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RESEARCH ARTICLE

# Increased PTOV1 expression is related to poor prognosis in epithelial ovarian cancer

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**Abstract** Altered expression of prostate tumor overexpressed-1 (PTOV1) is observed in various types of human cancers. However, the role of PTOV1 in epithelial ovarian cancer (EOC) remains unclear. PTOV1 messenger (m)RNA expression in EOC patients was evaluated by quantitative real-time PCR (qRT-PCR). PTOV1 protein expression was also analyzed in archived paraffin-embedded EOC tissues using immunohistochemistry (IHC), and its association with overall survival of patients was analyzed by statistical analysis. Results from qRT-PCR analysis show that the expression level of PTOV1 mRNA was significantly higher in tumor tissues of EOC, compared to that in adjacent noncancerous tissues ($P<0.001$). IHC staining showed that high expression of PTOV1 was detected in 57.2 % (87/152) of EOC cases. High expression of PTOV1 was significantly associated with pathological grade ($P=0.029$) and clinical stage ($P=0.001$). Moreover, the results of Kaplan-Meier analysis indicated that a high expression level of PTOV1 resulted in a significantly poor prognosis of EOC patients. Multivariate analysis showed that high expression of PTOV1 was an independent prognostic factor for overall survival ($P<0.001$). In conclusion, PTOV1 protein abnormal expression might contribute to the malignant progression of EOC. High expression of PTOV1 predicts poor prognosis in patients with EOC.

**Keywords** PTOV1 · EOC · Biomarker · Prognosis

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## Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy among women worldwide, and its incidence has been increasing persistently in Asian countries, including China [1, 2]. Approximately over 200,000 new cases of EOC occurred worldwide in 2011 [3]. EOC generally originates from the malignant transformation of the ovarian surface epithelium, which is a single continuous layer of epithelial cells surrounding the ovary. The majority of EOC patients are diagnosed at advanced stages because of asymptomatic characteristic and lack of susceptible detection at early stage [4]. Currently, surgery is still necessary for appropriate staging of EOC and for improving chemotherapy results and

survival rate. Chemotherapy is an important strategy in the treatment of EOC. Platinum-taxane combination has been used as the reference standard for the first-line chemotherapy of postsurgical EOC [5]. Although the standard platinum-taxane regimen shows effectiveness with a response rate of 80 % in advanced EOC patients, most of these patients relapse because of drug resistance [6, 7]. Therefore, the identification of novel and specific biomarkers that have clinicopathologic and prognostic significance in EOC is remarkably important.

Prostate tumor overexpressed-1 (PTOV1) was identified as a novel gene and protein during a screening for genes differentially expressed in prostate cancer [8]. PTOV1 is overexpressed in 71 % of prostate carcinomas and in 80 % of samples with prostate intraepithelial neoplasia, while it its barely detectable in normal prostate epithelium [9]. The PTOV1 gene is located on a region of chromosome 19 (19q13) that is associated with high risk of breast cancer [10, 11]. PTOV1 comprises 12 exons, and the encoded protein has two almost identical tandem arranged PTOV domains, each containing a potential nuclear localization signal [12]. PTOV1 expression is elevated in multiple cancers, including lung, endometrium, bladder, kidney, and ovary cancer [13]. Furthermore, high expression of PTOV1 has been found to promote tumor progression [14]. These results suggest that PTOV1 might play an important role in promoting tumorigenesis. However, relatively, little is known about the expression and clinical significance of PTOV1 in EOC. In this study, we therefore assessed the expression of PTOV1 in a series of EOC specimens and investigated its associations with clinicopathologic parameters and overall survival in patients with EOC.

