

# PTOV1 promotes cisplatin-induced chemotherapy resistance by activating the nuclear factor kappa B pathway in ovarian cancer

Hongwei Shen,<sup>1,4</sup> Bing Liao,<sup>1,4</sup> Zhiyong Wan,<sup>1,4</sup> Yunhe Zhao,<sup>1</sup> Zeshan You,<sup>1</sup> Jun Liu,<sup>1</sup> Jin Lan,<sup>1</sup> and Shanyang He<sup>2,3</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, Guangdong, China; <sup>2</sup>Department of Obstetrics and Gynecology, Guangdong Provincial People's Hospital and Guangdong Academy of Medical Sciences, Guangzhou 510080, Guangdong Province, China; <sup>3</sup>Second School of Clinical Medicine, Southern Medical University, Guangzhou 510280, Guangdong, China

**Chemotherapy resistance is a bottleneck for ovarian cancer treatment; therefore, revealing its regulatory mechanism is critical. In the present study, we found that prostate tumor overexpressed-1 (PTOV1) was upregulated significantly in ovarian cancer cells and tissues. Patients with high PTOV1 levels had a poor outcome. In addition, PTOV1 overexpression increased CDDP (cisplatin) resistance, while PTOV1 knock-down inhibited CDDP resistance, as determined using cell viability assays, apoptosis assays, and an animal model. Mechanistic analysis showed that PTOV1 increased nuclear factor kappa B (NF- $\kappa$ B) pathway activity, reflected by increased nuclear translocation of its p65 subunit and the phosphorylation of inhibitor of nuclear factor kappa-B kinase subunits alpha and beta, which are markers of NF- $\kappa$ B pathway activation. Inhibition of the NF- $\kappa$ B pathway in PTOV1-overexpressing ovarian cancer cells increased CDDP-induced apoptosis, suggesting that PTOV1 promoted chemotherapy resistance by activating the NF- $\kappa$ B pathway. In summary, we identified PTOV1 as a prognostic factor for patients with ovarian cancer. PTOV1 might be a target for inhibition of chemotherapy resistance.**

## INTRODUCTION

Ovarian cancer is the leading cause of mortality among gynecological malignancies. Many chemotherapy drugs have been used to treat ovarian cancer, such as CDDP (cisplatin),<sup>1</sup> oxaliplatin,<sup>2</sup> and ifosfamide;<sup>3</sup> however, chemotherapy resistance is a bottleneck for ovarian cancer treatment.<sup>4</sup> Understanding the regulatory mechanism of chemotherapy resistance is critical to treat ovarian cancer.

PTOV1 (encoding prostate tumor overexpressed-1) is located on 19q13.3–13.4 and is highly expressed in normal brain, heart, skeletal muscle, kidney, and liver but poorly expressed in normal prostate.<sup>5</sup> PTOV1 is overexpressed in prostate cancer cells and tissues, and high PTOV1 expression correlated positively with proliferation marker Ki67 levels. Shuttling of PTOV1 between the cytoplasm and the nucleus promotes cells to enter into the S phase, which promotes prostate cancer proliferation.<sup>6</sup> PTOV1 promotes prostate cancer invasion and growth by counteracting Notch pathway signaling.<sup>7</sup>

Another study showed that PTOV1 promotes prostate cancer growth and metastasis by directly promoting the translation of JUN (encoding C-Jun).<sup>8</sup> PTOV1 is highly expressed in high-grade prostatic intra-epithelial neoplasia and is a biomarker for studying prostate cancer progression.<sup>9</sup> It also has been reported to regulate many types of tumors and is a poor prognosis factor for squamous cell carcinoma, invasive urothelial carcinoma, nasopharyngeal carcinoma, bladder carcinoma, early-stage human laryngeal squamous cell carcinoma, and breast cancer.<sup>10–15</sup>

A previous study suggested that PTOV1 is a poor prognosis factor for epithelial ovarian cancer;<sup>16</sup> however, the role of PTOV1 in ovarian cancer progression has not been studied. The present study aimed to investigate the role of PTOV1 in ovarian cancer chemotherapy resistance. The results showed that PTOV1 regulates ovarian cancer resistance by activating the nuclear factor kappa B (NF- $\kappa$ B) pathway.

## RESULTS

### PTOV1 is upregulated in ovarian cancer cells and tissues

To analyze the role of PTOV1 in ovarian cancer progression and prognosis, we used data from gene expression profiles (GEO: GSE27651) generated from 6 ovarian surface epithelia, 8 serous borderline ovarian tumors, 13 low-grade serous ovarian carcinomas, and 22 high-grade serous ovarian carcinomas.<sup>17</sup> The results showed that PTOV1 was upregulated significantly in ovarian carcinoma (Figure 1A). We also used the KMplot dataset to analyze the relationship between PTOV1 expression and patient survival. Overall survival and post-progression survival showed that patients with high PTOV1 expression had poor survival compared with patients with low PTOV1 expression (Figure 1B), suggesting that PTOV1 might be a poor prognostic factor for ovarian cancer. To confirm the above

