancer prognosis and quality of life are poor. It is a threat to public health and safety since it brings a heavy mental and financial burden to the patients and their family[7,8]. At present, molecular targeted therapy has become a new hot spot on cervical cancer, but the mechanism of cervical cancer is still unclear.

Long non-coding RNA (lncRNA) was discovered in recent years[9]. A large number of transcripts of non-coding RNA at different lengths were found in eukaryotes. Furthermore, it was found that non-coding transcripts account for the

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vast majority of the human genome[10,11]. LncRNA is the transcripts over 200 nt in length, which is not considered to be involved in protein-coding and defined as "noise" of transcription. However, it was gradually found to be participating in epigenetic regulation, transcriptional regulation, and post-transcriptional regulation of gene expression[12,13]. In the genome, lncRNA is the most transcribed non-protein coding sequence and is considered to play a major regulatory role in the protein-coding gene[14]. Recent studies[15,16] show that lncRNA also plays an important role in tumorigenesis and development, especially in the three major malignant gynecologic cancers, including ovarian cancer, cervical cancer, and endometrial cancer. LncRNA NEAT1 is an important tumor cell growth and proliferation regulator that can promote neovascularization, tumor invasion and metastasis, and play a role in the gynecological tumors, such as endometrial cancer[17,18]. This study was to detect the expression of lncRNA NEAT1 in cervical cancer and to analyze its mechanism.

Patients and Methods

Patients

A total of 78 cases of patients with cervical cancer in The First Affiliated Hospital of Xinxiang Medical University from January 2017 to December 2017 were enrolled. All the patients with a mean age of 62.2 ± 7.9 (51-77) years old were treated with surgery. Inclusion criteria[17]: All patients were diagnosed as cervical cancer and received surgery for the first time without preoperative chemotherapy or radiotherapy; all subjects had signed informed consent. Exclusion criteria: recurrent cervical cancer; previous surgical treatment; previous radiotherapy or chemotherapy; combined with other diseases, such as infectious diseases, malignant tumors, severe diabetes mellitus, and other organ failures, autoimmune diseases, and malignant tumor complications. The tumor tissues and para-cancerous tissues were collected during operation and stored in liquid nitrogen. This study was approved by the Medical Ethics Committee of The First Affiliated Hospital of Xinxiang Medical University, Henan, China.

Main Instruments and Reagents

RNA extraction kit and reverse transcription kit were purchased from the RD Systems (Minneapolis, MN, USA). Other commonly used reagents were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). Real-time PCR reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Real-time PCR amplifier was purchased from the ABI (Foster City, CA, USA). DNA amplification instrument was purchased from the PE Gene Applied Biosystems. (Mode: 2400, Foster, CA, USA). Dulbecco's Modified Eagle Medium (DMEM) medium, fetal bovine serum (FBS), ethylene diamine tetraacetic acid (EDTA), and penicillin-streptomycin were purchased from Hyclone (Logan, UT, USA). Dimethylsulfoxide (DMSO) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) powder was purchased from Gibco BRL. Co. Ltd. (Grand Island, New York, USA). Trypsin-EDTA was purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinylidene difluoride (PVDF) membrane was purchased from Pall Life Sciences (Covina, CA, USA). Western blot related chemical reagents were purchased from Beyotime Biotech. (Shanghai, China). Enhanced chemiluminescence (ECL) reagent was purchased from Amersham Biosciences (Piscataway, NJ, USA). Rabbit anti-human nuclear factor κB (NF-κB), phosphorylated protein kinase B (p-AKT), phosphorylated phosphatidylinositol 3-kinase (p-PI3K), matrix metalloproteinase-2 (MMP2) protein kinase B (AKT), and phosphatidylinositol 3-kinase (p-PI3K) monoclonal antibodies, mouse anti-rabbit horseradish peroxidase (HRP) labeled IgG secondary antibody were purchased from the Cell Signaling Technology (Beverly, MA, USA). Transwell chamber was purchased from Corning (Corning, NY, USA). Caspase 3 activity kit was purchased Nanjing Jiancheng Bioengineer Institute (Nanjing, China). Thermo Forma 3110 5% CO2 incubator was purchased from Thermo Fisher Scientific (Hudson, NH, USA). Lab System Version 1.3.1 microplate reader was purchased from Bio-Rad Laboratories (Hercules, CA, USA).

