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Research article

ANGPTL3 diminishes the resistance of ovarian cancer to paclitaxel by blocking the PI3K-AKT-mTOR signaling pathway

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ABSTRACT

Angiopoietin-like protein 3 (ANGPTL3) is key in ovarian cancer (OC) cell growth and metastasis, notably by enhancing natural killer cells' capacity for inducing cell toxicity and apoptosis. However, its role in influencing chemotherapy resistance in OC remains ambiguous. In this study, we discovered a correlation between reduced ANGPTL3 levels and a less favorable outcome in OC patients using the Kaplan-Meier Plotter database. Lower levels of ANGPTL3 were detected in paclitaxel (PTX)-resistant OC tissues and cell lines via western blotting and immunohistochemistry. To investigate ANGPTL3's effects, we established SKOV3/PTX and 2780/PTX as PTX-resistant OC cell lines by incrementally increasing PTX exposure and then transfecting them with overexpress ANGPTL3 (OE-ANGPTL3) lentivirus. We conducted various assays such as CCK-8, colony formation, Edu staining, flow cytometry, and transwell to investigate the impact of ANGPTL3 on PTX resistance. Additionally, this effect was examined in a mouse subcutaneous xenograft model. Both *in vitro* and *in vivo* experiments demonstrated that ANGPTL3 overexpression mitigated PTX resistance in OC cells by inactivating the PI3K-AKT-mTOR pathway. In summary, our research reveals that ANGPTL3 enhances PTX sensitivity in OC by downregulating the PI3K-AKT-mTOR pathway. The study of this study suggest that ANGPTL3 could serve as a valuable therapeutic target for OC, signifying its clinical relevance in OC management.

1. Introduction

Ovarian cancer (OC) remains a leading cause of death from gynecological cancers globally, primarily due to its subtle onset and the swift emergence of resistance to chemotherapy treatments [1]. The advancement of OC, like many cancers, involves complex processes that facilitate cellular proliferation, invasion and, eventually, metastasis [2]. Traditional first-line therapy for OC often incorporates the use of Paclitaxel (PTX), but the onset of PTX-resistance severely limits its efficacy and ultimately, impacts patient survival rates unfavorably [3,4]. Addressing this challenge, scientists have dedicated significant research efforts to studying PTX-resistant OC cells. Notably, acquiring a more profound knowledge of the mechanisms that drive PTX resistance can guide the creation of more potent treatment approaches.

Abbreviations: OC, ovarian cancer; ANGPTL3, Angiopoietin-like protein 3; PTX, paclitaxel; OS, overall survival; HR, hazard ratio; PFS, progression-free survival; DAB, diaminobenzidine; ATCC, American Type Culture Collection; CCK-8, Cell counting kit-8; OD, optical density; EdU, 5-ethynyl-20-deoxyuridine; PI, propidium iodide; SD, standard deviation; ANOVA, one-way analysis of variance.

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The protein known as Angiopoietin-like protein 3 (ANGPTL3), which belongs to the ANGPTL family and is situated on chromosome 1p31.3, has been notably linked to disruptions in lipid metabolism [5]. Extensive research has revealed its vital roles in managing key physiological activities, including blood cell formation, the formation of new blood vessels, and the metabolism of fats [6,7]. Recent literature has highlighted the complex and often paradoxical role of ANGPTL3 in cancer progression. While elevated expression of ANGPTL3 has been linked to decreased survival in certain cancers, including esophageal cancer [8] and glioblastoma [9], its impact varies across different cancer types. In hepatocellular carcinoma, ANGPTL3 upregulation serves as a biomarker that sets it apart from benign liver conditions [10], and fosters the proliferation and spread of tumors in colorectal cancer [11]. In stark contrast, cervical cancer sees benefits from the suppression of ANGPTL3, which leads to decreased tumor aggressiveness [12]. Adding to this complexity, renal cell carcinoma demonstrates a negative correlation between ANGPTL3 levels and patient survival, suggesting a tumor-suppressive role where its overexpression impedes cancer cell proliferation and spread [13,14]. This contradictory behavior underscores the multifaceted nature of ANGPTL3 in cancer biology and its potential influences on the PI3K-AKT-mTOR pathway, a pivotal modulator of cell growth and a contributor to chemoresistance [15,16]. Intriguingly, recent findings by Wu et al. [17] hint at ANGPTL3 functioning as a tumor suppressor in OC, with elevated levels enhancing vulnerability to immune-mediated destruction. Given this context and the central role of PTX in treating OC, our study aims to demystify the convoluted relationship between ANGPTL3 expression levels and PTX resistance in OC.

Thus, this study explored the impact of ANGPTL3 on OC cells' resistance to PTX, using both laboratory and animal models for examination. Our research provides the initial insight into the role of the PI3K-AKT-mTOR signaling pathway in modulating PTX resistance through ANGPTL3. The results of this research may provide a new biomarker and a potential treatment target for OC.

2. Materials and methods

2.1. Survival analysis

The Kaplan-Meier plotter, accessible at kmplot.com, serves as a useful online tool offering extensive data on various cancers, including OC. To conduct survival analysis, this tool was used by first selecting the tumor type "OC", then inputting the gene "ANGPTL3", and choosing either OS (overall survival) or PFS (progression-free survival), along with all chemotherapy options. Following these inputs, the tool calculated and displayed the log-rank *p*-value and HR (hazard ratio), complete with 95 % confidence intervals, on the generated plot.

2.2. Clinical samples

Tissue samples in this study were obtained from patients diagnosed as OC during surgery, with the approval of the Institutional Review Boards of The First Hospital of Quanzhou Affiliated to Fujian Medical University (Approval No: FHQM-2020-32A, Fujian, China). Criteria for patient selection included: confirmation of OC through postoperative pathological examination, treatment history limited to PTX-based chemotherapy only, and the procurement of written informed consent from every participant before the commencement of the study. PTX-resistant cases (n = 18) were identified as those showing disease progression during postoperative chemotherapy or recurrence within six months after the primary chemotherapy. In contrast, PTX-sensitive cases (n = 26) were distinguished by either a recurrence occurring more than six months after primary chemotherapy or the absence of any recurrence.

2.3. Immunohistochemistry

Initially, the tumor tissues were separated and preserved in 10 % formalin for a period of 24 h. Following this, the tissues were dehydrated and set in paraffin, from which 5-μm thick slices were then prepared. These sections were then deparaffinized with xylene and rehydrated through a sequence of alcohol solutions of varying concentrations. For the staining process, the tissue slides underwent an initial incubation overnight at 4 °C with a rabbit anti-ANGPTL3 antibody (1:1000, ab118208, Abcam). Following this, they were labeled with a biotinylated serum derived from goats and a streptavidin-peroxidase complex for 15 min. The development of the sections was carried out using diaminobenzidine (DAB), followed by a counterstaining with hematoxylin. After a final dehydration and clearing in xylene, the prepared tissue sections were analyzed under a light microscope by two independent pathologists in a blinded manner.