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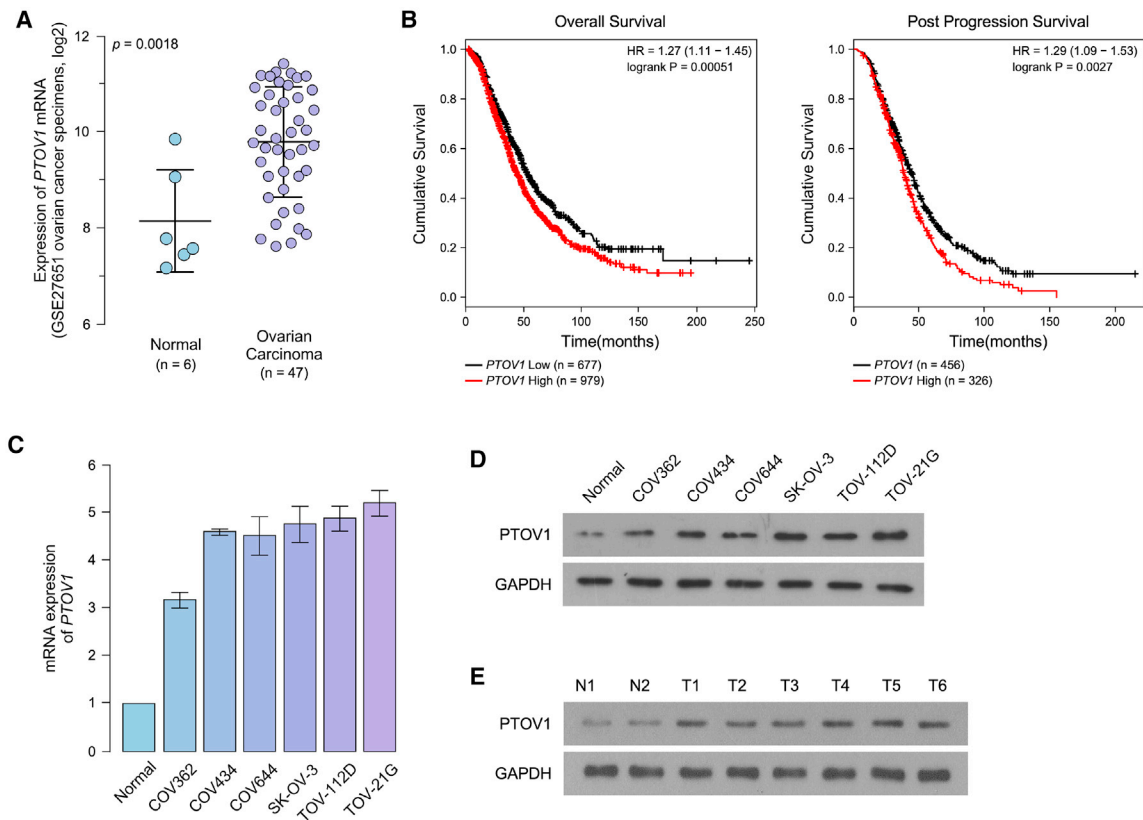
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<sup>4</sup>These authors contributed equally

**Correspondence:** Shanyang He, Department of Obstetrics and Gynecology, Guangdong Provincial People's Hospital and Guangdong Academy of Medical Sciences, No. 106 Zhongshan Er Road, Guangzhou 510080, Guangdong Province, China.

**E-mail:** drhsy8899@126.com





**Figure 1. PTOV1 is upregulated in ovarian cancer tissues and cells**

(A) PTOV1 was significantly upregulated in ovarian cancer tissues. Data come from GSE27651. (B) Overall survival and post-progression survival analysis showed patients with high PTOV1 expression had poor clinical outcome. (C and D) qPCR and western blot analysis showed PTOV1 was upregulated in ovarian cancer cells. (E) Western blot analysis showed PTOV1 was upregulated in ovarian cancer tissues. GAPDH was used as the loading control. All error bars represent mean  $\pm$  SEM.

results, we analyzed PTOV1 expression in ovarian cancer cells and tissues. Western blotting and qRT-PCR showed that PTOV1 was upregulated in ovarian cancer cells compared with that in normal ovarian epithelia cell (Figures 1C and 1D). Western blotting also showed that PTOV1 was upregulated in ovarian cancer tissues compared with that in normal ovarian tissues (Figure 1E). These results suggested PTOV1 was upregulated in ovarian cancer cells and tissues.

