T-cadherin in epithelial ovarian cancer: relationship with cancer progression and drug resistanceT-cadherin in epithelial ovarian cancer: relationship with cancer progression and drug resistance

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Abstract

Background: T-cadherin plays a crucial role in maintaining normal tissue structure by regulating specific cell adhesion, cellular recognition and signal transduction. The purpose of this study was to evaluate whether T-cadherin influences epithelial ovarian cancer (EOC) progression, differentiation and drug resistance and its possible mechanisms.

Methods: Epithelial ovarian carcinoma (EOC, n=63) and relevant contralateral normal ovarian (CNO, n=41) fresh tissues were collected from epithelial ovarian carcinoma patients, and benign ovarian tumour fresh tissues were collected from 55 patients with benign ovarian tumours. The human epithelial ovarian carcinoma cell line A2780 was cultured. T-cadherin expression was assessed by real-time RT-PCR and Western blotting, and the expression of matrix metalloproteinase-2 (MMP-2) was detected by Western blotting. pcDNA-T-cad plasmid technology was used to upregulate T-cadherin expression. In addition, A2780 cell migration and invasion ability, viability, colony formation, proliferation, apoptosis and sensitivity to paclitaxel were measured.

Results: T-cadherin mRNA and protein expression in EOC tissues from EOC patients was significantly downregulated, and there was no significant difference between the matched contralateral normal ovarian fresh tissue from the same patient and the benign ovarian tumour tissues from other patients. The migration and invasion abilities, viability, colony formation, and proliferation were attenuated by restoration of T-cadherin expression in A2780 cells via pcDNA-T-cad transfection; apoptosis, MMP-2 expression and sensitivity to Taxol were also enhanced by restored T-cadherin expression. The T-cadherin expression level was well correlated with the clinical characteristics and symptoms of EOC patients, including tumour stage, histology, lymph node metastasis, tumour size, distant metastasis and cisplatin resistance.

Conclusions: T-cadherin participates in the processes of epithelial ovarian carcinoma cell migration, invasion, proliferation, apoptosis and sensitivity to paclitaxel and can regulate the expression of MMP-2. Downregulation of T-cadherin expression may contribute to epithelial ovarian cancer progression, differentiation and drug resistance.

Background

Epithelial ovarian cancer (EOC) is the primary cause of unique cancer-related death in women. Patients with early stage, local tumours can be successfully treated with surgery combined with adjuvant therapy [1]. However, most ovarian cancer patients are diagnosed at an advanced stage with distant metastases that cannot be removed; the effects of these treatments are frequently temporary[2], and 80% of patients with late stage disease experience recurrence and advance to the fatal phase as a results of insensitivity to chemotherapy, distant metastasis and poor prognosis[3]. Current research indicates that the occurrence and development of EOC are closely related to various molecular and genetic changes [4]. Therefore, searching for the underlying mechanisms of cancer growth and for new markers that can

identify and treat high-risk recurrent and aggressive tumours at an early stage remains the main focus of EOC research.

Cadherin is a member of the transmembrane glycoprotein family and plays a crucial role in maintaining ordinary tissue structure by regulating specific cell adhesion, cellular recognition and signal transduction[5]. There is increasing evidence that calcium-mucin interference is associated with tumour development, especially invasion and metastasis[6]. T-cadherin, which is attached to the plasma membrane by a glycosylphosphatidylinositol anchor, lacks transmembrane and cytoplasmic domains and is the exclusive atypical member of the cadherin superfamily[7]. Recently, increasing attention has been paid to the function of T-cadherin in human malignant tumours[8]. Similar to the archetype of E-cadherin, a member of the superfamily, T-cadherin is considered a tumour suppressor that can regulate progression[9]. Indeed, decreased T-cadherin gene expression has been found in several human cancers, including bladder cancer[10], cervical cancer[11] and prostatic carcinoma[12]. Wu et al. confirmed that decreased T-cadherin protein expression increased the tumorigenicity of prostatic carcinoma and that overexpression of T-cadherin significantly decreased the invasion potential and growth rate of tumour cells[13]. T-cadherin can exert multiple effects to promote tumour growth via different mechanisms. Although previous studies have demonstrated that the frequent deletion of T-cadherin in gastric cancer is related to poor prognosis[14], little is understood about the biological role of T-cadherin in EOC.

In the present research, we demonstrated that T-cadherin is downregulated in EOC tissues and cell lines. Then, we further evaluated the effect of T-cadherin overexpression on the migration and invasion of EOC cells. Our results showed that T-cadherin participates in the uncontrolled migration and invasion of EOC cells and at least partially affects cell movement.

Materials And Methods

Ethical approval

This research conforms to and acts in accordance with the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) guidelines (http://www.equatornetwork.org/). All fresh human tissue specimens were collected with informed consent on the basis of the request of the Ningbo First Hospital Scientific Research Ethics Committee from December 2018 to August 2019.

Human tissue collection

Chinese-Han fresh ovarian tissue specimens, including benign ovarian tumour specimens (BOTs, n=55) from patients who underwent laparoscopic surgery and epithelial ovarian carcinoma (EOC, n=63) and relevant contralateral normal ovarian tissue (CNOs, n=41) specimens from patients who underwent surgery for pathologically confirmed epithelial ovarian carcinoma, were collected from the Department of Gynaecology and Obstetrics (Ningbo First Hospital) from October 2018 to August 2019 for protein and mRNA testing. The relevant contralateral normal ovarian tissues were obtained from the noninvaded ovary on the contralateral side. According to the 2014 classification of the International Federation of

Gynaecology and Obstetrics (FIGO), carcinoma patients were in stage I to IV. None of the patients were cured with any neoadjuvant chemotherapy or targeted therapy before radical operation.

