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Long noncoding RNA PCAT-1 promoted ovarian cancer cell proliferation and invasion by suppressing KLF6

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Abstract. – OBJECTIVE: Recently, the role of long noncoding RNAs (lncRNAs) in tumor progression has caught attention in numerous researchers. In our investigation, lncRNA PCAT-1 was studied to identify how it functioned in the progression of ovarian cancer.

PATIENTS AND METHODS: LncRNA PCAT-1 expression was detected by quantitative Real-time polymerase chain reaction (qRT-PCR) in both ovarian cancer cells and tissue samples. Furthermore, to identify the function of PCAT-1 in ovarian cancer, cell proliferation, tra[illegible] assay and Matrigel assay were conducted[illegible] dition, by performing qRT-PCR and Weste[illegible]ot assay, the underlying mechanism was explo[illegible]

RESULTS: PCAT-1 expression was remarka[illegible] higher in ovarian cancer samp[illegible] when co[illegible] pared with that in corresp[illegible]s. More[illegible] over, cell proliferation, mi[illegible]on a[illegible]nvasion were inhibited after PCAT[illegible]as kno[illegible]d down in ovarian cancer cells. H[illegible]ver, th[illegible] protein expression of [illegible]like f[illegible]r 6) was upregulated[illegible]er PC[illegible]as knocked down. Furthermo[illegible]he KLF6 e[illegible]sion level was negatively [illegible]to the PCAT[illegible]ression level in ovaria[illegible]he[illegible]mples.

CONCLUSIONS: We [illegible]d that PCAT-1 promotes the[illegible]gression of [illegible]n cancer through enhanci[illegible]ell metastasis a[illegible]roliferation via suppr[illegible]ng KL[illegible], which might be a novel therape[illegible]strate[illegible] ovarian cancer.

Key w[illegible]
Long [illegible]ding [illegible], PCAT-1, Ovarian cancer,

Introduction

[illegible]arian carcinoma is the second most general gy[illegible]ologic malignancy, which has the highest mortality accounting for 5-6% cancer-related deaths among the female. Almost 22,500 new patients w[illegible]ed with ova[illegible]n cancer and 14,100 o[illegible]an ca[illegible]patients died in America in 2017[1]. The mo[illegible]patients with ovarian can[illegible]usually diagn[illegible]at advanced stage. T[illegible]gh surgery and chemotherapy have been [illegible]ely utilized [illegible]tandard therapeutic strategies [illegible]varian cance[illegible]0% of these patients used to d[illegible]e resista[illegible] to therapies and metastasis, lead[illegible] to the patients' death[2,3]. This severe [illegible] underscores the urgency of early [illegible]tection and new treatment for the patients with [illegible]cancer.

[illegible] non-coding RNAs (lncRNAs) are a cluster of non-coding transcripts longer than 200 nucleotides. However, evidence has proved that lncRNAs play an important role in a variety of biological behaviors, including the tumorigenesis. For example, by regulating the expression of miR-335, lncRNA MSTO2P facilitates cell proliferation and colony formation in gastric cancer indirectly[4]. LncRNA HULC is overexpressed in gastric cancer that can predict prognosis of the patients and serve as a potential serum marker for diagnosis[5]. LncRNA APOC1P1-3 represses cell apoptosis in breast cancer through inhibiting the process of alpha-tubulin acetylation[6]. Moreover, by regulation of AKT-MDM2-p53 signaling axis, lncRNA LOC572558 depresses cell proliferation and tumor growth in bladder cancer[7]. However, how lncRNA PCAT-1 functions in the proliferation of ovarian cancer and the underlying mechanism remain unexplored. In our study, we found out that PCAT-1 was remarkably upregulated in ovarian cancer tissues. Moreover, PCAT-1 enhances cell proliferation and metastasis in ovarian cancer cell *in vitro*. Moreover, our further experiments explored the potential underlying mechanism how PCAT-1 functioned in ovarian cancer development.

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Patients and Methods

Tissue Specimens

Paired ovarian carcinoma were sequentially-enrolled from 52 ovarian carcinoma patients undergoing surgery between July 2016 and December 2017 in the 960th Hospital of the PLA Joint Logistics Support Force. The Ethics Committee of the 960th Hospital of the PLA Joint Logistics Support Force approved this study protocol, and all participants involved in this study given a written informed consent.

