# T-cadherin deficiency promotes endometriosis progression through the PI3K/AKT/mTOR signaling pathway

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# Abstract

**Background:** Endometriosis is a progressive and benign disease characterized by the presence of endometrial glands and stroma tissue outside of the uterine cavity. Though endometriosis is a benign disease, it has the characteristics of malignant tumour growth. Abnormal expression of T-cadherin is involved in the occurrence and progression of many tumours. We aimed to investigate whether T-cadherin promotes the migration and invasion of endometriosis cells through the PI3K/AKT/mTOR signaling pathway.

**Methods:** Ectopic and eutopic endometrial samples from 62 female patients with endometriosis and endometrial samples from 51 female patients without endometriosis were collected. The immortalized endometrial stromal cell line hEM15A was cultured. Real-time RT-PCR, immunohistochemistry and Western blot were used to detect the expression of T-cadherin, phospho-PI3K/Akt/mTOR and matrix metalloproteinase 2 (MMP-2). Transfection technology was employed to upregulate T-cadherin expression. The migration and invasion abilities of hEM15A cells were measured by the transwell assay with uncoated or Matrigel-coated membranes.

**Results:** The mRNA and protein expression of T-cadherin was significantly decresed in the ectopic tissues of the patients with endometriosis, while the mRNA and protein expression in the eutopic endometrial tissues of the same patients did not significantly differ from that in the patients without endometriosis. The migration and invasion ability and phospho-PI3K/Akt/mTOR and MMP-2 expression levels were decreased in hEM15A cells with high T-cadherin expression compared with the corresponding parameters in the normal control group. However, everolimus and BEZ235 inhibited cell migration and invasion in cells with low T-cadherin expression, and weakened overexpression of T-cadherin significantly attenuated MMP-2 protein expression.

**Conclusion:** Loss of T-cadherin promotes cell migration and invasion in endometriosis via the PI3K/AKT/mTOR signalling pathway.


# Background

Endometriosis is a progressive and benign disease characterized by the presence of endometrial glands and stroma tissue outside of the uterine cavity. The main clinical symptoms include severe dysmenorrhea, pelvic pain, and infertility, and endometriosis affects millions of women worldwide (Guan, et al., 2016). The actual causes of endometriosis remain largely controversial, although numerous studies have been conducted. The most widely accepted hypothesis is that endometrial tissue that was shed refluxed to the peritoneal cavity via the fallopian tubes during menstruation and that these ectopic endometrial cells infiltrated into the subperitoneum(Mate, et al., 2018). However, although up to 90% of women experienced retrograde menstruation during their menstrual cycles, most of them had no history of endometriosis (Basta, et al., 2014). This indicates that some other factors may participate in the pathogenetic process of endometriotic lesion formation.

An unusual characteristic of endometriosis is the strong invasive ability of endometriotic cells to migrate and invade into the pelvic organs and tissues(Lagana, et al., 2017). Though endometriosis is a benign disease, it has the characteristics of malignant tumour growth(Jiang, et al., 2018). Studies have demonstrated that cadherin, a large superfamily of cell-cell adhesion molecules crucial for cell tight connections, plays a vital function in cancer evolution and distant metastasis(Fulga, et al., 2015). Because there are many similarities between endometriosis and cancer, we hypothesize that cadherin is involved in the pathogenesis of endometriosis.

T-cadherin is a unique member of the cadherin superfamily that attaches to the plasma membrane by a glycosylphosphatidylinositol anchor, lacks an intracellular domain and is involved in adjusting and controlling tumour cell migration, invasion, neovascularization, metastatic potential and proliferation. Numerous studies have shown that abnormal expression of T-cadherin is involved in the occurrence and progression of many tumours (Nicolas, et al., 2017, Pfaff, et al., 2011, Tang, et al., 2012). The decreased of T-cadherin expression may be connected with an increased risk of malignant development and may therefore be a potential biomarker for certain types of cancer(Wang, et al., 2013). Re-expression of T-cadherin can inhibit cell proliferation and invasion, augment apoptosis susceptibility and inhibit tumour growth(Lin, et al., 2013). These results suggest that the deletion of T-cadherin may play an important role in the progression of multiple diseases.

