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Long noncoding RNA PCAT6 regulates cell growth and metastasis via Wnt/β-catenin pathway and is a prognosis marker in cervical cancer

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Abstract. – OBJECTIVE: Previously, long noncoding RNAs (lncRNAs) have been reported to have critical regulatory roles in the progression of human cancers. LncRNA prostate cancer-associated transcript 6 (PCAT6) has been reported to act as an oncogene in several tumors. However, its expression and function in cervical cancer (CC) have not been investigated. In this study, we aim to reveal the functions of PCAT6 and the underlying mechanisms in CC.

PATIENTS AND METHODS: We evaluated the expression levels of PCAT6 in CC tissues and cell lines using real-time PCR. The clinical data were interpreted by chi-square test, Kaplan-Meier survival analyses, univariate analysis, and multivariate analysis. The effect of PCAT6 on CC proliferation and metastasis was investigated by CCK-8 assay, EdU incorporation assay and transwell assay. The cell apoptosis was detected by apoptosis flow detection. RT-PCR and Western blotting were used to detect the expression levels of β-catenin, cyclin D1 and c-myc.

RESULTS: We found that PCAT6 expression was significantly up-regulated in human CC tissues and cell lines compared with their normal counterparts, and its high levels were associated with advanced FIGO stage, depth of cervical invasion and positively lymph node metastasis. Survival assays indicated that high PCAT6 expression had a negative influence on overall survival and disease-free survival. Moreover, multivariate analysis identified high PCAT6 expression as an unfavorable prognostic biomarker for CC patients. Functionally, knockdown of PCAT6 significantly suppressed CC cells proliferation, migration and invasion, and promoted apoptosis. Mechanistic investigation showed PCAT6 activates Wnt/β-catenin signaling in CC cell lines by promoting the expression of β-catenin, cyclin D1 and c-myc.

CONCLUSIONS: Our results indicated that PCAT6 played oncogenic roles and can be used as a therapeutic target for treating human CC.

Key Words
lncRNA PCAT6, Growth, Migration, Invasion, Wnt/β-catenin signaling, Prognosis, Cervical cancer.

Introduction

Cervical cancer (CC) ranks as the third leading cause of cancer mortality and is the second most common female malignancy in women[1,2]. Reports estimate that there are approximately 500,000 new cases of cervical cancer diagnosed each year. The etiology and pathogenesis of CC remains largely unclear. Human papillomavirus infection (HPV) causes more than 90% of cases[3]. At present, surgery, radiotherapy and chemotherapy remain the standard treatment for patients with CC[4]. In spite of recent progresses of chemotherapy, the prognosis of CC remains poor[5,6]. The major challenge for the treatment of CC patients is the delay of suspected patients who are diagnosed at advanced or metastatic stages[7]. Therefore, better understanding of the underlying mechanisms involved in CC development and progression is necessary for the development of prognsotic markers and novel effective therapies for CC patients. Long non-coding RNAs (lncRNAs) represent a diverse type of long RNA molecules lacking protein-coding capacity, with a length of larger than 200 nucleotides, and are characterized by diverse and complex sequences and mechanisms of action[8,9]. Growing shreds of evidence have indicated that lncRNAs display important regulatory function in several biological processes, such as cell proliferation, apoptosis, genomic imprinting, chromatin modification and post-transcriptional processing[9,10]. Increasing studies report that lncRNAs are implicated in diverse pathophysiological processes, particular in cancers, and more and more lncRNAs are reported to be frequently abnormally expressed in cancers, which suggest that lncRNAs could be used as promising biomarkers for tumor early detection and prognosis monitoring[11-13]. However, the carcinogenesis and pathological functions of

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most lncRNAs in CC are still unclear. LncRNA prostate cancer-associated transcript 6 (PCAT6), located at 1q32.1, was a newly identified lncRNA which was firstly reported to be involved in the regulation of prostate cancer[14]. Then, several studies reported that PCAT6 was abnormally expressed in several tumors, such as colon cancer[15], non-small-cell lung cancer[16] and gastric cancer[17], suggesting that PCAT6 may play an important role in progression of some tumors. However, little is known about the expression pattern and function of PCAT6 in CC.

Patients and Methods

Human CC Tissue Samples and Cell Lines

A total of 114 patients with CC were included in this study and had written informed consent. The paired tumor tissue samples and the corresponding adjacent normal tissues which were preserved at -80°C were obtained from the Zhejiang Cancer Hospital between March 2011 and April 2013. None of the patients received radiotherapy or chemotherapy before surgery. This study was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine. The relevant clinical information of the patients was shown in Table II. Cell lines used in this study including CC cell lines (Caski, SW756, HeLa, ME-180, SiHa and C33A) and cervical epithelial cells (Ect1/E6E7), were purchased from Shanghai Xiangf Bio Co., Ltd. (Hongkou, Shanghai, China). These cells were all cultured using RPMI-1640 medium (BeiNuo, Xuhui, Shanghai, China) supplemented with fetal bovine serum (FBS) (10%) as well as streptomycin-penicillin antibiotic solution (1%; Sangong, Songjiang, China) with 5% CO_2 at 37°C.