Cell culture

The human EOC cell line A2780 was purchased from Guangzhou Jennio Biotech Co., Ltd. and maintained in cell culture. Cells were cultured with DMEM (Gibco) containing 10% foetal calf serum (HyClone, USA) and double antibiotics (100 μg/ml streptomycin and 100 Units/ml penicillin) (HyClone). The cells were incubated in a humidified atmosphere with 5% CO2 at 37°C. The cell lines were authenticated.

T-cadherin is overexpressed in EOC cell lines

The pcDNA3.1 and pcDNA-T-cadherin (pcDNA-T-cad) plasmids were purchased and transfected into A2780 cell lines using Lipofectamine® 2000 according to the manufacturer's instructions. After 48 h of transfection, T-cadherin expression was detected by Western blotting.

Expression levels of the T-cadherin gene in ovarian tissues and cell lines were analysed by qRT-PCR

Total RNA levels in tissues or cultured cells were measured by real-time RT-PCR via the SYBR Green fluorescence signal detection kit according to the manufacturer's protocol. cDNA was amplified by qPCR as previously described[15]. Gene expression was normalized by endogenous housekeeping gene GAPDH level. The primer pair sequences for human T-cadherin were as follows: 5'-TTCAGCAGAAAGTGTTCCATAT-3' (forward) and 5'-GTGCATGGACGAACAGAGT-3' (reverse). The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal reference using the following primers: 5’-GGTGGTCTCCTCTGACTTCAACA-3’ (forward) and 5’-GTTGCTGTAGCCAAATTCGTTGT-3’ (reverse). The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression levels (fold changes), and all the tests were performed at least three times[16].

Protein extraction and Western blotting

Total protein was extracted from fresh tissue samples according to the manufacturer's instructions. The protein concentration was determined using the BCA Protein Assay Kit (SinoBio Biotech, China). Each equivalent sample protein was electrophoresed in 10% SDS-PAGE polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membrane was sealed with 5% nonfat milk at room temperature for 2 hours and incubated with monoclonal antibodies, including anti-T-cad (1:1,000; Abcam, Cambridge, MA, USA), anti-MMP-2 and anti-GAPDH at 4°C overnight. Subsequently, using the corresponding HRP-conjugated secondary antibodies, the membranes were incubated at room temperature for 2 h. After washing, proteins were quantified by the ChemiDoc XRS system (Bio-Rad, Philadelphia, USA). GAPDH was used as an internal control.

Transwell migration and invasion assays

Using 24-well Transwell chambers (8 μm; Corning Inc., USA), the cell migration assay was performed. At 48 h after transfection, 200 μl of suspension containing $4x10^5$ cells was added to the upper compartments in serum-free medium, and culture medium containing 10% FBS and 1% BSA was added to the lower chamber. After incubating at 37°C in humidified air for 24 h with 5% CO_2 and 95% O_2, the cells that migrated into the lower chamber were fixed with methanol for 2 min and stained with 1% crystal violet. Finally, microscopy with a CCD camera was used to photograph and count the cells. For the cell invasion assay, the procedures of the cell invasion experiment were similar to those of the cell migration experiment, except the Transwell chambers were coated with 24 μg/μl Matrigel (R&D Systems, USA).

Cell viability and colony formation assays

MTT assays were used to measure cell viability in A2780 cells daily over the following 5 days. Briefly, A2780 cells at a density of $1x10^4$ were inoculated into each well in 96-well plates and cultured with 10 μl MTT at a concentration of 0.5 mg/ml (Promega, USA) at 37°C for 4 h. Then, 150 μl dimethylsulfoxide (DMSO; Sigma, USA) was added to dissolve the purple formazan crystals for 1 h. Ultimately, an ELISA reader (Bio-Rad, Berkeley, CA, USA) was used at a wavelength of 595 nm to read the absorbance of each well. Each trial was performed in triplicate and repeated three times.

Cell proliferation assays

The proliferation ability of A2780 cell lines was detected by CCK-8 assay (Dojindo, Japan). Untransfected, transfected, and empty transfected A2780 cells were adjusted to a cell concentration of $5x10^3$ cells/ml and then inoculated with 100 μl diluted cell fluid per well in a 96-well plate with three identical wells per sample and time point. After culturing for 0, 24, 48 and 72 hours, 10 μL of CCK-8 solution was added to each well and incubated continuously for 2 hours at 37°C. Finally, the absorbance was measured at a wavelength of 450 nm by a fully automated microplate reader (Bio-Rad Laboratory, Irvine, CA, USA).

Cell apoptosis assays

Non-transfected, transfected and empty vector-transfected A2780 cells were collected, and serum-free medium was used to prepare a single cell suspension at a density of $3x10^4$ cells/ml. A 10 ml cell suspension was added to each well of the 6-well plate, and 0.25% trypsin digestion was performed. After 48 h of culture, an annexin V-FITC and propidium iodide (PI) double staining apoptosis kit (Dojindo, Japan) was applied according to the manufacturer's instructions. Apoptosis signals were detected by flow cytometry. Annexin V-FITC-positive cells were defined as apoptotic cells.

Paclitaxel sensitivity assays

To evaluate growth rates, A2780 cells were plated into 96-well plates at a density of $1x10^4$ cells/well. Growth medium (DMEM/10% FCS) with or without paclitaxel (1.8 μM; LC lab, Woburn, USA) was used to culture the cells overnight. The cells were cultured for another 4 days, rinsed gently with PBS, separated in PBS with 0.25% trypsin/1 mM EDTA, and counted with a Coulter counter. To evaluate the survival rate of

cells adhered on 96-well plates, an MTT assay was applied after exposure to paclitaxel for 0 h, 24 h, 48 h, 72 h, and 96 h.

Statistical analysis

All data are represented by the mean ± standard deviation (SD). One-way ANOVA was used for statistical comparison. Pearson's χ^2 test or Fisher's exact test was used to determine the significant difference in clinical characteristics and T-cadherin expression. SPSS 16.0 software was used for statistical analysis (SPSS Inc., Chicago, USA). *P* values <0.05 were considered significant.