The dysfunction of the PI3K/Akt/mTOR pathway plays a key role in regulating tumour cell growth, survival, invasion and migration, as well as drug resistance (Czyzykowski, et al., 2016). Previous studies have verified that the PI3K/Akt/mTOR signalling pathway participates in the pathophysiology of endometriosis (Pavlidou and Vlahos, 2014). However, the specific regulatory mechanism of PI3K/Akt in endometriosis has not been elucidated.

The function of T-cadherin in endometriosis development remains to be further clarified. In this study, the effect of T-cadherin on the migration and invasion of endometriotic endometrial stromal cells (ESCs) and the connection between T-cadherin and the PI3K/Akt/mTOR pathway were investigated.

# Methods

### Ethical approval

This research conforms to and was conducted in accordance with the Enhancing the QUAlity and Transparency Of health Research guidelines (http://www.equatornetwork.org/). All fresh human tissue specimens were collected after informed consent was obtained from the patients on the basis of the request of the Foshan First People's Hospital Scientific Research Ethics Committee in the years from December 2017 to August 2018.

### Tissue sample collection

According to the requirements of the First People's Hospital of Foshan Ethics Committee, all fresh tissue specimens were acquired with the patients' informed consent in the years from 2017 to 2018. Sixty-two Chinese-Han premenopausal women from 24-46 years of age with endometriosis who had a normal menstrual cycle of between 24 and 30 days were recruited for this study. All of the patients who were undergoing a laparoscopic procedure due to pain or other benign indications did not receive any hormone therapy or use an intrauterine device. Endometriosis was diagnosed by ultrasonography. The ectopic endometriosis lesion (ectopic) and endometrium (eutopic) tissues from surgical excision were confirmed by postoperative pathological examination. The study excluded patients with any other pelvic diseases that may affect the data.

Fifty-one Chinese-Han women without endometriosis underwent laparoscopic surgery for uterine fibroids and were included as a control group (mean age: 39.1, range: 36-45 years). All patients were confirmed to not have endometriosis by laparoscopic examination.

We make an appointment with patients and the menstrual cycle was determined in terms of the menstrual cycle date and endometrial histological analysis. On the day of the laparoscopic examination, 54.8% (34/62) and 47.1% (24/51) of the patients in the endometriosis group and in the control group were in the proliferative phases of the menstrual cycle, respectively. On the day of the laparoscopy, 45.2% (28/62) and 52.9% (27/51) of the women in the endometriosis group and the patients in the control group were in the secretory phases of the menstrual cycle, respectively. Menstruating women were not included in the study. Fresh samples were separated for immunohistochemistry, total RNA extraction and protein extraction.

### Immunohistochemistry

T-cadherin protein expression from a small series of ectopic, eutopic and control endometrial tissue specimens from the pathology file of the First People's Hospital of Foshan was explored by immunohistochemistry in accordance with the standard protocols as described previously. The primary antibodies used were mouse anti-T-cadherin antibodies (According to the product specification and our pre-test, the antibody was diluted 1:500; ab86192, Abcam, USA) and the secondary antibody incubated with the biotinylated (According to the product specification and our pre-test, the antibody was diluted 1:1000; Zhongshan Golden Bridge Inc., China). Immunohistochemical scoring in each section was performed using Image-Pro Plus.