HeLa Cell Cultivation and Grouping

HeLa cells stored in liquid nitrogen were sub-cultured after resuscitation. Cells in the 3rd-8th generation and logarithmic growth phase were used for the experiment. HeLa cells were randomly divided into three groups, including control group, siRNA-NC group, transfected by lncRNA NEAT1 negative control, and siRNA group transfected with lncRNA NEAT1 siRNA (silncRNA NEAT1 group).

LncRNA NEAT1 siRNA Transfection

Lnc RNA NEAT1 siRNA and siRNA-NC were transfected into HeLa cells. LncRNA

NEAT1 siRNA sequence was 5'-GCAGGAAUUCGGUGUUCA-3'. SiRNA-NC sequence was 5'-UCAGGUGAGAUUCUA-3'. The cell density in the 6-well plate was fused to 70%-80%. The lncRNA NEAT1 siRNA and negative control liposome were added into 200 µl serum-free DMEM medium and mixed at room temperature for 15 min, respectively. The mixed lipo2000 were mixed and incubated at room temperature for 30 min. Next, they were added to the cells with 1.6 ml serum-free DMEM medium and incubated in 5% CO_2 at 37°C for 6 h. At last, the cells were used for the experiment after 48 h.

Real-Time PCR

Total RNA was extracted from the tissue and cells by TRIzol and reverse transcribed to DNA. The primers were designed by Primer 6.0 and synthesized by Invitrogen (Table I). The reaction conditions included 55°C for 1 min, followed by 35 cycles of 92°C for 30 s, 58-60°C for 45 s, and 72°C for 35 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as loading control. According to the fluorescence quantification, the initial cycle number (CT) was calculated and 2-ΔCt method was used for semi-quantitative analysis.

MTT Assay

HeLa cells in logarithmic growth phase were collected in DMEM culture medium containing 10% FBS and seeded in 96-well plate at 5 × 103 cells. The supernatant was discarded after 24 h incubation and 20 µl of sterile MTT was added to the wells for 4 hours. Next, dimethyl sulfoxide (DMSO) was added to each well at 150 µl/well for 10 min until the purple crystal was completely dissolved. Then, the plate was tested at 570 nm to obtain the absorbance (A) value and calculate the proliferation rate.

Transwell Assay

According to the manual, the transwell chamber was coated by l:5 50 mg/l Matrigel for 24 h and air-dry at 4°C. Next, 500 µl of 10% FBS DMEM medium and 100 µl of tumor cell suspension prepared in serum-free medium were added to the lower and upper chamber, respectively. After 48 h cultivation, the membrane was washed by using PBS and fixed by ice-ethanol. After stained with crystal violet, the cells in the lower layer of the microporous membrane were counted. Each experiment was repeated for three times.

Caspase 3 Activity Detection

According to kit instructions, the cells were digested by trypsin and centrifuged at 600 ×g and 4°C for 5 min. The cells were added with lysate (150 mM NaCl, 1% Nonidet P 40 (NP-40), 0.1% sodium dodecyl sulfate (SDS), 2 µg/ml Aprotinin, 2 µg/ml Leupeptin, 1 mM phenylmethanesulfonyl fluoride (PMSF), 1.5 mM EDTA, 1 mM NaVanadate) and lysed on ice for 15 min. Then, the sample was centrifuged at 20000 ×g and 4°C for 5 min, and added with 2 mM Ac-DEVD-pNA to test OD value.