#### Clinical significance of PTOV1 expression in patients with primary ovarian cancer

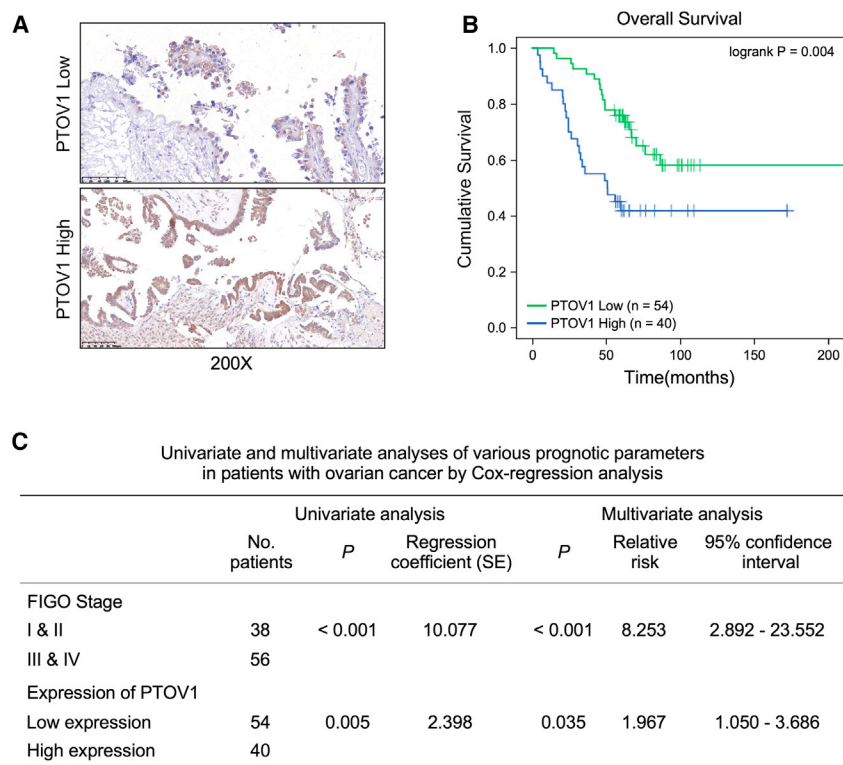
To determine the clinical significance of PTOV1, we used immunohistochemistry (IHC) to determine PTOV1 levels in samples from 268 patients with ovarian cancer (Figure 2A; Table S1); the isotype is shown in Figure S1. Kaplan-Meier survival analysis and the log-rank test showed that patients with high PTOV1 levels had significantly shorter survival times than those with low PTOV1 levels (Figure 2B). The chi-square test and Fisher's exact test showed that PTOV1 levels correlated significantly with lymphatic transfer ( $p < 0.001$ ), International Federation of Gynecology and Obstetrics (FIGO) stage ( $p < 0.05$ ), and vital status ( $p < 0.05$ ), which suggested

a correlation between higher PTOV1 expression and clinical progression of ovarian cancer. However, no significant correlation was found for other clinicopathological characteristics, such as age, differentiation, and pathological type (Table 1).

We also analyzed whether PTOV1 could serve as a prognostic factor for ovarian cancer. Univariate Cox regression analysis showed that PTOV1 expression ( $p = 0.005$ ) and FIGO stage were unfavorable prognostic factors (Figure 2C). To investigate whether PTOV1 is an independent prognostic factor for outcome, multivariate survival analysis was performed, which showed that PTOV1 expression and FIGO stage were independent prognostic factors for patients with ovarian cancer (Figure 2C).

#### PTOV1 promotes CDDP-induced chemotherapy resistance of ovarian cancer

To determine the role of PTOV1 in ovarian cancer progression, we overexpressed and knocked down *PTOV1* in ovarian cancer cell lines COV434 and SK-OV-3; qRT-PCR and western blotting were used to confirm the effects of overexpression and knockdown (Figure 3A). Cell viability assays showed that CDDP inhibited cell survival in a



dose-dependent manner. *PTOV1* overexpression reduced the effect of CDDP, while *PTOV1* knockdown increased the effect of CDDP (Figure 3B). Annexin V/propidium iodide (PI) apoptosis assay showed that *PTOV1* overexpression reduced apoptosis, while *PTOV1* knockdown increased apoptosis after CDDP treatment (Figure 3C). Terminal deoxynucleotidyl transferase nick-end-labeling (TUNEL) assays also showed that *PTOV1* overexpression reduced apoptosis, while *PTOV1* knockdown increased apoptosis after CDDP treatment (Figure 3D). These findings suggested that *PTOV1* increased chemotherapy resistance *in vitro*.

We used subcutaneous tumor transplantation in nude mice to confirm the above results. Without CDDP treatment, *PTOV1* overexpression significantly promoted tumor growth, while *PTOV1* knockdown significantly inhibited tumor growth (Figure S2). With CDDP treatment, *PTOV1* overexpression increased tumor growth compared with that of the vector control group, while *PTOV1* knockdown inhibited tumor growth compared with that of scramble group after treatment with CDDP (Figure 4A). Measurement of tumor volumes showed that *PTOV1* overexpression increased the tumor volume significantly, while *PTOV1* knockdown decreased the tumor volume significantly (Figure 4B). We also analyzed the apoptosis in the xenograft tumors. The TUNEL assay showed that *PTOV1* overexpression inhibited apoptosis significantly, while *PTOV1* knockdown promoted apoptosis significantly (Figure 4C). These findings suggested that *PTOV1* promoted chemotherapy resistance *in vivo*.

### Figure 2. PTOV1 is an independent prognostic factor for ovarian cancer patients

(A) Representative images for PTOV1 expression in ovarian cancer tissues, determined by IHC. (B) Overall survival analysis showed patients with high PTOV1 expression had poor outcome. (C) Univariate and multivariate Cox regression analysis showed PTOV1 was an independent prognostic factor for ovarian cancer patients. All error bars represent mean  $\pm$  SEM.

### PTOV1 promotes ovarian cancer chemotherapy resistance by activating the NF- $\kappa$ B pathway

To determine the regulatory mechanism by which PTOV1 promotes chemotherapy resistance, we used Gene Set Enrichment Analysis (GSEA) to analyze which pathway(s) were associated with PTOV1 expression. The results showed that PTOV1 expression correlated significantly with activation of the NF- $\kappa$ B pathway (Figure 5A). Luciferase reporter assays showed that *PTOV1* overexpression significantly increased luciferase activity expressed using the *NFKB1* (NF- $\kappa$ B) promoter, while *PTOV1* knockdown significantly inhibited *NFKB1*-controlled luciferase activity (Figure 5B), suggesting that