### Western blotting

Ectopic, eutopic and control endometrial tissue proteins were evaluated by using a BCA assay kit (SinoBio Biotech) to measure the protein concentration, which was performed following the manufacturer's instructions. Equal amounts of protein were analysed by electrophoresis on 8% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA). The membranes were blocked using 5% skim milk for 2 h at room temperature. Subsequently, the membranes were incubated with monoclonal mouse anti-T-cadherin (ab36905) and polyclonal antibody against PI3K (ab32089), p-PI3K

(ab138364), Akt (ab126433), p-Akt (ab8933) and MMP-2 (ab97779) (Cambridge, UK) or the mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (diluted 1:1000, Beyotime Biotechnology Inc.) overnight at 4°C. The membranes were then incubated in the HRP-conjugated secondary antibody for 2 h at room temperature. The proteins were measured by using the ECL system (CWBIOTECH, China) with the ChemiDoc XRS system (Bio-Rad, Philadelphia, USA).

### Quantitative real-time PCR

A consecutive series of 113 fresh ectopic, eutopic and control endometrial tissue samples were obtained from the Department of Gynecology, the First People's Hospital. The mRNA expression levels in the fresh cervical tissues and cultured cells were detected with real-time RT-PCR (CFX96 Real-Time System, Bio-Rad, USA) by using the SYBR Green fluorescence signal test kit according to the manufacturer's instructions. Real-time RT-PCR quantified the mRNA expression levels and was carried out in triplicate for each sample. The cycling conditions were set at 95°C for 5 minutes, followed by 40 cycles at 95°C for 10 s, 60°C for 5 s and 72°C for 15 s. The sequences of the primer pairs 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 amplified 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 measure the relative gene expression levels (fold change), and all the tests were performed at least three times(Guan, et al., 2019).

### Cell culture

The immortalized endometrial stromal cell line hEM15A was purchased from GuangZhou Jennio Biotech Co., Ltd. and was maintained in cell culture. Cells were cultured with DMEM (GIBCO) containing 10% foetal bovine serum (HyClone, USA) and two antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) (GIBCO). The cells were incubated in a humidified atmosphere with 5% CO2 at 37°C. The cell lines were authenticated.

### Transfection Experiments

The pcDNA3.1 and pcDNA-T-cadherin plasmids were purchased and transfected into hEM15A cell lines according to the manufacturer's instructions. After 48 h of cell transfection, T-cadherin expression was detected by qRT-PCR and Western blotting.

### Cell migration and invasion

For the migration assay, hEM15A cells were seeded into 24-well Transwell chambers at a density of $4x10^5$ per well with 200 μl FBS-free medium in the upper chambers (8 μm, BD, Bedford, MA, USA). For the invasion assays, in addition to precoating the transwell membranes with 24 μg/μl Matrigel (R&D Systems, USA), the rest of the steps were analogous to those used in the cell migration tests, and $4x10^5$ cells were plated in the upper compartment. The lower wells were filled with 500 μl culture medium, and

30% foetal bovine serum was added to induce cell migration and invasion. The cells on the above surface of the filter were wiped off with a cotton swab after 24 h of culture. The cells on the submembrane surface were fixed with 4% ethanol polyformaldehyde for 20 minutes, stained with 0.1% crystal violet for 2 minutes, and photographed with a CCD camera under a microscope. Five fields were selected at random, and the number of cells in each field was counted.

### Statistical analysis

All data are represented by the mean ± standard deviation (SD). ANOVA was used for statistical comparison. Statistical analysis was performed by using SPSS 16.0 software (SPSS Inc., Chicago, USA). *P* values <0.05 were considered significant.

## Results

### Decreased T-cadherin mRNA expression in endometriosis tissues

The gene expression levels of T-cadherin in ectopic, eutopic and normal endometrial tissues was measured by qPCR. As shown in Figure 1A, compared with the control and eutopic endometrial groups, T-cadherin mRNA expression was significantly decreased in the ectopic endometriotic specimens from the patients with endometriosis (n=62, $P < 0.05$). In total, 74.2% (46 out of 62 patients) of the ectopic endometrial samples showed reduced T-cadherin expression, and 25.8% of the ectopic samples (16/62) did not show obvious changes. Compared with the normal control group, there was no significant change in the expression of T-cadherin in the endometrium of patients with endometriosis (Figure 1A, $P>0.05$). These data indicate that the mRNA expression level of T-cadherin decreases in patients with endometriosis and is closely related to the development of endometriosis.

