

RESEARCH ARTICLE

Anti-oncogenic PTEN induces ovarian cancer cell senescence by targeting P21

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Abstract

Deletion and mutation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) are closely associated with the occurrence of tumors. Tumor suppressor gene PTEN mutation plays an important role in the pathogenesis of ovarian cancer. However, it has been unclear whether it can regulate the senescence of ovarian cancer cells. We speculated that PTEN might inhibit the occurrence and development of ovarian cancer by promoting the expression of P21. We found that the expression of TRIM39 in human ovarian cancer was significantly diminished. In SKOV3 cells treated with naringin, the expression of TRIM39, which binds P21 and inhibits P21 degradation, was significantly elevated. Real-time polymerase chain reaction (PCR), Western blot, and immunofluorescence were used to detect the expression of PTEN, p21, and TRIM39, β -galactosidase staining was used to detect cell senescence, Ki67 staining was used to observe cell proliferation, Trim39 interference or overexpression assay was used to detect its function. We speculated that PTEN might promote SKOV3 cell senescence by increasing TRIM39 expression and decreasing P21 degradation. Furthermore, by interfering with TRIM39 in SKOV3 cells, we found that the expression of P21 was downregulated, and the number of senescent SKOV3 cells decreased. With overexpression of TRIM39 in SKOV3 cells, the expression of P21 was upregulated, and the number of senescent SKOV3 cells increased. When naringin, a PTEN agonist, was added to SKOV3 cells in which TRIM39 protein was interfered with, the expression of P21 was significantly lower than that in the control group, and the number of senescent ovarian cancer cells was significantly diminished. Our results indicated that PTEN maintained the stability of P21 and decreased the degradation of P21 by increasing TRIM39 expression, thus promoting the senescence of SKOV3 cells, and PTEN maintained the stability of p21 and promoted the aging of SKOV3 cells might be a novel therapeutic target for ovarian cancer.

KEYWORDS

aging, ovarian cancer, P21, PTEN, TRIM39, β -galactosidase staining

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1 | INTRODUCTION

Malignant ovarian tumors are among the most common malignant tumors in female reproductive organs, with an incidence rate surpassed by those of only cervical cancer and uterine cancer (Aust et al., 2020; Nitecki et al., 2018). Among malignant ovarian tumors, epithelioma is the most common, followed by malignant germ cell tumors. The mortality is highest for epithelial ovarian cancer among all types of gynecological tumors; therefore, this cancer poses a serious threat to women's lives (Ramalingam, 2016). Because the ovaries are deep in the pelvic cavity and small, and typical symptoms are usually initially absent, detection in early stages is difficult. At the time of surgery, less than 30% of patients with epithelial ovarian cancer have tumors confined to the ovaries, and most tumors have spread to the pelvic and abdominal organs (Kapusta et al., 1990; Shazly et al., 2016). Therefore, early diagnosis and specific targeted therapy are key to early diagnosis and treatment. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is the first discovered tumor suppressor gene with phosphatase activity (Correia et al., 2014; Song et al., 2012). It is the main negative regulator of the PI3K pathway (Papa & Pandolfi, 2019). High frequency deletion and mutation of PTEN are commonly seen in a variety of advanced and metastatic tumors. phosphatidylinositol-3,4,5-biphosphate (PIP3) dephosphorylation and transformation into phosphatidylinositol-3, 4-biphosphate (PIP2) decreases the levels of PIP3 in cells, antagonizes the activity of phosphatidylinositol 3-kinase (PI3K), negatively regulates and inactivates the Akt signaling pathway, and further interferes with many tumor cells by regulating tumor proliferation, migration, and invasion (Milella et al., 2015). The PTEN signaling pathway plays an important role in tumor inhibition by inducing apoptosis; inhibiting the cell cycle, tumor cell invasion and metastasis, and tumor angiogenesis; and maintaining the stability of the immune system (Parsons, 2020). Beyond the classic PTEN/PI3K/Akt signaling pathway, might PTEN be involved in other signaling pathways regulating the occurrence and development of ovarian cancer? Many studies have found a close relationship between tumors and aging (Calcinotto et al., 2019). Most tumors occur during the aging of the body. Aging cells have difficulties in repairing mismatched genes, thus increasing the likelihood of activation of proto-oncogenes and inactivation of tumor suppressor genes (Herranz & Gil, 2018; Lasry & Ben-Neriah, 2015). However, the cell cycle in aging cells is blocked, thus causing the cells to remain in G0/G1 phase and inhibiting cell proliferation, thereby preventing cell proliferation and tumor occurrence. Many proteins regulating the cell cycle are involved in tumorigenesis and cell senescence, such as p53 and P21 (Chandler & Peters, 2013). We found that the expression of P21 protein was lower in ovarian cancer tissue than normal ovarian tissue, and the decreased expression of P21 promoted cell proliferation and delays aging, thus leading to tumor occurrence. Therefore, we further studied whether P21 might affect the occurrence and development of ovarian cancer, and whether PTEN might inhibit the proliferation of ovarian cancer cells by regulating P21. PTEN promoted the combination of tripartite motif 39 (TRIM39) and P21, thus preventing P21

ubiquitination and degradation, indirectly increasing the expression of P21, and ultimately promoting cell senescence and hindering the proliferation of ovarian cancer cells.

2 | MATERIALS AND METHODS

2.1 | Clinical sample collection and informed consent

From January 2016 to December 2019, we collected fresh tissue samples from 30 patients with ovarian cancer who underwent primary surgery at the Department of Obstetrics and Gynecology at the Central Hospital of Yangpu District Affiliated with Tongji University, and collected 30 fresh normal ovarian samples from patients after ovarian cyst resection. All fresh tissues were immersed into 4% paraformaldehyde at 4°C for 72 h for preparing paraffin section. The inclusion criteria were patients 35–65 years of age with no hormone therapy in the prior 3 months. The exclusion criteria were patients receiving preoperative chemotherapy. This study was approved by the ethics committee of the Central Hospital of Yangpu District Affiliated with Tongji University, and all patients provided written consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients provided signed informed consent for participation before sample collection and were informed that the samples would be used only for scientific research.