*PTOV1* activated the NF- $\kappa$ B pathway. Western blotting showed that *PTOV1* overexpression increased the nuclear translocation of NF- $\kappa$ B subunit p65, while *PTOV1* knockdown inhibited the nuclear translocation of p65 (Figure 5C). *PTOV1* overexpression increased the levels of phosphorylated inhibitor of NF- $\kappa$ B kinase subunit alpha (IKK $\alpha$ ) and inhibitor of NF- $\kappa$ B kinase subunit beta (IKK $\beta$ ), while *PTOV1* knockdown had the opposite effect (Figure 5C), suggesting that PTOV1 promoted NF- $\kappa$ B pathway activity. We also examined the expression of target genes of the NF- $\kappa$ B pathway, including *BCL2* (encoding BCL2 apoptosis regulator), *XIAP* (encoding X-linked inhibitor of apoptosis), *MYC* (encoding MYC proto-oncogene, BHLH transcription factor), *CCND1* (encoding cyclin D1), and *IL6* (encoding interleukin 6), which are also associated with apoptosis and proliferation. *PTOV1* overexpression increased the expression levels of these genes significantly, while *PTOV1* knockdown inhibited their expression significantly (Figure 5D), confirming that PTOV1 regulates the NF- $\kappa$ B pathway. To determine whether PTOV1 increased chemotherapy resistance by activating the NF- $\kappa$ B pathway, we inhibited the NF- $\kappa$ B pathway using the NF- $\kappa$ B inhibitor, SN50, in *PTOV1*-overexpressing cells that were treated with CDDP;<sup>18</sup> mutated I $\kappa$ B $\alpha$  was used as a negative control. Luciferase reporter assays showed that SN50 significantly inhibited NF- $\kappa$ B pathway activity (Figure 5E), and cell viability assays showed that inhibition of the NF- $\kappa$ B pathway increased the effect of CDDP on cell survival (Figure 5F). These findings suggested PTOV1 promoted chemotherapy resistance by activating the NF- $\kappa$ B pathway. We also confirmed this result in clinical samples. *BCL2*<sup>19</sup> and

**Table 1. Correlation between PTOV1 expression and clinicopathologic characteristics of ovarian cancer**

Characteristic	PTOV1		Chi-square test p value	Fisher's exact test p value
	Low No.	High No.		
<b>Age (years)</b>				
≤55	43	27	0.233	0.233
>55	11	13		
<b>Lymphatic transfer</b>				
Negative	44	18	< 0.001	< 0.001
Positive	10	22		
<b>Differentiation</b>				
G1	4	3	0.654	0.618
G2	18	17		
G3	32	20		
<b>Pathological type</b>				
Serous cystadenocarcinoma	37	33	0.052	0.048
Mucinous cystadenocarcinoma	9	2		
Clear cell carcinoma	4	1		
Endometrioid carcinoma	4	1		
Others	0	2		
<b>FIGO stage</b>				
I & II	29	9	0.003	0.03
III & IV	25	31		
<b>Vital status</b>				
Alive	34	17	0.049	0.048
Dead	20	23		

XIAP<sup>20</sup> are apoptosis suppressors and are also targets of the NF- $\kappa$ B pathway. We used qRT-PCR to analyze the expression of their encoding genes in eight ovarian cancer tissues (Figure 6A). Western blotting was used to analyze PTOV1 levels and the nuclear translocation of p65 in the same samples. We found the PTOV1 levels and *BCL2* and *XIAP* expression correlated positively, and PTOV1 levels correlated positively with the nuclear translocation of p65 (Figure 6B); these findings suggested that PTOV1 promotes the NF- $\kappa$ B pathway activity in clinical samples.

## DISCUSSION

In the present study, we revealed that PTOV1 was upregulated significantly in ovarian cancer cells and tissues. In addition, we observed that patients with high PTOV1 expression had shorter survival times than patients with low PTOV1 expression. PTOV1 was identified as an independent prognostic factor for ovarian cancer, which was consistent with the results of a previous study.<sup>16</sup>

CDDP resistance occurs frequently during ovarian cancer treatment,<sup>21</sup> and understanding the regulatory mechanism of CDDP resis-

tance generation is important for ovarian cancer therapy. We found that *PTOV1* overexpression reduced the effect of CDDP on apoptosis and tumor growth, while *PTOV1* knockdown had the opposite effect. GSEA analysis suggested that *PTOV1* expression correlated positively with NF- $\kappa$ B pathway activity.

The NF- $\kappa$ B pathway has been reported to regulate ovarian cancer chemotherapy resistance.<sup>22–24</sup> NF- $\kappa$ B is sequestered in the cytosol by Inhibitor of  $\kappa$ B (I $\kappa$ B). Cytokine or pathogen-associated molecular pattern (PAMP) ligation interacting with receptors initiates signaling cascades by activating the inhibitor of I $\kappa$ B kinase (IKK) complex. The IKK complex consists of three subunits: the catalytic subunits IKK $\alpha$  and IKK $\beta$ , and the regulatory subunit NEMO (IKK $\gamma$ ). IKK subunit phosphorylation activates the IKK complex kinase activity, which then phosphorylates I $\kappa$ B, promoting I $\kappa$ B polyubiquitination and degradation to liberate NF- $\kappa$ B. Free NF- $\kappa$ B enters the nucleus and regulates target gene expression.<sup>25–27</sup> We found that *PTOV1* overexpression increased the levels of phosphorylated IKK $\alpha$  and IKK $\beta$  and the translocation of p65, while *PTOV1* knockdown had the opposite effects, suggesting that PTOV1 increases NF- $\kappa$ B pathway activation. Luciferase activity assays also suggested that PTOV1 increases NF- $\kappa$ B pathway activation. Inhibition of the NF- $\kappa$ B pathway in *PTOV1*-overexpressing cells inhibited CDDP resistance, suggesting that PTOV1 promotes CDDP resistance by activating the NF- $\kappa$ B pathway.

