

LncRNA PCAT-1 regulates the proliferation, metastasis and invasion of cervical cancer cells

T.-T. MA^{1,2}, L.-Q. ZHOU^{1,2}, J.-H. XIA^{1,2}, Y. SHEN^{1,2}, Y. YAN^{1,2}, R.-H. ZHU³

¹Department of Radiotherapy, The Affiliated Huai'an Hospital of Xuzhou Medical University, Huai'an, China

²Department of Radiotherapy, Huai'an Second People's Hospital, Huai'an, China

³Department of Nuclear Medicine, Huai'an First People's Hospital, Huai'an, China

Abstract. – OBJECTIVE: To investigate the effects of long non-coding RNA (lncRNA) PCAT-1 on the proliferation, metastasis, and invasion of cervical cancer cells.

MATERIALS AND METHODS: LncRNA PCAT-1 small interfering RNA (siRNA) and negative siRNA were transfected into cervical cancer cell lines and the expression of lncRNA PCAT-1 in cells was confirmed by Real-time quantitative polymerase chain reaction (qPCR). Cell counting kit-8 (CCK-8) and colony formation assay were applied to detect the effect of lncRNA PCAT-1 on cell proliferation. The wound-healing assay was applied to test the effect of lncRNA PCAT-1 on cell metastasis. Matrigel cell invasion assay was performed to detect the impact of lncRNA PCAT-1 expression on invasion.

RESULTS: After transfected with the long non-coding PCAT-1 siRNA into cervical cancer cell lines for 48 h, the lncRNA PCAT-1 cells were significantly down-regulated. The results of CCK-8, clonogenic and wound-healing assay showed that the decreased expression of lncRNA PCAT-1 attenuated the proliferation and metastasis of cells. The results of matrigel cell invasion assay manifested that the decreased expression of lncRNA PCAT-1 could reduce the invasion ability. The up-regulation of lncRNA PCAT-1 was associated with poor prognosis of patients with cervical cancer.

CONCLUSIONS: LncRNA PCAT-1 siRNA transfected into cervical cancer cell lines can effectively lower the expression of lncRNA PCAT-1, while lncRNA PCAT-1 expression can inhibit the proliferation, metastasis and invasion abilities of cervical cancer cells.

Key Words:

lncRNA, lncRNA PCAT-1, Cervical neoplasms, Cell proliferation, Tumor invasion.

the health of them. The prognosis for cervical cancer, the second most common malignant tumor in women, is still poor. Current investigations^{1,2} have revealed that 99.8% of cervical cancer patients can be detected with human papillomavirus (HPV) infection. However, HPV infection alone is not enough to trigger cervical cancer. The reason is that the tumor development is a multi-stage and multi-step process of proto-oncogene activation or (and) inactivation of tumor suppressor genes. In this process, gene mutations and transcriptional abnormalities play important roles, and the corresponding changes must occur in cells themselves in order to eventually cause cervical cancer³. Therefore, studying the pathogenesis of cervical cancer is particularly important. Long non-coding RNA (lncRNA) was originally considered as the "noise" of genome transcription. It is a byproduct of RNA polymerase II transcription with no biological function with transcript length > 200 nt⁴. A number of papers have illustrated that lncRNA is related to many regulatory processes^{5,6}, including the development of tumors. Some abnormally expressed lncRNAs contribute to colon cancer⁷, breast cancer⁸ and liver cancer⁹. lncRNA-related databases (such as lncRNA disease database) were referred. Currently, lncRNAs related to cervical cancer include metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)¹⁰, maternally expressed gene 3 (MEG3)¹¹, and XLOC_010588¹². It has been reported that lncRNA PCAT-1 function in cancer needs to be determined. PCAT-1 regulates breast cancer susceptibility gene 2 (BRCA2) and controls homologous recombination in cancer¹³. In prostate cancer, lncRNA PCAT-1 can promote cell proliferation via regulating cMyc¹⁴. In this study, the expression of lncRNA PCAT-1 in cervical cancer cells was interfered, and it was

Introduction

Cervical cancer often occurs in young women with a high incidence rate, seriously threatening

found that the proliferation, metastasis and invasion of cells were significantly affected by the decreased expression of lncRNA PCAT-1.