### Decreased T-cadherin protein expression in endometriotic tissues

The gene and protein expression levels of T-cadherin were tested by immunohistochemistry and Western blotting, respectively. The data acquired from the paraffin-embedded tissue samples by immunohistochemistry showed that the T-cadherin protein expression levels were decreased in the ectopic endometrial samples (n=62, $P < 0.01$, Figure 1B, C) compared with the expression in the eutopic and normal control samples. T-cadherin protein expression was significantly decreased in 46 of the 62 patients.

The decreased T-cadherin protein expression was further confirmed by Western blotting (Figure 1D, E). Low protein expression of T-cadherin could be distinctly measured in the ectopic tissue samples. Conversely, T-cadherin proteins were expressed at high levels in the normal endometria of patients without endometriosis and those with endometriosis.

To summarize the above research results, these data indicate that T-cadherin expression decreased in ectopic endometrial tissues of patients with endometriosis, suggesting that T-cadherin may be involved in the progression of endometriosis.

### The correlation between T-cadherin expression and hEM15A cell migration

Our experiments confirmed that the ectopic tissues from patients with endometriosis express low levels of T-cadherin proteins. We wanted to determine whether there is a relationship between the enhanced migration of ectopic endometrial stromal cells and low expression of T-cadherin. To test this hypothesis, we investigated the relationship between T-cadherin expression and hEM15A cell migration using plasmid transfection technology. The migration ability of hEM15A cells was detected through the transwell migration assay under different treatment conditions (Figure 2). HEM15A cells were transfected with lipofectamine for 48 h in the control medium or transfected with pcDNA-Tcad or negative control pcDNA3.1 (NC). The results showed that the number of hEM15A cells transfected with pcDNA-Tcad decreased significantly ($P<0.01$ vs control or NC; Figure 2). The negative control pcDNA3.1 had no significant effect on cell migration ($P>0.05$ vs control). These results indicate that T-cadherin is a key protein that regulates ESC migration.

### The correlation between T-cadherin expression and hEM15A cell invasion

The relationship between T-cadherin expression and hEM15A cell invasion was analysed using the plasmid transfection technique and an invasion assay with Matrigel-precoated transwell filters under different treatment conditions (Figure 3). The hEM15A cells were incubated with the control medium or negative control pcDNA3.1 (NC) or transfected with 20 μM pcDNA-Tcad for 48 h in the presence of the transfection agent lipofectamine. As shown in diagram 5A, the number of hEM15A cells that had invaded across the Matrigel-precoated transwell filters was significantly reduced ($P<0.01$ vs NC and control; Figure 3). The negative control pcDNA3.1 had no significant effect on hEM15A invasion ability ($P>0.05$ vs control). These data proved that T-cadherin plays a vital role in ESC invasion.

### T-cadherin effects hEM15A cell migration and invasion via the PI3K/Akt/mTOR pathway

It was shown in this study that T-cadherin reduces hEM15A cell migration and invasion; therefore, we investigated the underlying molecular mechanism. T-cadherin is believed to affect cell function mainly through signal transduction because the molecule is attached to the cell membrane by glycosylphosphatidylinositol (GPI) molecules. Which signalling pathway is activated by T-cadherin? It has been reported that T-cadherin is involved in the regulation of the activity of the PI3K/Akt/mTOR signalling pathway in endothelial cells(Barra, et al., 2018), and on the other hand, AKT is known to take part in regulating cell migration and invasion(Yu, et al., 2017).

To confirm whether the PI3K/Akt/mTOR signalling pathway regulates the migration and invasion of hEM15A cells, we evaluated the antitumour effects of PI3K/Akt/mTOR using the mTOR inhibitor everolimus and the dual inhibitor BEZ235 in paired hEM15A cells with reduced T-cadherin expression. The two drugs inhibit cell migration and invasion in cells with low T-cadherin expression, indicating that the targeted action of the PI3K/Akt/mTOR pathway, especially double PI3K/mTOR inhibition, may be effective on hEM15A cells (Figure 4). These data indicate that T-cadherin, by deregulating the PI3K/AKT/mTOR signalling pathway, increases hEM15A migration and invasion.