Results

Decreased T-cadherin mRNA expression in EOC tissues

T-cadherin mRNA expression levels in BOT (n=55), CNO (n=41) and EOC (n=63) tissues were detected by real-time qPCR. As shown in **Figure 1A**, the mRNA expression of T-cadherin in EOC samples was significantly decreased (P < 0.05) compared with that in the relevant contralateral normal ovarian specimens from the identical patients and that in the BOT samples. In the 63 patients with EOC, 77.8% of EOC samples (49 out of 63 patients) presented reduced T-cadherin expression, and 22.2% of EOC samples (14/63) did not show obviously decreased T-cadherin mRNA expression. Compared to the BOT group, there was no significant difference in the level of T-cadherin mRNA expression in the relevant contralateral normal ovarian samples from the epithelial ovarian carcinoma patients (n = 41). These data indicated that the T-cadherin mRNA expression level is downregulated in EOC patients and is well correlated with the progression of EOC.

Decreased T-cadherin protein expression level in EOC tissues

The expression of T-cadherin protein in EOC tissue was assessed by Western blotting. The results showed that marked downregulation of T-cadherin proteins was observed in EOC specimens from 49 patients (out of 63 patients). In contrast, T-cadherin proteins were expressed at high levels in the BOT specimens and the relevant contralateral normal ovarian carcinoma samples from patients with EOC (Figure 1B and C).

Together with the real-time RT-PCR data, these results suggest that T-cadherin expression is downregulated in EOC tissues, indicating that low T-cadherin may be associated with the development of EOC.

Overexpression of T-cadherin suppressed EOC cell line migration ability

We successfully constructed stable T-cadherin-overexpressing EOC cells. After transfection for 48 h, the upregulation of T-cadherin in the EOC cell lines was verified via Western blotting. The migration ability of EOC cell lines was assessed by the Transwell migration assay. As shown in Figure 2 (A and B), the migration ability in the pcDNA-Tcad group was significantly decreased compared with that in the empty vector or control group A2780 cells (Figure 2A and B). The number of ovarian cancer cells migrating to

the lower membrane via Transwell filters increased significantly in the empty vector or control group compared with the pcDNA-Tcad group. The number of migrated cells in the empty vector and control groups was not significantly different. The results suggest that the migration ability of A2780 cells overexpressing the T-cadherin gene is decreased.

Overexpression of T-cadherin suppressed EOC cell line invasion ability

Using Matrigel-precoated polycarbonate Transwell filters, the invasion ability of A2780 cells obtained from the pcDNA-Tcad group, empty vector and control group was tested. In the invasion assay, the A2780 cells needed to dissolve the Matrigel before migrating across the Transwell filters. The results showed that the invasiveness of the pcDNA-Tcad group, empty vector and control group was different (Figure 2 C and D). The number of A2780 cells that invaded the Matrigel-precoated filters and reached the lower surface membrane layer was significantly reduced in the pcDNA-Tcad group compared with that in the empty vector and control groups ($P<0.01$). The number of invaded cells in the empty vector and control groups was not significantly different. The results suggest that T-cad-overexpressing A2780 cells possess higher invasion ability.

Overexpression of T-cadherin suppressed MMP-2 expression in EOC cells

The invasion ability of tumour cells is closely related to MMPs. MMP-2 plays a pivotal role in the progression of ovarian cancer. As shown above in our results, T-cadherin was downregulated in EOC tissues and was involved in A2780 cell invasion. To further study this relationship, the protein expression of MMP-2 and T-cadherin in different ovarian samples was assessed by using Western blotting technology. In contrast to the downregulated expression of T-cadherin, MMP-2 expression was upregulated in EOC tissue specimens from 63 EOC patients (out of 85 patients, Figure 3A and C). The mean MMP-2 expression level in the EOC tissues of the EOC group (63 cases) was higher than that in the paracarcinoma tissues of the same group (41 cases) and the ovarian tissues from the control group (55 cases) ($P<0.01$).

Interestingly, when the expression of T-cadherin was upregulated by pcDNA-Tcad in ovarian tumour cells, MMP-2 protein expression was prominently attenuated compared with that in the empty vector or control group ($P<0.01$; Figure 3B, D and E), indicating that T-cadherin can regulate MMP-2 protein expression.

Overexpression of T-cadherin suppressed EOC cell line proliferation

In the proliferation assays, the results showed that the proliferation ability of the pcDNA-Tcad group, empty vector and control group was different (Figure 4 A-C). A2780 cell proliferation was significantly reduced in the pcDNA-Tcad group compared with the empty vector and control groups ($P<0.01$). The number of proliferating cells in the empty vector and control group was not significantly different. The results suggest that T-cad-overexpressing A2780 cells possess lower proliferation ability.

Overexpression of T-cadherin facilitated EOC cell line apoptosis

To decipher the underlying mechanisms attributed to the T-cadherin-mediated inhibition of cell proliferation, we performed cell apoptosis analysis. In the assays, the results showed that the assay abilities of the pcDNA-Tcad group, empty vector and control group were different (Figure 4 D and E). The number of ovarian tumour cells was significantly increased in the pcDNA-Tcad group compared with the empty vector and control groups ($P<0.01$). The number of assay cells between the empty vector and control groups was not significantly different. The results suggest that ovarian tumour cells overexpressing T-cad possess higher apoptosis ability.

Overexpression of T-cadherin increases the sensitivity of EOC cells to paclitaxel

To determine whether the expression of T-cadherin is related to EOC progression and involved in the regulation of tumour sensitivity to chemotherapy, we investigated the effect of T-cadherin overexpression on the paclitaxel sensitivity of cultured EOC cells in vitro. By counting cells, EOC cell growth was evaluated with or without paclitaxel. Paclitaxel decreased the growth of T-cadherin-overexpressing EOC cells; however, the empty vector and control groups were markedly less sensitive to the drug inhibitory effects (Figure 5).