Cell Transfection

Small interfering RNA (siRNA) against PCAT6 (si-1 and si-2) and negative control siRNAs (si-NC) were purchased from Zoonbio Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). Then, we used a RFect transfection reagent kit (BioDai, Changzhou, Jiangsu, China) to transfect the siRNAs into the cells. Briefly, the HeLa or SiHa cells were placed into six-well plates and grown to 70% cell confluence using complete medium. Then, the medium was removed and the plates were added with fresh complete medium. Subsequently, 10 pmol siRNA was mixed with 10 μl RFect transfection reagent, and then the mixture was added into the cells. For plasmids transfection, 2 μg plasmids and 10 μl RFect transfection reagent were mixed and sequentially added into the cells.

RNA Purification and Real-Time Quantitative RT-PCR (qRT-PCR)

A GenElute total RNA extraction kit (Sigma-Aldrich, Pudong, Shanghai, China) was used to extract total RNA from the relevant tissues and cell lines. Subsequently, the reverse transcription to cDNA was conducted using a cDNA Reverse Transcription kit (MultiSciences, Hangzhou, Zhejiang, China). Then, the qRT-PCR analysis for PCAT6, β-catenin, cyclin D1 and c-myc determination was carried out using a UltraSYBR Mixture qRT-PCR kit (CWBiotech, Changping, Beijing, China) on a Bio-Rad iCyler iQ qRT-PCR apparatus (Hercules, CA, USA). The primer sequences used in this study were indicated in Table I. The expression of PCAT6 and mRNA were normalized to endogenous control glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The analysis of the relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Western Blot Analysis

Total protein samples were separately extracted from the indicated siRNAs-transfected HeLa and SiHa cells using RIPA lysis buffer (BinBio, Jinshan, Shanghai, China). Subsequently, equal amounts of protein (25 μg/lane) were loaded on 8-12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by being electrophoretically transferred onto polyvinylidene difluoride membranes. After being blocked using non-fat milk (5%), the membranes were then incubated with primary antibodies for 10-12 h at 4°C. After that, the membranes were washed by TBST buffer for three times and incubated with matched secondary antibodies. Finally, an ECL assay kit (TW-Reagent, Pudong, Shanghai, China) was applied to detect the protein bands. The anti-β-catenin and anti-c-myc antibodies were purchased from Abcam Co., Ltd.

Table I. The sequences of primers used in this study.

Names	Sequences (5′-3′)
PCAT6: forward	CAGGAACCCCCTCCTTACTC
PCAT6: reverse	CTAGGGATGTGTCCGAAGGA
GAPDH: forward	GTCAACGGATTTGGTCTGTATT
GAPDH: reverse	AGTCTTCTGGGTGGCAGTGAT

Table II. Clinicopathological features associated with lncRNA PCAT6 expression in 114 CC patients.

Clinicopathologic features	Total	lncRNA PCAT6 expression		p
		Low	High	
Age				0.572
< 45	58	30	28	
≥ 45	56	26	30	
Tumor size (cm)				0.240
< 4.0	67	36	31	
≥ 4.0	47	20	27	
Histologic grade				0.334
G1 + G2	64	34	30	
G3	50	22	28	
FIGO stage				0.038
Ib~IIa	77	43	34	
IIb~IIIa	37	13	24	
Lymph node metastasis				0.028
No	83	46	37	
Yes	31	10	21	
Depth of cervical invasion				0.020
<2/3	80	45	35	
≥2/3	34	11	23	

(Pudong, Shanghai, China). The anti-cyclin D1 and anti-GAPDH antibodies were obtained from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, Hubei, China).

Cell Counting Kit-8 (CCK-8) Assay

Briefly, twenty-four hours post transfection, the cells were planted into nighty-six-well plates and maintained at 37°C with 5% CO_2 for 4 days. At the indicated time period (0 h, 24 h, 48 h, 72 h and 96 h), each well was added with 10 µl CCK-8 reagent (GLPBIO, Montclair, CA, USA) and the plates were continued to be cultured for 1-2 h. Then, the cell viability was determined by measuring the optical density (OD) absorbance at the wavelength of 450 nm.

5-Ethynyl-20-Deoxyuridine (EdU) Incorporation Assay

We applied a Cell-Light EdU kit (RiboBio, Guangzhou, Guangdong, China) to conduct the EdU assays. In short, cells at proper density were planted in forty-eight-well plates for attachment. Then, 50 µM EdU was added into each well and maintained for 2 h. After being fixed with paraformaldehyde (4%), the cells were stained with Apollo Dye Solution as well as DAPI solution. Finally, the fluorescence was observed by a DMi8 fluorescence microscope (Leica, Mannheim, Germany).

Colony Formation Assay

HeLa or SiHa cells transfected with indicated siRNAs were maintained in twelve-well plates at a density of 500 cells per well. Next, the plates were maintained at 37°C with 5% CO_2 for 2-3 weeks. Subsequently, the cell colonies were fixed with paraformaldehyde (4%) and we used crystal violet (0.1%; Servicebio, Wuhan, Hubei, China) to stain these colonies. Finally, the stained colonies were photographed by a XSP-20CD microscope (Huxing, Pudong, Shanghai, China).