Materials and Methods

Materials

HeLa cells were preserved in our laboratory. C-33A cells (Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China), lncRNA PCAT-1 siRNA and negative siRNA (synthesized by Suzhou Gemma Gene, Suzhou, China), nucleic acid molecular weight markers SYBR Green dye (Bio-Rad, Hercules, CA, USA), cell counting kit-8 (CCK-8, Tongren Co., Ltd. Tokyo, Japan), medium and fetal calf serum (FCS) (HyClone, South Logan, UT, USA). Pancreatin (Gibco (Rockville, MD, USA), opti-MEM (Thermo Fisher Scientific, Waltham, MA, USA). Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA), TRIzol (Invitrogen, Carlsbad, CA, USA), PureLink RNA MiniKit (Thermo Fisher Scientific, Waltham, MA, USA), and DNase I (Thermo Fisher Scientific, Waltham, MA, USA), Matrigel (BD, Franklin Lakes, NJ, USA), 6/24/96 well cell culture plate (Nunc, Waltham, MA, USA), and primer synthesis (Beijing Aoke Ding Sheng Biotechnology Co., Ltd. Beijing, China).

Methods

Clinical Specimens

All the cancer specimens and adjacent non-tumor areas were obtained and then stored in liquid nitrogen. This work was approved by the Ethics Committee of Huai'an First People's Hospital. Signed written informed consents were obtained from all participants before the study.

Cell Culture and Transfection

HeLa and C-33A cervical cancer cells were seeded in 6-well plates. HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) F12 containing 10% fetal bovine serum (FBS), and C-33A was cultured in modified 1640 medium. Next, the cells were incubated in a humidified incubator at 37°C with 5% CO₂. When the cell density was about 40% confluence, the cells were transfected with Lipofectamine 2000. Next, 5 μL Lipofectamine 2000 containing 200 pmol lncRNA PCAT-1 was added in each well. The

same amount of Lipofectamine 2000 and negative siRNA were added into the control group and the experimental group, and siRNA sequence was 5'-GAGAAAGCAUCUGUACCCUUACAAU-3'.

Detection of Long Non-Coding RNA Expression of PCAT-1 by qPCR

The total RNA was extracted by the Pure-Link RNA Mini Kit. The DNA was digested with DNase I to remove the genomic contamination. The reverse transcription kit was used to reverse the total RNA, and SYBR Green Real-time quantitative PCR was used to detect lncRNA PCAT-1 expression.

The primers were as follows: PCAT-1 sense: 5'-AATGGCATGAACCTGGGAGGCG-3'; PCAT-1 antisense: 5'-GGCTTTGGGAAGT-GCTTTGGAG-3'. Reaction conditions: at 95°C for 2 min, 95°C for 10 s, 56°C for 10 s, 68°C for 12 s, 95°C for 1 min, 55°C for 1 min and 70°C for 6 s; a total of 40 cycles. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control. The GAPDH upstream primer was GAPDH-F: 5'-ACCCAGAAGACT-GTGGATGG-3', and the downstream primer was GAPDH-R: 5'-TTCTAGACGGCAG-GTCAG-GT-3'.

Detection of Cell Proliferation By Cell Counting kit-8 (CCK-8) Assay

The cells were transplanted into 96-well plates at a density of $2 \times 10^3/100 \mu\text{L}$ 12 h later, and the CCK-8 assay was performed after culturing for 24, 48, 72 and 84 h. During detection, serum-free medium was replaced, 10 μL CCK-8 were added to each well, and the optical density value at 450 nm (OD450) after incubating for 2 h at 37°C with 5% CO₂ was measured with 4 replicates in each group.