Cell Culture

Human ovarian cancer cell lines A2780, TOV112D, OVCAR-3 and SKOV3 and normal ovarian cell ISOE80 were maintained in the culture medium consisted of 10% fetal bovine serum (FBS; Gibco, Waltham, MA, USA), penicillin as well as Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Waltham, MA, USA) at 37°C in a humidified atmosphere with 5% CO_2.

Lentivirus Expressing Short-Hairpin RNA and Cell Transfection

Lentivirus expressing short-hairpin RNA (shRNA) directed against PCAT-1 was provided by GenePharma (Shanghai, China). The complementary DNA encoding PCAT-1 was amplified and inserted into pcDNA (GenePharma, Shanghai, China), which was then for transfection of ovarian cancer cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The detection of PCAT-1 expression level in these cells was conducted using quantitative Real-time polymerase chain reaction (qRT-PCR).

RNA Extraction and qRT-PCR

Total RNA from tissues and cells were separated by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then total RNA was reverse-transcribed to complementary deoxyribose nucleic acids (cDNAs) through PrimeScript RT reagent Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). Thermocycling conditions was as follows: 30 s at 95°C, 5 s for 40 cycles at 95°C, 35 s at 60°C. $2^{-\Delta\Delta Ct}$ method was used for calculating relative expression. The primer sequences are as follows: PCAT-1 forward 5'- TGAGAAGAGAAATCTATTGCC-3', PCAT-1 reverse 5'-GGTTTGTC-TCCTGCTTTA-3'; β-actin, forward 5'-GATGGAAATCGTCAGAGGCT-3' and reverse 5'- TGGCACTTAGTTGGAAATGC -3'.

Western Blot Analysis

Total proteins were collected from cells *via* radioimmunoprecipitation assay (RIPA) buffer and then quantified by using a protein assay (bicinchoninic acid method; Beyotime, Shanghai, China). The target proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Then the gels were incubated with antibodies after replacing the polyvinylidene difluoride (PVDF) membrane. Next, rabbit anti-β-actin (Cell Signaling Technology, CST, Danvers, MA, USA) and rabbit anti-KLF6 (Cell Signaling Technology, CST, Danvers, MA, USA) were used for incubation of these membranes. Pierce enhanced chemiluminescence (ECL) was utilized for visualizing Western Blotting Substrate Immunoreactive bands (Santa Cruz Biotechnology, Inc., Santa Cruz, USA).

Transwell Assay

24 h after transfection, 2×10^5 cells in 100 μl serum-free DMEM were transformed to top chambers of 8 μm culture inserts (Corning, Corning, USA) coated with or without 50 μg Matrigel (BD, Bedford, MA, USA). 20% DMEM was added to the lower chamber of the culture inserts. 24 h later, these inserts were treated by methanol for 30 min and stained by hematoxylin for 20 min. An inverted microscope (×20) was utilized for counting migrated and invaded cells in three random fields.

Statistical Analysis

All statistical analyses were carried out using Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA). The differences between two groups were compared by Student *t*-test. The statistically significance was defined as $p<0.05$.

Results

PCAT-1 Expression Level in Ovarian Cancer Tissues and Cells

First, qRT-PCR was conducted for detecting PCAT-1 expression in 52 patients' tissues and four ovarian cancer cell lines. As a result, PCAT-1 was significantly upregulated in tumor tissue samples (Figure 1A). PCAT-1 level of ovarian cancer cells was higher than that of normal ovarian cell ISOE80 (Figure 1B).