2.4. Cell culture and transfection

Two OC cell lines SKOV3 and A2780 were sourced from the ATCC (American Type Culture Collection, Manassas, USA). One normal ovarian epithelial cell line IOSE8 (Cat. No. MZ-2207) was procured from Mingzhou Biotechnology. All of them were grown in DMEM Medium (Gibco, Waltham, MA, USA), enriched with 10 % FBS (Gibco) and 1 % penicillin/streptomycin, and maintained at 37 °C in a 5 % CO_2 environment. To generate PTX-resistant OC cell lines (SKOV3/PTX and A2780/PTX), the parent SKOV3 and A2780 cells were progressively subjected to escalating doses of PTX. Following this, SKOV3/PTX and A2780/PTX were consistently treated with 50 nM PTX to maintain their resistance traits.

The lentiviral vector engineered to induce ANGPTL3 overexpression (OE-ANGPTL3) was synthesized by cloning the coding sequence of the ANGPTL3 gene (RefSeq NM_014495.4) into the viral construct. Subsequent packaging into lentiviral particles was carried out by Genechem Co. Ltd., (Shanghai, China). For lentiviral transduction, SKOV3/PTX and A2780/PTX cells were grown in six-

well plates, with each well containing a density of 5×10^4 cells/well and infected with OE-ANGPTL3 lentivirus or a control vector carrying no insert (empty vector), applying a MOI (multiplicity of infection) value of 10 for 48 h. Selection was performed using puromycin (2 mg/ml) for stable transfection.

2.5. PTX cytotoxicity assay

The SKOV3/PTX, A2780/PTX, along with their original parent cells, were exposed to different doses of PTX (spanning from 0 to 100 nM, specifically at 0, 1, 5, 10, 50, and 100 nM levels) for 24 h. To evaluate the cytotoxic impact of PTX on the cells, a Cell Counting Kit-8 assay kit (CCK-8, Doujindo Laboratories, Tokyo, Japan) was employed in conjunction with a microplate reader. The cells' resistance to PTX was assessed by calculating the concentration required to inhibit half of the maximum response, also known as the IC50 value.

2.6. CCK-8 assay

Transfected cells, at a count of 3,000 cells per well, were allocated into 96-well plates and further grown for 24 h, 48 h, and 72 h respectively. After these intervals, the cells underwent treatment with 10 μl of CCK-8 reagent and then underwent 2 h incubation at 37 °C. Subsequently, the optical density (OD) at 450 nm was determined using a microplate reader. To ensure precision, each sample underwent triple analysis in quintuplicate.

2.7. Colony formation assay

Transfected cells at a density of 600 cells were seeded in 6-well plates and then allowed to grow for two weeks. Post incubation, the resulting colonies were fixed and then stained using crystal violet (Solarbio, China) for a duration of 20 min. These colonies were then rinsed thrice with PBS, after which the total number of colonies was counted.

2.8. 5-ethynyl-20-deoxyuridine (EdU) assay

The growth of transfected cells was assessed with the KFluor488 EdU kit (KeyGene, Nanjing, China). Essentially, cells underwent treatment with a 50 μM EdU solution for 2 h at 37 °C and were then fixed using 4 % paraformaldehyde for 20 min. The cells were permeabilized using 0.5 % TritonX-100, followed by treatment with Apollo reagent for 30 min. Subsequently, the cell nucleus was marked with DAPI, and the stained cells were observed under a fluorescence microscope.

2.9. Flow cytometry analysis

For cell cycle detection, transfected cells were first dissociated with trypsin and subsequently fixed in 70 % ethanol for a period of 12 h at −20 °C. Post-fixation, the cells were gathered through centrifugation and treated with 40 μg/ml PI (propidium iodide) and 100 μg/ml RNase. The cell distribution across various stages of the cell cycle was subsequently analyzed using FACScan® flow cytometry. For apoptotic cell detection, transfected cells were first collected and resuspended in a binding buffer, after which they were stained with the Annexin V-FITC/PI Apoptosis Kit (KeyGEN Biotech, Nanjing, China). The analysis of apoptotic cells was then conducted using FACScan® flow cytometry.

2.10. Transwell assay

To evaluate cell migration and invasion, we used Transwell chambers, some of which contained Matrigel (Corning, New York, Madison, USA) and others that did not. Briefly, 2×10^4 transfected cells were mixed with 100 μl of serum-free medium and introduced into the upper chamber of the Transwell. The bottom chamber was filled with 600 μl of medium enriched with 10 % FBS serum, acting as a chemoattractant. After incubating for 24 h, the cells that migrated or invaded to the lower chamber were fixed with methanol, stained using crystal violet, and quantified across five random fields under an Olympus microscope (Tokyo, Japan).

2.11. In vivo tumor assay

Female BALB/c nude mice of five weeks old, acquired from Silaike Experiment Animal Company (Shanghai, China), were maintained under standard conditions free from pathogens. We established ovarian cancer (OC) xenograft tumors by subcutaneously injecting either stable Vector or OE-ANGPTL transfected SKOV3/PTX cells (1×10^7 cells in 100 μl PBS) into the right flank of each mouse. Tumor sizes were monitored on a weekly basis, and tumor volume was calculated using the formula: volume (mm^3) = (length $\times$ width^2)/2. Beginning one week after the cells were inoculated, the mice received intraperitoneal injections of PTX (5 mg/kg) diluted with PBS (Solarbio) twice a week for a duration of 4 weeks. Post a 35-day treatment period, the mice were anesthetized and subsequently euthanized, following which the tumor tissues were extracted for further investigation. The formula used to determine the tumor inhibition rate (TIR) is as follows: TIR (%) = [(Wc - Wt)/Wc] $\times$ 100, where Wc represents the average tumor weight in the control group (Vector), and Wt signifies the average tumor weight in the treated group (OE-ANGPTL). The research involving animals was sanctioned by the Animal Ethical and Welfare Committee at Fujian Medical University in Fujian, China, bearing the approval

number FMUA-F873.