In summary, we found that PTOV1 not only serves as a prognostic factor for patients with ovarian cancer but also represents a novel target for inhibition of chemotherapy resistance.

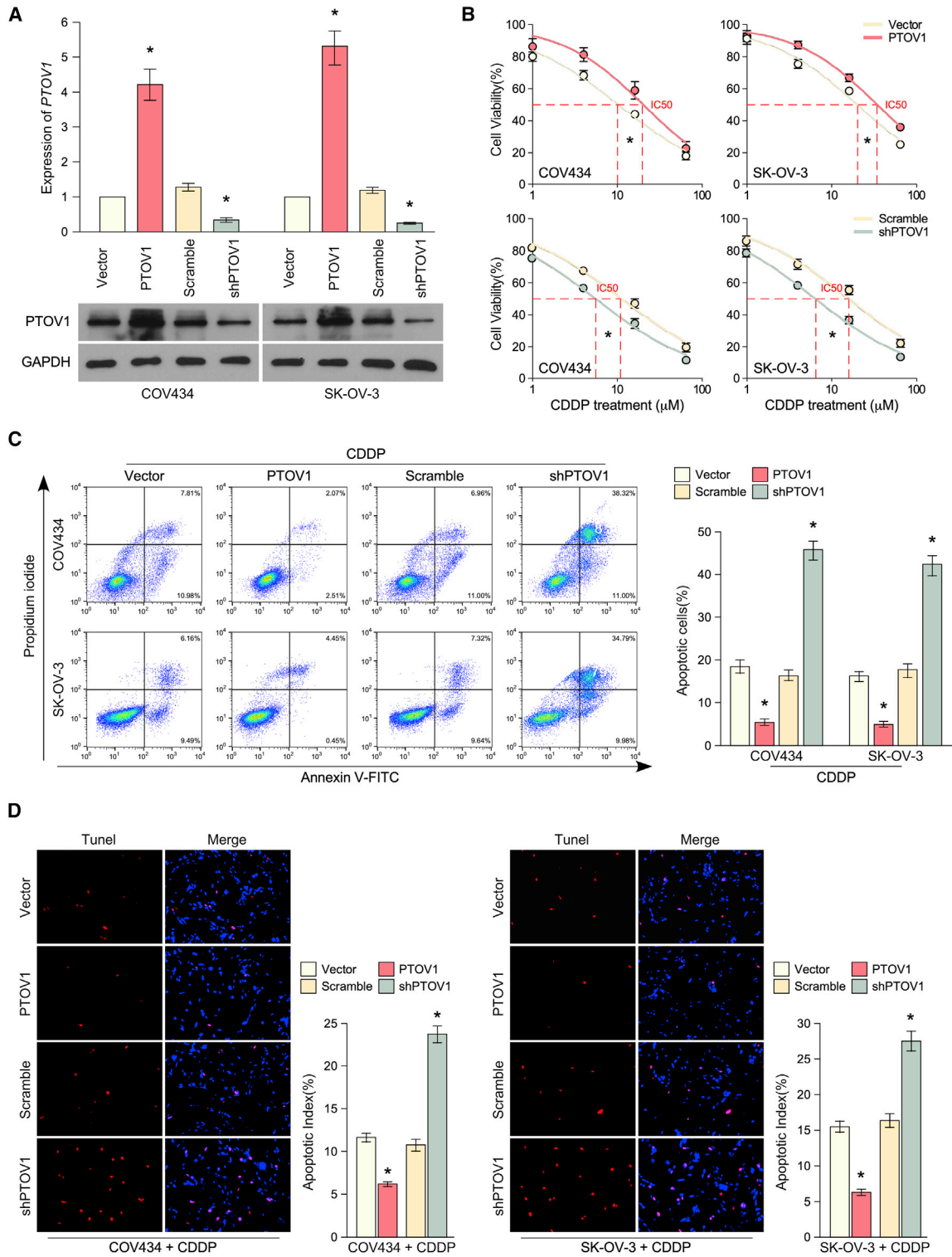
## MATERIALS AND METHODS

### Cell culture and clinical samples

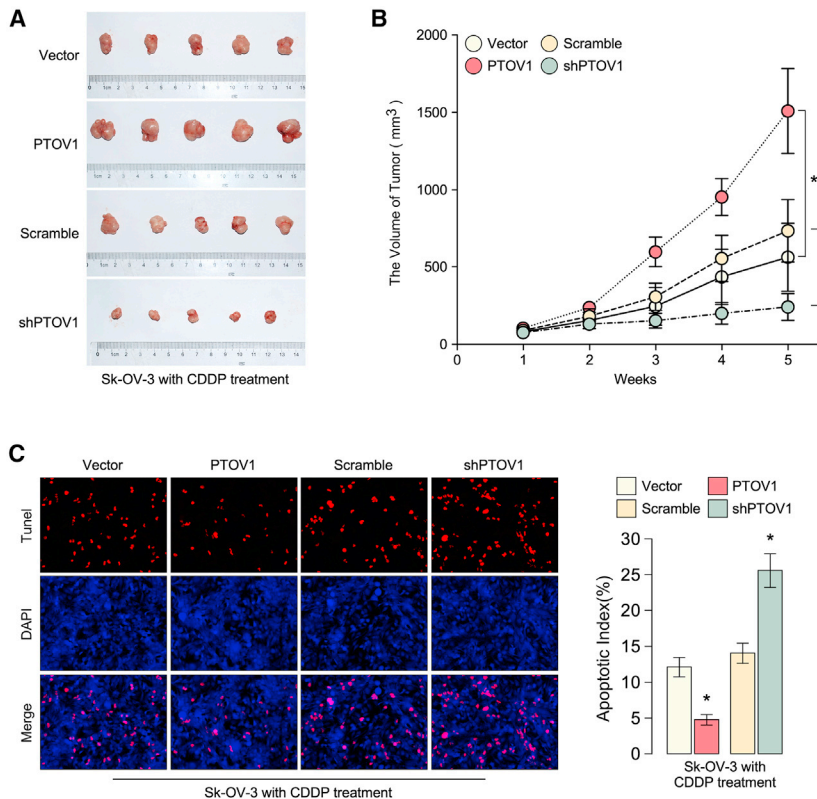
Normal ovarian epithelial cells were isolated from ovarian tissues in our lab and cultured using DMEM/high glucose (Hyclone) plus 10% fetal bovine serum (FBS) (Hyclone). Ovarian cancer cells COV362, COV434, COV644, SK-OV-3, TOV-112D, and TOV-21G were purchased from ATCC and were maintained using RPMI-1640 (Hyclone) supplemental 10% FBS (Hyclone). A cohort of 94 formalin-fixed and paraffin-embedded primary ovarian cancer tissues were obtained from the First Affiliated Hospital, Sun Yat-sen University. All tissues were confirmed by histological and clinical diagnosis. Two fresh normal ovarian tissues and eight fresh ovarian cancer tissues were also obtained from the First Affiliated Hospital, Sun Yat-sen University. For the use of these clinical tissues for research purposes, all tissues were obtained with written informed consent and approved by the Institutional Research Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University. The detailed clinical information for these tissues is shown in Table S1.

### Quantitative PCR (qPCR)

Total RNA was isolated using FastPure Cell/Tissue Total RNA Isolation Mini Kit (Vazyme) and reverse-transcribed into cDNA using HiScript II One-Step RT-PCR Kit (Vazyme). The relative



(legend on next page)



expression of genes was determined using AceQ Universal SYBR qPCR Master Mix (Vazyme) on a CFX-96 PCR Real-Time PCR Detection System (Bio-Rad). GAPDH was used as the internal control. Relative gene expression level was calculated using the  $2^{-\Delta\Delta C_t}$  method.<sup>28</sup>  $C_t$  represents the threshold cycle of each transcript.

