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PUM1 promotes ovarian cancer proliferation, migration and invasion

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ABSTRACT

Background: Abnormal expression of the *PUM1* gene (pumilio RNA binding family member 1) is closely related to chromosomal mutations and carcinogenesis. However, there is no report about expression or function of *PUM1* in ovarian cancer. The present study explored the role of *PUM1* in the development and progression of ovarian cancer.

Methods: Immunohistochemistry was used to detect the expression of *PUM1* in normal ovarian tissues and ovarian cancer tissues. The *PUM1* gene was silenced using small interfering RNAs in ovarian cancer cell line A2780. MTT, plate colony formation and EdU (5-ethynyl-2′-deoxyuridine) assays were used to detect cell growth, and cell apoptosis was detected by flow cytometry. Wound-healing and Transwell assays were performed to determine cell migration and invasion. Western blotting was used to detect the levels of cancer-related proteins.

Results: Immunohistochemistry showed that the level of *PUM1* in ovarian cancer tissues was higher than that in normal tissues. The cell proliferation, migration, and invasion ability decreased significantly, while cell apoptosis increased after silencing the *PUM1* gene. Moreover, western blotting showed that downregulation of *PUM1* decreased the levels of STAT3, BCL2, MMP2, and VEGFA.

Conclusions: Thus, *PUM1* promotes the development and progression of ovarian cancer, which may occur via the above-mentioned molecules.

1. Introduction

Ovarian cancer is a common malignant tumor of the female reproductive system, whose mortality rate ranks the highest among gynecological malignancies [1]. The ovaries are located in deep in the pelvic cavity; therefore, no obvious symptoms of ovarian cancer are found in the early stage. Accompanied by extensive implantation metastasis of the peritoneum, most patients are diagnosed in the late stage, and often suffer recurrence and further metastasis after surgery. As a result, the long-term prognosis of ovarian cancer is poor, with an average 5-year survival rate of about 46% [2,3].

It is generally accepted that dysregulation of the genome and epigenetics play a fundamental role in tumorigenesis and progression [4]. Recently, researchers have confirmed that anomalies in the genome and epigenetics are closely related to the histopathological type, grading, and staging of ovarian cancer, as well as a patient's drug sensitivity and prognosis [5]. Therefore, it is important to explore the abnormal expression of genes related to ovarian cancer and its molecular mechanism, which would help to establish new technology for early diagnosis, as well as new targets for individualized treatment.

As a member of RNA binding protein PUF family, pumilio RNA binding family member 1 is encoded by the *PUM1* gene located on human chromosome 1 p35.2. Initially found in Drosophila and nematodes, the human PUM1 and PUM2 proteins have been confirmed to affect the cell cycle [6], biological development [7], and the self-renewal of stem cells [8]. Previous studies noted that *PUM1* might serve as a housekeeping gene in various tissues, such as breast cancer [9] and uterine cervix cancer [10]. However, in human serous ovarian cancer and its paired normal tissue, *PUM1* shows variable expression and is not used for normalization in qRT-PCR [11]. Furthermore, recent studies have revealed the specific role of PUM1 is controversial, because it also regulates the expression of cancer-related genes and is closely related to carcinogenesis and progression [12–16].

However, there is no report about the expression or function of

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PUM1 in ovarian cancer. The present study explored the role of *PUM1* in the development and progression of ovarian cancer, showing that *PUM1* might represent a potential target for diagnosis and treatment.

2. Materials and methods

2.1. Ovarian cancer specimens

One hundred and thirty seven cases of epithelial ovarian cancer and 14 normal ovarian tissue specimens were collected by surgical resection at the Department of Gynecology of the First Affiliated Hospital of China Medical University (Shenyang, China). All the above 151 patients required removal of ovaries during surgery, with informed consent, according to ethical and legal standards. The patients received no preoperative chemotherapy or radiotherapy. These samples were frozen in liquid nitrogen and stored at −80 °C until use. The China Medical University Ethics Committee approved our study.

2.2. Cell culture and transfection

Ovarian cancer cell line A2780 was cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum (FBS) and 100 U/mL of penicillin/streptomycin. The incubator was set at 37 °C with 5% of CO2. Cells in the experimental group were transfected with a *PUM1* small interfering RNA using Lipofectamine 2000 according to the manufacturer's instructions. The *PUM1* siRNA sequences were GAUUGAUGCAGACGUCAAAdTdT (sense) and UUUGACGUCUCCAUCAAUCdTdT (antisense).