2.12. Quantitative real time PCR

Total RNA was isolated with the use of TRIzol reagent (Invitrogen), and from these RNA samples, 1 μg was transcribed into cDNA using the PrimeScript RT Master Mix Kit (Toyobo, Osaka, Japan). On an Applied Biosystems 7900HT Real-time PCR system, we performed quantitative real-time PCR with the SYBR Green Master Mix Kit (TaKaRa, Tokyo, Japan). The specific primer pairs employed were as follows: for ANGPTL3, forward primer 5′-CCAGACCGTGGAAGACCAAT-3′, and reverse primer, 5′-

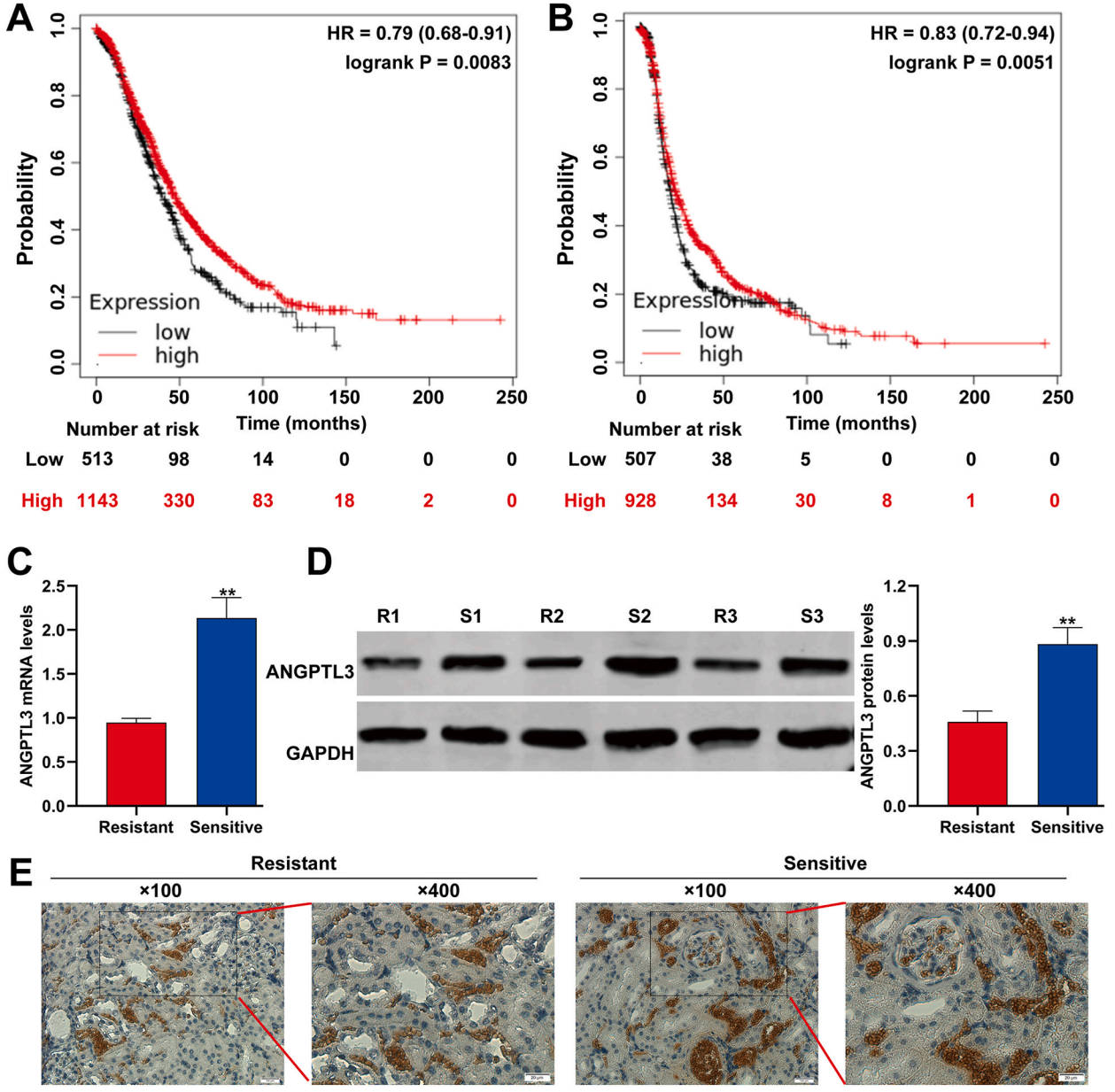

Fig. 1. Reduced ANGPTL3 expression was associated with poor prognosis and chemoresistance in OC. (A) Kaplan-Meier survival analysis illustrated the OS of patients with ovarian cancer between high and low ANGPTL3 expression. (B) Kaplan-Meier survival analysis illustrated the PFS of patients with ovarian cancer between high and low ANGPTL3 expression. OS, overall survival; PFS, progression free survival; HR, hazard ratio. (C) The mRNA levels of ANGPTL3 were determined in PTX-resistant and PTX-sensitive tissues using quantitative real time PCR. (D) Representative images of ANGPTL3 protein expression via Western blot analysis in PTX-resistant (R1/R2/R3) and PTX-sensitive (S1/S2/S3) tissues (n = 3; $^{**}p < 0.01$). (E) Representative immunohistochemical images are presented about ANGPTL3 expression in PTX-resistant and PTX-sensitive tissues; R, resistance; S, sensitive.

TTGGTGCTCTTGGCTTGGAA-3′; for GAPDH, forward primer, 5′- GAAAGCCTGCCGGTGACTAA-3′ and reverse primer, 5′-TGGAATTTGCCATGGGTGGA-3′. The relative expression levels of mRNA were determined using the $2^{-\Delta\Delta CT}$ method, with GAPDH used as the normalization control.

2.13. Western blot analysis

Proteins were isolated with RIPA lysis buffer, and the levels of protein were quantified using a BCA protein assay kit from Beyotime (Shanghai, China). The protein samples were subsequently subjected to separation via 10–12 % SDS-PAGE and were then transferred to PVDF membranes (Millipore). The membranes were then incubated with 5 % non-fat milk as a blocking agent for 2 h at ambient temperature. Following this, they were subjected to an overnight incubation at 4 °C with a variety of antibodies: ANGPTL3 (ab118208, Abcam), CDK4 (ab226474, Abcam), Cyclin D1 (#60186-1-1g, ProteinTech), Caspase-3 (#9661, Cell Signaling Technology), N-cadherin (ab76059, Abcam), E-cadherin (ab238099, Abcam), Vimentin (ab137321, Abcam), mTOR/p-mTOR (ab109268, Abcam), PI3K/p-PI3K (ab154598, Abcam), AKT/p-AKT (ab38449, Abcam), and GAPDH (10494-1-AP, Proteintech). Afterwards, the membranes were incubated with a horseradish peroxidase-tagged secondary antibody (ab205718; 1:5000; Abcam) for a duration of 2 h at ambient temperature. The protein bands were subsequently visualized utilizing an enhanced chemiluminescence system (Pierce, Rockford, IL, USA), with GAPDH used as the control for sample loading.

2.14. Statistical analysis

The experimental data consisted of at least three replicates and were presented as mean values accompanied by their respective standard deviations (SD). Statistical evaluations were performed using GraphPad Prism 8.0 software (California, USA). The assessment of differences between two groups was carried out employing Student's t-test, and for analyses involving more than two groups, one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc test was applied. A *p*-value below 0.05 was deemed statistically significant.