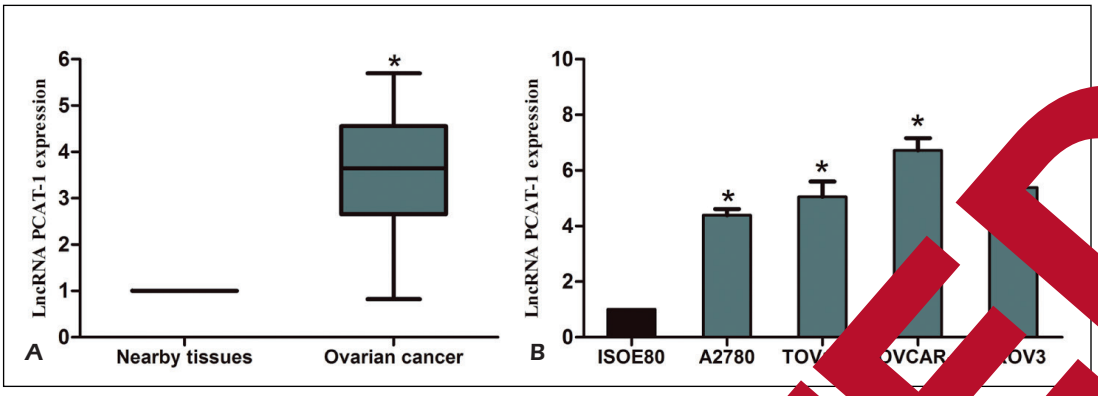


Figure 1. Expression levels of PCAT-1 were increased in ovarian cancer tissues a lines. AT-1 e ion was significantly increased in the ovarian cancer tissues compared with adjacent tissu Expression -1 relative to β-actin were determined in the human ovarian cancer cell lines and normal cell ISOE80 b R. Data are presented as the mean ± standard error of the mean. *p<0.05.

Knockdown of PCAT-1 Inhibited Cell Proliferation in OVCAR-3 Ovarian Cancer Cell

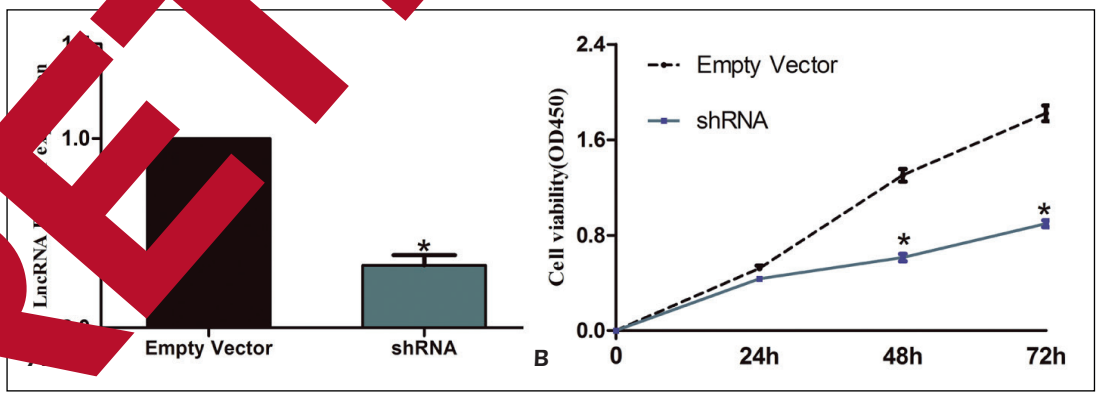
In our study, we chose OVCAR-3 cell lines for the knockdown of PCAT-1. Then qRT-PCR was utilized for detecting the PCAT-1 expression (Figure 2A). Moreover, we co ed CCK-8 assay and found that after was knocked down, the cell growth of OVCAR-3 cells was significantly repre (Figure 2B).

Knockdown of PCAT-1 I Cell Migration and Inv in in OVCAR-3 Ovarian cer C

The results of tra we after PCAT-1 was ked d he migrated abi varian cance s was significantly r ssed (Figure 3A). The outcome of Matri- assay also aled that after PCAT-1 was ked down i arian cancer cells, the num- b invaded s was remarkably decreased (Fig

The Interaction Between KLF6 CAT-1 in OVCAR-3 Ovarian Cells

Then qRT-PCR results showed that expression level of KLF6 in ovarian cancer cells was higher in PCAT-1 shRNA (shRNA) group when compared with the KLF6 level in empty vector group (Figure 4A). Western blot assay found out that after PCAT-1 was knocked down, KLF6 could be upregulated at protein level (Figure 4B).



e 2. Knockdown of PCAT-1 inhibited OVCAR-3 ovarian cancer cell proliferation. **A**, PCAT-1 expression in OVCAR-3 ov cancer cells transduced with PCAT-1 shRNA (shRNA) and the empty vector was detected by qRT-PCR. β-actin was used as an internal control. **B**, CCK-8 assay showed that knockdown of PCAT-1 significantly inhibited cell growth in OVCAR-3 ovarian cancer cells. The results represent the average of three independent experiments (mean ± standard error of the mean). *p<0.05.