Clone Formation Experiment

The cells were transplanted into 6-well plates at a density of $1 \times 10^3/100 \mu\text{L}$ 12 h later, and cultured at 37°C with 5% CO₂ for 2 days. Then, the medium was discarded and washed twice with phosphate-buffered saline (PBS). 1 mL 0.1% crystal violet solution was added to each well. 30 min later, the crystal violet solution was discarded and washed with PBS until the eluate became clear. Finally, pictures were taken and counted.

Wound-Healing Assay

HeLa and C-33A cells were seeded in 6-well plates and transfected until the cells reached a

density of about 50%. After 6 h of transfection, the medium was changed and continued to grow for 2 days until the cells grew well. The 200 μ L pipette tip was placed perpendicular to Scratches on the cell culture surface. The cells were washed three times with PBS, and the medium was changed. The scratch point was recorded at time 0 and photographed every 12 h, and the scratch distance was measured.

Invasion Assay

Based on the manufacturer's protocol¹⁵, cell invasion experiment was conducted with BioCoat Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) and chambers (8- μ m pore size, Millipore, Eschborn, Germany). 1×10^5 cells were seeded into 24-well plates with serum-free medium, and then cultured in the upper chamber at 37°C with 5% CO₂. Cells under the filter were completely fixed, and then stained by crystal violet. Analysis results were analyzed using NIS Elements image software (Nikon, Tokyo, Japan).

Statistical Analysis

SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. All quantitative data were expressed as mean \pm standard deviation. Two-tailed *t*-tests were used for pairwise comparisons. The survival curve was detected by Kaplan-Meier. The log-rank test was used for the prognosis of patients. $p < 0.05$ represented that the difference was statistically significant.

Results

Effect of the Down-Regulation of lncRNA PCAT-1 on Cervical Cancer Cell Proliferation

LncRNA PCAT-1 siRNA was interfered with the expression of lncRNA PCAT-1 in HeLa and C-33A cells. The results showed that the expression of lncRNA PCAT-1 in cells was significantly lower than that in the control group (Figure 1A), indicating that lncRNA PCAT-1 siRNA can effectively interfere with the expression of lncRNA PCAT-1 and significantly reduces lncRNA PCAT-1 in cells. CCK-8 results showed that the OD450 values of HeLa and C-33A cells transfected with lncRNA PCAT-1 siRNA were lower than that of cells transfected with siRNA negative control, indicating that the cell proliferation is inhibited, and the difference was statistically significant ($p < 0.01$, Figure 2A).

Effect of the Down-Regulation of lncRNA PCAT-1 on Cervical Cancer Cell Cloning Ability

The result of clone formation revealed that the ability of individual cells to form cell clusters was diminished as the expression of lncRNA PCAT-1 in HeLa and C-33A cells was knocked down compared with that of cells transfected with siRNA negative controls. Both cell clone formation test and CCK-8 cell proliferation test results manifested that lncRNA PCAT-1 could promote the proliferation of cervical cancer cells (Figure 2B).

Effect of the Down-Regulation of lncRNA PCAT-1 on Cervical Cancer Cell Metastasis Ability

Scratch cell assay results indicated that the healing for scratches slowed down in HeLa and C-33A cells with the knock-down expression level of lncRNA PCAT-1 compared with that in cells transfected with siRNA negative controls. In other words, the ability of cell metastasis was weakened, indicating that the lncRNA PCAT-1 can promote comprehensive metastasis ability of cervical cancer cells (Figure 3).

Effect of the Down-Regulation of lncRNA PCAT-1 on the Invasion Ability of Cervical Cancer Cells

Matrix gel cell invasion assay was performed by placing serum-free cell suspension on the top of a chamber covered with a matrix gel and adding a medium containing 10% FBS on the lower layer. The invasion ability of cells would be reflected by the number of cells entering from the matrix gel to the lower layer. The results of Matrix gel cell invasion assay evidenced that the number of cells entering the lower cell compartment was decreased after the interference with lncRNA PCAT-1 in HeLa and C-33A cells compared with that of cells transfected with siRNA negative controls (Figure 4). The results revealed that lncRNA PCAT-1 could promote cell invasion.