3. Results

3.1. Lowered ANGPTL3 levels correlated with adverse outcomes and chemotherapy resistance in OC

To investigate the predictive significance of ANGPTL3 in OC patients, we examined survival data via Kaplan-Meier plotter database. Our findings, as illustrated in Fig. 1A, revealed that low ANGPTL3 levels correlated with reduced OS ($p = 0.0083$, HR = 0.79). Additionally, low ANGPTL3 expression was linked to decreased PFS in OC patients (Fig. 1B, $p = 0.0051$, HR = 0.83). Collectively, these results indicate ANGPTL3 as a potential prognostic indicator in OC. Moreover, we assessed ANGPTL3 expression in ovarian cancer samples from patients treated with PTX at mRNA and protein levels. PTX-resistant specimens exhibited notably reduced levels of ANGPTL3 protein in contrast to those sensitive to PTX (Fig. 1C–D). This observation was further supported by reduced immunohistochemical staining of ANGPTL3 in PTX-resistant samples relative to PTX-sensitive samples (Fig. 1E).

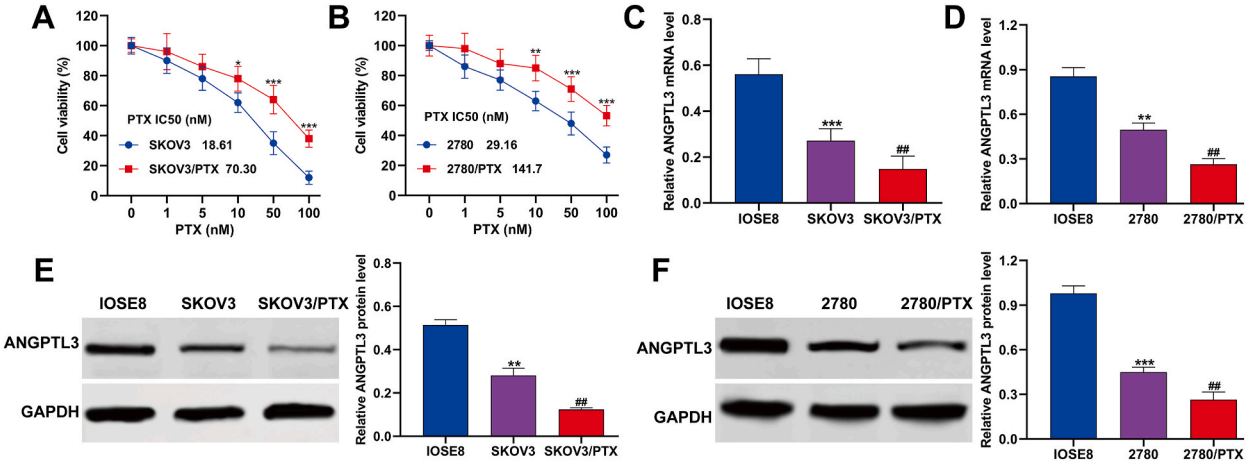

Fig. 2. ANGPTL3 was lowly expressed in PTX-resistant OC cells. (A–B) CCK-8 assay was employed to detect cell viability after PTX treatment (0, 1, 5, 10, 50, and 100 nM) in SKOV3 and 2780 cells and PTX-resistant SKOV3 and 2780 cells. (C–D) Quantitative real time PCR and (E–F) Western blot analysis were performed to determine ANGPTL3 expression in IOSE-80 cells, SKOV3 and 2780 cells as well as PTX-resistant SKOV3 and 2780 cells. Each value represents the mean ± SD from three independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with IOSE8; $\#\#p < 0.01$, compared with SKOV3 or 2780.

3.2. ANGPTL3 expression was diminished in PTX-resistant OC cells

Our investigation into the role of ANGPTL3 in PTX resistance within ovarian cancer began with an analysis of PTX-resistant OC cell lines derived from SKOV3 and 2780 cells. The resistance to PTX in these cell lines was confirmed by exposing both the original ovarian cancer cell lines (SKOV3 and 2780) and their PTX-resistant derivatives (SKOV3/PTX and 2780/PTX) to a range of increasing PTX doses (0, 1, 5, 10, 50, and 100 nM). CCK-8 assay revealed a markedly higher cell viability in SKOV3/PTX and 2780/PTX cells compared to their respective parent SKOV3 (Fig. 2A) and 2780 cells (Fig. 2B). Furthermore, a significant downregulation of ANGPTL3 mRNA expression was observed in SKOV3/PTX and 2780/PTX cells relative to the SKOV3 and 2780 cells (Fig. 2C–D). Consistent results were also obtained from Western blot analysis (Fig. 2E–F). Collectively, these findings suggest a potential link between ANGPTL3 expression and OC cell sensitivity to PTX.

3.3. Elevated ANGPTL3 levels inhibited growth in PTX-resistant OC cells

The research further investigated if ANGPTL3 plays a role in influencing PTX sensitivity in OC cells. To explore this, the study introduced either the OE-ANGPTL3 or a control empty vector plasmid into PTX-resistant SKOV3 and 2780 cell lines. The successful overexpression of ANGPTL3 in these cells is depicted in Fig. 3A. Following this, a noticeable reduction in the viability of SKOV3/PTX and 2780/PTX cells was observed with the overexpression of ANGPTL3, as indicated in Fig. 3B. To delve deeper into the growth characteristics of PTX-resistant ovarian cancer cells, colony formation assays along with Edu staining procedures were implemented. The findings, illustrated in Fig. 3C, showed a significant reduction in colony numbers for the OE-ANGPTL3 group compared to the control vector group in both SKOV3/PTX and 2780/PTX cells. In a similar trend, there was a notable decrease in the percentage of Edu-positive cells within both SKOV3/PTX and 2780/PTX cells after overexpressing ANGPTL3, as depicted in Fig. 3D.

3.4. Elevated ANGPTL3 levels triggered G0/G1 phase arrest and cell apoptosis in PTX-resistant OC cells

Following this, we investigated the influence of ANGPTL3 on cell cycle distribution and apoptosis rate in PTX-resistant OC cells. Results from flow cytometry and PI staining demonstrated that the overexpression of ANGPTL3 notably amplified the proportion of SKOV3/PTX (Fig. 4A) and 2780/PTX (Fig. 4B) cells in the G0/G1 phase (SKOV3/PTX cells: 73.13 % ± 55.26 *vs.* 59.71 % ± 33.16, $p < 0.01$; 2780/PTX cells: 61.69 % ± 4.95 *vs.* 47.41 % ± 4.12, $p < 0.01$), while concurrently diminishing the fraction in the S phase (SKOV3/PTX cells: 14.43 % ± 2.45 *vs.* 24.90 % ± 2.86, $p < 0.05$; 2780/PTX cells: 23.93 % ± 3.15 *vs.* 33.89 % ± 3.56, $p < 0.05$) and G2/M phase (SKOV3/PTX cells: 12.43 % ± 3.29 *vs.* 15.39 % ± 2.06, $p < 0.05$; 2780/PTX cells: 14.10 % ± 2.75 *vs.* 18.70 % ± 2.49, $p < 0.05$). Additionally, flow cytometry using Annexin V/PI double staining demonstrated that ANGPTL3 overexpression significantly increased apoptosis in SKOV3/PTX and 2780/PTX cells (Fig. 4C–D). Western blot assays also confirmed that the increased expression of ANGPTL3 resulted in the decreased presence of G0/G1 phase indicators, including CDK4 and Cyclin D1 and an increase in pro-apoptotic caspase-3 expression in these cells (Fig. 4E). These findings suggest that the G0/G1 arrest and apoptosis induced by ANGPTL3 might contribute to its enhancing effects on PTX sensitivity in OC cells.