#### Western blot and IHC

Cells were lysed using RIPA buffer (Beyotime Biotechnology) supplemented with protease inhibitors (Complete, Roche). The following antibodies were used: anti-PTOV1 (ab81173, Abcam), p65 (#8242, Cell Signaling Technology [CST]), IKK $\beta$  (#8943, CST), IKK $\alpha$  (#2682, CST), p-IKK $\beta$  (ab59159, Abcam), p-IKK $\alpha$  (ab38515, Abcam), p84 (ab487, Abcam), and GAPDH (AC033, Abclonal) antibodies.

IHC was performed according to standard methods as described previously.<sup>29</sup> The results of staining were scored independently by three pathologists blinded to clinical outcome, based on both the proportion of positively stained tumors cells and the intensity of

#### Figure 4. PTOV1 overexpression increased CDDP-induced chemotherapy resistance *in vivo*

(A) Representative images of tumors removed from mice who were xenografted tumor cells with PTOV1 overexpression or knockdown. (B) Tumor volume assay showed the effect of PTOV1 overexpression or knockdown on CDDP-induced chemotherapy. (C) TUNEL assay showed the effect of PTOV1 overexpression or knockdown on CDDP-induced chemotherapy in xenograft derived from tumor cells. All error bars represent mean  $\pm$  SEM. \* $p < 0.05$ .

staining. The proportion of tumor cells was scored as follows: score 0, no positive cells; score 1, up to 10% positive cells; score 2, 10% to ~50% positive cells; score 3, 50%–80% positive cells; score 4, over 80% positive cells. The intensity of protein expression was shown as follows: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellowish brown), and 3 (strong staining, brown).