Up-regulated lncRNA PCAT-1 Could Help Predict the Poor Prognosis of Patients With Cervical Cancer

Expression in patients with cervical cancer was also examined by qRT-PCR. lncRNA PCAT-1 expression was significantly increased in cervical cancer tissues compared with that in adjacent non-tumor tissues ($p < 0.05$, Figure 1B). The high expression level of lncRNA

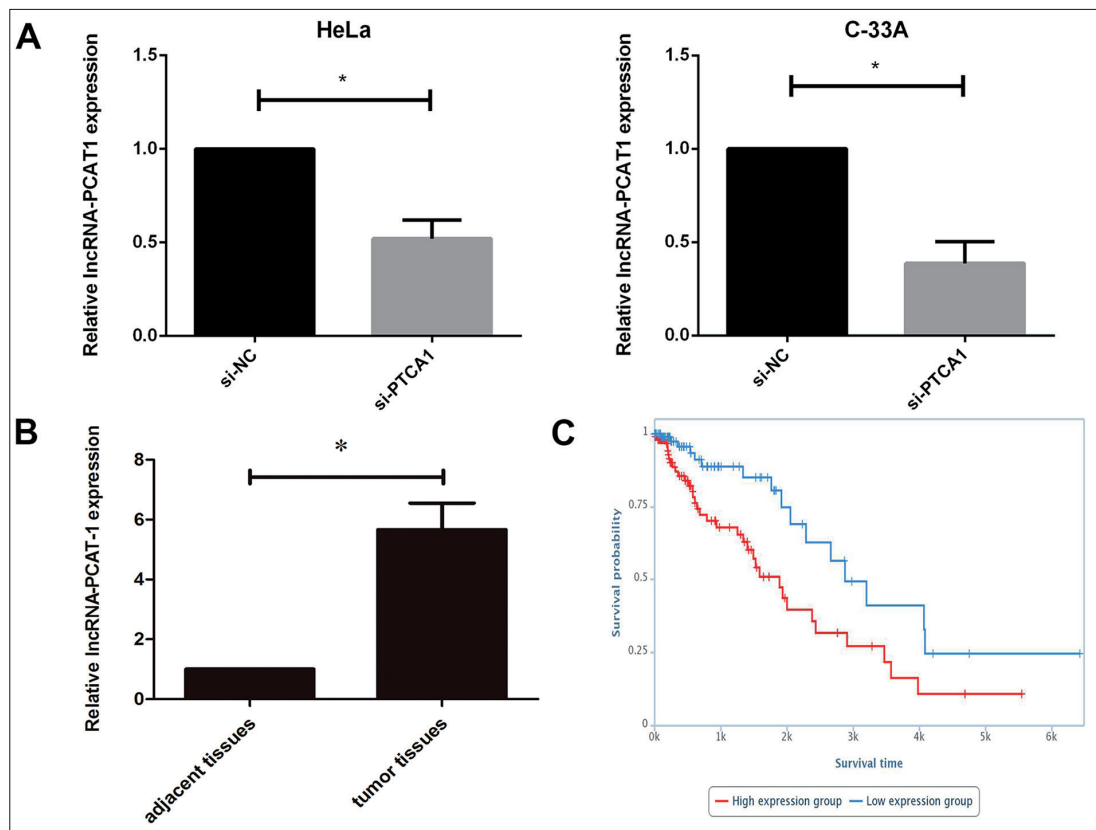


Figure 1. **A**, IncRNA-PCAT1 is reduced by siRNA in cervical cancer cell lines (HeLa and C-33A), si-NC: siRNA-negative control and si-PTCA1: siRNA-PTCA1. **B**, IncRNA-PCAT1 is increased in tumor tissues as compared to adjacent tissues by RT-PCR. **C**, IncRNA PCAT-1 up-regulation is associated with the poor prognosis of patients with cervical cancer by Kaplan-Meier analysis and log-rank test. $*p < 0.05$.