Western Blot

Total protein was extracted by lysis buffer and stored at -20°C after quantification. The separated proteins were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the gel was transferred to PVDF membrane by semi-dry transfer method at 100 mV for 1.5 h. The primary antibodies were incubated at adilution of 1:2000 for p-AKT, 1:2000 for p-PI3K, 1:1000 for MMP2, 1:1500 for AKT, and 1:2000 for PI3K. Then, the membrane was incubated in goat anti-rabbit secondary antibody at 1:2000 dilution for 30 min. After washed by PBS Tween 20 (PBST), the membrane was treated by chemiluminescence and analyzed by protein image analysis system software and Quantity one software. The experiment was repeated four times (n=4).

Statistical Analysis

All data analyses were performed on SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were presented as mean

Table I. Primer sequence.

Gene	Forward 5′-3′	Reverse 5′-3′
GAPDH	AGTAGTCACCTGTTGCTGG	TAATACGGAGACCTGTCTGGT
cyclin D1	ACTCCATGGAGCCAAACG	ATGAAGCTGGTAGCGCAG
CDK4	CTTACATGTGACCTGCC	CCGGTTCAACTCTCCTT
lnc RNA NEAT1	ATGCTTTCTCAACTTGTTGG	TCACCG CTCTTGGCCGTCACA

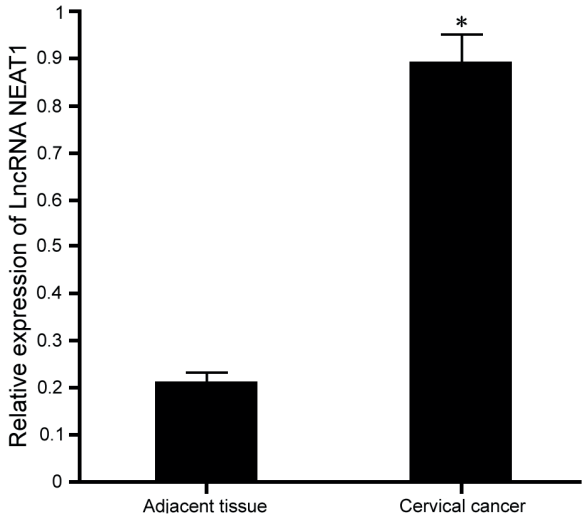

Figure 1. LncRNA NEAT1 expression in cervical cancer. *p<0.05, compared with adjacent tissue.

± standard deviation ($\bar{x}$ ± SD). Student's t-test was used to compare the differences between two groups. Tukey's post hoc test was used to validate the ANOVA for comparing measurement data between groups. p<0.05 was considered statistically significant.

Results

LncRNA NEAT1 Expression in Cervical Cancer

Real-time PCR was used to test lncRNA NEAT1 expression in cervical cancer. It was showed that lncRNA NEAT1 expression in cervical cancer was significantly higher than that in adjacent tissue (p<0.05) (Figure 1).

Effects of lncRNA NEAT1 siRNA on HeLa cells

After lncRNA NEAT1 siRNA transfection, lncRNA NEAT1 expression markedly reduced in HeLa cells compared with control and siRNA-NC group (p<0.05) (Figure 2).

Effects of lncRNA NEAT1 siRNA on HeLa Cell Proliferation

After lncRNA NEAT1 siRNA transfection, HeLa cell proliferation was tested by MTT assay. It was found that HeLa cell proliferation was markedly suppressed after lncRNA NEAT1 siRNA transfection for 48 h (p<0.05) (Figure 3).

Effects of lncRNA NEAT1 siRNA on Caspase 3 Activity in HeLa Cells

After lncRNA NEAT1 siRNA transfection, caspase 3 activity in HeLa cells was assessed by caspase 3 detection kit. It was observed that caspase 3 activity was apparently enhanced after lncRNA NEAT1 siRNA transfection for 48 h (p<0.05) (Figure 4).

Effects of lncRNA NEAT1 siRNA on HeLa cell invasion

After lncRNA NEAT1 siRNA transfection, HeLa cell invasion was evaluated by transwell assay. It was demonstrated that cell invasion was significantly restrained after lncRNA NEAT1 siRNA transfection for 48 h (p<0.05) (Figure 5).