2.2 | Immunofluorescence staining

Ovarian cancer tissue samples and normal ovarian tissue samples were cut into 1 cm³ tissue with ophthalmic scissors and fixed with 4% paraformaldehyde (Sinopharm) at 4°C for 72 h. The ovarian tissue was embedded in liquid paraffin, then sectioned and stored at room temperature. For immunofluorescence staining, the paraffin sections were dewaxed with xylene (Sinopharm), hydrated with 100%, 90%, and 70% ethanol (Sinopharm), then placed in a beaker filled with sodium citrate antigen repair solution (Beyotime) and covered with antigen repair solution. The sections were then heated at 95°C for 15 min, cooled to room temperature and washed with 0.01 M phosphate-buffered saline (PBS) (Beyotime) three times for 5 min each. Mouse antibodies to PTEN (1:500, Abcam), P21 (1:500, Abcam) and TRIM39 (1:500, Abcam), and rabbit antibody to Vimentin (1:500, Abcam) were diluted in primary antibody diluent for 30 min, mixed well, deposited on the surface of the tissue, incubated at 4°C overnight and washed with 0.01 M PBS three times for 10 min each. Goat anti-mouse Alexa Fluor 488 antibody and goat anti-rabbit Alexa Fluor 555 antibody (Thermo Scientific) were diluted in secondary antibody diluent at a ratio of 1:1000, mixed well, deposited on the surfaces of tissues or cells, incubated at 37°C for 1 h and washed with 0.01 M PBS three times for 10 min each. 4',6-diamidino-2-phenylindole (Thermo Scientific) staining solution was deposited on the tissue sections,

and the slides were sealed. The staining was observed under a fluorescence microscope (Olympus).

2.3 | Cell culture and viral infection

SKOV3 cells were purchased from the cell bank of Shanghai Chinese Academy of Sciences. McCoy's 5 A cell culture medium (Sigma) + 10% fetal bovine serum were used as the complete culture medium. When the cell fusion density reached 90%, the SKOV3 cells were passaged. According to the experimental requirements, SKOV3 cells were seeded in 6-cm dishes and 24-well plates. The 24-well plates were used for β -gal and TdT-mediated dUTP Nick-End Labeling (TUNEL) staining to detect aging and apoptotic cells. The lentivirus for overexpression of PTEN and P21 and the lentivirus interfering with and overexpressing TRIM39 (RNA oligo sequence: ss 5'-UUCU AACAGACAAGAAAGGAA-3', as 5'-CCUUU CUUGUCUGUUAGAAAC-3') were purchased from Shanghai Gene Chemical Technology Co., Ltd. with a titer of 1×10^8 TU/. SKOV3 cells with a density of 5×10^6 cells/were prepared in McCoy's 5 A medium and seeded in 6-cm culture dishes. When the cell density exceeded 90%, 400 μ l virus infection enhancer HitransG A and 100 μ l lentivirus or negative control (neg-control) with a titer of 1×10^8 TU/were added. After incubation at 37°C for 24 h, the medium was replaced with fresh culture medium, and the culture medium was subsequently changed every other day. At approximately 72 h after infection, the infection efficiency (the number of green fluorescent protein positive cells) was observed. When the efficiency reached or exceeded 90%, the infection was considered successful, and follow-up experiments were performed.

2.4 | Real-time polymerase chain reaction (PCR)

The samples were collected according to the traditional method of RNA extraction, and 1 TRIzol (Invitrogen) was added, followed by 200 μ l chloroform (Sinopharm group), and then incubated for 10 min. After centrifugation at 12,000 rpm at 4°C for 15 min, the upper aqueous phase was transferred to a new RNase-free ependorf tube, 500 μ l isopropanol was added, and the mixture was incubated for 10 min and then centrifuged for 10 min at 12,000 rpm. The supernatant was discarded, and the white RNA deposited at the bottom of the tube was retained. After cleaning with 75% ethanol, the RNA was dried. After the white RNA became colorless and transparent, it was dissolved in 30 μ l diethyl pyrocarbonate (DEPC) and stored at -80°C. TB Green® Premix Ex Taq™ kit (Takara) was used for quantitative PCR. A 20- μ l reaction system was used, with predenaturation at 95°C for 30 s, followed by 95°C \times 5 s and 60°C \times 30 s for 40 cycles. After the reaction, the amplification curve and melting curve were confirmed. The relative gene expression was calculated according to the $2^{-\Delta\Delta C_t}$ method and then compared between groups. PTEN primer sequence: forward: 5'-TGGATTCGA CTTAGACTTGACCT-3'; reverse: 5'-GGTGGGTTATGGTCTTCAAA AGG-3', P21 primer sequence: forward: 5'- TGCAACTACTACAGA AACTGCTG-3'; reverse: 5'-CAA

AGTGGTCGGTAGCCACA-3', TRIM39 primer sequence: forward: 5'-GAAAGGGCGAGTT

GACTCCAC-3'; reverse: 5'-GGCTGCATATTTGTCCCCATT-3', GAPDH primer sequence: forward: 5'-CTTGCTCAAGCTTAGTTCTAG G-3'; reverse: 5'-GAGTGCTCAGTGGTAT TGC-3'.