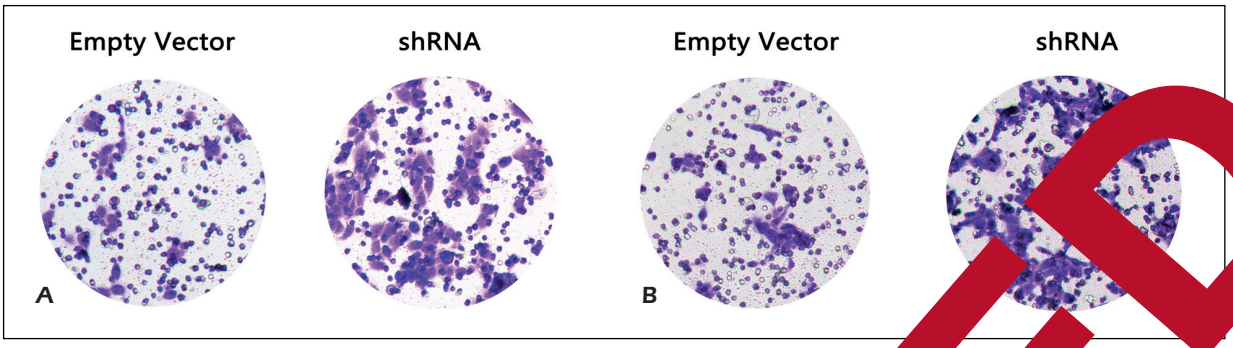


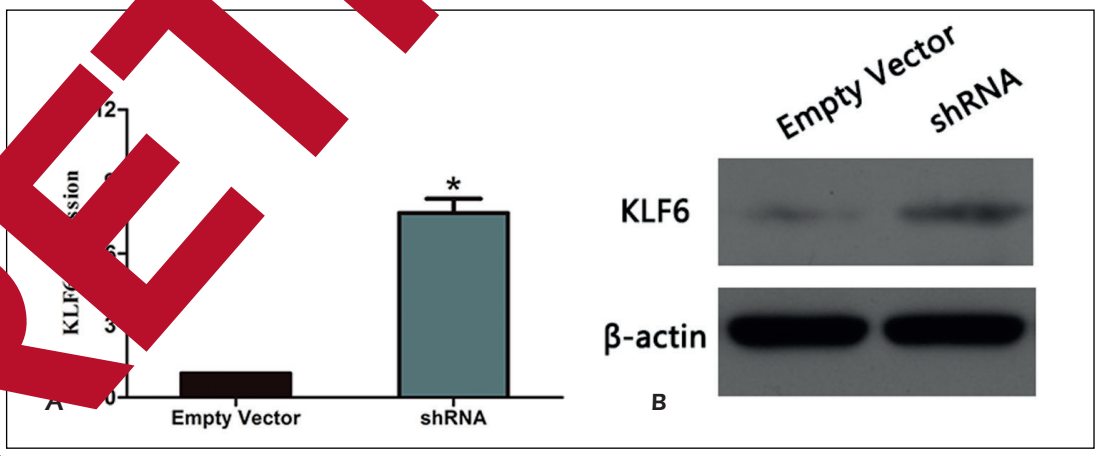
Figure 3. Knockdown of PCAT-1 inhibited OVCAR-3 ovarian cancer cell migration and in[illegible]. **A**, T[illegible]well assa[illegible]howed that knockdown of PCAT-1 significantly decreased cell migration in OVCAR-3 ovarian [illegible]er c[illegible]trigel as[illegible]howed that number of invaded cells was significantly decreased via knockdown of PCAT-1 [illegible]VCAR-[illegible]an can[illegible]ells. The results represent the average of three independent experiments (mean ± standard e[illegible]f the mean). [illegible]

The Interaction Between KLF6 and PCAT-1 in OVCAR-3 Ovarian Cancer Tissues

We further found that KLF6 expression of ovarian cancer tissues was significantly lower compared with that of adjacent tissues (Figure 5A). Correlation analysis revealed that the negative association was seen between KLF[illegible] pression level and PCAT-1 expression in [illegible] cancer tissues (Figure 5B).

Discussion

Evidence proved that [illegible]NAs [illegible]ticipate in the development of o[illegible] cance[illegible]ld be potential indicate[illegible]d therapeutic target for[illegible] cancer. For[illegible]ance, lncRNA AF-A[illegible]AS1 promotes cell proliferation and atten-[illegible]s cell apopt[illegible] in ovarian cancer[8]. LncRNA [illegible]E1-AS inhi[illegible] the progression of ovarian c[illegible]oma stem[illegible]ls and functions as a novel targ[illegible] ovarian cancer[9]. Upregulated by oestr[illegible]ncRNA ElncRNA1 functions as an oncogene in the proliferation of epithelial ovarian cancer cell[10]. LncRNA ZFAS1 promotes cell [illegible]ion, migration and chemoresistance in epithelial ovarian cancer by interacting with miR-150-5[11]. In addition, downregulation of lncRNA SPRY4-IT1 enhances cell metastasis in ovarian cancer by partly regulating epithelial-mesenchymal transition[12].