The relationships between T-cadherin expression and clinicopathological characteristics in EOC patients

These above findings raise the question of whether T-cadherin is related to clinicopathological characteristics. The correlation between the expression of T-cadherin protein and the clinicopathological characteristics of 63 EOC patients with a mean age of 54.7±8.3 years was analysed (Table 1). The data showed that T-cadherin downregulation was well correlated with tumour stage, histology, lymph node metastasis, tumour size, distant metastasis and cisplatin resistance ($P<0.05$). Downregulated expression of T-cadherin was detected in 92.6% of cisplatin-resistant EOC patients. The T-cadherin downregulation rate in the EOC patients with distant metastasis was significantly higher than that in the EOC patients without distant metastasis (77.8% vs. 52.4%, $P=0.001$, Table 1). This suggests that the T-cadherin gene is closely associated with the development of EOC.

Discussion

T-cadherin, a novel adhesion molecule, has been reported as a crucial independent prognostic predictor factor for human ovarian cancer[17]. However, its potential biological role in human epithelial ovarian cancer is not entirely clear. In this research, we determined T-cadherin gene and protein expression in a group of human epithelial ovarian cancer tissue samples. We found that T-cadherin mRNA and protein expression levels were markedly lower in human epithelial ovarian carcinoma tissues and cell lines than in controls and that the expression levels were well correlated with the progression of endometriosis. Furthermore, functional analysis of genes was carried out on EOC cells to study the biological functions of T-cadherin in cell migration and invasion in vitro. The results showed that the overexpression of T-cadherin decreased the motor activity of EOC cells. These data indicate that the decrease in T-cadherin protein may be the driving factor for the occurrence of ovarian tumours, while the increase in T-cadherin protein may inhibit tumour development.

Although there have been many studies on the role of T-cadherin in the pathogenesis of carcinoma[18], the mechanism of T-cadherin in the occurrence and development of ovarian cancer is not entirely clear. The T-cadherin gene is genetically or epigenetically involved in several cancers[17]. Tumour cell migration is one of the key processes in tumour metastasis and is often accompanied by increased mobility of tumour cells[19]. Classical cadherins are usually located at the site of intercellular connections and contribute to the formation of intercellular junctions[20]. Loss of intercellular adhesion promotes tumour cell movement and growth. T-cadherin regulates cell migration and target finding by reducing homologous and heterophilic interactions involved in adhesion[21]. Downregulation of T-cadherin has been implicated in the progression of various carcinomas[22]. The upregulation of T-cadherin reduces the malignant characteristics of human prostate cancer, melanoma, breast cancer and liver cancer cell lines. Human breast cancer cells overexpressing T-cadherin injected into the adipose tissue of the breast in nude mice reduced tumour growth and pleomorphism compared with that in the control group[23]. Moreover, melanoma cells with restored expression of T-cadherin showed a reduced tumour growth rate in a nu/nu mouse tumour model[24]. In the present study, we demonstrated that T-cadherin plays a crucial role in the migration and invasion of human epithelial ovarian carcinoma cells and may be related to the development of human epithelial ovarian cancer.

Interestingly, this study revealed that the expression of T-cadherin was negatively correlated with the expression of MMP-2. The downregulation of T-cadherin expression was correlated with the pathogenesis of human epithelial ovarian cancer, very likely due to the augmented expression of MMP-2. Studies have confirmed that the downregulated expression of T-cadherin increases the invasion of bladder transitional cell carcinoma by enhancing cell-extracellular matrix adhesion and upregulating the expression of MMP-2[25]. MMP-2 is a matrix metalloproteinase (MMP) family protein that can degrade the extracellular matrix and play a crucial role in physiological and pathological processes such as migration and invasion of malignant tumours[26]. Elevated MMP-2 expression has been discovered in human epithelial ovarian cancer, and it has been suggested that human epithelial ovarian cancer tissue may be inherently more aggressive and metastatic[27]. It is now well believed that MMP-2 may be involved in the process of tumour cell invasion and metastasis[28]. We demonstrated in this work that the expression of MMP-2 can be regulated by T-cadherin in human epithelial ovarian cancer. This result indicated that T-cadherin may take part in the invasion process of ovarian cancer cells by regulating MMP-2 expression; however, the specific underlying mechanism still needs further clarification.

Though chemotherapy is still the main treatment option for epithelial ovarian cancer, its survival benefit is limited because of the common development of chemical resistance[29]. There is continuing evidence that the effectiveness of treatment depends on the state of differentiation of tumour cells[30]. However, chemotherapeutic drugs are preferred to target differentiated cells, as progenitor cells are insensitive to chemotherapy and may maintain tumour growth[31]. This research revealed that T-cadherin affects the sensitivity of ovarian cancer cells to paclitaxel, a diterpenoid alkaloid compound with anticancer activity that is widely used in the treatment of a range of tumours, including ovarian carcinoma. T-cadherin gene re-expression in ovarian carcinoma cell lines with low expression of T-cadherin notably reduced the cell sensitivity to paclitaxel by decreasing viability. These data indicate that T-cadherin expression is not only

closely associated with the progression of epithelial ovarian cancer but also directly affects the survival of ovarian cancer cells and may affect the efficacy of epithelial ovarian cancer therapy. Much deeper research is needed to determine the specific molecular mechanisms of T-cadherin affecting the differentiation and plasticity of ovarian cancer cells and to clarify T-cadherin as a potential therapeutic molecular target and prognostic biomarker for ovarian cancer.

Conclusion

In summary, our study provided original clinical and laboratory evidence primarily showing that T-cadherin expression promotes the development of ovarian cancer by regulating the expression of MMP-2. T-cadherin may be a potential novel therapeutic target for ovarian cancer therapy.