Apoptosis Detection

A BD Bioscience Annexin V-FITC/PI detection kit (FuShen, Xuhui, Shanghai, China) was used to conduct the cell apoptosis detection. The cells were first trypsinized using Trypsin-EDTA Solution (MultiSciences, Hangzhou, Zhejiang, China) and washed using ice-cold PBS buffer twice. Afterwards, the cells were stained with Annexin V-FITC as well as PI, followed by being placing in the dark for 20 min at room temperature. Finally, the apoptosis was determined by a BD Accuri C6 Plus flow cytometer (ZLW Biological, Fengtai, Beijing, China).

Wound-Healing Assay

The cells were transfected with indicated siRNAs, harvested and re-plated at high density in six-well plates. Twenty-four hours later (the cell

confluence reached more than 95%), the cell monolayers were scraped with a 200 μl pipette tip (time: 0 h) and photographed by a microscope (XSP-20CD; Huxing, Pudong, Shanghai, China). After culturing for 48 h, the wounded areas were also photographed.

Transwell Invasion Assay

The indicated siRNAs-transfected cells were trypsinized into single cells and resuspended in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (without FBS). Then, 1×10^5 treated cells were seeded into the upper chamber of each transwell insert pre-coated with BD Biosciences Matrigel (100 μl; FuShen, Xuhui, Shanghai, China) to form a matrix barrier, and the lower chamber of each well was added with serum-free RPMI-1640 medium (500 μl). After incubation for 24 h at 37°C, the invaded cells adhering to the lower sides of the transwell inserts' membranes were stained using a dye solution containing 0.1% crystal violet (Servicebio, Wuhan, Hubei, China). Finally, the invaded cells were photographed by a microscope (XSP-20CD; Huxing, Pudong, Shanghai, China).

TOPFlash Luciferase Assay

The activity of Wnt/β-catenin signaling was determined by TOPFlash luciferase assay. In brief, the indicated siRNAs-transfected cells were trypsinized into single cell-suspensions and then placed in twenty-four-well plates at the density of 5×10^4 cells per well. Subsequently, TOPFlash reporter plasmid (Addgene, Cambridge, MA, USA), FOPFlash reporter plasmid (Addgene, Cambridge, MA, USA) and Renilla TK-luciferase vector (Promega, Dongcheng, Beijing, China) were co-transfected into the treated cells using the above described cell transfection methods. Finally, the luciferase activity was determined by a Promega dual luciferase reporter assay kit (Promega, Madison, WI, USA).

Statistical Analysis

All statistical analysis was conducted using SPSS 19.0 (SPSS, Armonk, NY, USA) and GraphPad Prism 7 (GraphPad, La Jolla, CA, USA) software. Statistical differences between two groups of data were determined using the unpaired two-tailed Student *t*-test. The Chi-square test and Fisher's exact test were used to examine the association between PCAT6 expressions and various clinicopathological parameters. The Kaplan-Meier method was employed to plot the overall survival curve and the log-rank test was applied to evaluate survival difference. Cox proportional hazards regression model was used for univariate and multivariate analyses of prognostic significance of PCAT6. A $p<0.05$ signified statistical significance.

PCAT6 is upregulated in CC Tissues and Cell Lines

In order to explore the possible role of PCAT6 in CC, we firstly detected the expression of PCAT6 in CC patients by RT-PCR. As shown in Figure 1A, we found that PCAT6 expression was significantly up-regulated in CC tissues compared to matched normal cervical tissues ($p<0.01$). Then, we further detected the expression levels of PCAT6 in a panel of CC cell lines (Caski, SW756, HeLa, ME-180, SiHa, and C33A) and normal cervical cells (Ect1/E6E7), finding that as compared to Ect1/E6E7, PCAT6 were markedly upregulated in all evaluated CC cell lines (Figure 1B). Thus, our results indicated that increased expression of PCAT6 may be associated with progression of CC. Given the relatively higher expression of HeLa and SiHa among all CC cell lines, it was decided to use the HeLa and SiHa cells for subsequent experiments.

Prognostic Values of PCAT6 Expression in CC Patients

All patients with cervical cancer were assigned into two groups (high PCAT6 group and low PCAT6 group) based on the median expression level of PCAT6. Then, we used chi-square test to explore whether PCAT6 expression was associated with the clinicopathological factors of patients with CC. As shown in Table II, PCAT6 expression was observed to be closely correlated with advanced FIGO stage ($p = 0.038$), positively lymph node metastasis ($p = 0.028$) and depth of cervical invasion ($p = 0.020$). However, there were no significant correlations between PCAT6 expression and other clinicopathologic features including age, tumor size and histologic grade ($p > 0.05$). To determine the relationship between PCAT6 expression and the prognosis of CRC patients after surgery, we used a Kaplan-Meier survival analysis and log-rank tests. As shown in Figure 2A and 2B, we found that patients with high expression of PCAT6 had shorter overall survival ($p = 0.0024$) and disease-free survival ($p = 0.0042$) as compared with the PCAT6-low group (Table II). In univariate analysis, high expression of PCAT6, FIGO stage, lymph

