

TCP1 Regulates PI3K/AKT/mTOR Signalling Pathway to Promote Ovarian Cancer Cell Proliferation

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Research

Keywords: TCP1, ovarian cancer, prognostic biomarker, PI3K/AKT/mTOR signalling pathway

Posted Date: October 1st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-80647/v1>

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Abstract

Objective

TCP1 is one of the eight subunits of the TCP1 ring complex (TRiC) or the multi-protein mammalian cytosolic chaperone complex. TRiC participates in protein folding and regulates the expression of multiple signalling proteins and cytoskeletal components in cells. Although the clinical importance of its subunits has been clarified in various carcinomas, the function of TCP1 in ovarian cancer (OC) remains unclear. We aimed to identify the association between the expression of TCP1 and epithelial ovarian cancer (EOC) development and patients' prognosis, and explore the underlying mechanisms of TCP1 on the tumour progression of ovarian cancer cells.

Methods

TCP1 protein expression was tested in the various ovarian tissues by immunohistochemistry (IHC), and the correlation between TCP1 expression and clinical physiologic or pathologic parameters of EOC patients was analyzed in this study. The relationship between TCP1 expression and ovarian cancer patients' prognosis was collected and analyzed using the Kaplan-Meier (KM) Plotter online database. Then, the expression levels of TCP1 was tested in different OC cell lines by Western blot. Furthermore, a model using ovarian cancer cell line A2780 was constructed for studying the functions of TCP1 in human EOC cell growth, migration, and invasion. Finally, possible regulated signalling pathways were discussed.

Results

TCP1 protein expression in ovarian cancer or borderline tissue was significantly higher compared to that in benign ovarian tumours and normal ovarian tissue. The upregulated expression of TCP1 in ovarian cancer was positively associated with and the differentiation grade and FIGO stage, which predicted poor clinical outcomes. Compared with IOSE-80 cells, TCP1 protein was overexpressed in the A2780 cells. TCP1 knockdown using shRNA lentivirus inhibited cell viability in A2780 cells. Western blot showed that the phosphatidylinositol-3 kinase (PI3K) signalling pathway was activated in the tumour invasion of EOC driven by TCP1.

Conclusion

The protein level of TCP1 is overexpressed in aggressive histologic types of epithelial ovarian cancer. Upregulated TCP1 is correlated with poor prognosis of patients and TCP1 may serve as a novel prognostic biomarker. The mechanism of cancer progression promoted by TCP1 upregulation may be linked to the PI3K signalling pathway activation and TCP1 may serve as a novel target for ovarian cancer treatment.

Introduction

EOC is the most common histotype of OC [1-3], which is a highly aggressive and lethal cancer in gynecologic cancers. Some statistical data indicated that close to 70% of patients with OC remain undetected until the advanced stage [4]. Currently, the standard treatment for advanced OC is primary cytoreductive surgery followed by combination chemotherapy using platinum and taxane [4-6]. Tumour recurrence may ultimately occur in approximately 75% of patients with advanced OC, 20% of which becomes resistant [7]. The early diagnosis and new treatment strategies of OC have been carried out over the last three decades. However, discovering effective anticancer drugs for OC treatment is not successful and much work is warranted [2, 8-10]. Therefore, clarifying the underlying biological mechanism of EOC and developing effective novel anticancer drugs for EOC are necessary. A previous study has shown that the expression of TRiC mRNAs was increased over 4-fold after ovarian carcinoma became resistant to cisplatin when the cells were exposed to cisplatin in the in-vitro study [11].

TRiC is an important eukaryotic chaperonin [12-14]. It has a double-ring that are stacked back-to-back with an empty central cavity [15]; each ring contains 8 different, yet paralogous subunits (CCT 1–8) [16, 17]; each subunit is ~60 kDa [12] and can recognize proteins in different polarities and hydrophobic subunits [18]. A recent study suggested that the TRiC subunit, TCP1, was essential for the breast cancer survival and TCP1 is regulated by oncogene activation driven by PI3K signalling [19].

However, the functional role of TCP1 in EOC is still unclear. Therefore, we tested the protein expression of TCP1 in EOC tissues and analyzed its prognostic value for EOC patients to demonstrate the function of TCP1 in OC cell growth and survival. Then, we knocked down TCP1 using related shRNA and evaluated its functions on the EOC cell proliferation, invasion, and migration. Finally, we explored the possible mechanism of TCP1 in the development of OC cells.

Materials And Methods

Tissue samples and patient data

The ethics approval for present study was obtained from the Ethics Committee of the Fujian Medical University Union Hospital. We collected 109 formalin-fixed paraffin-embedded ovarian tissue, including 13 normal ovarian tissue chips, 26 ovarian cystadenoma chips, 8 border ovarian tumour chips, and 62 ovarian malignant tumour chips from patients treated initially at the Fujian Medical University Union Hospital between 2016 and 2019. Relevant clinical parameter data were collected from the hospital medical record system and the definite histological diagnosis and grading came from the pathological reports. The clinical-stage were determined based on the International Federation of Gynecology and Obstetrics, 2009 (FIGO, 2009).

IHC and quantitative analysis

Serial 3-µm sections from all samples were deparaffinized and rehydrated through xylenes and serial graded ethanol to water followed by antigen retrieval. These samples then were incubated overnight at 4 °C with TCP1 alpha primary antibody (Abcam Corporation; 1:200). The washed tissue samples were

incubated with secondary antibody IgG (Merck Millipore; 1:300) for 30 min at room temperature (RT). Tissue slices were stained with 3,3'-diaminobenzidine and hematoxylin, and observed under an optical microscope. Finally, all images were analyzed integrated optical density (IOD) to calculate the average IOD /TCP1 positive staining area (μm^2) using Image-pro plus software.