## Materials and methods

### Sample collecting

Formalin-fixed, paraffin-embedded tumor tissues and corresponding tumor-adjacent specimens undergoing surgical therapy were obtained from 152 patients with EOC treated at the Tianjin Medical University General Hospital between January 2006 and January 2008. The stage of tumors was evaluated according to the International Federation of Gynecology and Obstetrics (FIGO) system. Tumors were graded according to the Silverberg grading system. All the cases were reevaluated for grade and histological type by two independent pathologists. When a conclusion differed, the final decision was made by consensus. The original clinical data were obtained from hospital medical records. In addition, 30 self-pairs of EOC specimens and adjacent noncancerous tissues were snap frozen in liquid nitrogen and stored at −80 °C following surgery for quantitative real-time PCR (qRT-PCR) analysis. None of the patients had received radiotherapy, chemotherapy, or immunotherapy before surgery. Written informed consent was obtained from the patients for publication of this study and any accompanying images. Study protocol was approved by the Ethics Committee of Tianjin Medical University General Hospital, and all experiments were performed in accordance with approved guidelines of Tianjin Medical University General Hospital.

### RNA extraction and real-time quantitative RT-PCR

PTOV1 gene expression in 30 paired tumor tissue samples and adjacent noncancerous tissues were confirmed by real-time quantitative RT-PCR. Total RNA was extracted according to the manufacturer's instructions (TRIzol, Invitrogen, USA). RNA (2 μg) was reverse transcribed into cDNA (Promega, Madison, WI). Quantitative PTOV1 messenger (m)RNA levels were assessed using Mastercycler® ep realplex (Eppendorf, Hamburg, Germany) with an iQ™ SYBR Green Supermix Kit (BioRad, Berkeley, CA) according to the manufacturer's protocol. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The qPCR primers were as follows: PTOV1, sense 5′-TGGCTCCACTTGCCACATCATC-3′ and antisense 5′-ATTCTGCCGAACCTTGCGCTC-3′ and GAPDH, sense 5′-TGA AGGTCGGAGTCAACGG-3′ and antisense 5′-CTGGAAGATGGTGATGGGATT-3′. The cycling conditions were as follows: 95 °C for 2 min, then 40 cycles of 95 °C for 15 s, 59 °C for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 5 min. Each reaction was performed in triplicate, and the mean PTOV1 mRNA level for each tumor was compared with its matched noncancerous tissue. The expression level of PTOV1 was expressed as $2^{-\Delta\Delta Ct}$, where $\Delta Ct = Ct(PTOV1) - Ct(GAPDH)$.

### Immunohistochemistry

Isolated tumors were fixed in 10 % neutral buffered formalin for 48 h and embedded in paraffin according to standard protocols. Sections (thickness, 2 μm) were deparaffinized and rehydrated in a graded series of 100, 95, 90, 80, and 70 % ethanol. For antigen retrieval, slides were boiled in EDTA (1 mM, pH 8.0) for 15 min in a microwave oven. Endogenous peroxidase activity was blocked in 3 % $H_2O_2$ at room temperature for 10 min. Sections were then stained with anti-PTOV1

(rabbit anti-PTOV1 monoclonal antibody; 1:500 dilution; Abcam, Cambridge, UK) antibodies at 4 °C overnight. After three washes in phosphate buffered saline (PBS), sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Envision Detection Kit, GK500705, Gene Tech, Shanghai, China) for 30 min at room temperature. After washing in PBS, antibody complexes were colored with 3,3′-diaminobenzidine (DAB) and then counterstained with hematoxylin. Slides were dehydrated and evaluated.

### Immunohistochemistry evaluation

The specimens were analyzed by two pathologists who were blinded to the patients' clinical outcomes. The total PTOV1 immunostaining score was calculated as the sum of the positively stained tumor cells and staining intensity. Briefly, the percentage of positive staining was scored as "0" (0 to 9 %, negative), "1" (10 to 25 %, sporadic), "2" (26 to 50 %, focal), or "3" (>50 %, diffuse). The staining intensity was scored as "0" (no staining), "1" (weak staining), "2" (moderate staining), or "3" (strong staining). The total immunostaining score was calculated as the value of percent positivity score×staining intensity score and ranged from 0 to 9. The expression level of PTOV1 was defined as follows: "-" (score 0 to 1), "+" (2 to 3), "++" (4 to 6), and "+++" (>6). Based on their levels of PTOV1 expression, patients were divided into two groups: low PTOV1 ("-" and "+") and high PTOV1 ("++" and "+++").