3.5. Elevated levels of ANGPTL3 curtailed the mobility and invasive potential of PTX-resistant OC cells

Beyond assessing cell cycle dynamics and apoptosis, we further scrutinized the impact of ANGPTL3 on the metastatic potential of

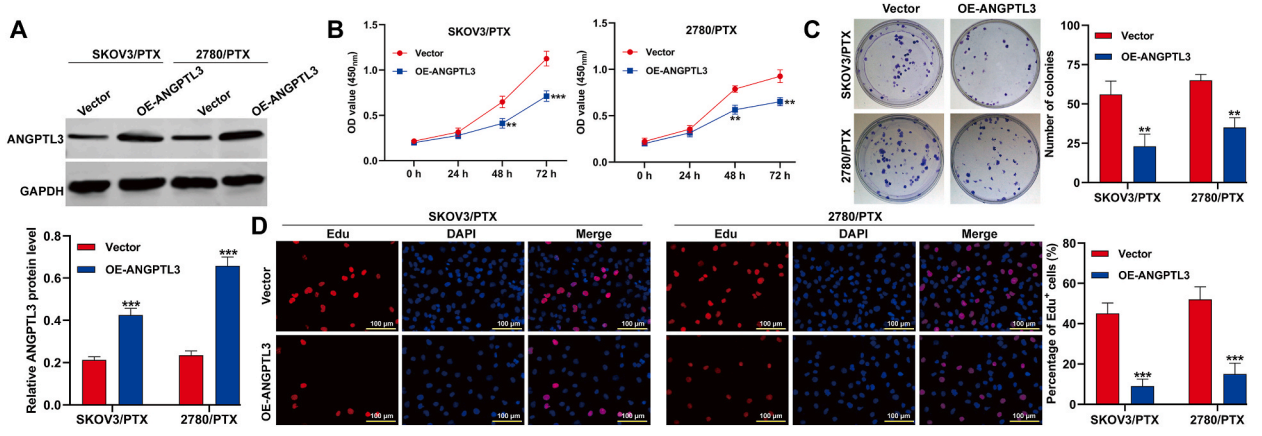

Fig. 3. Overexpression of ANGPTL3 suppressed the proliferation of PTX-resistant OC cells. (A) Western blot analysis of ANGPTL3 in SKOV3/PTX and 2780/PTX cells after transfection with OE-ANGPTL3 or Vector plasmids. (B) Cell viability was determined by CCK-8 assay in transfected SKOV3/PTX and 2780/PTX cells. (C) Colony formation assay and (D) Edu staining were conducted to assess cell proliferation ability of transfected SKOV3/PTX and 2780/PTX cells. Each value represents the mean ± SD from three independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with Vector.

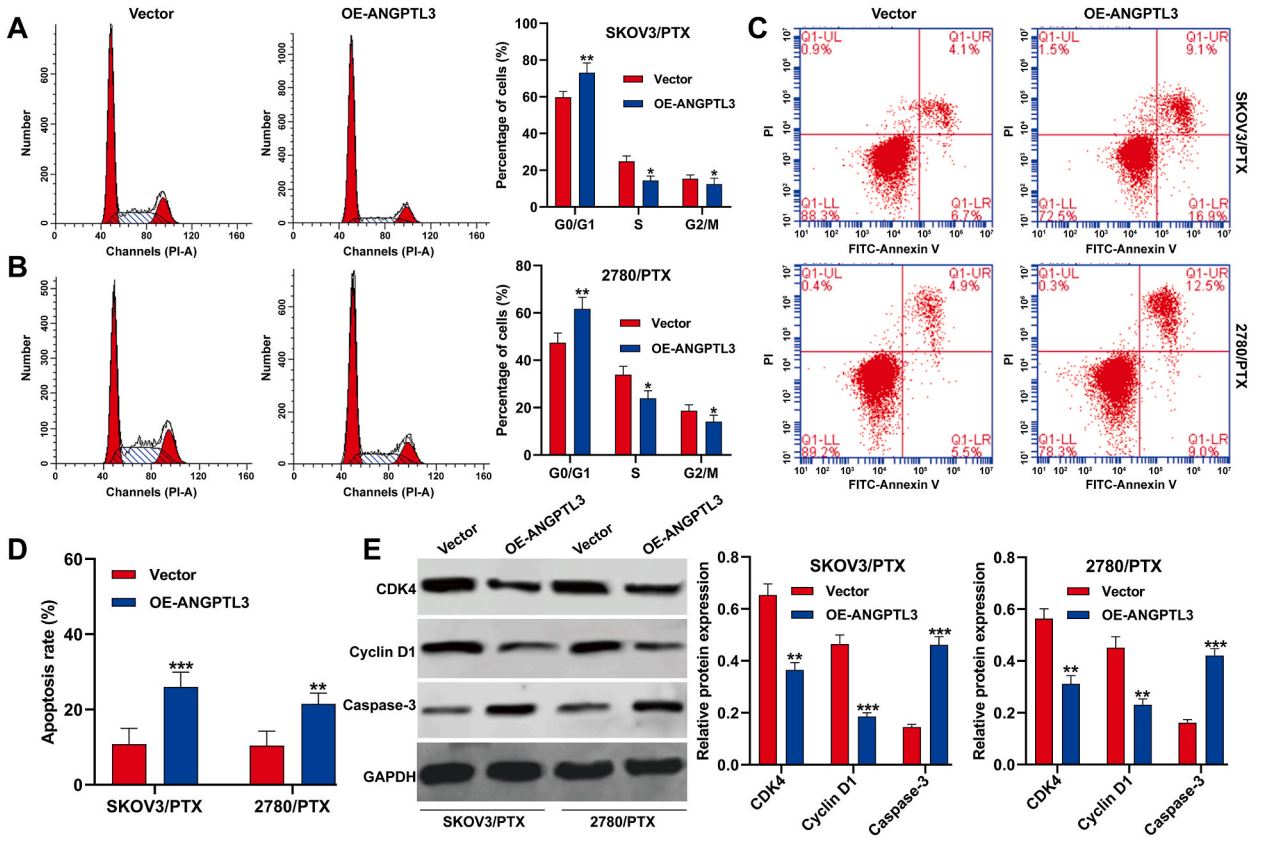

Fig. 4. Overexpression of ANGPTL3 induced G0/G1 arrest and apoptosis in PTX-resistant OC cells. SKOV3/PTX and 2780/PTX cells were transfected with OE-ANGPTL3 or Vector for 48 h. (A–B) The effects of OE-ANGPTL3 transfection on cell cycle distribution were determined in SKOV3/PTX and 2780/PTX cells. The representative results of cell cycle were shown in left panel and corresponding quantification of cell cycle distribution was depicted in right panel. (C–D) The effects of OE-ANGPTL3 transfection on apoptotic rate were determined in SKOV3/PTX and 2780/PTX cells. (E) The protein expression of CDK4, Cyclin D1 and caspse-3 was detected using Western blot analysis in transfected SKOV3/PTX and 2780/PTX cells. Each value represents the mean ± SD from three independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, compared with Vector.