Survival analysis using KM Plotter

The correlation between TCP1 expression and prognosis of OC was analyzed using the KM Plotter database (<http://kmplot.com>). In the database, 1657 OC cases with clinical data and mRNA expression were downloaded from Gene Expression Omnibus. Patients were divided into two subgroups according to TCP1 low or high expression using the optimal cut-off value. The overall survival (OS), progression-free survival (PFS), hazard ratio (HR), and log-rank P of TCP1 in OC patients were analyzed online using the Affymetrixmetrix ID (222010_at) approach.

Cell culture

The human EOC cell line A2780 and normal ovarian cell IOSE-80 were purchased from the Bena Culture Collection (Kunshan, Jiangsu Province, China) and cultured in 5% CO₂ at 37°C in dulbecco's modified eagle medium (DMEM; Gibco) added with 10% fetal bovine serum (FBS; Gibco). Another EOC cell SKOV3 was obtained from Guangzhou Cellcook Biotech Company and cultured in 5% CO₂ at 37°C in McCoy's 5a supplemented with 10% FBS. Cell line authentication by short tandem repeat (STR) profiling

Western blot assay

Total protein (20 μg /lane) was separated by polyacrylamide gel electrophoresis and then transferred to a PVDF membrane. Then, the membrane was blocked with 5% non-fat dry milk solution and incubated with the various primary antibodies at 4 °C overnight. Next day, the washed membrane using tris buffered saline tween (TBST) was incubated with horse radish peroxidase (HRP) conjugated secondary antibodies at RT for 2 h followed by visualized using chemiluminescent HRP substrate (Merck Millipore) on a Western blot imaging system. The band intensity was detected using Image Lab software. The protein expression was normalized to glyceraldehydes 3-phosphate dehydrogenase (GAPDH) expression. The primary antibodies used were anti-TCP1 alpha Rabbit Monoclonal (Abcam Corporation; 1:1,000), anti-mTOR Rabbit antibody (Abcam Corporation; 1:1,000), Akt antibody (CST Corporation, 1:1,000), phospho-Akt(Ser473) Rabbit mAb (CST Corporation, 1:1,000), and anti-GAPDH Mouse (TransGen Biotech, 1:2,000). Anti-mouse (Merck Millipore, 1:20,000) and anti-rabbit (Merck Millipore, 1:20,000) secondary antibodies were used.

Construction of stable TCP1-knockdown cell line

The pLKO.1 Puro vector was used to construct lentiviruses for TCP1 RNA interference (shTCP1) and negative control (shCtr) experiments. The sequences targeting TCP1 were designed based on the human TCP1 gene (Table 3) and synthesized according to the pLKO.1 Puro vector specification. To prepare

lentiviral particles, 8 µg of the shTCP1 vector (pLKO.1 Puro) and the packaging plasmids (5 µg pMDL, 3 µg pVSVG, and 2 µg pREV) were cotransfected into 293T cells. The TCP1-NC group was transfected with negative lentivirus. Lentivirus-containing medium was collected after 48 h of transfection and used to culture A2780 cells. After 48 h of transfection, the medium was replaced with complete medium. Then, puromycin with a final concentration of 2.0 µg/mL was added for stable cell line screening for 72 h. Then, the survived cells were collected for TCP1 expression analysis. The stable cell lines were constructed in A2780/TCP1- negative control group (NC) and A2780/TCP1- knockdown group (KD), which were used for subsequent experimentation.

MTT assay

The proliferation of A2780/TCP1-NC and A2780/TCP1-KD cells was detected by MTT assay. The cells were firstly inoculated to 96-well plates (1,000 cells/well) and cultured in a humidified 5% CO₂ incubator at 37°C. Then, the plates were added with methyl thiazolyl tetrazolium (MTT, 0.5 mg/mL, 10 µL/well) at 24, 48, 72, and 96 h. After 4 h of normal culture, the supernatant was removed and purple formazan crystals were dissolved using a 150 µL dimethyl sulfoxide (DMSO) solution. The plate oscillated for 10 min at RT. The optical density at 490 nm (OD₄₉₀) of each well was measured by microplate reader using wells without cells as blanks. The cell viability curve was drawn by the abscissa of the time point and the ordinate of OD value. Each experiment was performed in triplicate.

Colony formation assay

Infected cells were routinely harvested, resuspended, and then placed in 6-well plates (1000 cells/well) to analyze cell colony formation. After 10 days of incubation with each 3-day medium changes, the surviving cells were washed using cold phosphate buffered solution (PBS) and fixed by 4% polyformaldehyde and dyed with 1% crystal violet. The colonies with more than 50 cells were counted. We divided colony number by plated cell number to calculate the colony forming efficiency (CFE, %). The experiments were repeated three times.

Wound-healing assay

Approximately 2×10^6 cells were seeded in 6-well plates. The cell monolayers were scratched using sterile 200-µl pipette tips after reaching 80% confluence. Serum-free medium was added into the plates after washing the floating cells. Cells were cultured at 37°C for 48 h. The wound width was photographed and recorded every 24 h. The results were observed using the Image J software. Wound closure was computed according to the ratio of districts uncovered by cells before and after wound scratching.

Cell invasion and migration assay

Transwell (8-µm pore size) chambers (Falcon) were coated with matrigel, placed in 24-well cell culture plates, and then air-dried in the incubator for 4h. The 50 µL complete medium was added into each pore at 37°C for 30 min. A2780/TCP1-NC and A2780/TCP1-KD cells were cultured with 5% FBS medium.

Suspended cells (15×10^4 cells/200 μ L) were added into the upper chamber and 600 μ l 15% FBS medium as a chemoattractant was put into the lower chamber. The invasive cells on the outside of the chamber were stained with 0.5% crystal violet after 48h incubation. The cell slides were photographed under an inverted microscope in 5 randomly-selected fields at $\times 200$ magnification.