2.5 | Western blot

For western blot analysis, the concentrations of extracted protein from cultured cells were measured quantitatively with bicinchoninic acid protein assays. Then 5 \times loading buffer (Beyotime) was added to the extracted protein. After mixing, the protein was denatured at 100°C for 5 min. The protein was then stored at -80°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 20 μ g total protein per lane. The protein was transferred to poly vinylidene fluoride membranes (Millipore), which were blocked for 30 min at room temperature and then incubated with the relevant antibody: mouse polyclonal antibodies to PTEN (1:2000, Abcam), P21 (1:1000, Abcam) or TRIM39 (1:2000, Abcam), or rabbit polyclonal antibody to GAPDH (1:5000, Abcam). The corresponding secondary antibodies, goat anti-mouse HRP (1:5000, CST) or goat anti-rabbit HRP (1:5000, CST), were used to detect the protein bands. The protein was quantified in IPP software, and the differences between groups were compared.

2.6 | The β -gal staining

The senescent cells were identified with a β -galactosidase Staining Kit (Beyotime), which uses X-gal as the substrate and produces dark blue products under the catalysis of aging-specific β -galactosidase. Blue cells expressing β -galactosidase can easily be observed under a light microscope. The cells were stained with β -galactosidase, fixed at room temperature for 10 min and washed with 0.01 M PBS (Beyotime) three times for 5 min each. Then, a working dye solution comprising a mixture of β -galactosidase staining solution A (10 μ l), β -galactosidase staining solution B (10 μ l), β -galactosidase staining solution C (930 μ l), and X-gal (50 μ l) was added, and the mixture was incubated at 37°C for 4 h. The samples were observed, photographed and counted under an ordinary optical microscope. If photos were not taken sufficiently quickly, the dye solution was discarded, an equivalent volume of PBS was added, and the cells were stored at 4°C for several days. The proportion of positive cells was determined as the number of β -galactosidase positive cells in 10 fields divided by the total number of cells and was compared through statistical analysis between groups.

2.7 | TUNEL staining

After removal of the culture medium, 4% paraformaldehyde (Sinopharm) was added to fix the cells at room temperature for 15 min.

The cells were then washed with 0.01 M PBS (Beyotime) three times for 5 min each. PBS containing 0.3% Triton X-100 was then added, and the cells were incubated at room temperature for 5 min and washed with 0.01 M PBS three times for 5 min each. TUNEL staining solution was prepared according to a 1:9:10 ratio of terminal deoxynucleotidyl transferase enzyme:fluorescent labeling solution:TUNEL detection solution. After addition of the TUNEL staining solution to each well, the cells were incubated at 37°C for 2 h in the dark and washed with 0.01 M PBS three times for 5 min each. Then the number of TUNEL positive staining cells was observed and counted under a fluorescence microscope. Ten fields were selected from each well, and photographs were taken. The number of TUNEL positive cells in 10 fields was counted and divided by the total number of cells to determine the proportion of positive cells. This proportion was compared through statistical analysis between groups.

2.8 | Statistical analysis

All statistical analysis was computed in SPSS 18.0 (SPSS Inc). All quantitative indexes are expressed as the mean \pm SD. The experimental data were analyzed with one-way analysis of variance (ANOVA) and Tukey's post hoc test. $p < .05$ was considered to indicate a statistical difference between the groups.

3 | RESULTS

3.1 | Downregulation of PTEN and P21 expression in human ovarian cancer

We first detected the expression of PTEN and P21 in human ovarian cancer tissues and normal human ovarian tissues. Real-time PCR results showed that the expression of PTEN and P21 was downregulated at the mRNA level in human ovarian cancer tissues compared with normal human ovarian tissues (Figure 1a). Western blot analysis showed that the protein expression of PTEN and P21 in human ovarian cancer tissues was downregulated compared with that in the normal ovarian tissues (Figure 1b). Immunofluorescence staining showed that the protein expression of PTEN and P21 in human ovarian cancer tissues was downregulated compared with that in normal ovarian tissues (Figure 1c). According to the above results, we speculated that the downregulation of PTEN and P21 might be associated with the pathogenesis of ovarian cancer

3.2 | P21 promotes the senescence of human ovarian cancer cells

Because the expression of P21 in ovarian cancer was low, we speculated that P21 downregulation might promote the proliferation

of ovarian cells, to prevent mutant ovarian cells from undergoing aging or apoptosis, and promoting the occurrence and development of ovarian cancer. Therefore, we examined the role of P21 in the aging and proliferation of ovarian cancer cells. β -gal staining showed that the number of senescent cells in SKOV3 cells overexpressing P21 was significantly greater than the number of normal ovarian cancer cells (Figure 2a), whereas the proliferation ability was significantly lower (Figure 2b), thus indicating that P21 was involved in the occurrence and development of ovarian cancer. We detected the changes in PTEN mRNA (Figure 2c) and protein levels (Figure 2d) in SKOV3 cells overexpressing P21 and found that P21 did not affect the expression of PTEN.

3.3 | PTEN promotes cell senescence and P21 expression in human ovarian cancer cells

As a tumor suppressor gene, PTEN inhibits the occurrence and development of ovarian cancer. The classic tumor suppressor pathway is inhibition of PI3K/Akt signaling, thereby inhibiting cell proliferation. Whether PTEN participates in the pathogenesis of ovarian cancer through regulating cell aging required further study. Therefore, we overexpressed PTEN in SKOV3 cells to examine cell aging. Overexpression of PTEN promoted aging (Figure 3a) and inhibited proliferation (Figure 3b) in SKOV3 cells. In addition, we examined the expression of P21 in SKOV3 cells overexpressing PTEN and found that the expression of P21 was higher than that in the control group at both the mRNA (Figure 3c) and protein levels (Figure 3d). Thus, we speculated that PTEN might promote the senescence of ovarian cancer cells and inhibit the proliferation of ovarian cancer cells by promoting the expression of P21.