Effects of lncRNA NEAT1 siRNA on Cyclin D1 and CDK4 Expressions in HeLa Cells

After lncRNA NEAT1 siRNA transfection, cyclin D1 and CDK4 expressions in HeLa cells were detected by Real-time PCR. It was revealed that cyclin D1 and CDK4 expressions were significantly inhibited after lncRNA NEAT1 siRNA transfection for 48 h (p<0.05) (Figure 6).

Effects of lncRNA NEAT1 siRNA on AKT/PI3K Signaling Pathway

After lncRNA NEAT1 siRNA transfection, AKT/PI3K signaling pathway activity in HeLa cells was determined by Western blot. It was showed that AKT/PI3K signaling pathway phosphorylation was markedly suppressed after lncR-

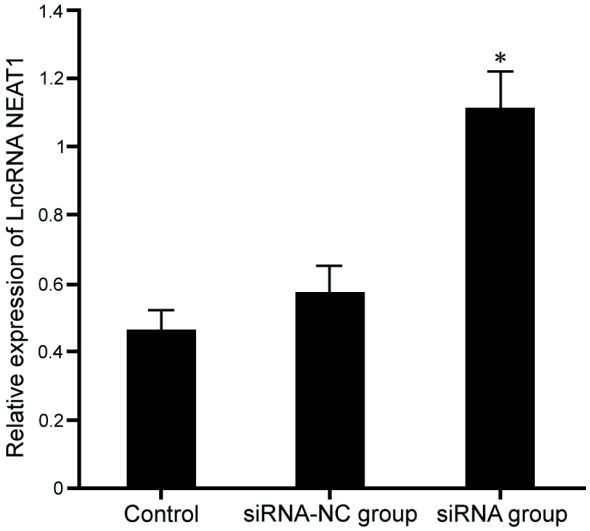

Figure 2. LncRNA NEAT1 expression in HeLa cells. *p<0.05, compared with control.

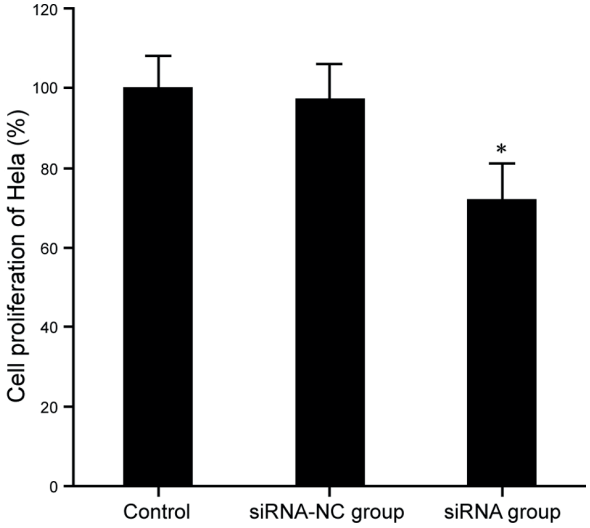

Figure 3. The influence of lncRNA NEAT1 siRNA on HeLa cell proliferation. $^{*}p<0.05$, compared with control.

NA NEAT1 siRNA transfection for 48 h ($p<0.05$) (Figure 7).

Effects of lncRNA NEAT1 siRNA on MMP2 Expression

After lncRNA NEAT1 siRNA transfection, MMP2 expression in HeLa cells was detected by Western blot. It was found that MMP2 expression was apparently inhibited after lncRNA NEAT1 siRNA transfection for 48 h ($p<0.05$) (Figure 8).