For migration assays, the Transwell chambers were not coated with matrigel and the follow-up procedure was consistent with the invasion assays. Each experiment was conducted three times.

Statistical analysis

All the data were represented as the mean $\pm$ standard deviation (SD). Statistical analyses were carried out by the SPSS software (version 20.0; SPSS) and Graph Pad Software (Graph Pad Prism 8.0.1). The independent *t*-test was used to the comparison of two groups. The one-way analysis of variance (ANOVA) followed by post-hoc test was applied for comparing multiple groups. $P < 0.05$ was considered as statistically significant.

Results

TCP1 was abundantly expressed in EOC tissues and its expression level had a significant association with the grade of differentiation and FIGO stage

Compared to that in non-EOC tissues, the staining intensity of TCP1 in EOC tissues was significantly higher based on IHC assay ($P < 0.05$) (Table 1) (Fig. 1A). The relationships between the EOC clinicopathologic variables and TCP1 expression were summarized in Table 2. The ANOVA analyses showed that the level of TCP1 expression correlated significantly with FIGO stage ($P = 0.001$) and the grade of differentiation ($P = 0.001$), but not with the tumour size, age, pathological type, lymph node metastasis, and volume of ascites. In summary, the TCP1 upregulation may be associated with the advanced OC.

High expression of TCP1 confers a poor prognosis in OC patients

Using the probe for TCP1 (Affymetrix ID: 222010_at) analyzed the relationship between TCP1 and progress-free survival rate of OC. The cut-off date was set as PFS, and the number of eligible cases in the database was 1435 KM Plotter. The results showed that the HR value was 1.25, and the log-rank *P* was 0.0022 (Fig. 1B). Then, the cut-off date was set as OS, and the number of eligible patients in the database was 1656 KM plotter. The results showed that the HR value was 1.27, and the log-rank *P* was 0.00088 (Fig. 1C). The above results dedicated that the TCP1 was a risk factor for the prognosis of the patients with OC.

The expression of TCP1 protein in A2780 EOC cells is upregulated

TCP1 protein expression was tested by Western blot in EOC cells A2780 and SKOV3. TCP1 was overexpressed in A2780 ($P < 0.01$) but not in SKOV3 cells compared with normal cells IOSE-80 (Fig. 2A and

2B).

Inhibition of TCP1 expression in A2780 cells

Based on the different levels of two EOC cells detected by western blotting (Fig. 2A and 2B), A2780 cells with high levels of TCP1 expression were selected for transfection with TCP1 shRNA lentivirus and the stable TCP1 knockdown cells were cultured for studying the TCP1 function and its mechanism in EOC cells. After infection, knockdown of TCP1 protein expression in A2780 cells was confirmed by Western blotting (Fig. 2C and 2D).

TCP1 knockdown inhibited cell growth in vitro

Cell proliferation assay using MTT and colony formation assays were carried out to uncover the roles of TCP1 in the cancer cell growth. The cell viability in A2780/TCP1-KD was clearly suppressed compared with that in A2780/TCP1-NC (Fig. 3A), as well as colony forming abilities ($P < 0.01$, Fig. 3B and 3C). The results of these assays demonstrated that TCP1 was essential for OC cell growth.

TCP1 silence inhibited the invasion and migration of A2780 cells.

The wound-healing and transwell assays were performed to examine the EOC migrated and invasive abilities after TCP1 silence. The wound-healing assay showed that stable TCP1 knockdown inhibited the migration rate of A2780 cells compared with controls (Fig. 4A and 4D). Similar results were obtained with the transwell migration (Fig. 4B and 4E) and invasion (Fig. 4C and 4F) assays. Collectively, these results demonstrated that TCP1 promoted cell migration and invasion in ECO.

TCP1 regulated PI3K/AKT/mTOR signalling through p-AKT

Protein was extracted from A2780/TCP1-NC and A2780/TCP1-KD cells. The results showed that phospho-AKT (p-AKT) and mTOR were decreased after TCP1 downregulation using Western blot (Fig. 5A and 5B). The GAPDH served as an internal control. Therefore, inhibition of TCP1 via decreasing p-AKT of PI3K/AKT/mTOR pathway to inhibit the development of OC, which was a central regulator of metabolism, survival, and proliferation in normal tissues and cancers.

Discussion

In this study, we demonstrated that TCP1 protein, a member of TRiC, is commonly altered in OC, essential in the occurrence of OC cells, and as well related to prognosis of OC patients. Our study findings indicate roles of TCP1 in OC and suggest TRiC may be used as a novel target for OC therapy. Survival data collected from the KM Plotter with over 1000 patients' prognosis data demonstrates that TCP1 upregulation is related to poor prognosis of OC patients when compared with those patients with wild-type expression. To further determine the roles of TCP1 in clinical studies, the formalin-fixed paraffin-embedded samples were stained for TCP1 via IHC and TCP1 expression was analyzed. Interestingly, TCP1 was over-expressed in EOC samples especially in higher grade and FIGO stage samples. The

findings suggested cancer in OC patients with upregulated TCP1 expression was more aggressive. Inhibitors targeting TCP1 or TRiC complex may be beneficial to prognosis of OC patients with higher TCP1 expression if TCP1 decisively affects the phenotypes.

TRiC, the most complex of the chaperonins, is constituted by 8 distinct subunits encoded by different genes [12]. Originally, TRiC was identified and characterized by its essential role in folding the cytoskeletal proteins such as tubulin and actin [14, 20, 21], cell cycle regulators Cdc20 [22], Cdh1 [22], p21ras oncoproteins [23], and the Von Hippel-Lindau tumour suppressor protein [24]. Also, a previous study estimated that 5% of all cytoplasmic proteins in the eukaryotic cells are considered as components for the folding functions of TRiC [25]. Tubulins, one of the TRiC substrates, are studied the most thoroughly and are the target of taxanes [26, 27], the common drugs for OC chemotherapy [28, 29]. Taxanes' resistance is a challenge for OC therapy. The facts that TRiC is essential for folding of tubulin and OC patients with upregulated TCP1 expression have poor survival suggest TCP1 may affect the sensitivity of patients to taxanes. Future studies need to be performed to clarify the underlying targets of TRiC to enhance the taxane sensitivity for the OC therapy and possibly overcome the taxane resistance.