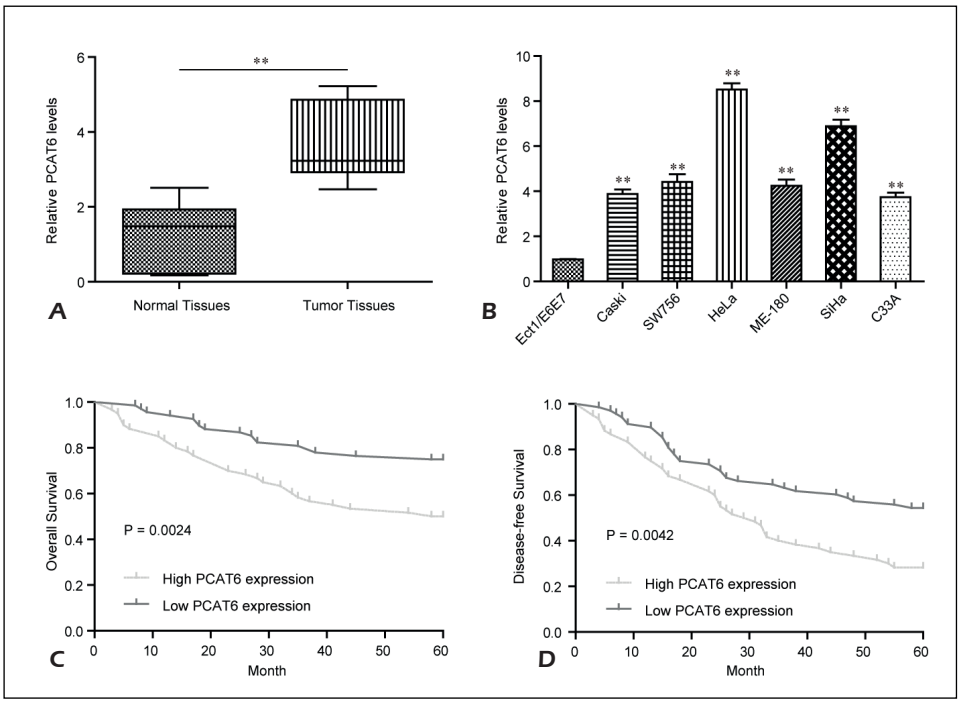

Figure 1. Expression levels of PCAT6 in CC tissues and cell lines and clinical significance. **A**, Difference in the average PCAT6 relative level between CC tissues and matched paracarcinoma normal tissues. **B**, Relative PCAT6 expression levels in six CC lines (Caski, SW756, HeLa, ME-180, SiHa and C33A) and a normal cervical epithelial cell line (Ect1/E6E7) were determined with RT-PCR. Overall survival (**C**) and disease-free survival (**D**) in CC patients with low or high serum concentrations of PCAT6. Statistical analysis was performed using log-rank test. *p<0.05, **p<0.01.

node metastasis and depth of cervical invasion were evaluated to correlate poor overall survival. More importantly, multivariate analysis confirmed that PCAT6 was an independent poor prognostic factor for both overall survival (RR = 2.942, $p < 0.05$; Table II) in CC (Table III). In addition, the similar results of the effects of PCAT6 was found in disease-free survival of CC patients (Table IV). Thus, we firstly provided evidence that PCAT6 could be a new cancer biomarker for CC patients.

Silence of PCAT6 Impaired Cervical Cells Proliferation and Accelerated Cell Apoptosis

To gain insight into the potential role of PCAT6 in CC, we first synthesized two siRNAs specific targeting PCAT6 (si-1 and si-2) and separately transfected them into two CC cell lines: HeLa and SiHa cells. Then, we conducted qRT-PCR analysis and found that PCAT6 expression was knocked down in HeLa and SiHa cells after transfection with si-1 and si-2 (Figure 2A). There-

Table III. Prognostic value of lncRNA PCAT6 expression for overall survival of patients with CC in univariate and multivariate analyses by Cox Regression.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	1.346 (0.655-1.892)	0.462	–	–
Tumor size	1.644 (0.794-2.218)	0.155		–
Histologic grade	1.472 (0.842-2.128)	0.124	–	–
FIGO stage	3.162 (1.362-4.476)	0.011	2.898 (1.138-3.994)	0.017
Lymph node metastasis	3.325 (1.488-4.873)	0.007	3.024 (1.219-4.348)	0.009
Depth of cervical invasion	3.462 (1.348-5.012)	0.005	2.895 (1.148-4.337)	0.015
PCAT6 expression	3.357 (1.489-4.667)	0.008	3.018 (1.287-4.238)	0.012