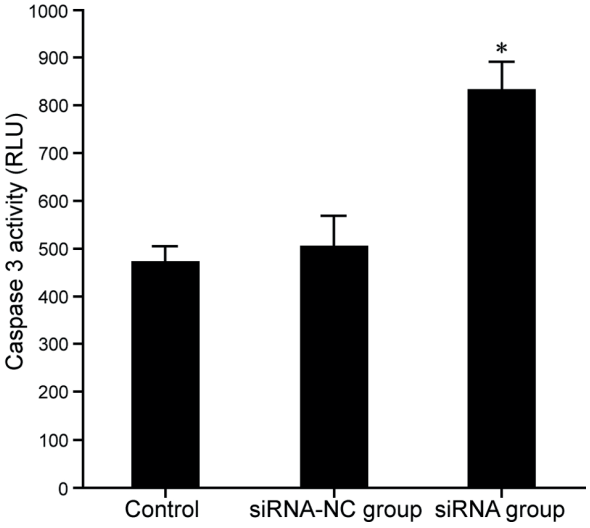

Figure 4. The effect of lncRNA NEAT1 siRNA on caspase 3 activity in HeLa cells. $^{*}p<0.05$, compared with control.

Discussion

Human papillomavirus (HPV) infection, unhealthy sexual life and eating habits, smoking, work environment, and pressure lead to an increasing incidence of cervical cancer. In recent years, it was found the younger trend of cervical cancer, which was the majority at 30-55 years old[19]. Cervical cancer incidence and mortality in China account for about 25% of the world. Currently, there is still lack of deficiency for the prevention and treatment of cervical cancer[20]. It is urgently needed to find a new molecular target for diagnosis and treatment of cervical cancer.

LncRNA can regulate gene expression and participate in the physiological activities in many ways, including chromatin modification, genomic imprinting, nuclear transport, chromosomal gene silencing, and transcriptional activation[21]. LncRNA can regulate the normal and pathological conditions of cells, including growth, proliferation, cell cycle, and apoptosis. Thus, lncRNA is an important regulatory factor in the development and progression of human diseases[22]. Recently, lncRNA expression was detected abnormally in neoplasia formation and progression. Moreover, it was found that some lncRNAs play roles in tumors[23]. LncRNA NEAT1 was confirmed to be expressed in many normal tissues but significantly increased in many kinds of tumors, including lung cancer, liver cancer, colorectal cancer, meningioma, and even leukemia. This suggests that the lncRNA NEAT1 may be involved in tumor development and can be used as a predictor of tumor prognosis[17,18,24]. However, the role and mechanism of lncRNA NEAT1 in cervical cancer have not been elucidated. Therefore, we first detected the expression of lncRNA NEAT1 in cervical cancer tissues and confirmed that the expression of lncRNA NEAT1 was significantly increased in cervical cancer. Therefore, we transfected lncRNA NEAT1 siRNA into HeLa cells to reduce the lncRNA NEAT1 expression. The results showed that it inhibited tumor cell proliferation, promoted tumor cell apoptosis, reduced invasive ability, restrained cell cycle-related genes cyclin D1 and CDK4 expressions, which suggests that lncRNA NEAT1 regulates the proliferation and invasion of NSCLC. MMP-2 is a member of the matrix metalloproteinase family that may promote tumor cell invasion and metastasis[25]. AKT/PI3K signaling pathway is closely related to tumorigenesis, which leads to tumor cell proliferation, cell cycle, and progression. It