Currently, there are no available data about the functional roles of TRiC or TCP1 in OC cells. We decided to study the TCP1 functions in the cell growth and survival of OC. In our study, we detected TCP1 is differentially expressed in the various OC cells. TCP1 was upregulated in the A2780 cells line, but not in SKOV3 cells, compared with IOSE-80, which is consistent with other study findings [30]. The reason for the different expressions of TCP1 in the various ovarian cancer cells could be that EOC has several pathologically distinct subtypes, such as endometrioid, serous, clear cell, etc [31]; although the two cell lines used in this study are derived from OC patients with adenocarcinoma type, they may come from tissues with different subtypes preserving individual phenotypes. For instance, the ovarian cancer cell line SKOV3 showed greater resistance to cisplatin by 10-fold and by 5.8-fold to carboplatin when compared with that in A2780 cells [32]. Also, TCP1 or TRiC activity is found not to always correlate with their expression levels [30]. Therefore, we selected the suitable cell line, A2780, and successfully constructed stable cell lines of the A2780/TCP1-NC group and A2780/TCP1-KD group by using specific lentiviral shRNA targeting TCP1, which were used for further analysis. The functional studies confirmed that the silencing of TCP1 inhibited the motility and aggression of OC cells. The results suggested that TCP1 can accelerate the malignancy of OC, which is consistent with the findings of other studies that involved other tumours.

One study has confirmed the effect of TCP1 is achieved via the PI3K/AKT pathway [19]. Based on the recent studies of cancer genomics, several genes in the major cell signalling pathways were dysregulated in OC [8, 9]. Among these abnormal signalling pathways in OC, the PI3K/AKT/mTOR pathway was altered frequently with a great chance for OC therapeutic intervention [33, 34]. AKT, a known PI3-kinase target, is a serine-threonine kinase that regulates numerous downstream target genes [35, 36], ultimately regulating metabolic processes and cellular survival [37]. To investigate the underlying molecular mechanisms of TCP1 for improving proliferation, invasion, and migration in OC cells, some proteins involved in PI3K/AKT/mTOR signalling pathways were tested in A2780 cells. As shown in Fig. 5, compared to total

AKT contents, p-AKT at serine 473 (Ser473), one of the two phosphorylation sites on AKT for its activation, was lowered in A2780/TCP1-KD cells [38]. Levels of p-Akt are typically determined in cells to measure PI3K activity. Altogether, the results showed that knockdown of TCP1 may inhibit phosphorylation of AKT at Ser473 and its activation. In summary, TCP1 can regulate OC cell proliferation, invasion, and migration via the PI3K/AKT/mTOR pathway.

One possible limitation of this study is that data were generated and analyzed from in-vitro experiments. In future studies, the specific mechanisms underlying the differential expression of TCP1 in the various ovarian cancer cells, and also the TCP1 possible regulation of p-AKT expression in ovarian cancer requires further investigation

Conclusion

In conclusion, TCP1 is overexpressed in OC and is dependent prognostic biomarkers for overall survival in ECO. TCP1 may upregulate p-AKT, thereby promote OC cell proliferation.

Abbreviations

TRiC: TCP1 ring complex; TCP1: T-complex protein 1; OC: ovarian cancer; EOC: epithelial ovarian cancer; PI3K: phosphatidylinositol-3 kinase; p-AKT: phospho-AKT; OS: overall survival; PFS: progression-free survival; shRNA: short hairpin RNA; shTCP1: lentiviruses for TCP1 RNA interference; shCtr: lentiviruses for negative control;

Declarations

Acknowledgements

Not applicable.

Authors' contributions

HW designed the experiments and wrote the paper. ZZ, YL arranged the clinical

data. HW, YL analyzed the data. HW, XF drafted the paper. ZZ, XF revised the final paper. The authors read and approved the final manuscript.

Funding

Fujian Provincial Department of Science and Technology (CN) (2100201)

Availability of data and materials

The datasets of the overall survival (OS), progression-free survival (PFS), risk ratio (HR), and log-rank P of TCP1 in OC patients analyzed during the current study are available in the Kaplan-Meier Plotter repository,

[<http://kmplot.com>].

The datasets of clinical parameter data used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate.

This study was approved by the Ethics Committee of Fujian Medical University Union Hospital.

Consent for publication

Consent was obtained from all individual participants included in the study.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table1. The average optic density of TCP1 in ovary tissues in different histological subtype groups (IOD/area)

Group	n	IOD/area
Normal	13	0.003±0.004
Cystadenoma	28	0.011±0.015
Borderline	8	0.059±0.050 ^{a,b}
Carcinoma	62	0.077±0.042 ^{a,b}

Data presented as mean ± SD

^a $p \leq 0.05$ vs. normal

^b $p \leq 0.05$ vs. cystadenoma

Table 2. Relationship between TCP1 expression and clinicopathological features of EOC patients.