### Statistical analysis

Comparisons between two groups were done using Student's *t* test for continuous data and the chi-square test for categorical data. The correlation between the PTOV1 expression and clinicopathologic characteristics was analyzed with the chi-square test. The overall survival was calculated by Kaplan-Meier method and compared by log-rank test. The prognostic varieties in predicting overall survival were assessed by multivariate Cox proportional hazards regression analysis. Results were given as mean±S.D. All statistical tests were two-sided, and a significant difference was considered when $P<0.05$.

## Results

### Overexpression of PTOV1 mRNA in EOC tissues

Real-time quantitative RT-PCR was performed to detect the expression of PTOV1 mRNA in 30 pairs of EOC and adjacent noncancerous tissues. Overall, 24 of the 30 patients (80 %) showed a higher expression level of PTOV1 mRNA in EOC tissue specimens compared to noncancerous tissue specimens. The mean expression value of PTOV1 mRNA in cancer tissues was significantly higher than the value in relevant normal tissues (2.26±0.54 vs 1.07±0.23, $P<0.001$, Fig. 1).

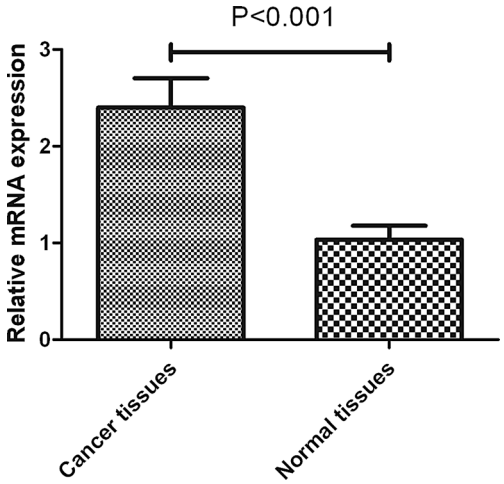


**Fig. 1** PTOV1 mRNA expression in EOC tissues. PTOV1 mRNA level in EOC tissues was significantly higher compared to that in adjacent noncancerous tissues (2.26±0.54 vs 1.07±0.23, $P<0.001$)

### Overexpression of PTOV1 protein in EOC tissues

Immunohistochemistry was performed in all 152 paraffin-embedded, archival EOC tumor samples and in available 152 adjacent noncancerous samples. High expression of PTOV1 was detected in 87/152 (57.2 %) of tumor tissues, and only 26/152 (17.1 %) in adjacent noncancerous tissues (Fig. 2). The protein expression level of PTOV1 was significantly higher in EOC tissues than the level in adjacent noncancerous tissues ($P<0.001$).

### Correlation of PTOV1 protein expression with the clinicopathologic characteristics

The association between PTOV1 protein expression and clinicopathologic characteristics of EOC was explored by the chi-square test. As it is shown in Table 1, high expression of PTOV1 was significantly associated with pathological grade ($P=0.029$) and clinical stage ($P=0.001$). However, no significant relationship was found between PTOV1 protein expression and variables such as age, histological type, and residual tumor after surgery (Table 1).