2.3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The cells were counted and seeded in 96-well plates at a density of 3000 cells per well. After adherence, the cells in the experimental group were transiently transfected with the *PUM1* siRNA. MTT solution (20 μL) was added into each well at specific time points (0, 24, 48, and 72 h) after transfection. The plates were cultured for a further 4 h in an incubator. The supernatant was removed and 150 μL of dimethyl sulfoxide was added. The absorbance was measured at 490 nm using a microplate spectrophotometer.

2.4. Plate colony formation assay

The cells were counted and seeded into six-well plates at a density of 500 cells per well, and cultured for 14 d until the colonies were visible. The cells were washed with phosphate-buffered saline (PBS) and fixed with paraformaldehyde for 15 min. The colonies were stained with Giemsa's solution for 15 min and washed with tap water. After air-drying, the colonies with more than 50 cells were counted.

2.5. EDU (5-ethynyl-2′-deoxyuridine) assay

The mock group and si-PUM1 transfected cells were seeded into 24-well plates and then incubated with 2× EdU solution for 8 h. After fixing with 4% formaldehyde and washing with 3% bovine serum albumin (BSA), the cells were incubated with 0.5% Triton-X-100 for 20 min. The cells were then incubated with 200 μL Click-iT for 30 min. The reaction solution was removed and the cells were washed with 3% BSA and PBS. After staining with 5 μg/mL Hoechst 33342 solution for 30 min in the dark, the cells were observed and photographed under a fluorescence microscope.

2.6. Apoptosis assay

After digestion with EDTA-free trypsin, the cells were centrifuged at 1500 r for 5 min and washed with PBS. The cells were then resuspended in 100 μL of 1 × Binding Buffer with 5 μL of Annexin V-fluorescein isothiocyanate (FITC) and 5 μL of propidium iodide (PI) Staining Solution in the dark. After dilution with 400 μL of 1 × Binding Buffer, cell apoptosis was detected using flow cytometry within 1 h.

2.7. Wound-healing assay

Cells were digested and seeded in 6-well culture plates with mitomycin C in medium to inhibit cell proliferation. After being cultured to 80% confluence, the cells were scratched with a 200 μL pipette tip, washed with PBS, and replaced with FBS-free medium. Wounds were photographed under a microscope after scratching at 0 and 48 h.

2.8. Cell invasion assay

The filters of Transwell chambers were coated with 30 μL of basement Matrigel at a dilution of 1:10 by serum-free medium. Cells were counted (25000) and resuspended in 200 μL serum-free medium, and then seeded in the upper chamber. The lower chamber received 600 μL of 10% FBS medium as a chemoattractant. After 48 h of incubation, cells above the filter were removed. The invaded cells at the bottom of filter were fixed in 4% paraformaldehyde, and stained with 1% crystal violet. The cells were photographed and counted under an Olympus fluorescence microscope.

2.9. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from ovarian carcinoma tissues and cell lines using the Trizol reagent (Takara, Shiga, Japan). After detecting the $OD_{260/280}$ using a spectrophotometer (Unico, Shanghai, China), the quantified RNA was reverse transcribed into cDNA using random primers and avian myeloblastosis virus transcriptase. The cDNAs were amplified by real-time quantitative PCR. The comparative expression level of *PUM1* was determined using the value of threshold cycle (Ct) normalized to the expression of the 18s rRNA gene.

2.10. Western blotting

Total cell proteins were extracted from lysed cells, denatured, and concentrated into 30 μg for each sample. The samples were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and electrotransferred onto Hybond membranes (Amersham, Munich, Germany). After blocking with 5% fat-free milk for 1.5 h, the membranes were incubated with primary antibodies recognizing PUM1, STAT3 (signal transducer and activator of transcription 3), BCL2 (B-Cell CLL/lymphoma 2), MMP2 (matrix metalloproteinase 2) and VEGFA (vascular endothelial growth factor A) (1:1000; Proteintech, USA) overnight at 4 °C, with anti-βactin (1:3000, Proteintech, USA) as the internal control. The membranes were washed three times with TBST the next day, and then incubated with anti-rabbit secondary antibodies (1:5000) for 2 h. After washing with TBST, the immunoreactive protein bands were visualized under enhanced chemiluminescence (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

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