Characteristics	N	IOD/area	t/F value	Pvalue
Age (years)			-1.125	0.271
<60	47	0.074±0.043		
≥60	15	0.087±0.037		
Tumour size			-0.363	0.72
<5cm	14	0.074±0.037		
≥5cm	48	0.078±0.043		
FIGO Stage ²⁰⁰⁹			-3.497	0.001 *
I+II	29	0.060±0.033		
III+IV	32	0.094±0.043		
Histological subtype			2.728	0.052
Serous	38	0.086±0.044		
Mucinous	17	0.065±0.026		
Clear cell	4	0.014±0.007		
Endometrioid	3	0.091±0.069		
Grade of differentiation ^a			4.262	0.001 *
Low	27	0.050±0.023		
High	35	0.091±0.042		
Lymph node metastasis ^b			1.617	0.137
Positive	9	0.100±0.048		
Negative	47	0.073±0.040		
The volume of ascites			1.112	0.27
Positive	29	0.083±0.039		
Negative	33	0.072±0.044		

N number of patients

^a Low = G1; High = (G2 + G3);

^b Additional six cases did not undergo retroperitoneal lymphadenectomy

* $P<0.05$.

Table 3. The TCP1 shRNA sequences

Name	Sequence(5'-3')
shTCP1 sense	CCGGGGTGTACAGGTGGTCATTATTCAAGAGATAATGACCACCTGTACACCTTTTTTG
shTCP1 anti-sense	AATTCAAAAAAGGTGTACAGGTGGTCATTATCTCTTGAATAATGACCACCTGTACACC3

Figures

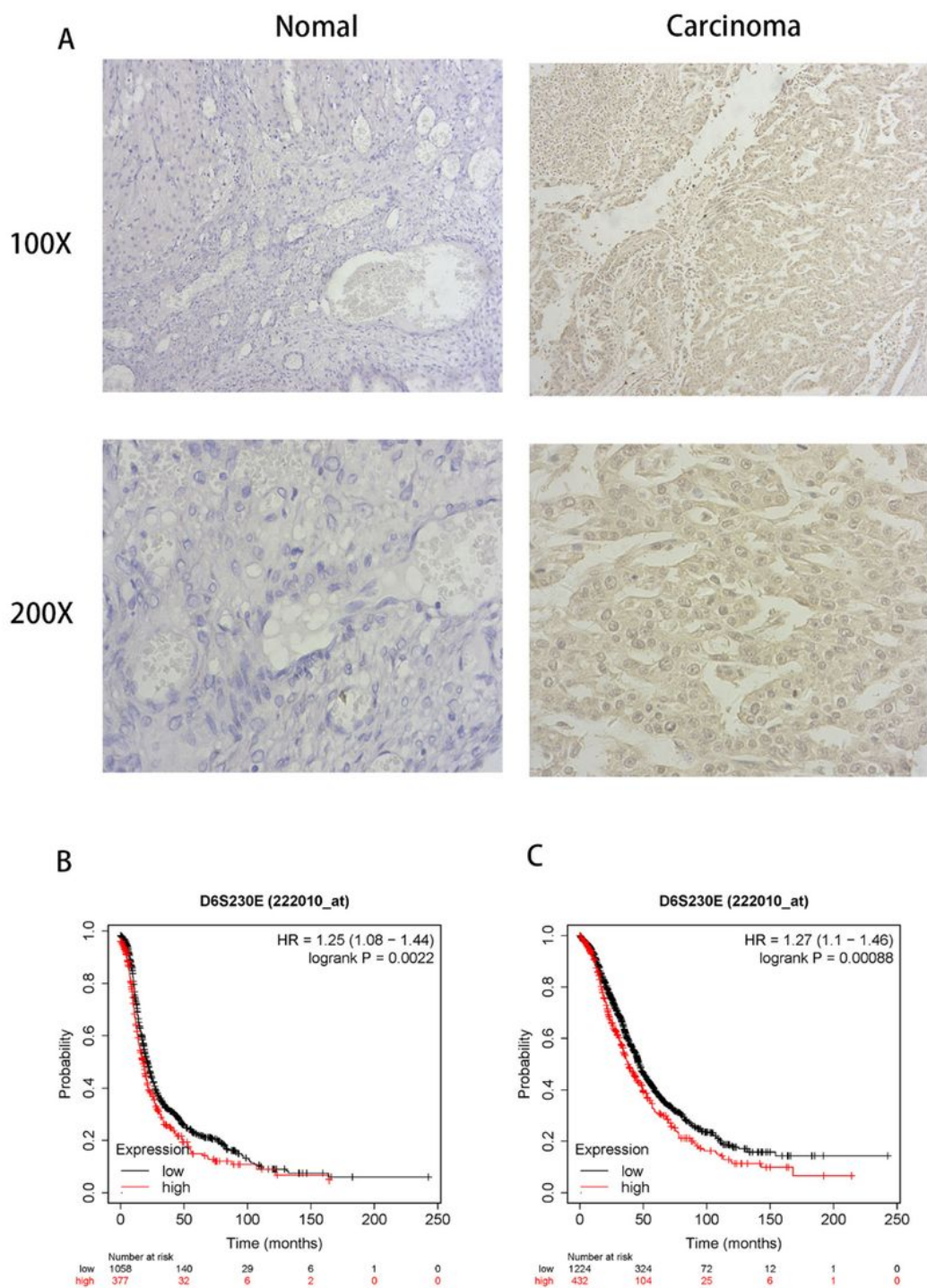


Figure 1

IHC analyses of TCP1 protein expression in different ovarian tissues specimens and Kaplan-Meier survival analyses of the EOC patients (A) The expression of TCP1 in the normal and tumor samples detected by IHC. (B) The cut-off data was set as PFS, and the number of eligible cases in the database was 1435 KM Plotter. The HR value was 1.25 and the log-rank P was 0.0022. (C) The cut-off data was

set as OS, and the number of eligible patients in the database was 1656 KM plotter. The HR value was 1.27 and the log-rank P was 0.00088.