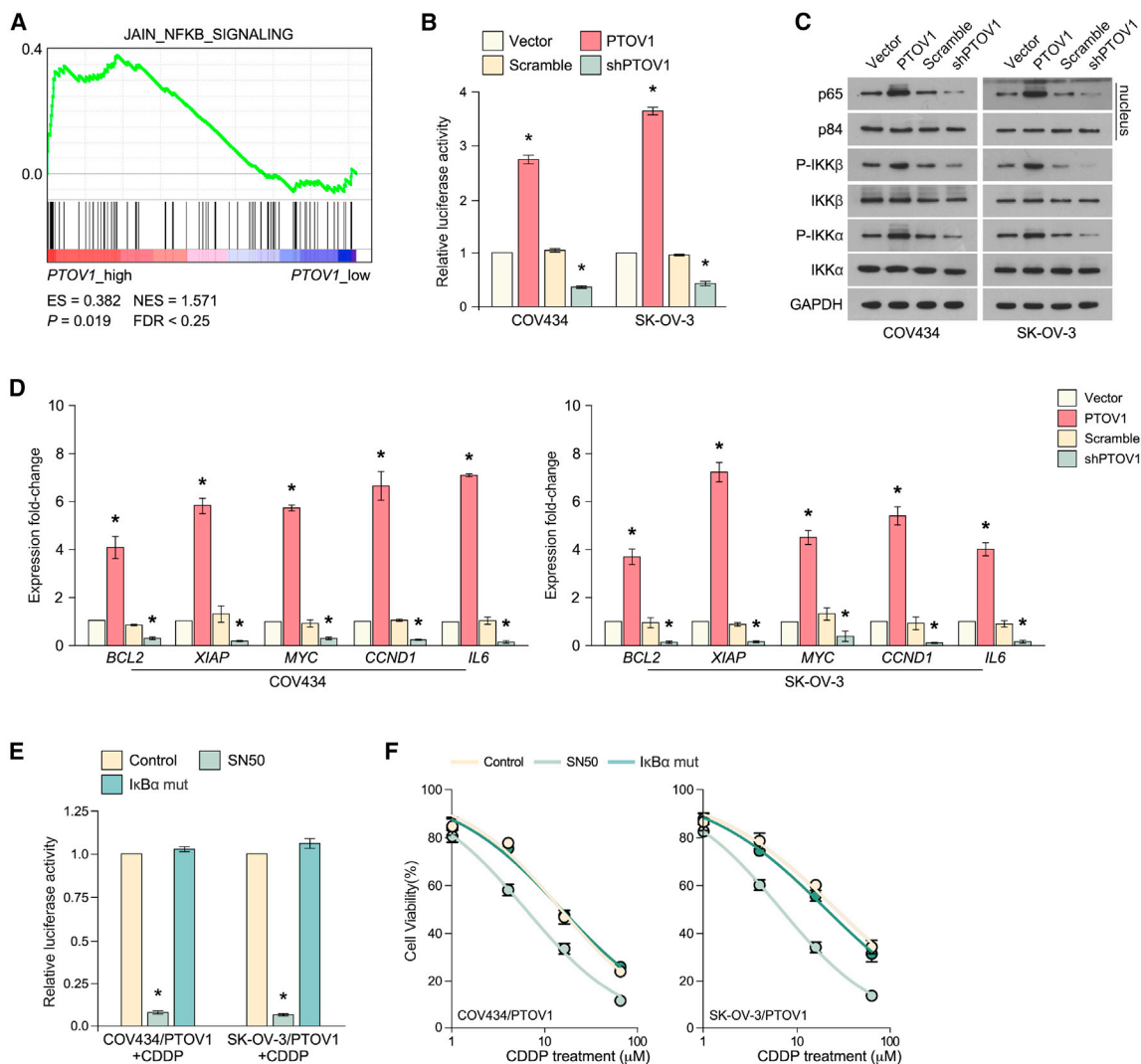
The staining index (SI) was calculated as the product of the staining intensity and the proportion of positive cell scores (scored as 0, 1, 2, 3, 4, 6, 8, 9, or 12). Cut-off values for PTOV1 expression were chosen based on a measurement of heterogeneity using the log-rank test with respect to overall survival. The optimal cut-off was identified as an SI score of greater than or equal to 4, which was considered as having high PTOV1 expression, and a score less than 4 was considered as having PTOV1 expression.

#### Vector construction and compounds

To overexpress PTOV1, the CDS sequence of PTOV1 was cloned into lentiviral vector pSin-EF2-Puro vector; the empty vector was used as the negative control. To knock down PTOV1, short hairpin RNA (shRNA) of PTOV1 was cloned into lentiviral vector PLKO.1-Puro vector. The target sequence for PTOV1 was 5'-CAG ATCGTCAACAACAAGTTT-3'. The Scramble was used as the negative control. The lentivirus was generated using Lipofectamine 2000 (Thermo) in 293T according to the manufacturer's instruction. Lentivirus supernatant was used to infect ovarian cancer cells, and puromycin (Thermo) was used to screen stable cell lines. Small molecule compound CDDP was purchased from Selleck. NF- $\kappa$ B inhibitor SN50 was purchased from MCE; 0.5  $\mu$ g/mL was used. The sequence of mutated I $\kappa$ B $\alpha$  was subcloned into pcDNA 3.1 vector.

#### Figure 3. PTOV1 overexpression increased CDDP-induced chemotherapy resistance *in vitro*

(A) qPCR and western blot analysis confirmed the effect of PTOV1 overexpression or knockdown. GAPDH was used as the loading control. (B) MTT assay showed the effect of PTOV1 overexpression or knockdown on cell viability induced by different concentrations of CDDP. (C) Apoptosis assay showed the effect of PTOV1 overexpression or knockdown on CDDP-induced chemotherapy. Apoptosis was determined by flow cytometry after Annexin V-FITC/PI dual staining. (D) TUNEL assay showed the effect of PTOV1 overexpression or knockdown on CDDP-induced chemotherapy. All error bars represent mean  $\pm$  SEM. \* $p < 0.05$ .



