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Long non-coding RNA PVT1 promotes cell proliferation and invasion through regulating miR-133a in ovarian cancer

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ABSTRACT

The long non-coding RNA, plasmacytoma variant translocation 1 (PVT1), was reportedly to be highly expressed in a variety of tumors including ovarian cancer (OC). However, the role and mechanism of action of PVT1 in the carcinogenesis and progression of OC remains largely unknown. PVT1 and miR-133a expression were detected by quantitative real time PCR(qRT-PCR) assays in OC tissues and cell lines. Cell Counting Kit-8 (CCK-8), flow cytometer, wound healing and transwell invasion assays were performed to evaluate cell proliferation, cycle, migration and invasion abilities, respectively. qRT-PCR and luciferase reporter assays demonstrated PVT1 regulated miR-133a expression. Here, we discovered that PVT1 shows higher expression in OC tissues than in normal ovarian tissues, and patients who show higher expression of PVT1 have worse progression-free and overall survivals compared to lower expression of PVT1. Additionally, we observed that knockdown of PVT1 significantly inhibited OC cell proliferation, and decreased the migration and invasion capabilities of OC cells. Mechanistically, miR-133a was identified to serve as a direct downstream target of PVT1 in OC. Knockdown of PVT1 inhibited cell proliferation, migration and invasion through negative regulating miR-133a in OC cells. Taken together, our finding shows that PVT1 may be a novel biomarker for prognosis and a promising therapeutic target for OC.

1. Introduction

Ovarian cancer (OC) is the most fatal gynecological malignancy and the fourth common cause of cancer death among women [1,2]. Although recent improvements in surgery and chemotherapy, the long-term prognosis of OC patients is still poor mainly due to tumor-cell-unlimited proliferation and strong metastasis ability [3]. Therefore, exploring the underlying molecular mechanisms of carcinogenesis and progression of OC is essential for the development of diagnosis marker and therapy agent for this disease.

Long non-coding RNAs (lncRNAs) were defined as a novel class of RNA transcripts consisting of more than 200 nucleotides in length without encoding proteins capacity [4]. It is well established that lncRNAs are involved in various biological events, such as cell proliferation, cycle arrest, cell differentiation and cancer progression [5]. Accumulating evidence has suggested that lncRNAs are generally dysregulated and play critical roles tumorigenesis and development of various types of cancers, including OC [6–8]. For example, LncRNA HOXD cluster antisense RNA 1 (HOXD-AS1) promoted OC cell proliferation and colony formation, and enhanced the migration and invasion capabilities of OC cells through regulating miR-608/ frizzled family receptor 4 [9]. Long noncoding RNA lncBRM promoted the proliferation, migration and invasion of ovarian cancer cells *via* upregulation of Sox4 [10]. High expression of lncRNA nuclear enriched abundant transcript1 (NEAT1) accelerated ovarian cancer cell growth and decreased cell apoptosis through regulating by miR-34a-5p/BCL2 pathway [11]. LncRNA MEG3 inhibited tumorigenesis and progression of epithelial ovarian carcinoma by regulating activity of ATG3 [12]. These studies suggested that lncRNAs might act as tumor suppressors or oncogenes in OC and might be developed as diagnostic and prognostic biomarkers and therapy target for OC.

Plasmacytoma variant translocation 1 (PVT1) locates at chromosome8q24, a region shared with well-known oncogene *c-myc* in various types of cancers [13]. PVT1 has been reported to be abnormally upregulated, and exerts an carcinogenic effect in multiple malignancies, including renal cancer [14] prostate cancer [15], non-small cell lung cancer [16], breast cancer [17], cervical cancer [18], gastric cancer [19],colorectal cancer [20], and pancreatic cancer [21]. Recently studies demonstrated that PVT1 expression was upregulated in OC tissues [22], and that PVT1 promoted cisplatin resistance in OC cells by

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regulating apoptotic pathways [23]. However, the role and mechanism of action of PVT1 in the carcinogenesis and progression of OC remains largely unknown. In the present study, the expression level and clinical significance of PVT1 in OS tissues were investigate, and the effects of PVT1 on cell proliferation, migration and invasion were evaluated in OC by a series of *in vitro* and *in vivo* experiments. Furthermore, the underlying mechanism of PVT1 in regulating proliferation and invasion of OC was also investigated.

2. Materials and methods

2.1. Human OC clinical specimens

A total of 42 paired OC tissues and corresponding adjacent normal tissues were obtained from the First Hospital of Jilin University. All patients had never subjected to preoperative chemo- or radio-therapy. All tissues were frozen in liquid nitrogen until use. This study was approved by the ethical committee of the Jilin University, and written informed consent was obtained by each patient before surgery.

2.2. Cell culture

Three human OC cell lines (HEY, SKOV-3 and OVCAR-3) and a normal human ovary cell line (IOSE80) were brought from Chinese Academy of Sciences (Shang-hai, China), were cultured in RPMI 1640 medium (Gibco, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) at 37 °C in a humidified incubator with 5% CO_2.