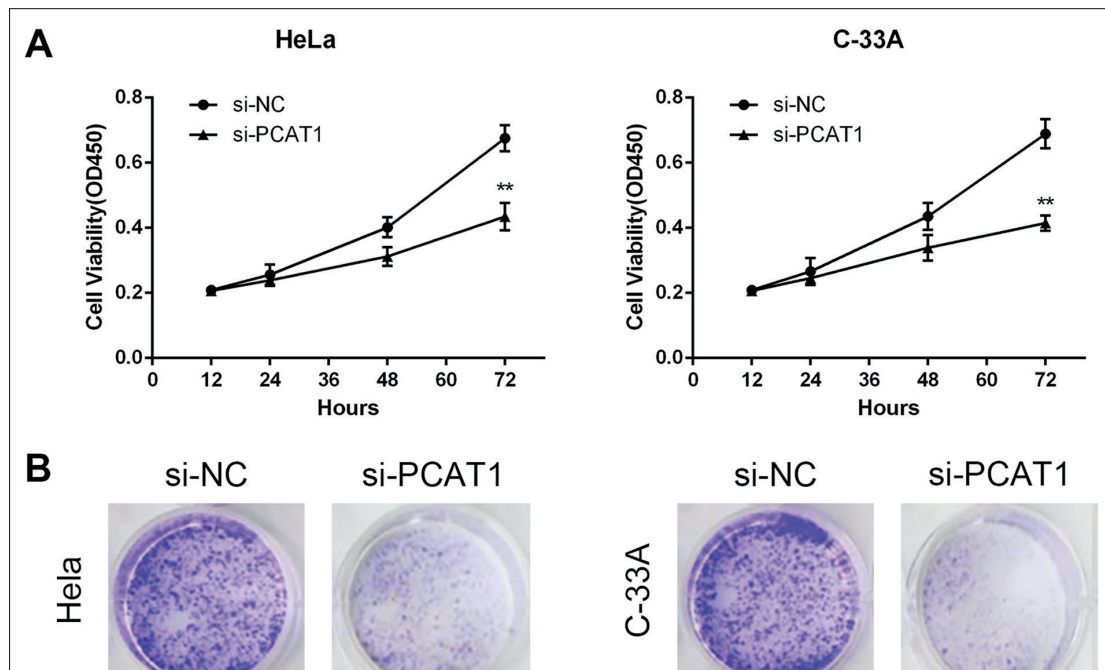


Figure 2. **A**, Comparison of the proliferative ability of HeLa and C-33A cell lines between si-NC and si-PCAT1 group at 12 h, 24 h, 48 h and 72 h is detected by CCK-8. $*p < 0.05$. **B**, Cell cloning ability is detected in si-NC and si-PCAT1. $*p < 0.05$.

Figure 3. Cell metastasis is detected by wound-healing assay at 0 h and 48 h. $*p < 0.05$.