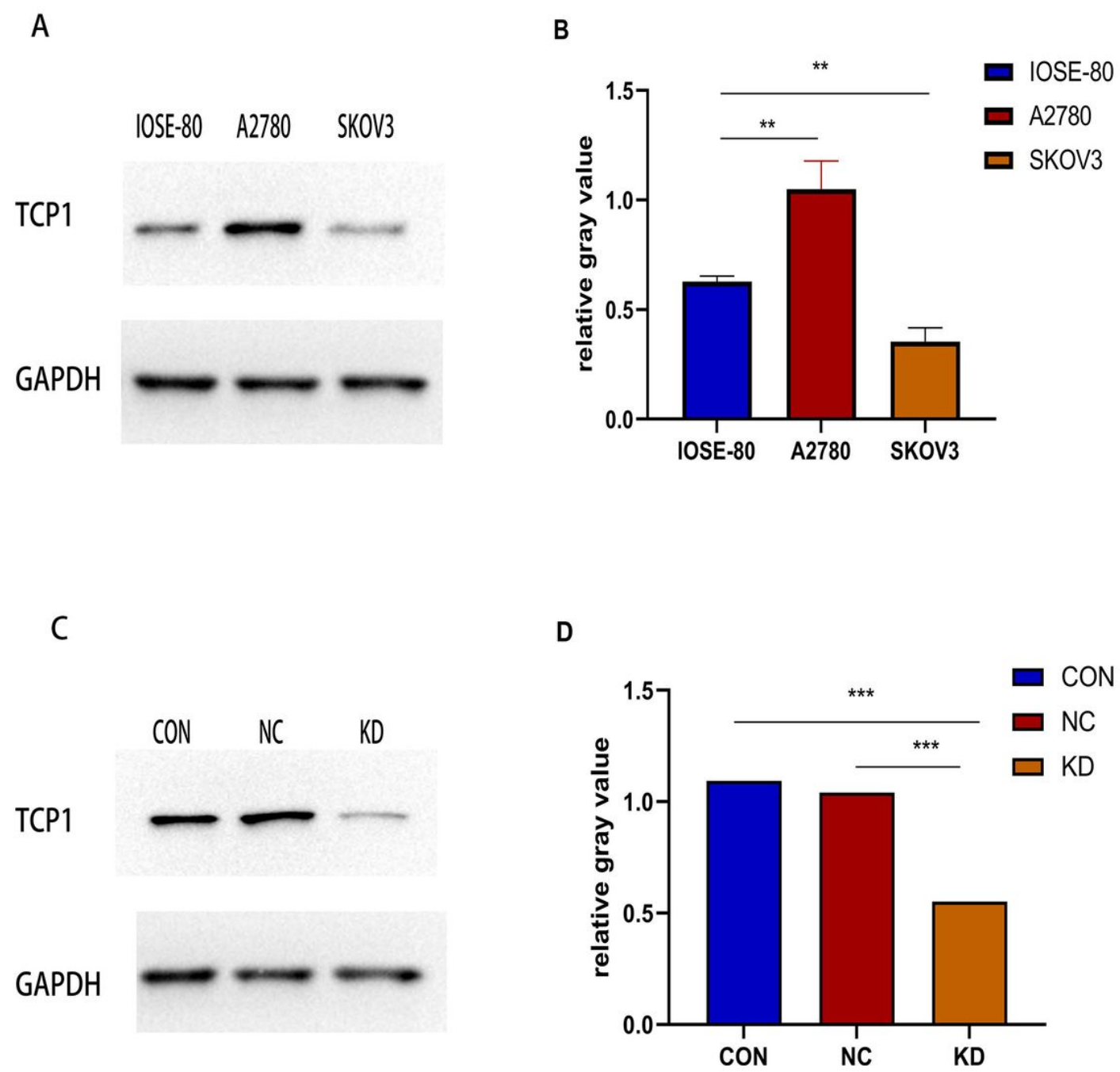


Figure 2

The expression level of TCP1 protein in human EOC cell lines as evaluated by western blotting and the knockdown effect by shRNA against TCP1. (A) TCP1 protein was up-regulated in A2780 cells, but not in SKOV3 cells, compared with IOSE-80 cells. (B) Histogram plotted with three relative gray values from Fig. 2A. Each cell line was conducted in triplicate. **P<0.01 vs. IOSE-80. (C) The TCP1 expression levels of A2780/TCP1- KD cells that tested shRNA against TCP1 was markedly decreased compared with

A2780/TCP1-NC cells (NC: negative control) and A2780 cells control. (CON: controls). (D) Histogram plotted with three relative gray values from Fig. 2C. Each cell line was conducted in triplicate. ***P<0.001 vs.A2780.