Table IV. Prognostic value of lncRNA PCAT6 expression for disease-free survival of patients with CC in univariate and multivariate analyses by Cox Regression.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	1.467 (0.582-2.128)	0.288	–	–
Tumor size	1.231 (0.637-2.042)	0.126		–
Histologic grade	1.148 (0.669-2.356)	0.109	–	–
FIGO stage	3.326 (1.214-4.665)	0.013	2.652 (1.231-3.546)	0.034
Lymph node metastasis	3.134 (1.338-4.769)	0.011	3.123 (1.166-4.459)	0.016
Depth of cervical invasion	3.554 (1.547-5.241)	0.002	3.123 (1.436-4.889)	0.008
PCAT6 expression	3.3465 (1.654-5.125)	0.003	3.221 (1.345-4.556)	0.009

after, we performed CCK-8 assays to evaluate the cellular growth curves and the results revealed that repressing the expression of PCAT6 remarkably suppressed the cell proliferation of HeLa and SiHa cells (Figure 2B). Similarly, results of another classical cellular proliferation detecting meth-

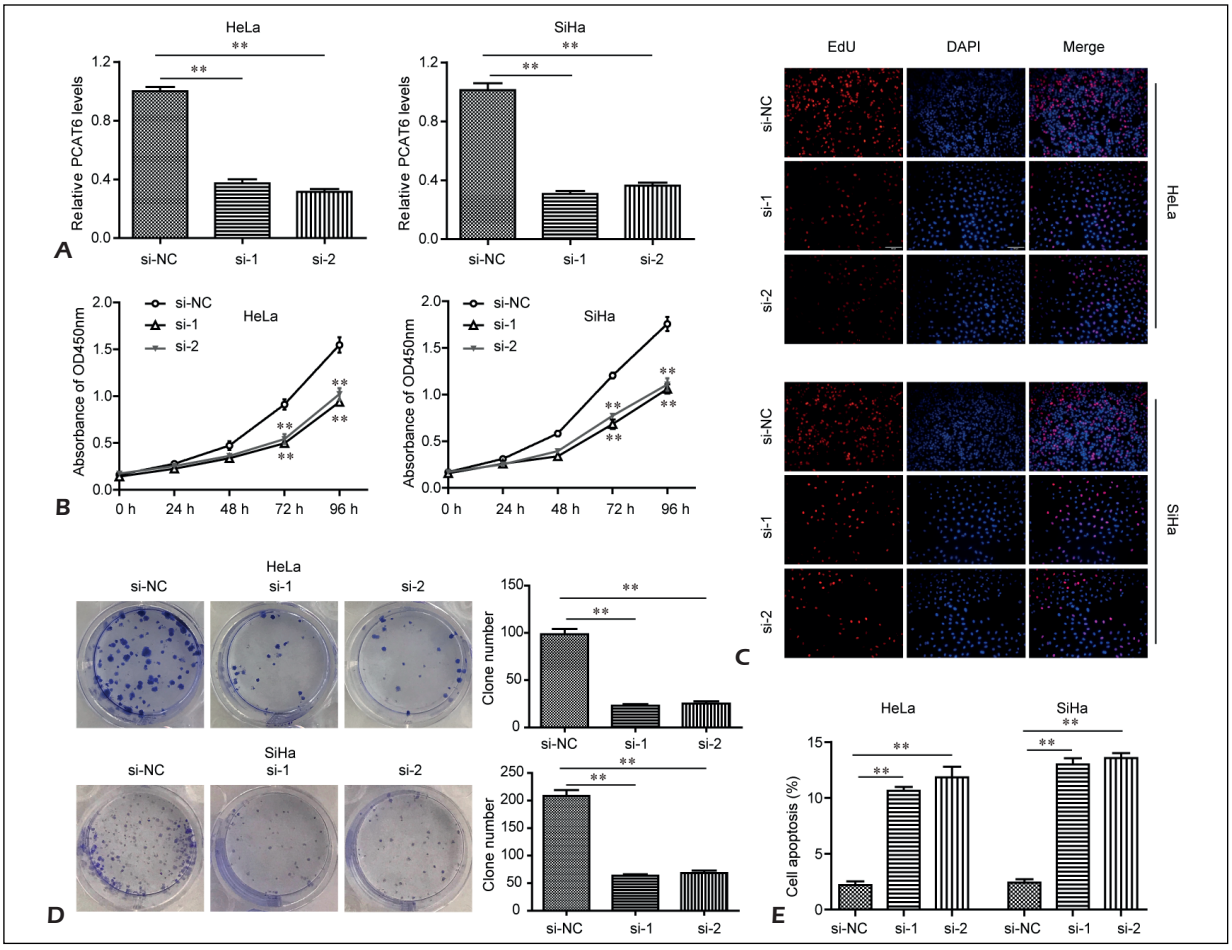

Figure 2. The effects of PCAT6 on the proliferation and apoptosis of HeLa and SiHa cells. **A**, Relative expression levels of PCAT6 in HeLa and SiHa cells when transfected with PCAT6 siRNAs (si-1 and si-2) and negative control siRNAs (si-NC). **B**, CCK-8 assays were conducted to examine the proliferation of HeLa and SiHa cells. **C**, EdU assays were utilized to determine the cell proliferation. The proliferative cells were labeled red and the DAPI stained cell nuclei were blue. **D**, Colony formation assays detected the colony formation ability of HeLa and SiHa cells after treatment with PCAT6 siRNAs or si-NC. **E**, Flow cytometry analysis was performed to evaluate the apoptotic rates of PCAT6 siRNAs or si-NC-transfected HeLa and SiHa cells. **p*<0.05, ***p*<0.01.