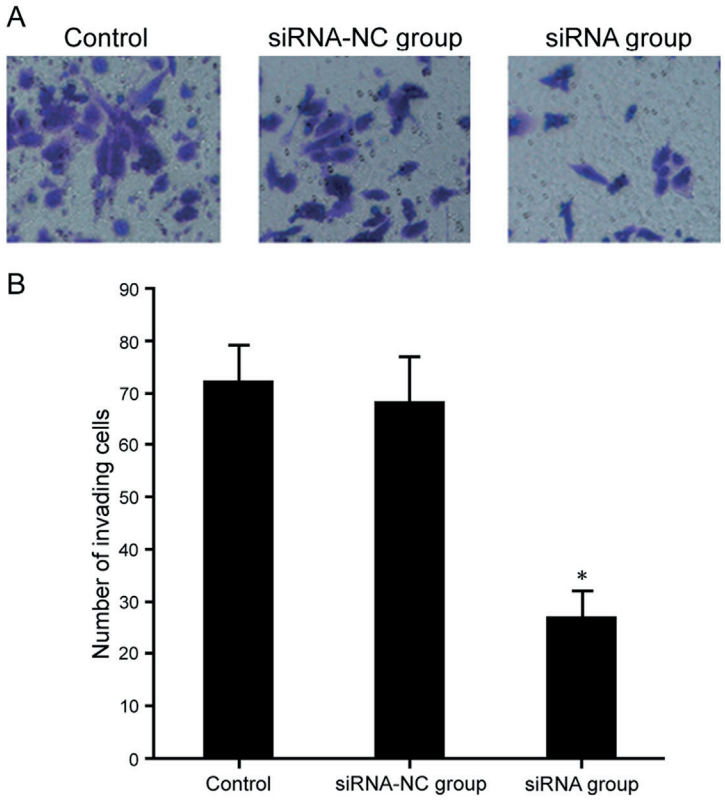

Figure 5. The impact of lncRNA NEAT1 siRNA on HeLa cell invasion. ***A,*** Transwell chamber assay analysis of the impact of lncRNA NEAT1 siRNA on HeLa cell invasion. ***B,*** HeLa cell invasive ability statistical analysis. *p<0.05, compared with control.

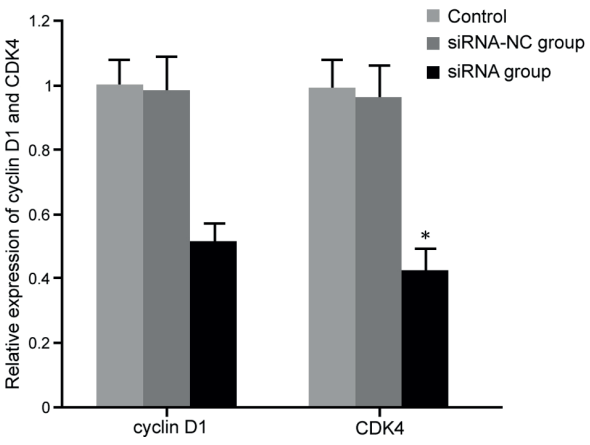

Figure 6. The influence of lncRNA NEAT1 siRNA on cyclin D1 and CDK4 expressions in HeLa cells. *p<0.05, compared with control.

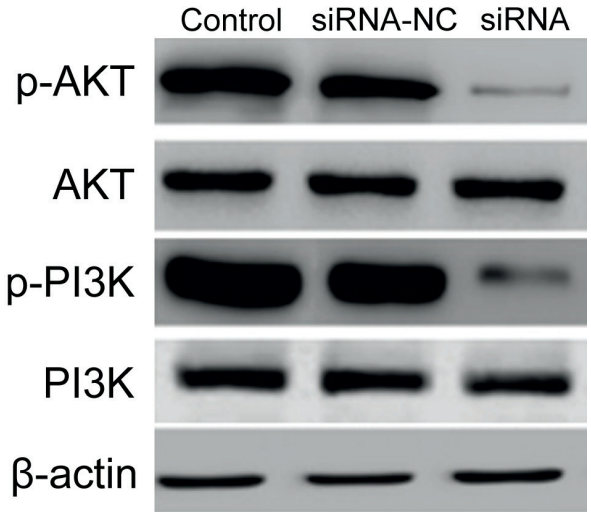

Figure 7. The effect of lncRNA NEAT1 siRNA on AKT/PI3K signaling pathway.

was confirmed that cervical cancer is related to AKT/PI3K signaling pathway[26]. In this study, we observed that lncRNA NEAT1 siRNA inhibited the expression of MMP-2 and suppressed the activation of AKT/PI3K pathway. We observed the expression of lncRNA NEAT1 in cervical cancer and related mechanisms. The clinical significance of lncRNA NEAT1 in cervical cancer as a molecular marker would be investigated in the future.