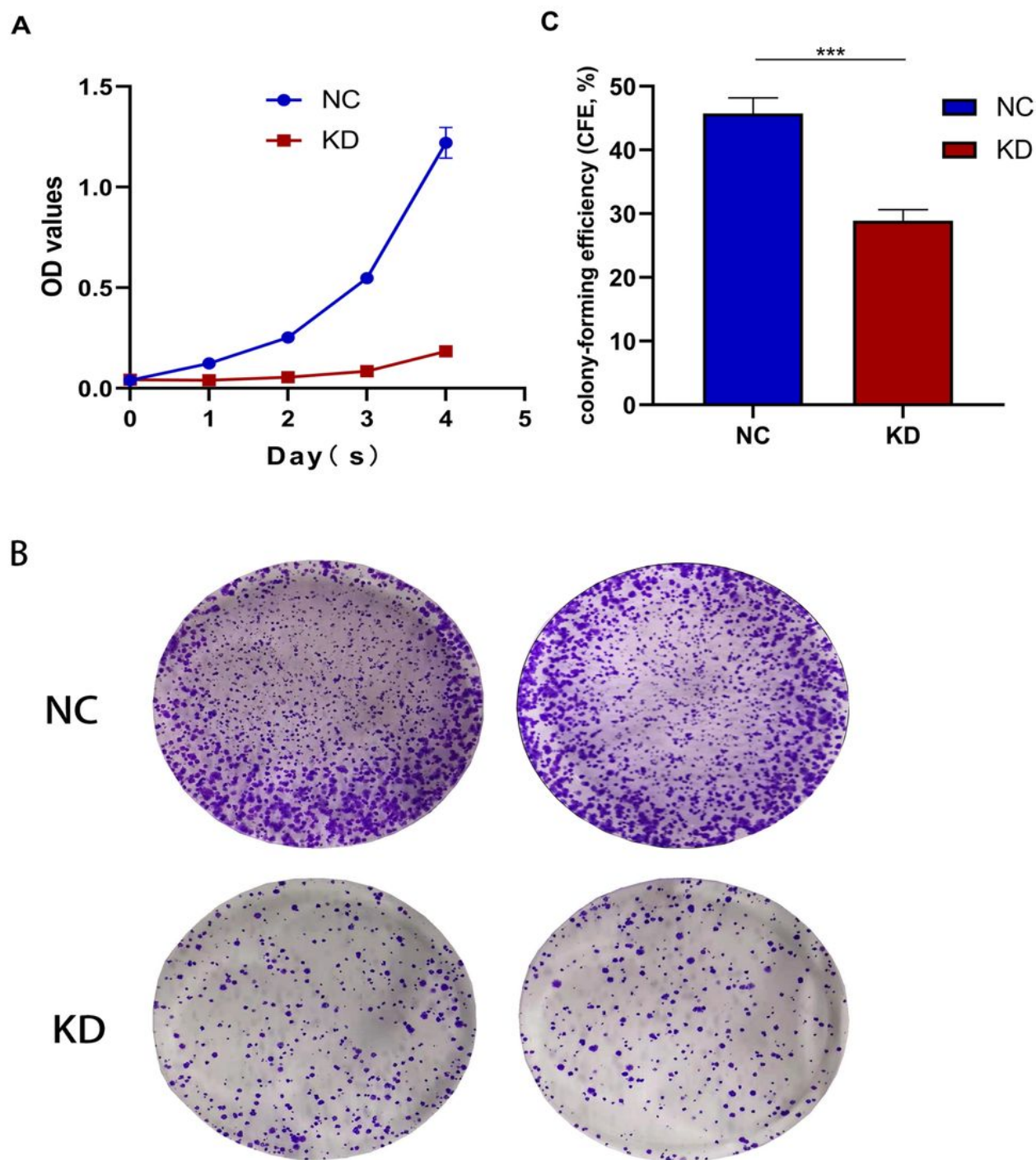


Figure 3

Growth-inhibiting role of knockdown TCP1 in A2780 cell lines. (A)The MTT assay showed that with a dramatic decrease of TCP1 expression, the proliferation of A2780 cells was significantly inhibited as

shown by a nearly flat growth curve. The difference arose from Day 1 and persisted until Day 4. (B) Colony-formation assays indicated decreased growth rates in TCP1-KD A2780 cell line. (C) The colony-formation abilities from Fig. 3B. One independent experiment was carried out in triplicate. Values are shown as the mean $\pm$ standard deviation (SD). Use independent Student's t-test to calculate P-values. ***P< 0.001 vs. the NC.