2.3. siRNA and transfection

For functional analysis, two small interfering RNA (siRNA) against PVT1 (si-PVT1#1, 5′- GCUUGGAGGCUGAGGAGUUTT-3′; si-PVT1#2, 5′-GCUUGGAGGCUGAGGAGUUTT -3′) and siRNA scrambled control (si-Ctrl, 5′-UUC UCC GAA CGU GUC ACG UTT-3′) were chemically synthesized and purified by GenepharmaCo. Ltd., (Shanghai, China).miR-133a mimic, negative control mimic (miR-Ctrl), and miR-133a inhibitor were brought from RiboBio Co., Ltd (Guangzhou, China). Cells were maintained on 6-well plates to 70% confluence and transfected with oligonucleotides or plasmids using the Lipofectamine RNAi MAX (Invitrogen) according to the manufacturer's instructions. Cells were harvested 24 h after transfection for further analysis.

2.4. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissues and cultured cells using TRIZOL regent (Invitrogen). 1 μg of total RNA was reversely transcribed to complementary DNA (cDNA) using the Prime Script™ RT reagent kit according to the manufacturer's instructions (Takara, Dalian, China). Then PVT1 and miR-133a expression were determined by PCR with a SYBR Green PCR kit (Takara) and TaqMan MicroRNA Assay Kit (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 7900 real-time PCR system (Applied Biosystems), respectively. The relative expressions of PVT1 and miR-133a were calculated using $2^{-\Delta\Delta CT}$ method and normalized to GAPDH or U6 snRNA, respectively. The primers used in this study were as follows: PVT1: 5′-TGAGAACTGTCCTTACGTG ACC-3′ (sense) and 5′-AGAGCACCAAGACTGGCTCT-3′ (anti-sense); GAPDH:5′-GTCAACGGATTTGGTCTGTATT-3′ (sense); 5′-AGTCTTCTG GGTGGCAGTGAT-3′(anti-sense); miR-133a: 5′-TGCTTTGCTAGAGCTG GTAAAATG-3′(sense) and 5′-AGCTACAGCTGGTTGAAGGG-3(anti-sense); U6: 5′-CTCGCTTCGGCAGCACA (sense) and 5′- AACGCTTCAC GAATTTGCGT-3′ (anti-sense).

2.5. Cell proliferation assays

Cells were seeded in a 96-well plate at a density of 2×10^3 cells/well for 24 h, then transfected with si-PVT1#1,si-PVT1#2, or si-Ctrl. Cell proliferation was determined using Cell Counting Kit-8 (CCK-8) kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. The absorbance of each well at a wavelength of 450 nm was monitored by a spectrophotometer (Bio-Tek Instruments, Germany).All experiments were performed at least three times.

2.6. Flow cytometric analysis

Transfected cells were seeded in 6-well plates at density of 2×10^5 cells per well, and cultured for 48 h. Then cell cycle arrest was determined using BD Cycle test Plus DNA Reagent Kit (BD Biosciences, Franklin Lakes, NJ,USA) by FACScan flow cytometer (BD Biosciences). The percentage of the cells in G0/G1, S, and G2/M phases were counted using CellQuest software 3.2 (BD Biosciences).

2.7. Wound healing assay

Transfected cells were cultured in RPM1640 medium with 10% FBS to full confluence. After that, the cell monolayer was scraped using pipette and washed twice with medium to form a wound. Cells were incubated in RPM1640 medium free- serum at 37 °C for 24 h. The wound were observed at 0 and 24 h after scraping under an inverted microscope (Nikon, Tokyo, Japan) and corresponding photographs were taken.

2.8. Transwell assay

Transfected cells in serum-free RPM1640 were added to upper chamber (Corning Inc., USA) pre-coated the Matrigel (BD Biosciences), and 600 μl of medium with 10% FBS was added into the lower chamber. After incubation for 48 h, the cells on the upper layer of the membrane were removed, and those invaded through the membranes were fixed with 4% paraformaldehyde, and stained with 1% crystal violet (Sigma). Five randomly selected fields were observed under an inverted microscope (Nikon), and the average was calculated.

2.9. Luciferase reporter assay

The putative miRNA binding sites on PVT1 sequences were predicted by software StarBase V2.0 (http://starbase.sysu.edu.cn/). The PVT1 sequence containing the predicted wild-type or mutated-type miR-133a binding sites were amplified by PCR and subcloned into a pmirGLO reporter vectors (Promega Corporation, Madison, WI, USA) to generate the reporter vectors pmirGLO-PVT1-WT (PVT1-Wt) and pmirGLO -PVT1-Mut (PVT1-Mut).

For luciferase reporter assay, SKOV3 cells were seeded into 24-well plates and co-transfected with 100 ng of constructed luciferase plasmids together with 100 nM miR-133a or miR-Ctrl. Luciferase activity was measured at 48 h post-transfection using the dual-luciferase reporter assay system (Promega, Madison, WI, USA).

2.10. Statistical analysis

The experimental results were expressed as mean ± standard deviation and analyzed using SPSS statistical software for Windows Version 19 (SPSS, Chicago, IL, USA). Difference between groups was compared by the Student's *t*-test or one-way ANOVA. Kaplan-Meier survival and log-rank test were used for overall survival. Correlation between PVT1 expression and miR-133a expression in OC tissues was analyzed using Pearson's correlation analysis. P values < 0.05 are considered statistically significant.