Abbreviations

EOC: Epithelial ovarian cancer; MMP-2: Matrix metalloproteinase-2; CNO: Contralateral normal ovarian; BOT: Benign ovarian tumour; DMEM: Dulbecco's modified Eagle's medium; PVDF: Polyvinylidene fluoride; HRP: Horseradish peroxidase; qRT-PCR: Quantitative real-time PCR; SD: Standard deviation; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Declarations

Author Contributions

Yu-tao Guan, Zhong-qiu Lin, Lining Cui and Gang Wang designed the research. Yi Lin, Fubin Zhang, Xiaojiao Zheng performed experiments and analyzed data. Yang-ping Chen contributed to sample collection. Lingling Zhou performed pathology detection. All authors read and approved the final manuscript.

Competing interests

All authors read and approved the final version of the manuscript, and the authors declare that there are no conflicts of interest.

Availability of data and materials

All data and materials can be provided upon request.

Consent for publication

The authors agree to publish this manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Ningbo First Hospital.

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Tables

Table 1. Epithelial ovarian cancer patient characteristics and T-cadherin expression

Characteristic	Total (N=63)	No. of T-cadherin (-) cases(%)[a]	P[b]
Age, years			
≤55	35	26 (74.3)	0.456
55	28	23 (82.1)	
Stage			
I+II	15	8 (53.3)	0.009
III+IV	48	41 (85.4)	
Grade			
Low and moderate (G1+G2)	27	17 (66.7)	0.066
High (G3)	36	32 (86.1)	
Lymph node metastasis			
Negative	28	18 (64.3)	0.021
Positive	35	31 (88.6)	
Histologic type			
Serous adenocarcinoma	47	38 (80.9)	0.351
Non-serous adenocarcinoma	16	11 (68.8)	
Tumor size (cm3)			
10	18	10 (55.6)	0.007
≥10	45	39 (86.7)	
Distant metastasis			
Absent	21	11 (52.4)	0.001
Present	42	38 (77.8)	
Cisplatin resistance			
Yes	27	25 (92.6)	0.014
No	36	24 (66.7)	

a. T-cadherin (-) denotes low expression of T-cadherin protein.

b. Pearson's $\chi 2$ test or Fisher's exact test was done to determine the statistical significance.

Figures

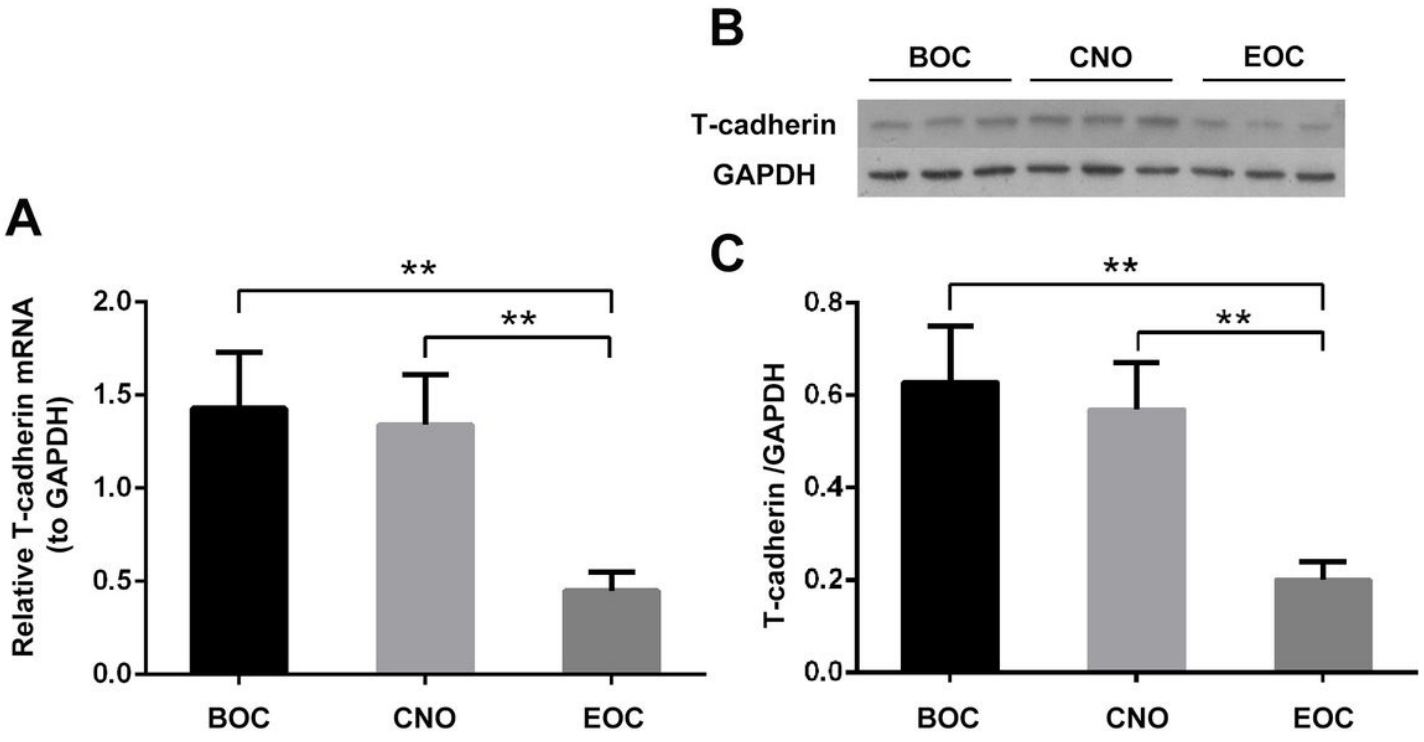

Figure 1

Decreased expression of T-cadherin mRNA and protein in epithelial ovarian cancer tissues from patients with epithelial ovarian cancer. (A) T-cadherin mRNA expression levels detected by quantitative real-time RT-PCR in epithelial ovarian cancer tissues from 41 patients with epithelial ovarian cancer, in relevant contralateral normal ovarian (CNO) tissues from 29 patients with epithelial ovarian cancer and in benign ovarian tumour fresh tissues (BOC) from 35 patients with benign ovarian tumour. (B) Representative Western blot of T-cadherin protein and GAPDH from different tissues. (E) Densitometric analysis of T-cadherin protein levels in different tissues detected by Western blotting. Data are presented as the mean ± SD. ** $P < 0.01$.