3.4 | TRIM39 inhibits the degradation of P21 and promotes the senescence of ovarian cancer cells

The interaction between TRIM39 and P21 prevents the binding of Cdt2 and P21, thus inhibiting the ubiquitination of P21 by CRL4Cdt2E3 complex and promoting cell-cycle arrest. Therefore, we first examined the expression of TRIM39 in human ovarian cancer tissues. Real-time PCR (Figure 4a), western blot analysis (Figure 4b), and immunofluorescence staining (Figure 4c) showed that the expression of TRIM39 in ovarian cancer tissues was significantly lower than that in normal ovarian tissues. Furthermore, by interfering with TRIM39 in SKOV3 cells, we found that the expression of P21 was downregulated, and the number of senescent SKOV3 cells decreased. With overexpression of TRIM39 in SKOV3 cells, the expression of P21 was upregulated, and the number of senescent SKOV3 cells increased (Figure 4d,e). These results suggested that PTEN promoted SKOV3 cell senescence by increasing TRIM39 expression and decreasing P21 degradation.

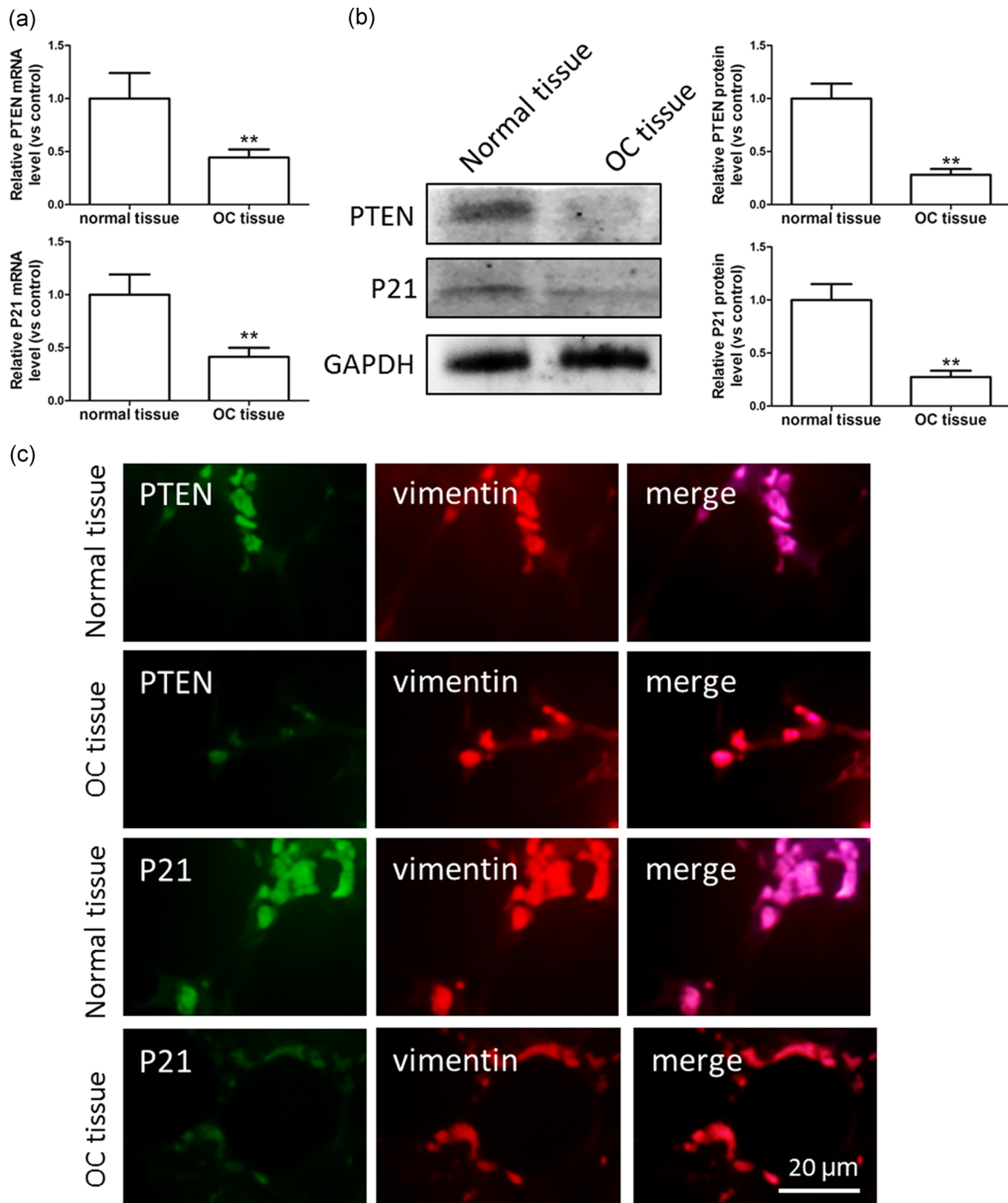


FIGURE 1 Downregulation of PTEN and p21 expression in human ovarian cancer. Real-time PCR results showed that compared with normal human ovarian tissue, PTEN and p21 mRNA expression in human ovarian cancer tissue were downregulated (a), the results of Western blot showed that the protein expression of PTEN and p21 in human ovarian cancer tissues were downregulated compared with normal ovarian tissues (b). Immunofluorescence staining showed that the protein expression of PTEN and p21 in human ovarian cancer tissues were downregulated compared with normal ovarian tissues (c). ** $p < .01$ (vs. control). Scale bar, 20 μm . PTEN, phosphatase and tensin homolog deleted on chromosome10; PCR, polymerase chain reaction