**Figure 5. PTOV1 promoted chemotherapy resistance through activating the NF-κB pathway**

(A) GSEA assay showed PTOV1 expression was positively correlated with NF-κB pathway. (B) Luciferase reporter assay showed PTOV1 overexpression increased NF-κB pathway activity, while PTOV1 knockdown reduced NF-κB pathway activity. (C) Western blot analyzed the expression of NF-κB pathway-associated genes in cells with PTOV1 overexpression or knockdown; p84 was used as the loading control for nuclear proteins, and GAPDH was used as the loading control for total proteins. (D) qPCR analysis of the expression of the downstream target genes of the NF-κB pathway; these genes also are associated with apoptosis. (E) Luciferase reporter assay showed that inhibition of the NF-κB pathway significantly inhibited NF-κB pathway activity in PTOV1-overexpressing cells. (F) MTT assay showed that inhibition of the NF-κB pathway increased the effect of CDDP on cell survival in PTOV1-overexpressing cells. All error bars represent mean ± SEM. \**p* < 0.05.

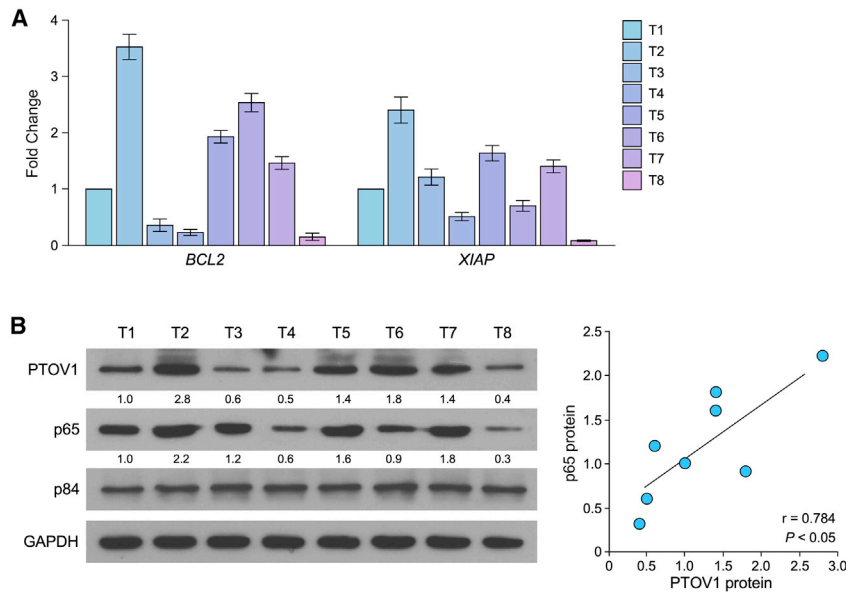
**Cell viability assay and apoptosis**

MTT assay was used to examine the cell viability. Briefly, cells were seeded on 96-well plates at a density of  $5 \times 10^3$  cells per well and treated with different concentrations of CDDP; at the point times, MTT was added, and then cells were incubated at 37°C for 4 h. The absorbance of formazan product was measured by a microplate reader (BioTek) at 490 nm. Each experiment was performed in triplicate. Annexin V/PI and TUNEL assay were used to determine the effect of PTOV1 on chemotherapy resistance. Annexin V-FITC

Apoptosis Detection Kit and One Step TUNEL Apoptosis Assay Kit were purchased from Beyotime Biotechnology. Apoptosis assay was performed according to the manufacturer’s instruction.

**Tumor xenografts**

6-week-old BALB/c nude mice were purchased from Charles River and randomly divided into four groups (n = 5 per group);  $1 \times 10^6$  SK-OV-3 with vector control, PTOV1 overexpression, Scramble control, or PTOV1 knockdown per mouse were inoculated



**Figure 6. PTOV1 expression is positively correlated with NF- $\kappa$ B pathway activity**

(A) qPCR analysis of BCL2 and XIAP expression in ovarian cancer tissues. (B) Western blot analysis of PTOV1 and p65 expression in ovarian cancer tissues. p84 was used as the loading control for nuclear proteins, and GAPDH was used as the loading control for total proteins. Statistical analysis showed PTOV1 expression is positively correlated with NF- $\kappa$ B pathway activity. All error bars represent mean  $\pm$  SEM.

subcutaneously into the inguinal folds of the nude mice. 12 days after, the mice were injected intraperitoneally with 2 mg/kg CDDP every 5 days for 5 weeks. Tumor volume was determined using an external caliper. On day 40, animals were euthanized, and the tumors were excised and photographed. The tumors were used for IHC assay.

#### Luciferase reporter assay

NL3.2.NF- $\kappa$ B-RE vector was purchased from Promega and transfected into cells using Lipofectamine 2000 (Thermo). Nano-Glo Luciferase Assay System (Promega) was used to analyze luciferase activity.

#### Statistical analysis

All data were analyzed using SPSS 19.0 software and presented as mean  $\pm$  SEM. Student's t test was used to examine statistical differences between two groups. The chi-square test was used to analyze the correlation between PTOV1 expression and clinicopathological characteristics. Survival curve was plotted using Kaplan-Meier survival analysis and compared by the log-rank test. Univariate and multivariate Cox regression analyses were used to estimate the significance of various variables for survival.  $p < 0.05$  in all cases was considered significant. All the experiments were repeated three times. GSEA was performed using a webtool, <https://www.gsea-msigdb.org/gsea/index.jsp>.

#### SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.omto.2021.02.008>.

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#### AUTHOR CONTRIBUTIONS

S.H. and H.S. conceived and designed the study. S.H., H.S., B.L., and Z.W. performed the study. Z.W., Y.Z., and Z.Y. contributed to data acquisition and analysis. H.S., Jun Liu, Jin Lan, and L.W.Z. contributed to the figures and statistical analysis. S.H. wrote the manuscript. All authors read and approved the final manuscript.

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

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