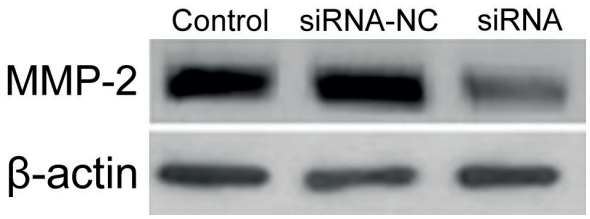

Figure 8. The impact of lncRNA NEAT1 siRNA on MMP2 expression.

Conclusions

We showed that LncRNA NEAT1 siRNA transfection suppressed cervical cancer proliferation, promoted cell apoptosis, and restrained cell invasion by regulating the AKT/PI3K signaling pathway. Therefore, the lncRNA NEAT1 may be used as a potential molecular for the diagnosis and treatment of cervical cancer through regulating AKT/PI3K signaling pathway, which would be confirmed in the following study.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Mei J, Wang DH, Wang LL, Chen Q, Pan LL, Xia L. MicroRNA-200c suppressed cervical cancer cell metastasis and growth via targeting MAP4K4. Eur Rev Med Pharmacol Sci 2018; 22: 623-631.
2) Jing L, Zhong X, Zhong Z, Huang W, Liu Y, Yang G, Zhang X, Zou J, Jing C, Wei X. Prevalence of human papillomavirus infection in Guangdong Province, China: a population-based survey of 78,355 women. Sex Transm Dis 2014; 41: 732-738.
3) Rahimi S, Marani C, Gardner F, Yeoh CC, Akaev I, Votano S. Endocervicoscopy and biopsy to detect cervical intraepithelial squamous neoplasia in nonvisible squamocolumnar junction with unsatisfactory colposcopy: a pilot study. Technol Cancer Res Treat 2018; 17: 1533034617753811.
4) Bandyopadhyay A, Mukherjee U, Ghosh S, Ghosh S, Sarkar SK. Pattern of failure with locally advanced cervical cancer–A retrospective audit and analysis of contributory factors. Asian Pac J Cancer Prev 2018; 19: 73-79.
5) Guo KY, Han L, Li X, Yang AV, Lu J, Guan S, Li H, Yu Y, Zhao Y, Yang J, Zhang H. Novel proteasome inhibitor delanzomib sensitizes cervical cancer cells to doxorubicin-induced apoptosis via stabilizing tumor suppressor proteins in the p53 pathway. Oncotarget 2017; 8: 114123-114135.
6) Ju Y, Tian D, Tan Y, Fu Z. Palliative care with cervical intrathecal infusion and external pump for a late-stage cancer patient with refractory pain: a case report. Medicine 2018; 97: e9714.
7) Kong SY, Huang K, Zeng C, Ma X, Wang S. The association between short-term response and long-term survival for cervical cancer patients undergoing neoadjuvant chemotherapy: a system review and meta-analysis. Sci Rep 2018; 8: 1545.
8) Brodersen J. High incidence of cervical cancer in women over 60 is likely due to less intensive cervical screening in this generation of women. BMJ Evid Based Med 2018; 23: 37.
9) Ye H, Liu K, Qian K. Overexpression of long noncoding RNA HOTTIP promotes tumor invasion and predicts poor prognosis in gastric cancer. Onco Targets Ther 2016; 9: 2081-2088.
10) Wang SH, Wu XC, Zhang MD, Weng MZ, Zhou D, Quan ZW. Upregulation of H19 indicates a poor prognosis in gallbladder carcinoma and promotes epithelial-mesenchymal transition. Am J Cancer Res 2016; 6: 15-26.
11) Zhang SR, Yang JK, Xie JK, Zhao LC. Long noncoding RNA HOTTIP contributes to the progression of prostate cancer by regulating HOXA13. Cell Mol Biol 2016; 62: 84-88.
12) Li P, Zhang G, Li J, Yang R, Chen S, Wu S, Zhang F, Bai Y, Zhao H, Wang Y, Dun S, Chen X, Sun Q, Zhao G. Long noncoding RNA RGMB-AS1 indicates a poor prognosis and modulates cell proliferation, migration and invasion in lung adenocarcinoma. PLoS One 2016; 11: e0150790.
13) Chang L, Qi H, Xiao Y, Li C, Wang Y, Guo T, Liu Z, Liu Q. Integrated analysis of noncoding RNAs and mRNAs reveals their potential roles in the biological activities of the growth hormone receptor. Growth Horm IGF Res 2016; 29: 11-20.
14) Wu ZJ, Li Y, Wu YZ, Wang Y, Nian WQ, Wang LL, Li LC, Luo HL, Wang DL. Long non-coding RNA CCAT2 promotes the breast cancer growth and metastasis by regulating TGF-beta signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 706-714.
15) Cheng N, Guo Y. Long noncoding RNA NEAT1 promotes nasopharyngeal carcinoma progression through regulation of miR-124/NF-kappaB pathway. Onco Targets Ther 2017; 10: 5843-5853.
16) Shi Y, Ye P, Long X. Differential expression profiles of the transcriptome in breast cancer cell lines revealed by next generation sequencing. Cell Physiol Biochem 2017; 44: 804-816.
17) Wang J, Zhao X, Guo Z, Ma X, Song Y, Guo Y. Regulation of NEAT1/miR-214-3p on the growth, migration and invasion of endometrial carcinoma cells. Arch Gynecol Obstet 2017; 295: 1469-1475.
18) Zhang M, Wu WB, Wang ZW, Wang XH. lncRNA NEAT1 is closely related with progression of breast cancer via promoting proliferation and EMT. Eur Rev Med Pharmacol Sci 2017; 21: 1020-1026.
19) Yao JJ, Zhou GQ, Wang YQ, Wang SY, Zhang WJ, Jin YN, ZhangF, Li L, Liu LZ, Cheng ZB, Ma J, Qi ZY, Sun