### Upregulation of MMP-2 expression and its negative correlation with T-cadherin via the PI3K/Akt/mTOR pathway in hEM15A

Cell invasion ability is closely related to MMPs. MMP-2 plays a vital role in the progression of endometriosis (Jin, et al., 2014). Our research showed that T-cadherin expression decreased in the ectopic tissues of the endometriosis patients and was involved in hEM15A cell invasion. Further studies revealed the MMP-2 protein expression in different endometrial specimens, and its relationship with T-cadherin was examined by Western blot and transfection technology. Contrary to the downregulation of T-cadherin expression, the expression of MMP-2 protein was increased in the ectopic tissue specimens from 46 patients with endometriosis (out of 62 patients, Figure 5A, B). The average expression level of MMP-2 protein in the ectopic specimens (62 endometriosis patients) was higher than that in the eutopic tissues from the patients with endometriosis and the control endometrial specimens from the patients without endometriosis ($P<0.01$).

Using pcDNA-Tcad to overexpress T-cadherin in hEM15A cells, the levels of phospho-PI3K, phospho-AKT and mTOR phosphorylation were significantly reduced compared with the levels in the control ($P<0.01$; Figure 5 C-G). Moreover, the level of MMP-2 was markedly reduced ($P<0.01$; Figure 5 H). In contrast, the level of phospho-PI3K/Akt/mTOR was not significantly different in the pcDNA-NC group compared with the normal control group ($P>0.05$; Figure 5 I, J). Thus, T-cadherin clearly regulates the PI3K/AKT/mTOR signalling pathway. However, everolimus and BEZ235 weakened the overexpression of T-cadherin and significantly increased MMP-2 protein expression, suggesting that T-cadherin can regulate the protein expression level of MMP-2 via the PI3K/Akt/mTOR pathway in hEM15A cells. These data indicate that T-cadherin, by deregulating the PI3K/AKT/mTOR signalling pathway, reduces hEM15A cell migration and invasion.

## Discussion

Despite its potent and extensive tumour inhibition effects related to a series of tumour cellular activities, the functions of T-cadherin in the progression of endometriosis have not been illuminated. In the present study, we identified a novel T-cadherin/PI3K/AKT/mTOR functional axis as a neoteric pathogenic pathway in endometriosis. T-cadherin downregulation may accelerate endometriosis development by expediting the migration and invasion of ESCs via the T-cadherin/PI3K/AKT/mTOR signalling pathway.

T-cadherin silencing has been shown to be a pivotal regulator of the evolution of tumour types, including hepatic, prostate, skin and breast cancers, by increasing cancer cell proliferation, migration, invasion and metastasis(Dasen, et al., 2017, Kong, et al., 2017, Philippova, et al., 2013, Yan, et al., 2008). However, the potential molecular biological function of T-cadherin in endometriosis is not completely clear. In this study, we discovered that the mRNA and protein expression levels of T-cadherin were dramatically lower in endometriotic cell lines and tissues compared with normal and eutopic endometrial tissues. Furthermore, we investigated the function of ectopic endometrial stromal cells to explore the biological functions of T-cadherin on cell migration and invasion in vitro. We found that T-cadherin regulated the

migration and invasion ability of immortalized endometrial stromal cells via the PI3K/Akt/mTOR pathway, indicating that T-cadherin is strongly related to the development of endometriosis.