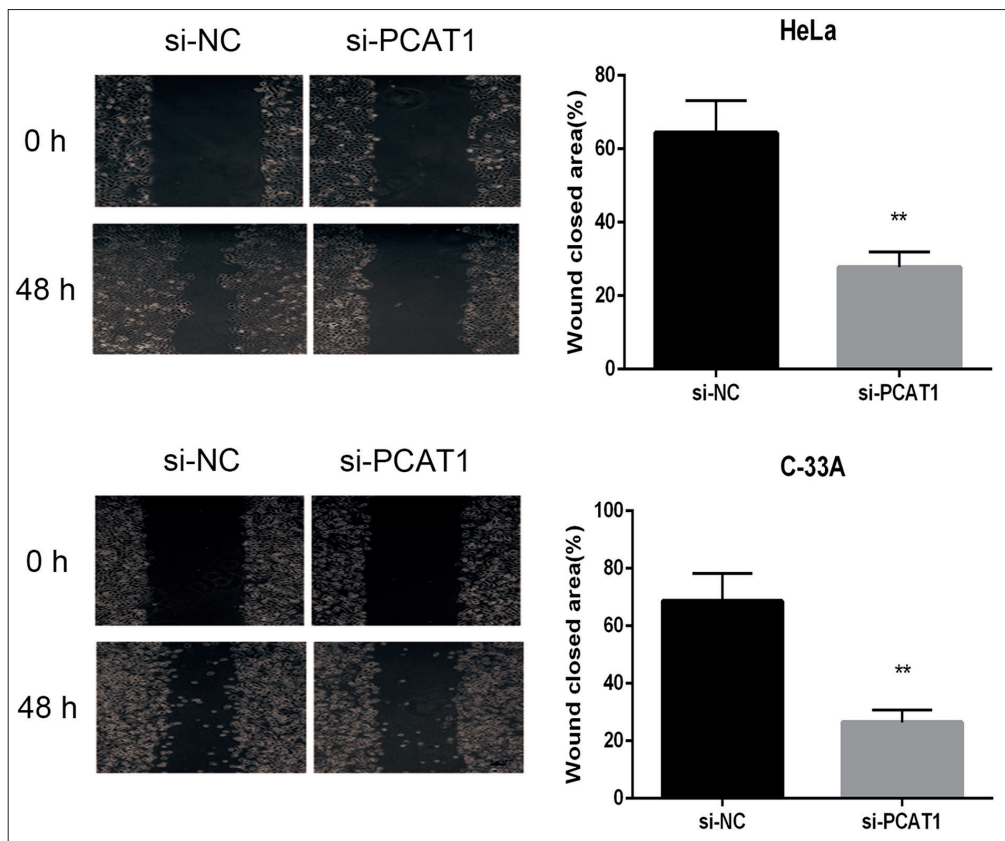


Figure 4. Cell invasion is detected by transwell invasion assay. $*p < 0.05$.