PTX-resistant OC cells. As depicted in Fig. 5A, the overexpression of ANGPTL3 significantly diminished the quantity of migratory cells in both SKOV3/PTX and 2780/PTX cells. Similarly, the invasive potential of these cells was remarkedly diminished following OE-ANGPTL3 transfection compared to the control Vector transfection, as evidenced in Fig. 5B. Additionally, our examination of EMT markers revealed that overexpression of ANGPTL3 substantially elevated the presence of E-cadherin, while concurrently reducing the levels of N-cadherin and vimentin in these cells, as shown in Fig. 5C.

3.6. Overexpression of ANGPTL3 downregulated the PI3K-AKT-mTOR signaling pathway in PTX-resistant OC cells

The PI3K-AKT-mTOR signaling cascade is broadly acknowledged as a fundamental mechanism for cell survival and is pivotal in mediating drug resistance throughout cancer therapies. In light of this, our study investigated the potential impact of ANGPTL3 on this pathway in PTX-resistant OC cells. The results aligned with our expectations, showing that the heightened expression of ANGPTL3 successfully suppressed the expression ratios of p-PI3K/PI3K, *p*-AKT/AKT, and *p*-mTOR/mTOR in both SKOV3/PTX and 2780/PTX cells, as illustrated in Fig. 6. Consequently, our findings suggest that the diminished PTX resistance observed in OC cells may be partly due to the deactivation of the PI3K-AKT-mTOR signaling pathway, triggered by the overexpression of ANGPTL3.

3.7. ANGPTL3 overexpression repressed PTX resistance in OC cell in vivo

To gauge the impact of ANGPTL3 on the resistance of ovarian cancer to PTX in a live setting, we developed a xenograft tumor model with the use of nude mice. SKOV3/PTX cells, which had been transfected with either a standard Vector or OE-ANGPTL3, were subcutaneously introduced into BALB/c nude mice. One week after cell injection, the mice were given intraperitoneal PTX injections (5 mg/kg) twice weekly for four weeks. Our findings indicated that tumors in the OE-ANGPTL3 group grew more slowly compared to those in the Vector group (Fig. 7A). By the 35th day, both the size (Fig. 7B) and weight (Fig. 7C) of the tumors in SKOV3/PTX cells

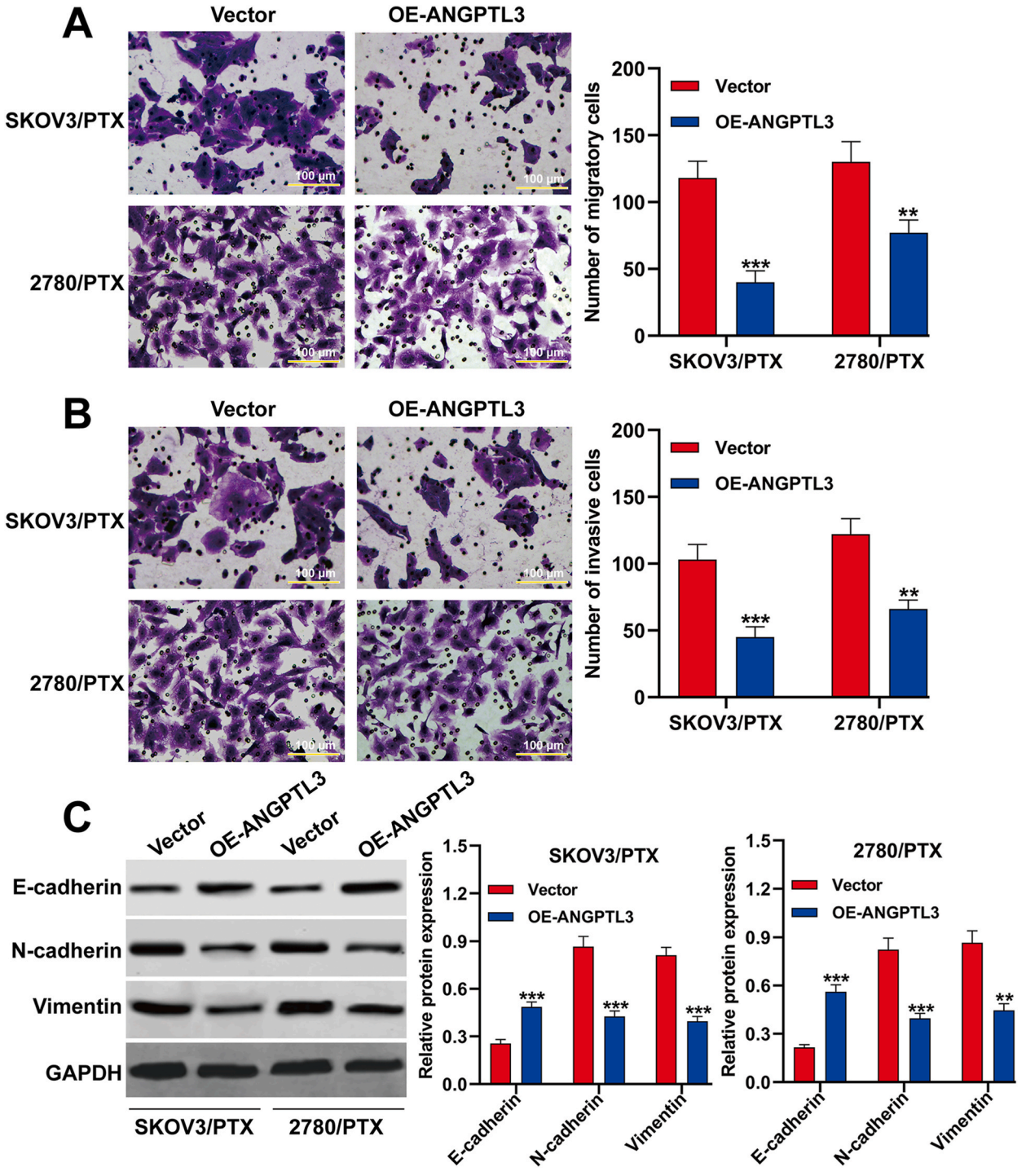

Fig. 5. Overexpression of ANGPTL3 inhibited the migration and invasion capacities of PTX-resistant OC cells. SKOV3/PTX and 2780/PTX cells were transfected with OE-ANGPTL3 or Vector for 48 h. Transwell assay was utilized to analyze cell migration (A) and invasion (B) in transfected SKOV3/PTX and 2780/PTX cells. (C) The protein levels of E-cadherin, N-cadherin and Vimentin were detected in transfected SKOV3/PTX and 2780/PTX cells. Each value represents the mean ± SD from three independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with Vector.