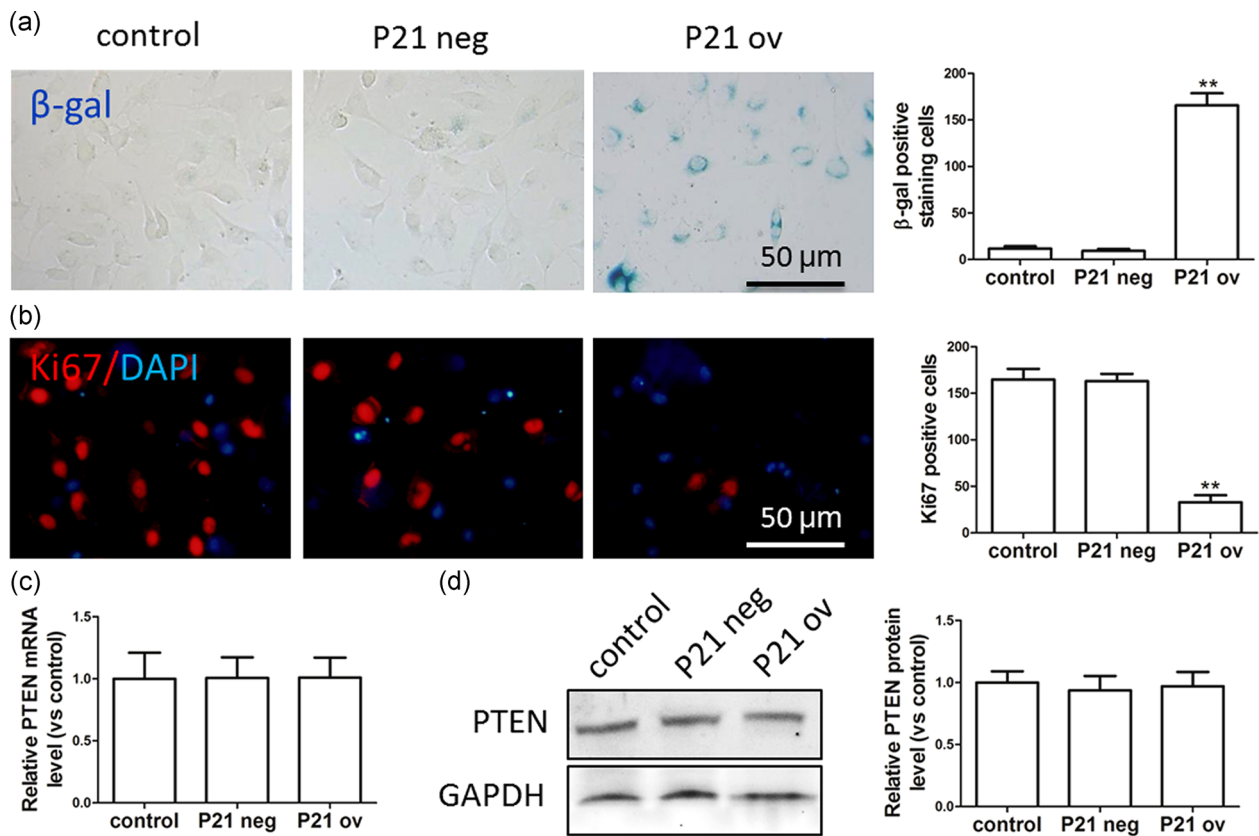


FIGURE 2 P21 promotes the senescence of human ovarian cancer cells. β -gal staining showed that the number of senescent cells in SKOV3 cells overexpressing P21 was significantly greater than the number of normal ovarian cancer cells (a), and the proliferation ability was significantly decreased (b). We detected the changes of PTEN mRNA and protein levels in SKOV3 cells overexpressing p21 and found that p21 did not affect the expression of PTEN (c,d). $**p < .01$ (vs. control). Scale bar, 50 μ m. PTEN, phosphatase and tensin homolog deleted on chromosome10

3.5 | PTEN promotes ovarian cancer cell senescence by increasing TRIM39 expression and decreasing P21 degradation

In SKOV3 cells treated with naringin, an agonist of PTEN, the expression of TRIM39, which binds P21 and inhibits the degradation of P21, significantly increased, thus suggesting that PTEN maintains P21 stability by promoting the expression of TRIM39 (Figure 5a,b). On the basis of the above results, we added naringin to SKOV3 cells in which TRIM39 was interfered with and found significantly lower expression of P21 (Figure 5c) and significantly fewer senescent cells (Figure 5d) in the ovarian cancer group than the control group, thus indicating that PTEN maintained the stability of P21 and decreased the degradation of P21 by increasing TRIM39 expression, thereby promoting the senescence of SKOV3 cells.

4 | DISCUSSION

Malignant ovarian tumors are among the most common malignant tumors in female reproductive organs; their incidence rate ranks third after cervical cancer and endometrial cancer. Epithelial tumors

account for 50%–70% of primary ovarian tumors, and their malignant types account for 85%–90% of ovarian malignant tumors (Hauptmann et al., 2017; Webb & Jordan, 2017). Surgery and chemotherapy can cure most patients in early stages but cannot save the lives of many late stage patients (Cobb & Gershenson, 2018). The main types of ovarian cancer are borderline tumors, serous cancer, endometrioid cancer, clear cell cancer and mucinous cancer. In the past 10 years, to improve the cure rate of ovarian cancer, a new classification dividing ovarian cancer into Type I and Type II has been proposed (Kurman & Shih Ie, 2010, 2011). Type I is low-grade endometrial, mucinous and clear cell carcinoma with v-raf murine sarcoma viral oncogene homolog B (BRAF), V-Ki-ras2 Kirsten rat-sarcoma viral oncogene homolog (KRAS), and PTEN mutations. Type II is a high-grade serous carcinosarcoma with mutations in the p53, Breast Cancer Susceptibility Protein (BRCA)1 and BRCA2 genes (Prahm et al., 2015; Stasenکو et al., 2019). Because of the polygenic mutations in ovarian cancer, current treatment methods include targeted therapy, antiangiogenesis therapy, and hormone replacement therapy in addition to traditional chemotherapy drugs.