od, EdU assay, demonstrated that a decrease in PCAT6 expression also greatly inhibited the proliferative rates of both HeLa and SiHa cells (Figure 2C). In addition, we also carried out cell colony formation assays and we found that PCAT6 knockdown had a significant repression on colony formation abilities of both HeLa and SiHa cells (Figure 2D). Furthermore, we next conducted flow cytometric analysis to assess the effects of PCAT6 knockdown on the apoptosis of CC cells, and the data showed that the cell apoptosis of PCAT6 siRNAs-transfected HeLa and SiHa cells was notably elevated when compared with cells transfected with si-NC (Figure 2E). Collectively, our data confirmed that depressing expression of PCAT6 inhibited the proliferation of CC cells and accelerated cell apoptosis, which indicated that PCAT6 might serve as a tumor promoter in the development of CC.

Depression of PCAT6 Attenuated the Migration and Invasion of CC Cells

Since the above findings demonstrated that PCAT6 affected the cellular growth and apoptosis of CC cells, we wondered that whether PCAT6 was also capable to modulate the metastatic potentials of CC cells. Hence, we separately carried out wound healing and transwell invasion assays to determine the migratory as well as invasive capacity of HeLa and SiHa cells. The results from wound healing assays revealed that, after transfection with PCAT6 siRNAs, the migratory ability of HeLa and SiHa cells was significantly reduced because the wounded areas were notably wider in the PCAT6 siRNAs-transfected cell groups (Figure 3A and B). Additionally, transwell invasion assays validated that transfection of PCAT6 siRNAs resulted in a marked decline of invasive cell number of HeLa and SiHa cells, which indicated that silence of PCAT6 was able to impair the invasion of CC cells (Figure 3C). Therefore, these data clarified that PCAT6 had crucial influence on the metastasis of CC.

Knockdown of PCAT6 Inhibited the Activity of Wnt/β-Catenin Signaling in CC Cells

Our above results had certified that PCAT6 exhibited oncogenic property in CC, hence, we next aimed to discover the exact molecular mechanism behind this. Since several signaling pathways including Wnt/β-catenin signaling played central roles in regulating diverse aspects of numerous cancer types, we next determine the activity of Wnt/β-catenin signaling in CC cells after PCAT6 was knocked out. Firstly, we carried out TOP/FOP flash reporter assays in HeLa cells after they were transfected with PCAT6 siRNAs or si-NC. According to the data, silence of PCAT6 contributed to a remarkable reduction of the relative luciferase activities when compared with the controls, which indicated that PCAT6 knockdown had significant influence on Wnt/β-catenin signaling (Figure 4A). Subsequently, the qRT-PCR analysis was also performed to evaluate the expressing changes of several key genes involved in Wnt/β-catenin signaling. As the data presented in Figure 4B, PCAT6-depleted HeLa and SiHa cells exhibited obviously lower expression of β-catenin, cyclin D1 and c-myc, than si-NC-transfected cells. Similarly, the results from Western blot analysis showed that depression of PCAT6 caused dramatically decreased protein levels of β-catenin, cyclin D1 as well as c-myc in HeLa and SiHa cells (Figure 4C and D). Therefore, our data suggested that PCAT6 was able to modulate the activity of Wnt/β-catenin signaling pathway in CC.

Discussion

CC is a common gynecological malignancy worldwide, accounting for 8% of the total cases and total deaths from cancer[18]. Improvements in CC outcomes are dependent on better screening, diagnosis, prognosis, prediction and treatment[19]. With the rapid advancement of RNA microarray screening technique, more and more dysregulated lncRNAs were identified and were reported to influence the progression of CC[20,21]. In the current study, we firstly reported that the expression levels of PCAT6 were significantly upregulated in CC tissues and cell lines, which was consistent with the levels of PCAT6 in other tumors. Then, we further explored the clinical significance of PCAT6 in CC patients and the results indicated that higher expression of PCAT6 was significantly associated with advanced FIGO stage, depth of cervical invasion and positively lymph node metastasis. Moreover, the results of Kaplan-Meier assays indicated that CC patients with higher PCAT6 expression level had shorter overall survival and disease-free survival than those with low PCAT6 expression level. Finally, multivariate Cox hazard regression analysis identified high PCAT6 expression as an independent indicator of unfavorable prognosis for CC patients. Taken together, we provided first evidence that PCAT6