overexpressing ANGPTL3 were notably reduced following PTX treatment, compared to those in cells lacking ANGPTL3 overexpression. This resulted in average TIR of 52.17 %. Furthermore, we verified elevated levels of ANGPTL3 mRNA in the xenograft tumor tissues belonging to the OE-ANGPTL3 group (Fig. 7D). Additionally, Western blot analysis showed that the overexpression of ANGPTL3 led to

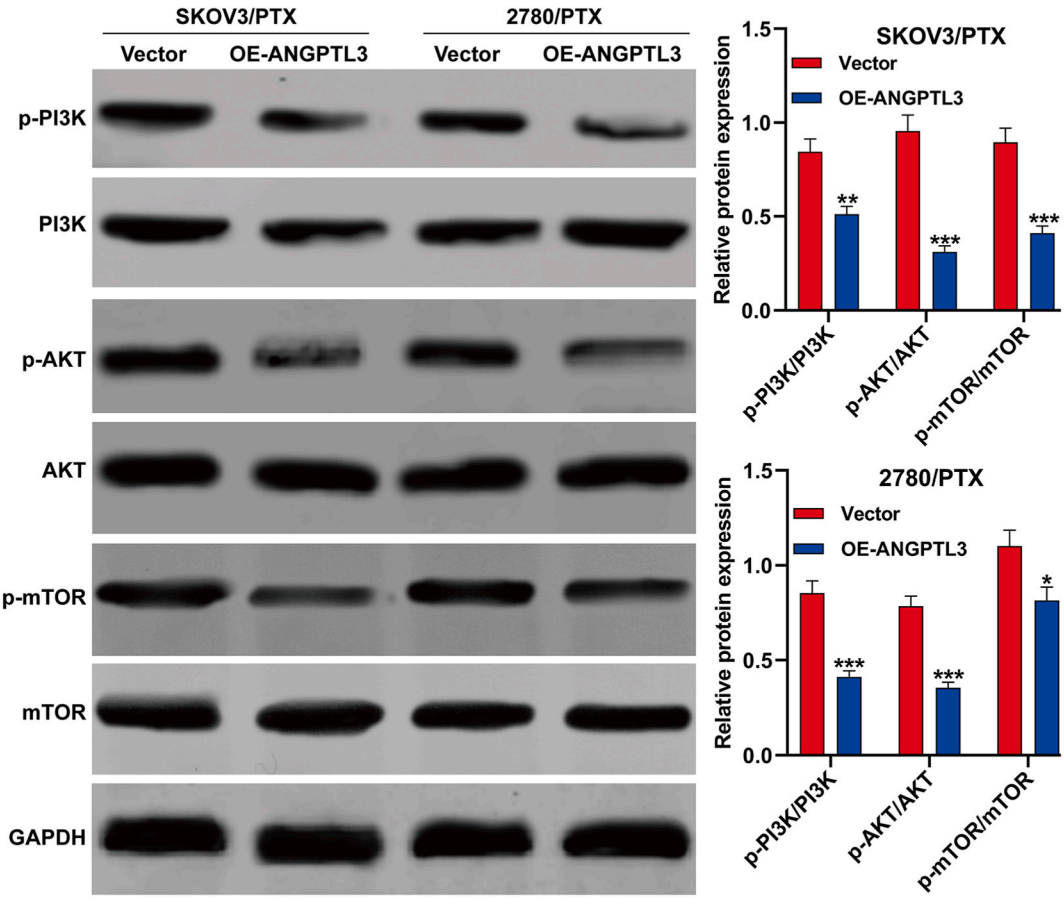

Fig. 6. Overexpression of ANGPTL3 downregulated the PI3K-AKT-mTOR signaling pathway in PTX-resistant OC cells. The protein levels of PI3K, p-PI3K, AKT, *p*-AKT, mTOR, and *p*-mTOR were determined in SKOV3/PTX and 2780/PTX cells after transfection with OE-ANGPTL3 or Vector. Each value represents the mean ± SD from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with Vector.

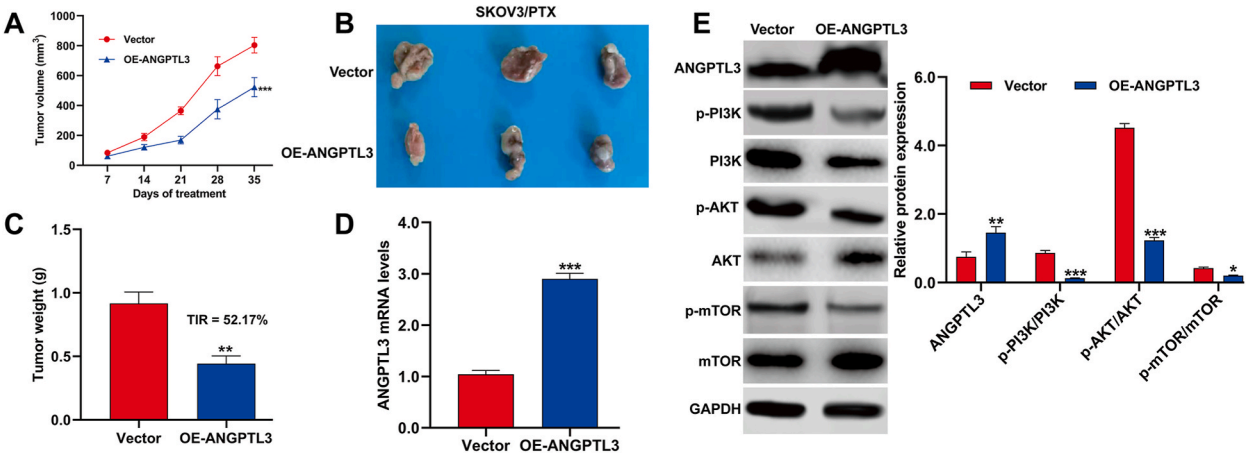

Fig. 7. ANGPTL3 overexpression repressed PTX resistance in OC cell *in vivo*. BALB/c nude mice were injected with SKOV3/PTX cells (1×10^7) transfected with OE-ANGPTL3 or Vector (n = 3 per group). After one weeks, the mice were intraperitoneally administered with PTX (5 mg/kg) twice a week for 4 weeks. (A) Tumor volume was monitored continuously for 35 days. On Day 35, the xenografts were resected (B) and weighed (C). (D) The mRNA expression of ANGPTL3 was determined by quantitative real time PCR in xenograft tumor tissues. (E) The protein levels of ANGPTL3, p-PI3K, PI3K, *p*-AKT, AKT, *p*-mTOR and mTOR were determined in xenograft tumor tissues. Each value represents the mean ± SD from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with Vector; TIR, tumor inhibition rate.

a significant reduction in the protein levels of p-PI3K/PI3K, *p*-AKT/AKT, and *p*-mTOR/mTOR within the xenograft tumor tissues (Fig. 7E). These findings from live studies align with our laboratory results, highlighting the significant role of ANGPTL3 in modulating PTX resistance in OC.