The tumor suppressor gene PTEN (MMAJC1/TEPI) is located on chromosome 10q23.3, and its mutation and deletion are associated with tumorigenesis. Through its dual substrate-specific phosphatase

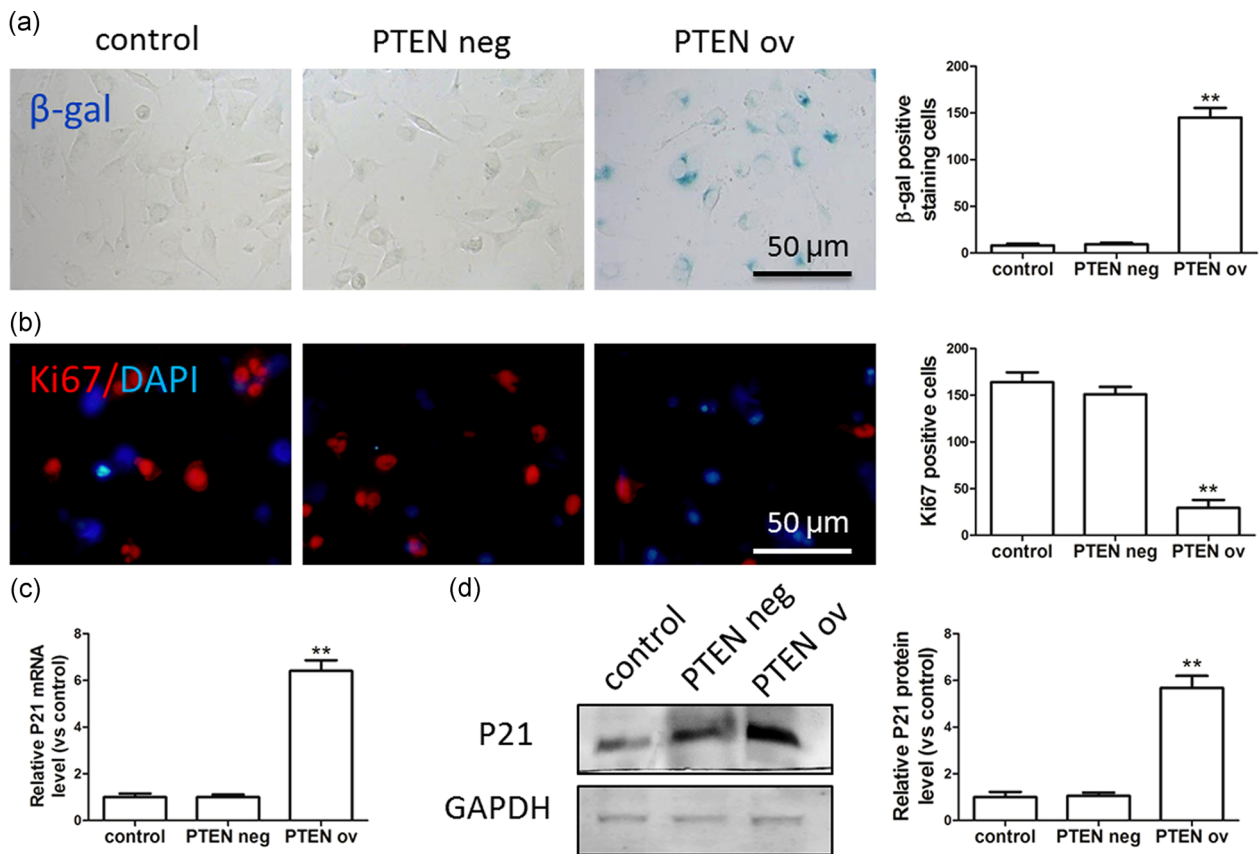


FIGURE 3 PTEN promotes senescence and p21 expression in human ovarian cancer cells. We overexpressed PTEN in SKOV3 cells and observed cell senescence. The results showed that overexpression of PTEN promoted the senescence of SKOV3 cells (a) and inhibited the proliferation of SKOV3 cells (b). At the same time, we detected the expression of p21 in SKOV3 cells overexpressing PTEN, and found that the expression of p21 was higher than that in the control group at both mRNA and protein levels (c,d). ** $p < .01$ (vs. control). Scale bar, 50 μm. PTEN, phosphatase and tensin homolog deleted on chromosome10

activity mediated by the PI3K and Mitogen-activated protein kinases (MAPK) signaling pathway, PTEN mainly plays a tumor suppressor role and affects cell growth, apoptosis, proliferation, and adhesion. Recent research has confirmed that PTEN is closely associated with the occurrence and development of endometrial cancer, ovarian cancer, cervical cancer, and other gynecological tumors (Cybulska et al., 2019; Wang et al., 2015). However, beyond the classic PI3K/Akt pathway, whether PTEN inhibits the occurrence of ovarian cancer through other pathways remains to be further studied. Here, we found that the expression of PTEN, P21, and TRIM39 was significantly diminished in human ovarian cancer tissues. P21 is involved in the regulation of the cell cycle, whereas TRIM39 is responsible for the stability of P21. As a tumor suppressor gene, P21 is a cyclin-dependent kinase inhibitor. It regulates the cell cycle through its protein levels, thus inhibiting the occurrence and development of tumors. P21 is associated with tumor proliferation, differentiation, metastasis, and invasion and is an indicator of prognosis. P21 also regulates cell senescence by inhibiting the cell cycle (Abbas & Dutta, 2009; Skirnisdottir & Seidal, 2013). In this study, we examined whether P21 promotes ovarian cancer cell senescence and inhibits the occurrence and development of ovarian cancer by regulating the

expression of P21. Our results showed that P21 increased the senescence of ovarian cancer cells, and thus promoted cell-cycle arrest and inhibited the proliferation of ovarian cancer cells. This effect was achieved through regulation of the expression of PTEN; thus, PTEN inhibits the occurrence and development of tumors by promoting P21 expression. However, PTEN cannot directly regulate the expression of P21. Therefore, we speculated that PTEN might affect P21 expression by regulating the P21 binding protein TRIM39. PTEN increased the expression of TRIM39 and thus promoted the binding of TRIM39 to P21, prevented the ubiquitination and degradation of P21, and thus indirectly promoted the expression of P21 and the senescence of ovarian cancer cells.