The abnormal expression or function of cadherin is related to alterations of cellular migration and invasion and the transduction of cellular signalling pathways. Studies on cell adhesion molecules in endometriosis mainly focus on E- and N-cadherin, which are closely related to the process of epithelial-mesenchymal transition (EMT) in endometriosis(Mai, et al., 2019, Nguyen, et al., 2017). T-cadherin is a novel adhesion molecule member in the cadherin family that anchors glycoproteins on the cell surface and that is responsible for cell selective recognition and adhesion; in addition, T-cadherin plays a crucial role in cell migration and invasion(Lin, et al., 2017). Experiments have shown that T-cadherin was downregulated in ovarian cancer, lung cancer, cervical cancer, bladder cancer and prostate cancer(Andreeva and Kutuzov, 2010). Moreover, T-cadherin can regulate cancer progression by regulating cancer cell proliferation and migration (Bosserhoff, et al., 2014). Research has shown that the re-overexpression of T-cadherin can suppress the migration and invasion of gastric cancer cells by upregulating the expression of E-cadherin and downregulating vimentin and MMP-2 expression(Lin, Chen, Huang, Chen, Ye, Lin and Wang, 2017). Compared with vascular smooth muscle cells (SMCs) expressing T-cadherin, SMCs lacking T-cadherin showed increased adhesion to extracellular matrix substrata and decreased expression of matrix metalloproteinases, several integrins, and collagens(Frismantiene, et al., 2018). Our present research demonstrated that T-cadherin plays a crucial role in the migration and invasion of ESCs in endometriosis, which may be related to the progression of endometriosis.

Cell migration and invasion are the main steps of endometriosis and are largely dependent on matrix degradation through matrix metalloproteinase (Hu, et al., 2018). How is T-cadherin involved in the progression of endometriosis? It is thought that cell function is affected by T-cadherin mainly via its signalling pathway rather than its adhesion properties because the molecule is attached onto the membrane via a glycosylphosphatidylinositol (GPI). T-cadherin mediates its effects by regulating cell signalling pathways. The in vitro findings suggested that the PI3K/Akt/mTOR pathway is a pivotal cellular signalling pathway that influences various cellular functions, including cell proliferation, growth, migration and invasion (Li, et al., 2018). PI3K regulates cell function by the phosphorylation of Akt and mTOR. This signalling pathway plays a vital role in migration and invasion and has been shown to be activated in multiple diseases including endometriosis (McKinnon, et al., 2016). It is widely believed that the PI3K/AKT/mTOR pathway is an ideal therapeutic target for various diseases(Barra, Ferro Desideri and Ferrero, 2018). In this study, we verified that the deletion of T-cadherin in endometrial cells can significantly increase the protein expression of p-Akt and p-mTOR, thus expediting the PI3K/Akt/mTOR signalling transduction cascade and facilitating endometriotic cell migration and invasion by increasing the expression of MMP-2 protein. This result is reversed when T-cadherin protein expression is increased. This finding indicates that the PI3K/Akt/mTOR signalling pathway participates in the pathogenesis of endometriosis-related migration and invasion and that loss of T-cadherin might be an initiating factor of endometriosis. Because the peritoneal microenvironment of EM patients has changed, the endometriotic cells can escape from immune surveillance and grow in ectopic sites(Herington, et al., 2011). Akt plays a key role in helping cells escape immune surveillance(Russell, et al., 2018). Increased expression of

phosphorylated AKT protein has been found in ectopic endometrial tissue in women with endometriosis, and the aberrant AKT signalling pathway induced the formation of ectopic lesion(Kim, et al., 2014). Excessive activation of AKT may also lead to decreased expression of progesterone receptor (PR) protein in ectopic endometriosis(Courtoy, et al., 2018). The expression of phosphorylated mTOR protein in ectopic lesions was higher than that in normal endometrial tissues in patients with endometriosis. The overactivation of the mTOR pathway may also be a function involved in changing the pelvic microenvironment in patients with endometriosis(McKinnon, Kocbek, Nirgianakis, Bersinger and Mueller, 2016). The activation of the PI3K/Akt/mTOR signalling pathway significantly upregulated the expression of MMP-2 to promote melanoma cell invasion and migration(Zhang, et al., 2017). Inhibition of PI3K/Akt/mTOR signalling pathway activation can suppress cell growth, migration, and invasion(Bi, et al., 2018).