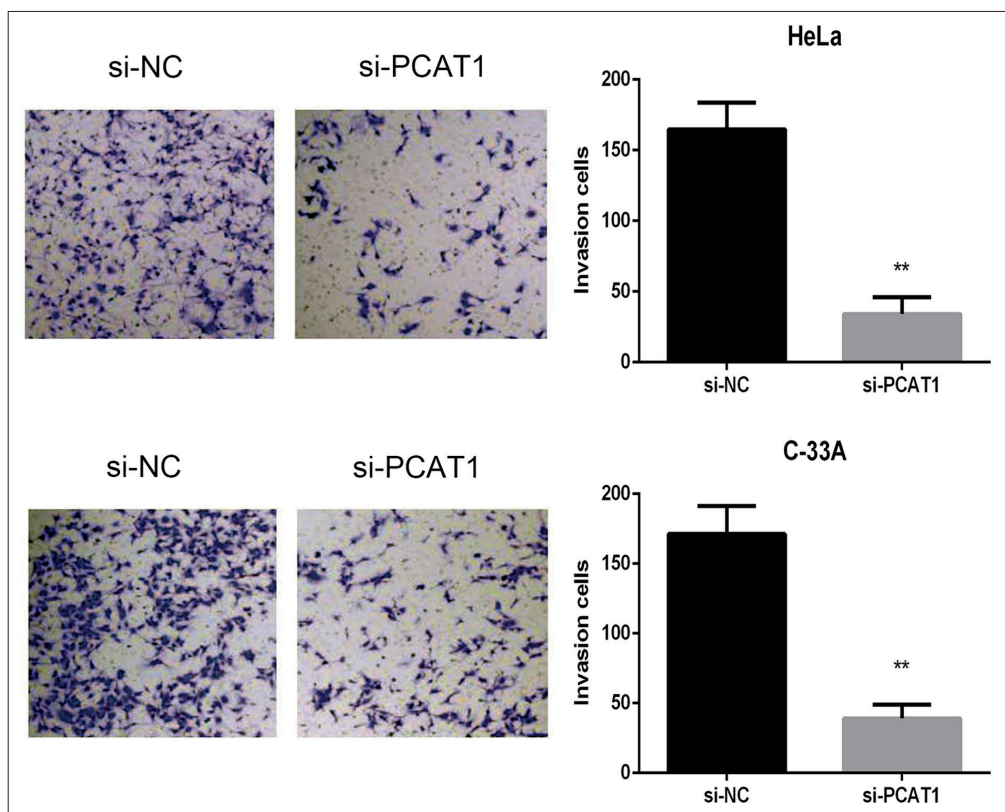


Table I. lncRNA-PCAT-1 expression and clinical factors of cancer patients.

Factor	NO.	lncRNA-PCAT-1		p
		High	Low	
NO.	75	38	37	
Age (years)	< 55	33	16	0.738
	≥ 55	42	20	
Tumor size (cm)	< 4	31	11	0.027
	≥ 4	44	27	
Lymph nodes metastasis	Negative	48	18	0.002
	Positive	27	7	
HPV infection	Negative	29	19	0.041
	Positive	46	27	
FIGO stage	I-II	42	15	0.003
	III-IV	33	23	

PCAT-1 was correlated with FIGO stage, tumor size and metastasis ($p < 0.05$, Table I), which indicated that increased expression of lncRNA PCAT-1 is involved in the progression of cervical cancer. Kaplan-Meier analysis was used to detect the correlation between lncRNA PCAT-1 expression and patients' survival. The patients with highly expressed lncRNA PCAT-1 tended to have shorter overall survival than those with lowly expressed lncRNA PCAT-1 (Figure 1C).

Discussion

Cervical cancer is a common tumor around the world that threatens women's health. Studying its molecular mechanism of development is of great importance in light of its poor prognosis. More and more studies have shown that non-coding RNA plays an important role in regulating the cancer development. Some of these microRNAs (miRNAs) are abnormally expressed in cervical cancer and affect the proliferation and metastasis. However, some special miRNAs can be used as molecular factors for the cervical cancer diagnosis¹⁶⁻¹⁹. In recent years, it has been found that some lncRNAs play an important role in the development of cervical cancer. Qin et al¹¹ found that MEG3 is significantly down-regulated in cervical cancer tissues in the comparison of 18 pairs of cervical cancer tissues with adjacent normal tissues. Overexpression of MEG3 *in vitro* inhibits the proliferation of cervical cancer cells, HeLa and C-33A, thus leading to G2/M cell arrest. However, Liao et al¹² found that XLOC_010588 was down-regulated in the comparison with 218 cervical cancer tissues.

Overexpression of XLOC_010588 can inhibit the proliferation cells, while knocking down XLOC_010588 inhibit the growth of HCC94 cells. The molecular mechanism of the study suggested that XLOC_010588 and c-Myc could reduce c-Myc expression. These findings indicate that lncRNA plays an important role in cervical cancer and has potential application value. To find a new functional lncRNA involved in cervical cancer progression, the expression of lncRNA PCAT-1 was detected in the study, which showed that it was highly expressed in cervical cancer. Therefore, it was speculated that lncRNA PCAT-1 might affect the biological process of cervical cancer, but there is no report on the related function of PCAT-1 in cervical cancer.

Conclusions

The effects of lncRNA PCAT-1 on proliferation, metastasis and invasion of cervical cancer cells were investigated using RNAi technique. The results demonstrated that lncRNA PCAT-1 expression could inhibit the proliferation of cervical cancer cells and weaken the metastasis and invasion abilities, and lncRNA PCAT-1 could help predict the poor prognosis of cervical cancer patients, which is of great significance for further revealing the pathogenesis of cervical cancer. Further exploring the molecular mechanism in which lncRNA PCAT-1 plays a role and identifying its target genes and signaling pathways will provide new molecular markers and therapeutic targets for the diagnosis and treatment of cervical cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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