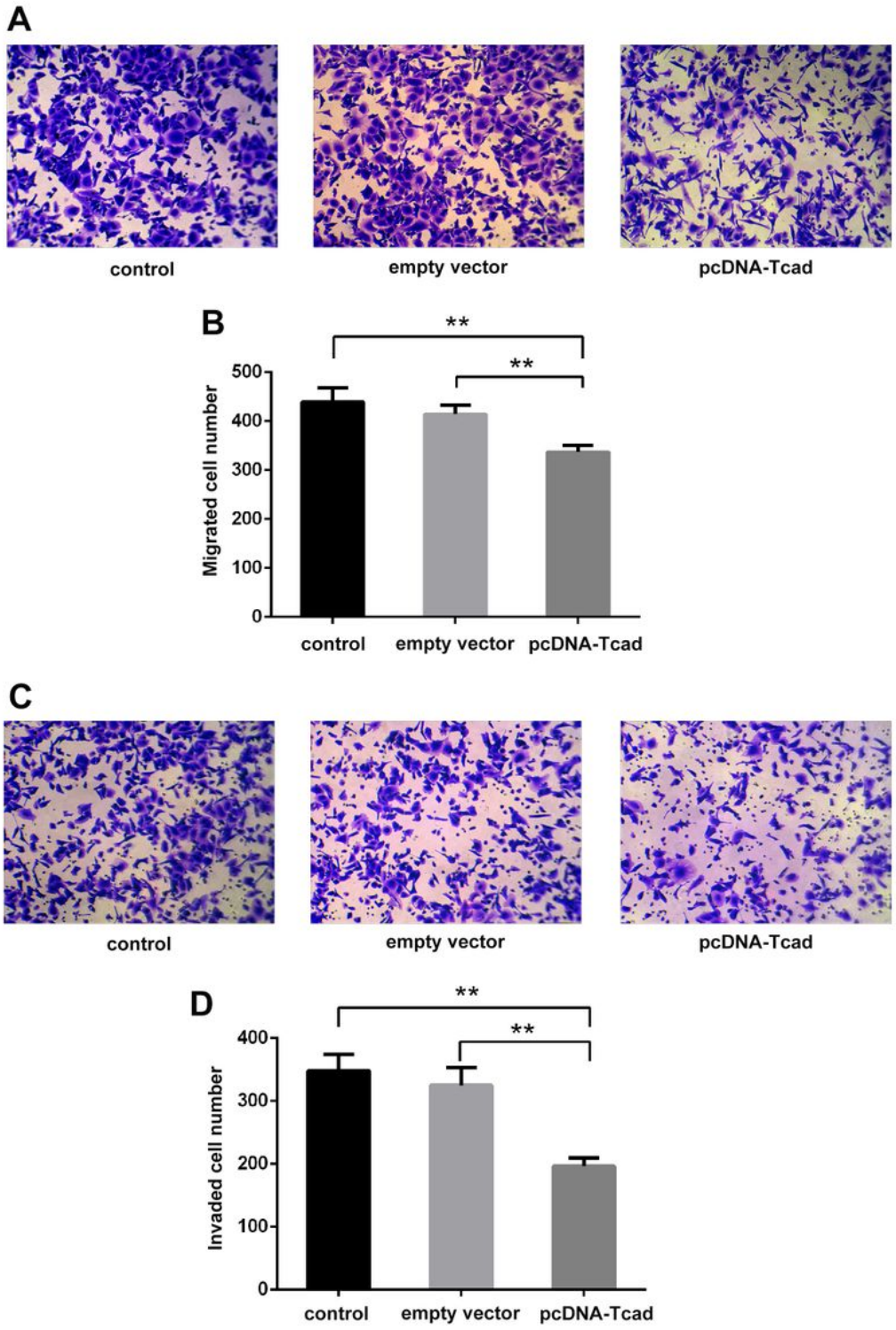

Figure 2

Decreased migration and invasion ability of the epithelial ovarian cancer cell line A2780 in response to overexpression of T-cadherin with pcDNA-T-cadherin. The A2780 cells were incubated in the control medium (control, no additives) or transfected with 20 μM pcDNA-T-cadherin (pcDNA-Tcad) or pcDNA3.1 (empty vector) in the presence of the transfection agent lipofectamine (5 μl/ml) for 48 h before detection of migration ability by the Transwell assay. (A) Images showing the migrated A2780 cells on the lower

surface of the Transwell membranes. (B) Number of migrated A2780 cells in different groups in five random fields under the microscope. (C) Images showing the migrated A2780 cells on the lower surface of the Transwell membranes. (D) Number of invaded A2780 cells in different groups in five random fields under the microscope. ** P < 0.01.