TRIM39 was cloned in the year 2000, but its biological function remains unclear (Huang et al., 2012; Lee et al., 2009). P21 is an inhibitor of cyclin-dependent kinase. It participates in the regulation of cell growth, differentiation, aging, and death by modulating the cell cycle. It plays an important role in the response of cells to stress and the occurrence and development of tumors. TRIM39 interacts with P21, thus preventing Cdt2, a substrate recognition protein in the CRL4Cdt2 E3 complex, from binding to P21. Consequently, the ubiquitination

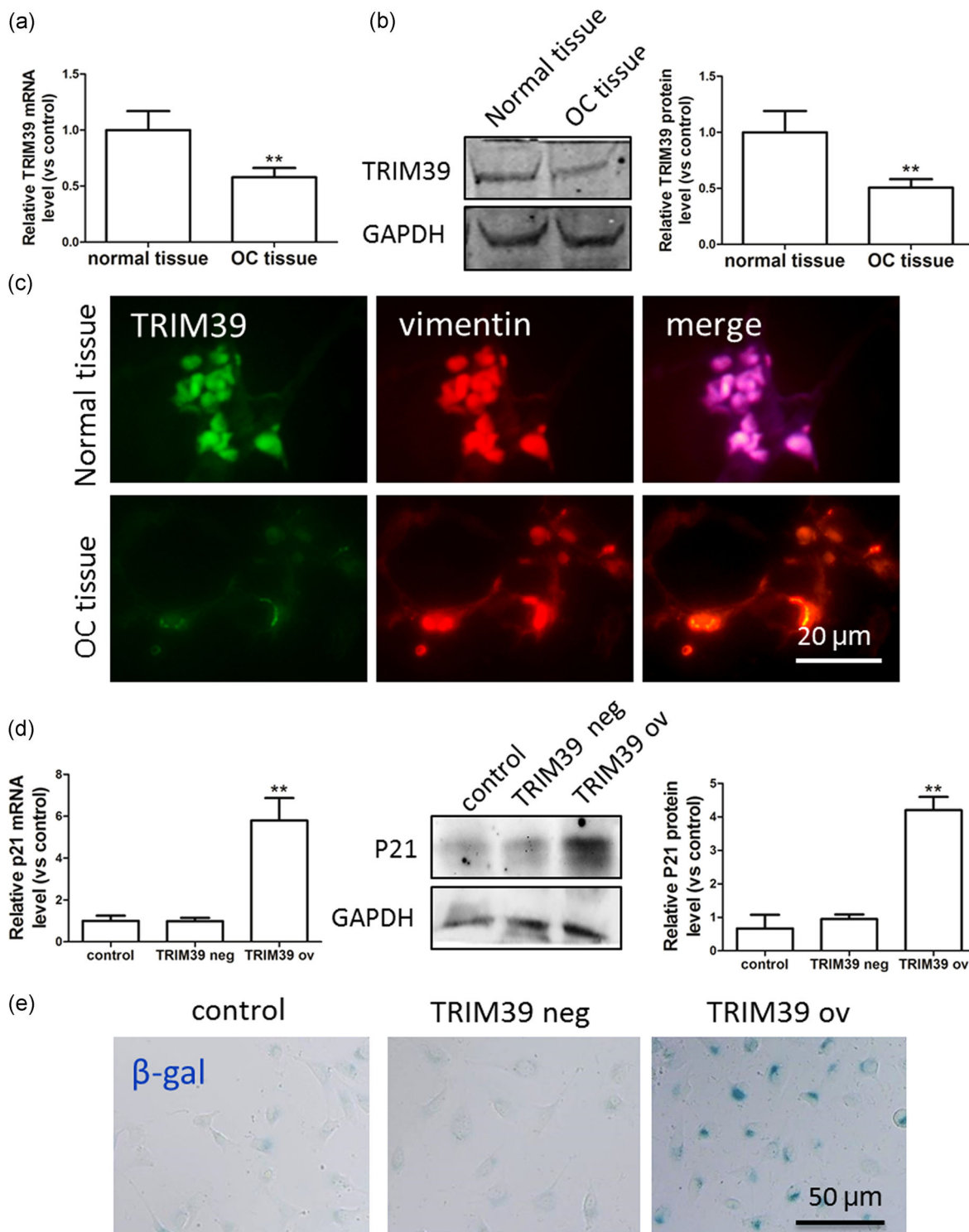


FIGURE 4 TRIM39 inhibits P21 degradation and promotes ovarian cancer cell senescence. We detected the expression of TRIM39 in human ovarian cancer tissues. Real-time PCR (a), Western blot (b), and immunofluorescence staining (c) showed that the expression of TRIM39 in ovarian cancer tissues was significantly lower than that in normal ovarian tissues. Furthermore, by interfering with TRIM39 in SKOV3 cells, we found that the expression of P21 was downregulated and the number of senescent SKOV3 cells was decreased. Overexpression of TRIM39 in SKOV3 cells showed that the expression of P21 was upregulated (d) and the number of senescent SKOV3 cells was increased (e). ** $p < .01$ (vs. control). Scale bar, 20 or 50 μm . PCR, polymerase chain reaction