4. Discussion

ANGPTL3, a versatile secreted protein mainly synthesized in the liver, is subject to several post-translational modifications including cleavage and glycosylation, positioning it as an emerging biomarker and a potential therapeutic target for associated diseases [18]. Our research aimed to assess the influence of ANGPTL3 on drug resistance in OC cells. The initial data showed a reduced expression of ANGPTL3 in OC tissues that were resistant to PTX, in contrast to those sensitive to PTX, which was associated with a less favorable outcome in OC patients. This is consistent with findings that levels of ANGPTL3 are remarkedly decreased in RCC specimens, when compared with non-tumor tissues, with patients exhibiting lower ANGPTL3 levels experiencing shorter periods of disease-free and overall survival [13]. Zhang et al. [14] reinforced this trend in RCC, observing that patients with diminished ANGPTL3 expression had reduced overall and disease-free survival compared to those exhibiting elevated ANGPTL3 levels. Intriguingly, ANGPTL3 was found to be upregulated in sorafenib-responsive RCC cases, suggesting its potential as a predictor for sorafenib therapy response in RCC [19]. These pieces of evidence led us to hypothesize that ANGPTL3 might play a crucial role in OC cell drug resistance.

In our gain-of-function assays, we further established that ANGPTL3 enhances PTX sensitivity in OC cells. This is achieved by reducing cell proliferation, inhibiting G0/G1 phase, and decreasing migration and invasion, while also increasing apoptosis in PTX-resistant OC cells. It is well-known that G0/G1 phase control is crucial in many cancers, and dysregulation of the CDK/cyclin complex can lead to a loss of this control [20,21]. Activation of caspase-3, known to boost chemosensitivity, is a common phenomenon in various cancers [22–24]. Bao et al. [19] reported that ANGPTL3 induces cellular apoptosis and enhances the response to sorafenib. In a similar vein, ANGPTL3 has been implicated in PAN-induced reduction of podocytes, influencing both detachment and apoptosis in laboratory conditions [25]. Furthermore, EMT is vital in tumor cell infiltration, metastasis, the development of drug resistance, and formation of tumor stem cells [26]. Targeting EMT is significant in overcoming drug resistance in cancer [27]. Here, we observed that ANGPTL3 overexpression in PTX-resistant OC cells resulted in heightened expression of E-cadherin and a decrease in the expression levels of N-cadherin and Vimentin, markers of EMT. This is consistent with the known regulatory role of ANGPTL3 in podocyte EMT [28], leading us to speculate that ANGPTL3 reduces PTX resistance in OC cells partly by suppressing the EMT process. Our findings align with observations where ANGPTL3 overexpression in RCC cells inhibited cell growth, movement, and invasion, while enhancing cell apoptosis and reducing Cyclin D1 levels [14]. However, this contrasts with findings in oral cancer, where reducing ANGPTL3 levels resulted in diminished cell growth and arrest in the G1 phase of the cell cycle, a process linked to the increased expression of the cyclin-dependent kinase inhibitor p21 [29]. These evidence explored the complex role of ANGPTL3 across different cancers, suggesting that its contradictory effects might be due to: 1) the tumor microenvironment, where varying levels of cytokines, growth factors, and extracellular components could alter ANGPTL3's activity across cancers like ovarian, esophageal, or glioblastoma; 2) cancer cell heterogeneity, where genetic and epigenetic differences within tumors affect ANGPTL3's signaling, impacting tumor growth, metastasis, and survival differently; and 3) the interaction of ANGPTL3 with other signaling pathways, where its role in cancer progression may shift based on the balance of pro-tumorigenic and anti-tumorigenic signals, varying by cancer type. This underscores the need for further research into ANGPTL3's multifaceted role in cancer biology.

The PI3K-AKT-mTOR signaling pathway, a fundamental and universally conserved mechanism within eukaryotic cells, is essential for promoting cell growth and survival [30]. The pathway's importance in cell survival and its frequent activation in OC highlight the potential clinical benefits of targeting it with inhibitors [31]. In line with this, our study demonstrates that overexpressing ANGPTL3 markedly diminishes the levels of crucial indicators within the PI3K/Akt/mTOR pathway, namely p-PI3K, *p*-AKT, and *p*-mTOR, in PTX-resistant OC cells, across both laboratory and living organism experiments. Of note, the study's focus on AKT phosphorylation at T308, a pivotal process within the PI3K/AKT pathway that's vital for cellular proliferation and survival, chosen due to its primary role in activating AKT. T308 phosphorylation initiates key downstream signals, distinct from S473 phosphorylation, which further enhances activity. This focus is also driven by the availability of specific T308 antibodies and the experimental model's strong response to T308 stimuli, allowing for precise monitoring and investigation of AKT activation dynamics. Our findings align with other studies, such as the observation that silencing CXCR4 decreases cancer stem cells (CSCs) and EMT, thereby enhancing chemotherapy sensitivity in OC cells [16]. Similarly, PTPRZ1 (protein tyrosine phosphatase receptor type Z1) has been found to mitigate cisplatin resistance and increase cytotoxicity in OC via inhibiting the PI3K/AKT/mTOR pathway [32]. These findings propose that ANGPTL3 might function as a suppressor of the PI3K-AKT-mTOR signaling pathway, possibly contributing to the control of PTX resistance in OC.

Our research reveals that low ANGPTL3 expression correlates with worse prognoses in ovarian cancer, suggesting its role as a biomarker. Enhancing ANGPTL3 can reduce paclitaxel resistance by downregulating the PI3K/AKT/mTOR pathway, highlighting its therapeutic potential. Strategically targeting ANGPTL3 to modulate this pathway could transform OC treatment paradigms, potentially improving response rates and survival outcomes, especially for patients with resistant disease profiles.

Ethical approval statement

This research received the approval from the Ethical Committee at Fujian Medical University (Approval Number: FHQM-2020-32A, Fujian, China) and was conducted according to the Declaration of Helsinki's guidelines. Consent was acquired from all participants. Additionally, the Fujian Medical University's Animal Ethical and Welfare Committee granted its approval for the animal research involved (Approval No. FMUA-F873, Fujian, China).

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Data availability statement

Data are available from the corresponding author upon a reasonable request.

CRediT authorship contribution statement

Dandan Wu: Writing – original draft, Funding acquisition, Data curation, Conceptualization. **Jia Liu:** Methodology, Investigation, Formal analysis, Data curation. **Xin Yang:** Software, Investigation, Formal analysis. **Zhifen Wu:** Visualization, Validation, Investigation, Formal analysis. **Tingzhao Wang:** Writing – original draft, Software, Methodology, Formal analysis. **Meiqin Xiao:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31520.

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