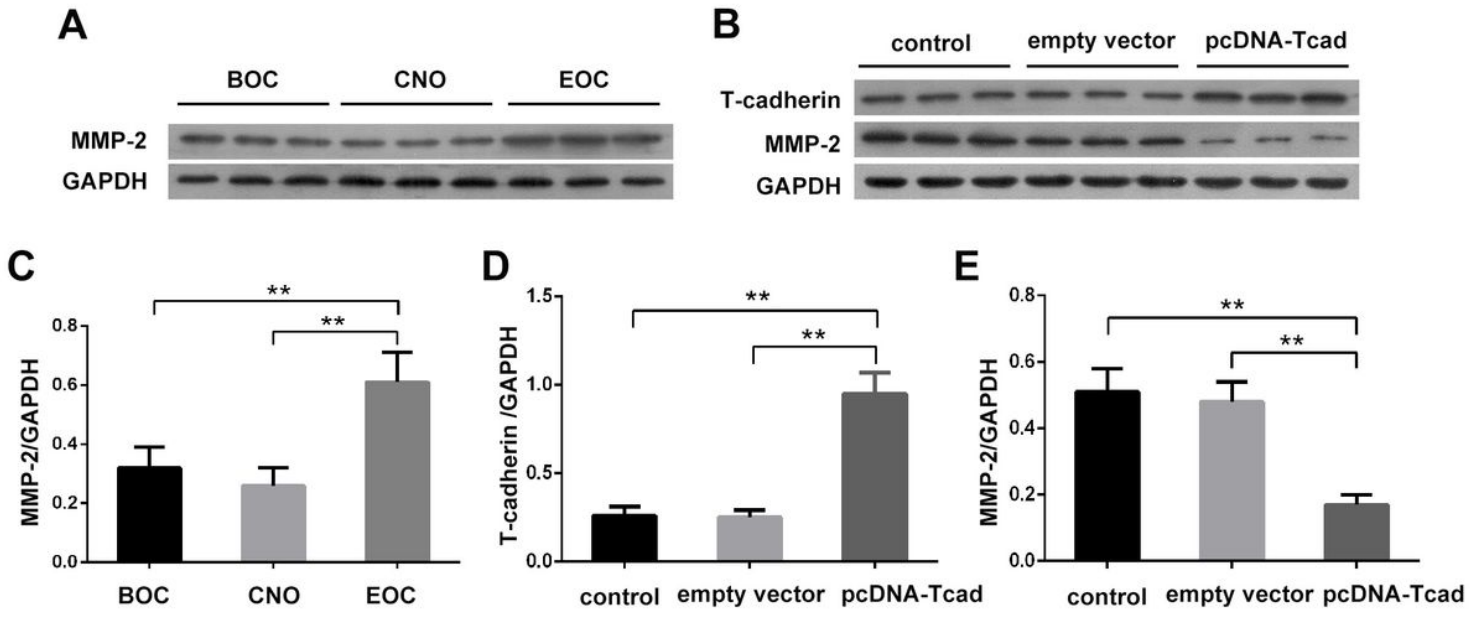

Figure 3

Upregulation of MMP-2 expression in the epithelial ovarian cancer tissues of epithelial ovarian cancer patients. Overexpression of T-cadherin suppressed MMP-2 expression in EOC cells. (A and C) Representative Western blot and densitometric analysis of the expression levels of T-cadherin and MMP-2 proteins in BOC, CNO and EOC tissues. (B, D and E) Representative Western blot and densitometric analyses of the protein expression levels of T-cadherin and MMP-2 in A2780 epithelial ovarian cancer cells overexpressing T-cadherin and pcDNA-T-cadherin (mean ± SD, ** P < 0.01).