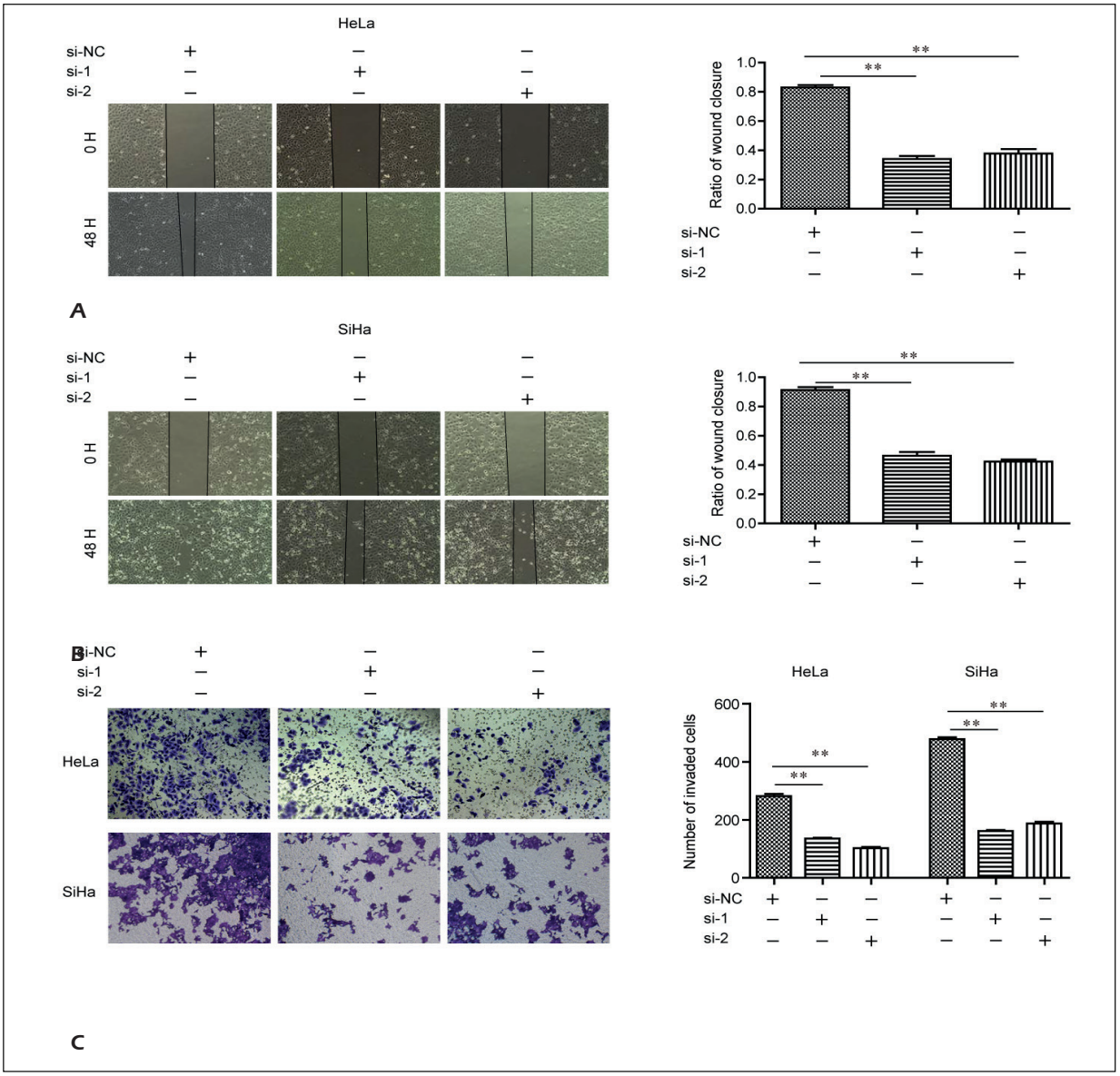

Figure 3. The influence of PCAT6 on the migration and invasion of HeLa and SiHa cells. (**A** and **B**) Representative images of the wound healing assays. The data suggested that knockdown of PCAT6 inhibited the migration of HeLa and SiHa cells. **C**, Depressing expression of PCAT6 suppressed the invasion of HeLa and SiHa cells. *p<0.05, **p<0.01.

was highly expressed and could be a potential new prognostic biomarker for CC patients.

Since PCAT6 was firstly identified by Du et al[14], PCAT6 has been demonstrated to exert oncogenic activity in a variety of carcinomas. For instance, Xu et al[17] reported that PCAT6 was overexpressed in gastric cancer and correlated with advanced clinical stages and shorter overall survival. Functionally, they found that up-regulation of PCAT6 suppressed the proliferation, migration and invasion by endogenously competition with miR-30. Cui et al[22] showed that PCAT6 was highly expressed in non-small cell lung cancer and its knockdown could inhibit the proliferation, migration and invasion of tumor cells through regulating miR-330-5p. Huang et al[15] found that increased expression of PCAT6 was associated with a worse survival status and promoted colon cancer cell apoptosis by modulation of anti-apoptotic protein ARC levels. These results revealed that PCAT6 acted as a tumor-related lncRNA. However, the functional role of PCAT6 in CC remains unclear. In this study, we performed *in vitro* assays and found that knockdown of PCAT6 inhibited the