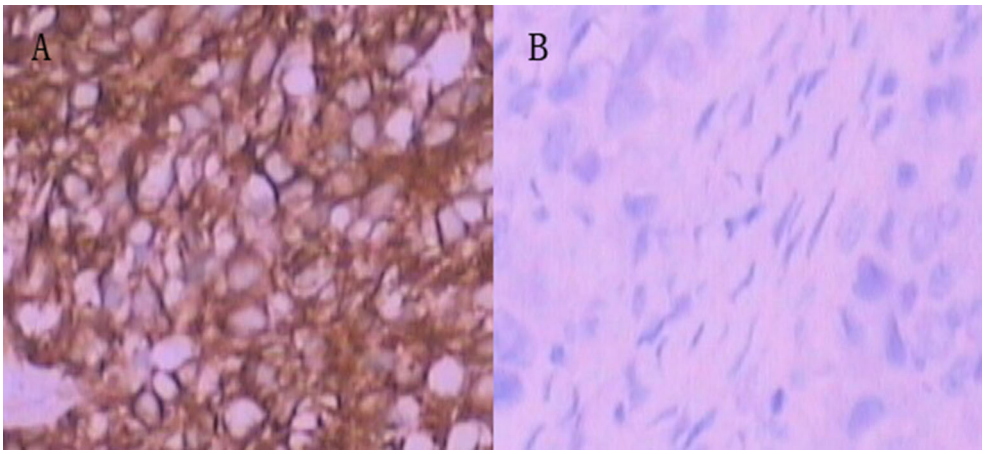


**Fig. 2** IHC analysis of PTOV1 in EOC patients. **a** High PTOV1 expression. **b** Low PTOV1 expression

Correlation of PTOV1 protein expression with survivals

Kaplan-Meier survival curves were used to evaluate overexpression of PTOV1, and they showed that the 5-year overall survival rate was significantly lower in patients with high PTOV1 expression than in those with low PTOV1 expression ($P<0.001$, Fig. 3). Table 2 shows the results of univariate and multivariate analyses of the factors related to patient prognosis. Univariate regression analyses revealed that histological grade, tumor stage, and PTOV1 expression significantly affected postoperative outcome. Multivariate analysis indicated that PTOV1 expression was one of the independent prognostic factors, along with pathological grade and tumor stage (Table 2).

**Table 1** Correlations of PTOV1 expression with the clinicopathologic features of EOC

| Variable | No. of cases | PTOV1 expression | | *P* value |
|---|---|---|---|---|
| | 152 | Low | High | |
| Age (years) | | | | |
| ≤50 | 74 | 34 | 40 | 0.44 |
| >50 | 78 | 31 | 47 | |
| Pathological grade | | | | |
| 1–2 | 53 | 29 | 24 | 0.029 |
| 3 | 99 | 36 | 63 | |
| Clinical stage | | | | |
| I–II | 57 | 34 | 23 | 0.001 |
| III–IV | 95 | 31 | 64 | |
| Histological type | | | | |
| Serous | 112 | 47 | 65 | 0.739 |
| Nonserous | 40 | 18 | 22 | |
| Residual tumor after surgery | | | | |
| <1 cm | 96 | 40 | 56 | 0.156 |
| ≥1 cm | 56 | 25 | 21 | |

## Discussion

Epithelial ovarian cancer (EOC) is the most lethal gynecological malignancies and the second leading cause of cancer-related death in women worldwide [15]. In spite of the development of diagnostic and therapeutic technologies, the clinical outcome of EOC patients remains poor, because the majorities of patients are diagnosed at an advanced stage of disease due to mild and diffuse symptom and develop recurrence [2]. The 5-year overall survival of patients with advanced-stage EOCs is only 40~45 % [3]. Thus, it is of great clinical significance to identify and validate tumor-specific markers for early-stage diagnosis in order to improve the therapeutic levels and individualize therapeutic strategies.