Fig[illegible]4. Interaction between PCAT-1 and KLF6 in OVCAR-3 ovarian cancer cells. **A**, qRT-PCR results showed that KLF6 expr[illegible]ion was higher in PCAT-1 shRNA (shRNA) compared with the empty vector. **B**, Western blot assay revealed that KLF6 protein expression was increased in PCAT-1 shRNA (shRNA) compared with empty vector. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

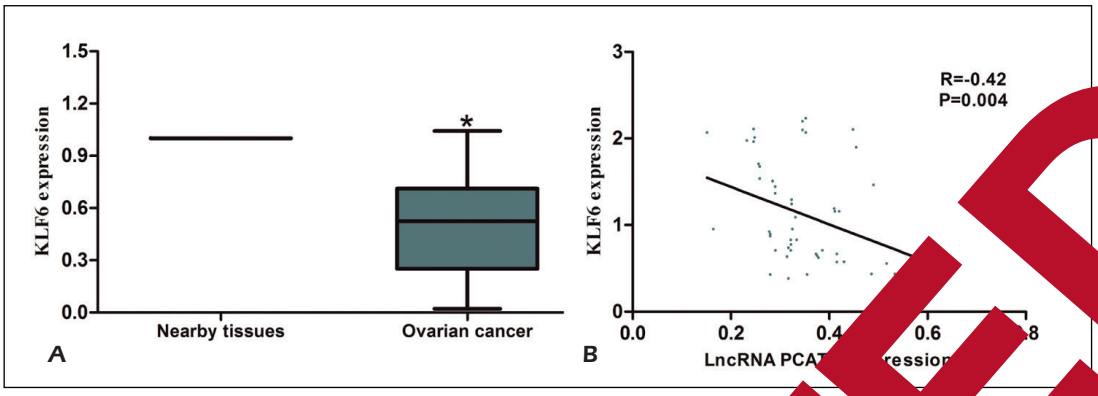


Figure 5. Interaction between PCAT-1 and KLF6 in ovarian cancer tissues. **A**, KL as sig ly dow ated in ovarian cancer tissues compared with adjacent tissues. **B**, The linear correlation b en the expre KLF6 and PCAT-1 in ovarian cancer tissues. The results represent the average of three ind experiments. presented as the mean ± standard error of the mean. *$p<0.05$.

LncRNA PCAT-1 (Prostate cancer-associated ncRNA transcripts 1) was initially discovered in prostate cancer[13]. PCAT-1 was highly expressed in bladder cancer and might be a novel therapeutic target[14]. Overexpressed PCAT-1 promoted cell proliferation and cell metastasis in non- ll cell lung cancer[15]. Bi *et al*[16] reported upregulating CDKN1A, PCAT-1 facilitate ll metastasis in gastric cancer. In addition, R *al*[17] proposed that the PCAT-1 played an imp ant role in tumorigenesis of h llular ca cinoma *via* TP53-miR-215-P L axis. In our research, we found that P T-1 was upregulated in both ova ancer nd cells. Besides, after PCAT- ked ovarian cancer cell iferat d metastasis were found inhi . Above f indicated that PCAT-1 p umorigenes ovarian cancer and n act oncogene.

KLF6 (Krüppel-like f 6) is widely known as a tum ppressor in ma cinomas, which regula iverse iological pro esses. For example, F6 co ns the progression of hepatocell ar na dis ination by regulating a VAV signa axis[18]. KLF6-E2F1 axis activate as ssive clear cell renal cell ca ma a 6 functions as an anti-onco *via* trans riptional depression of E2F1[19]. K glioblastoma, which is related to r prognosis for the patients through targeting F6[20]. Moreover, as a target of miR-630, KLF6 ces cell proliferation and cell invasion in epithe ovarian cancer[21]. In the present study, KLF6 expression could be upregulated after knockdown of PCAT-1. Moreover, KLF6 expression in ovarian c r tissues was negatively related to PCAT-1 ession. All results above suggested that T-1 might p ote tumorigenesis of ovarian c *via* supp ng KLF6.

Conclusions

We identified that PCAT-1 was remarkably higher-expressed in ovarian cancer tissues and cells. Besides, PCAT-1 could enhance ovarian cancer cell migration and invasion through targeting KLF6. These findings suggest that PCAT-1 may contribute to therapy for ovarian cancer as a candidate target.

Conflict of Interests

The authors declared no conflict of interest.

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