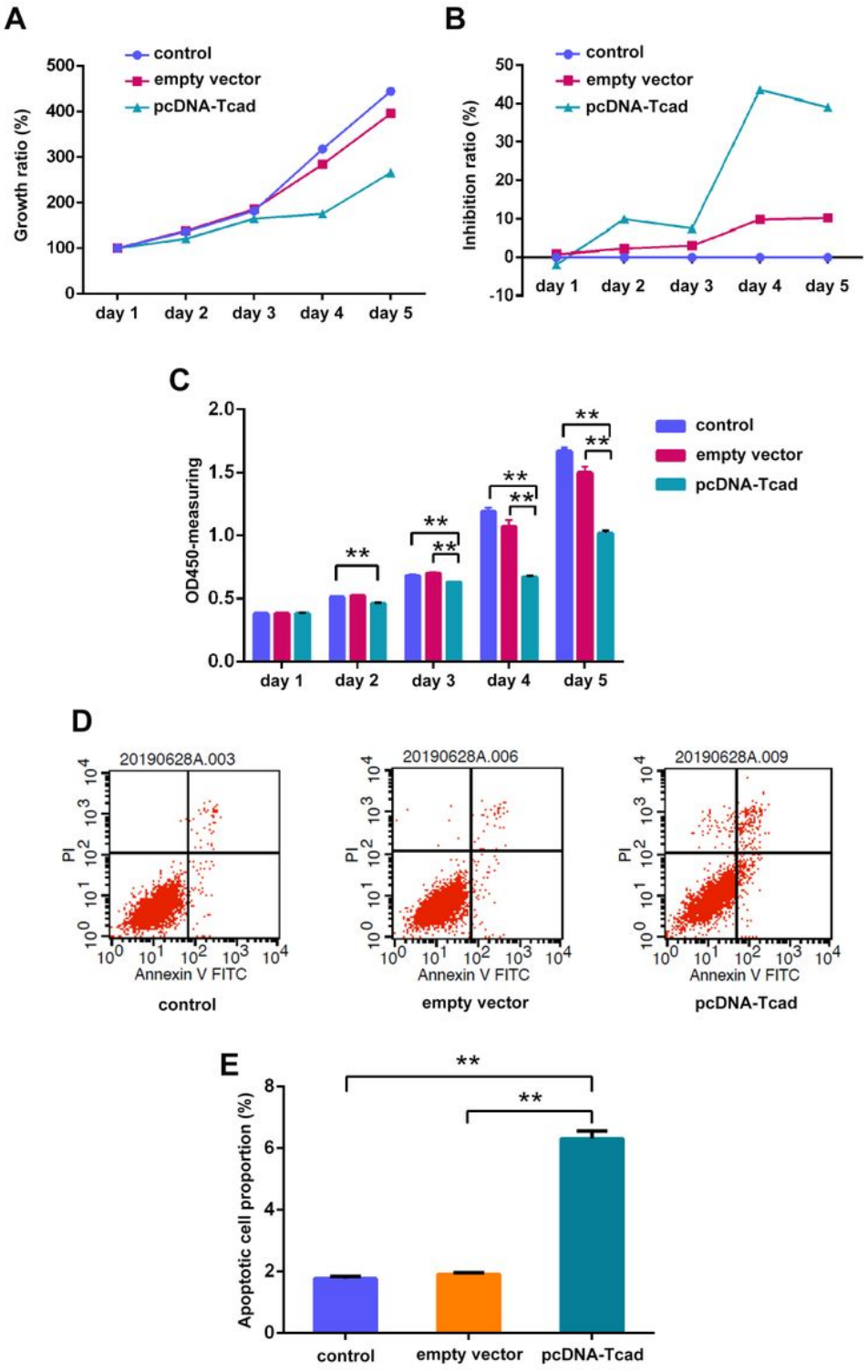

Figure 4

Overexpression of T-cadherin suppressed EOC cell line proliferation and facilitated EOC cell line apoptosis. (A) The growth rate and inhibition rate of A2780 cells with or without paclitaxel in the control, empty vector and pcDNA-Tcad groups with or without paclitaxel were detected by CCK-8 assay. (B) Colony formation assay and quantification analysis of A2780 cells in the control, empty vector and pcDNA-Tcad groups. (C) Quantification of proliferation A2780 cells in the control, empty vector and

pcDNA-Tcad groups. (D) Flow cytometry analysis of annexin V and PI staining in A2780 cells in the control, empty vector and pcDNA-Tcad groups. (E) Quantification of apoptotic A2780 cells in the control, empty vector and pcDNA-Tcad groups. **P < 0.01.

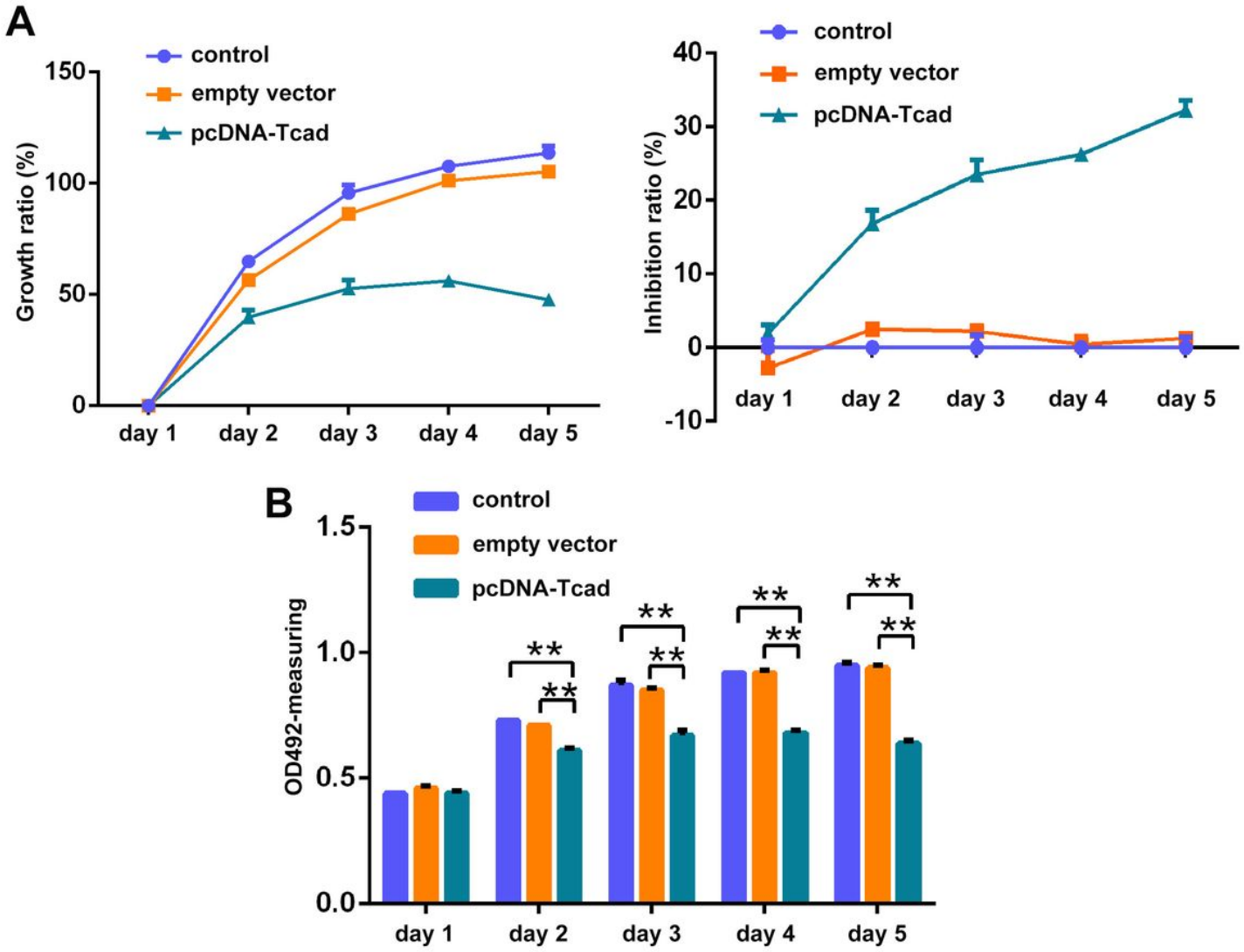

Figure 5

Overexpression of T-cadherin increases the sensitivity of EOC cells to paclitaxel. (A) The growth and inhibition rates of A2780 cells with or without paclitaxel in the control and the empty vector and pcDNA-Tcad groups with or without paclitaxel were detected. (B) Quantification of A2780 cells with or without paclitaxel in the control, empty vector and pcDNA-Tcad groups. **P <0.01.