PTOV1 was identified as a novel gene and protein during a screening for genes differentially expressed in prostate cancer. PTOV1 protein consists of two repeated blocks of 151 and 147 amino acids, joined by a short linker peptide, and is

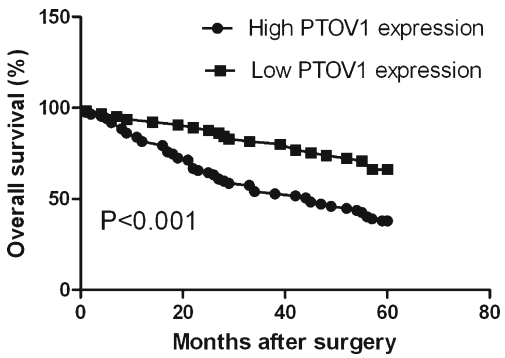


**Fig. 3** Kaplan-Meier analyses of overall survival periods among 152 curatively resected EOC patients are shown stratified according to PTOV1 expression

**Table 2** Univariate and multivariate analyses showing the overall survival rate for patients with EOC

| Variables | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | RR | 95 % CI | *P* value | RR | 95 % CI | *P* value |
| PTOV1 | 1.973 | 0.786–2.654 | < 0.001 | 1.861 | 0.907–2.765 | < 0.001 |
| Age | 1.458 | 0.696–2.808 | 0.564 | 1.565 | 0.706–2.862 | 0.498 |
| Pathological grade | 1.646 | 0.866–2.632 | 0.021 | 1.743 | 0.896–2.886 | 0.042 |
| Clinical stage | 1.798 | 0.648–2.865 | 0.018 | 1.759 | 0.945–2.861 | 0.012 |
| Histological type | 1.853 | 1.138–2.674 | 0.643 | 1.8074 | 0.734–2.943 | 0.567 |
| Residual tumor after surgery | 1.675 | 0.786–2.445 | 0.706 | 1.796 | 0.896–2.754 | 0.643 |

*RR* relative risk, *95 % CI* 95 % confidence interval

encoded by a 12-exon gene localized in chromosome 19q13.3 [12]. Previous studies have shown that PTOV1 is highly expressed in various human neoplasms, including prostate, breast, lung, endometrium, bladder, kidney, and ovary cancer [9–11]. Recently, Lei et al. reported that PTOV1 protein is overexpressed in breast cancers and is a novel predictive biomarker for patient survival [14]. However, the role of PTOV1 in tumorigenesis and progression is limited. In this study, we examined PTOV1 expression in EOC tissues, analyzed the relationship between PTOV1 expression and clinicopathologic factors, and determined the potential role of PTOV1 in EOC prognostic prediction. Our data indicated that PTOV1 was remarkably overexpressed in EOC and could be served as a potential biomarker of prognosis.

In this study, we demonstrated that PTOV1 mRNA level in EOC tissues was significantly higher compared to that in adjacent noncancerous tissues. It suggested that PTOV1 might play a role in the tumorigenesis of EOC. To investigate whether PTOV1 can accurately predict the outcome in patients with EOC, immunohistochemistry (IHC) was performed in 152 archived paraffin-embedded EOC samples. Interestingly, the expression of PTOV1 in EOC was closely correlated with pathological grade ($P$=0.029) and clinical stage ($P$=0.001).

Kaplan-Meier analysis was used to evaluate the survival of patients with EOC. Patients with high PTOV1 expression were likely to be with significantly shorter overall survival. We next evaluated the PTOV1 expression and other clinicopathologic factors on prognosis of EOC using univariate analyses. Results indicated that pathological grade, clinical stage, and PTOV1 expression as significant predictors of cancer-specific survival. Furthermore, PTOV1 expression and those clinicopathologic variables significant in univariate analysis were further evaluated in multivariate analysis. Results indicated that pathological grade, clinical stage, and PTOV1 expression were also independent predictor for overall survival of EOC patients (Table 2).

In conclusion, we showed that PTOV1 is overexpressed in EOC tissues. Moreover, our study provides the clinical evidence that PTOV1 is independently prognostic for outcome in EOC. Independent validation of these clinical findings, examination of PTOV1 expression in other kinds of cancers, and further investigation of the cell biology of PTOV1 and its potential as a therapeutic target are clearly warranted.

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**Conflicts of interest** None

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