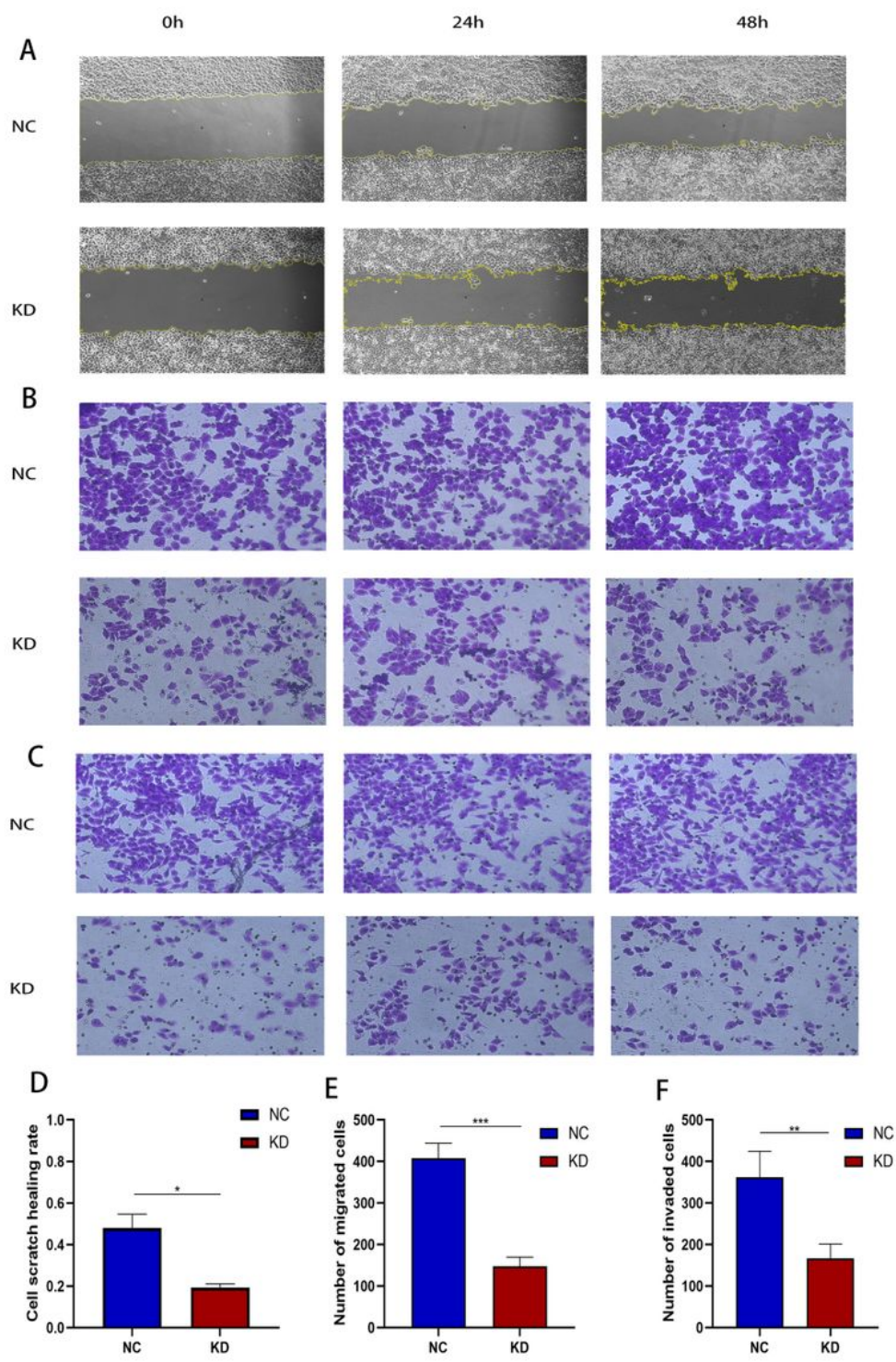


Figure 4

Suppression of EOC cell migration and invasion ability by TCP1 silencing. (A and D) TCP1 knockdown in A2780 cell line inhibited cell migration, as revealed by a wound healing assay. (B and E) Transwell assays revealed that shRNA-TCP1 knockdown decreased the migration of A2780 cells (C and F) TCP1 knockdown remarkably attenuated the invasion ability of A2780 cells. Data are presented as the mean $\pm$ SD of three independent experiments. P-values were obtained with the independent Student's t-test. *P<0.05 vs. the NC; **P<0.01 vs. the NC; ***P <0.001 vs. the NC. Magnification: 200 $\times$.

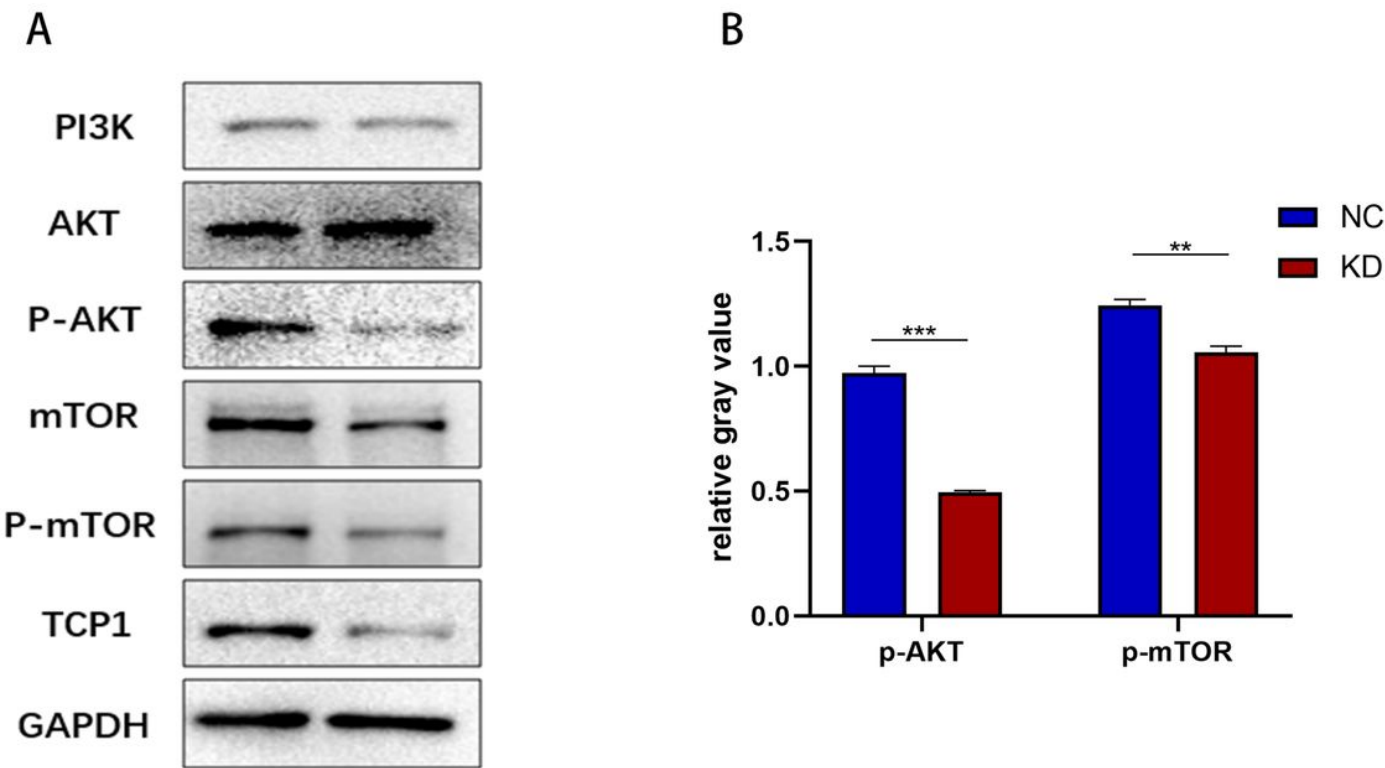


Figure 5

Knockdown of TCP1 inhibited PI3K/AKT/mTOR pathway. (A) Phosphorylation levels of PI3K, AKT and mTOR by Western blot analysis. The GAPDH was used as an internal control. (B) Histogram plotted with three relative gray values from Fig. 5A. Data are presented as the mean $\pm$ SD of three independent experiments. **P<0.01 vs. the NC; ***P<0.001 vs. the NC.