Y. Prognostic values of the integrated model incorporating the volume of metastatic regional cervical lymph node and pretreatment serum Epstein-Barr virus DNA copy number in predicting distant metastasis in patients with N1 nasopharyngeal carcinoma. Chin J Cancer 2017; 36: 98.

20) Zhang J, Hao X, Yang Y, Yan CS, Ma C, Xiao M, Gu LS, Wang Y. Evaluation of supplementary diagnostic value of contrast-enhanced ultrasound for lymph node puncture biopsy. J Thoracic Dis 2017; 9: 4791-4797.

21) Chen JX, Chen M, Zheng YD, Wang SY, Shen ZP. Up-regulation of BRAF activated non-coding RNA is associated with radiation therapy for lung cancer. Biomedi Pharmacother 2015; 71: 79-83.

22) Shi Y, Liu Y, Wang J, Jie D, Yun T, Li W, Yan L, Wang K, Feng J. Downregulated Long Noncoding RNA BANCR Promotes the Proliferation of Colorectal Cancer Cells via Downregualtion of p21 Expression. PLoS One 2015; 10: e0122679.

23) Dong S, Qu X, Li W, Zhong X, Li P, Yang S, Chen X, Shao M, Zhang L. The long non-coding RNA, GAS5, enhances gefitinib-induced cell death in innate EGFR tyrosine kinase inhibitor-resistant lung adenocarcinoma cells with wide-type EGFR via downregulation of the IGF-1R expression. J Hematol Oncol 2015; 8: 43.

24) Zhang Y, Lun L, Li H, Wang Q, Lin J, Tian R, Pan H, Zhang H, Chen X. The value of lncRNA NEAT1 as a prognostic factor for survival of cancer outcome: a meta-analysis. Sci Rep 2017; 7: 13080.

25) Wu MH, Lin CL, Chiou HL, Yang SF, Lin CY, Liu CJ, Hsieh YH. Praeruptorin A inhibits human cervical cancer cell growth and invasion by suppressing MMP-2 expression and ERK1/2 signaling. Int J Mol Sci 2017; 19: E10.

26) Dong P, Hao F, Dai S, Tian L. Combination therapy Eve and Pac to induce apoptosis in cervical cancer cells by targeting PI3K/AKT/mTOR pathways. J Recept Signal Transduct Red 2018; 38: 83-88.