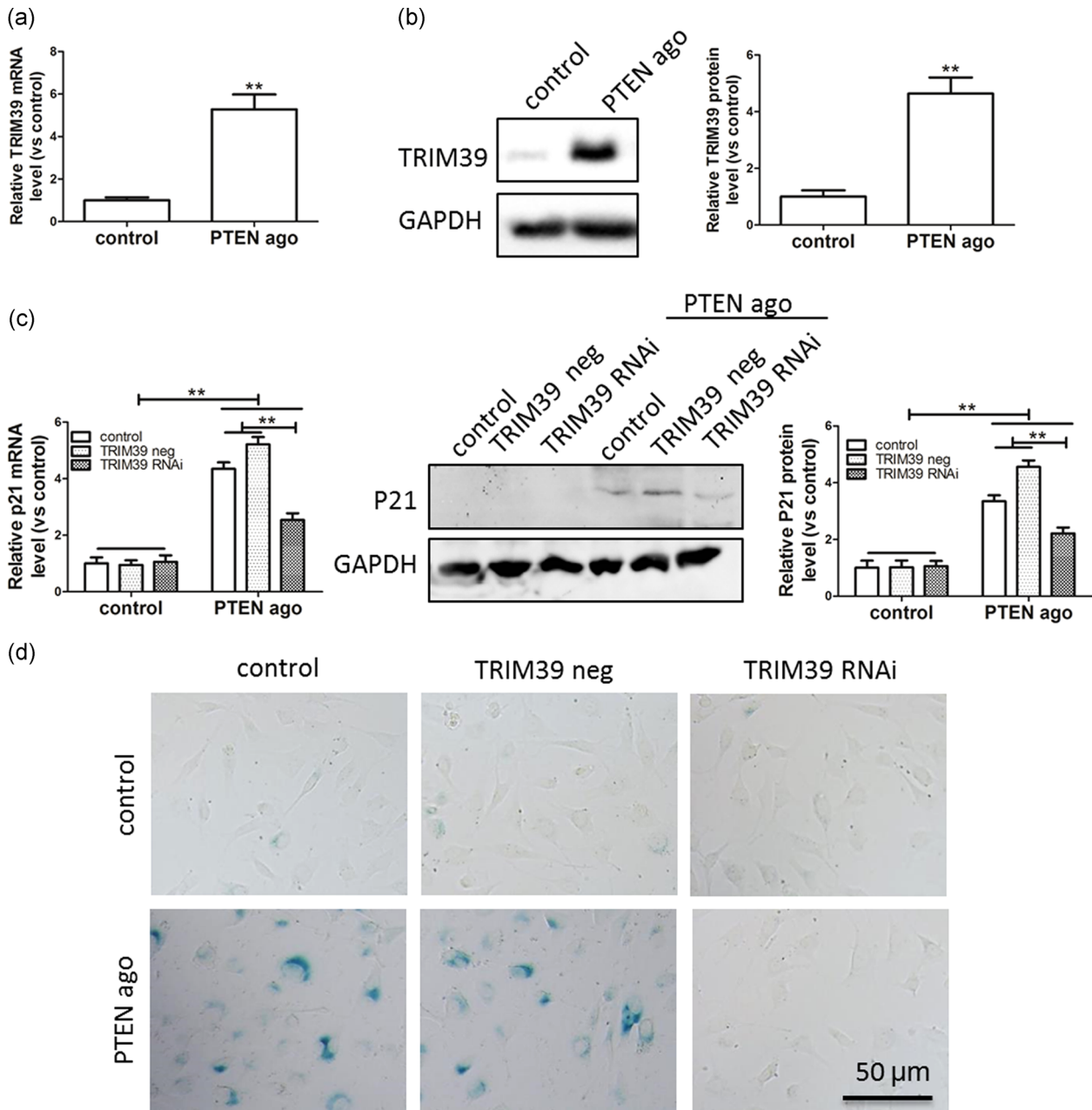


FIGURE 5 PTEN promotes ovarian cancer cell senescence by promoting TRIM39 expression and reducing P21 degradation. In SKOV3 cells treated with naringin, the expression of TRIM39, which binds to P21 and inhibits the degradation of p21, was significantly increased, suggesting that PTEN can maintain the stability of P21 by promoting the expression of TRIM39 (a,b). When naringin, a PTEN agonist, was added to SKOV3 cells that interfered with TRIM39 protein, the expression of P21 was significantly reduced and the number of senescent ovarian cancer cells was significantly reduced compared with the control group (c,d). $**p < .01$ (vs. control). Scale bar, 50 μ m. PTEN, phosphatase and tensin homolog deleted on chromosome10

proteasome degradation of P21 mediated by the CRL4Cdt2 E3 complex is inhibited, thereby affecting the cell cycle and cell response to DNA damage (Shibata et al., 2011; Zhang, Mei, et al., 2012). TRIM39 is an important molecule that regulates the cell cycle. In physiological conditions, TRIM39 and P21 negatively regulate the cell cycle (Zhang, Huang, et al., 2012). In the presence of DNA damage, TRIM39 plays an important role in cell-cycle arrest.

5 | CONCLUSIONS

In this study, we found that PTEN inhibited the degradation of P21 by increasing the expression of TRIM39, thus promoting cell-cycle arrest in G0/G1 phase and cell aging, and inhibiting the occurrence and development of ovarian cancer. Accordingly, PTEN maintained the stability of p21 and promoted the aging of SKOV3 cells might be a novel therapeutic target for ovarian cancer. However, PTEN may be

involved in a variety of regulatory pathways that inhibit tumorigenesis. Further exploration of downstream target genes for tumor targeted therapy is therefore needed.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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