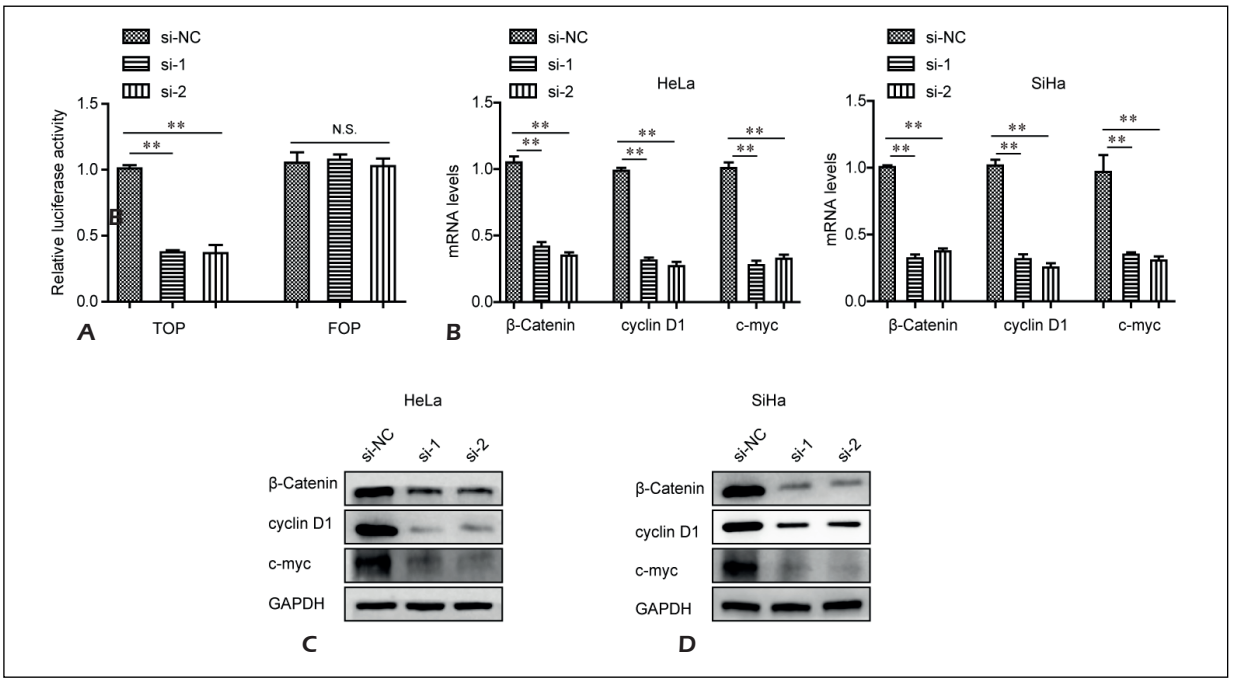

Figure 4. The activity of Wnt/β-catenin signaling pathway was affected by PCAT6 in HeLa and SiHa cells. **A**, TOPFlash luciferase assays were employed to evaluate the activity of Wnt/β-catenin signaling in HeLa cells. **B**, The qRT-PCR assays were conducted to determine the mRNA changes of β-catenin, cyclin D1 and c-myc in HeLa and SiHa cells. (**C** and **D**) Western blot assays detected the protein levels of β-catenin, cyclin D1 and c-myc. **p*<0.05, ***p*<0.01.

ability of cell proliferation, colony formation and metastasis, whereas promoted cell apoptosis. Our results showed that PCAT6 may act as an oncogenic lncRNA in the development and progression of CC. Wnt/β-catenin pathway acts as a critical regulator in regulation of cell proliferation, fate specification, cell death and differentiation in several developmental stages and body homeostasis[23]. The abnormal activations of Wnt/β-catenin signaling pathway have been proved to be an essential issue in origination and progression of CC[24,25]. Genetic mutations of the critical factors of Wnt/β-catenin pathway are primarily responsible for this dysregulated activation[26]. Recently, more and more studies reported that some tumor-related lncRNAs could display their roles by modulating Wnt/β-catenin pathway. For instance, lncRNA EZR-AS1 was reported to promote cells proliferation and migration by regulating Wnt/β-catenin pathway in breast cancer[27]. lncRNA XIST was shown to act as a tumor promoter in colon cancer by sponging miR-34a via nt/β-catenin signaling pathway[28]. In CC, it was reported that down-regulation of lncRNA NNT-AS1 suppressed tumor growth and metastasis through Wnt/β-catenin signaling pathway[29]. In the current study, we also explored whether PCAT6 exhibited its tumor-promotive role by promoting Wnt/β-catenin signaling pathway in CC. we found that down-regulation of PCAT6 significantly suppressed the expression levels of β-catenin, cyclin D1 and c-myc, indicating that Wnt/β-catenin pathway was inactivated. Taken together, these data revealed that PCAT6 positively promotes CC cell proliferation and metastasis through modulation of Wnt/β-catenin pathway.

Conclusions

Our present study identified a novel CC-associated lncRNA, PCAT6, and its high expression was significantly associated with the poor survival rate of CC patients. Suppression of PCAT6 could suppress the proliferation and metastasis of CC cells by modulating Wnt/β-catenin pathway. Our study may facilitate the development of lncRNA-directed diagnostics and therapeutics against CC.

Conflict of Interests

The authors declare no conflicts of interest.

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