# Conclusion

In summary, we demonstrated that decreased T-cadherin expression can be detected in human endometriotic tissues. Our study not only confirmed the importance of low T-cadherin in promoting the progression and metastasis of endometriosis but also provided detailed evidence for its pathogenesis. Low T-cadherin increases cell migration and invasion by upregulating the expression of MMP-2 by activating the PI3K/Akt/mTOR signalling pathway. These findings further our understanding of the complex mechanisms of endometriosis and provide potential new targets for the therapy of patients with endometriosis.

# Abbreviations

PI3K：Phosphatidylinositol-3-hydroxykinase; AKT: Threonine kinase; mTOR: mammalian target of rapamycin; ESCs: Endometriotic endometrial stromal cells; RNA: Ribonucleic acid; SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis; PCR: Polymerase chain reaction; GPI: Glycosylphosphatidylinositol; MMP: Matrix metalloproteinase; EMT: Epithelial-mesenchymal transition; SMCs: Smooth muscle cells; EM: Endometriosis; PR: Progesterone receptor.

# Declarations

### Ethics approval and consent to participate

Human studies were approved by the First People's Hospital of Foshan Ethics Committee.

### Consent for publication

Not applicable.

### Availability of supporting data

All data generated or analysed during this study are included in this published article and its supplementary information files.

### Competing interests

No conflicts of interest, financial or otherwise, are declared by the authors.


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### Authors' contributions

Yu-tao Guan, Li-ning Cui, Yong Xie and Gang Wang designed the research. Fu-bin Zhang, Wei-feng Li, Xiao-lu Zhang, Yang-ping Chen and Li-jiang Xu performed experiments and analyzed data. Yan-qing Huang Li-wei Wang and Li-xin Chen contributed to sample collection. Ling-ling Zhou performed pathology detection. All authors read and approved the final manuscript.


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# References


Andreeva AV, Kutuzov MA. Cadherin 13 in cancer. *Genes, chromosomes & cancer* 2010;49: 775-790.

Barra F, Ferro Desideri L, Ferrero S. Inhibition of PI3K/AKT/mTOR pathway for the treatment of endometriosis. *British journal of pharmacology* 2018;175: 3626-3627.

Basta P, Koper K, Kazmierczak W, Wisniewski M, Makarewicz A, Dutsch-Wicherek M, Kojs Z, Popiela TJ, Slusarz R, Dubiel M *et al.* The biological role of Treg cells in ectopic endometrium homeostasis. *Histol Histopathol* 2014;29: 1217-1233.

Bi QJ, Men XJ, Han R, Li GL. LHX6 inhibits the proliferation, invasion and migration of breast cancer cells by modulating the PI3K/Akt/mTOR signaling pathway. *Eur Rev Med Pharmacol Sci* 2018;22: 3067-3073.

Bosserhoff AK, Ellmann L, Quast AS, Eberle J, Boyle GM, Kuphal S. Loss of T-cadherin (CDH-13) regulates AKT signaling and desensitizes cells to apoptosis in melanoma. *Molecular carcinogenesis* 2014;53: 635-647.

Courtoy GE, Donnez J, Marbaix E, Barreira M, Luyckx M, Dolmans MM. Progesterone Receptor Isoforms, Nuclear Corepressor-1 and Steroid Receptor Coactivator-1 and B-Cell Lymphoma 2 and Akt and Akt Phosphorylation Status in Uterine Myomas after Ulipristal Acetate Treatment: A Systematic Immunohistochemical Evaluation. *Gynecologic and obstetric investigation* 2018;83: 443-454.

Czyzykowski R, Polowinczak-Przybylek J, Potemski P. Nicotine-induced resistance of non-small cell lung cancer to treatment--possible mechanisms. *Postepy Hig Med Dosw (Online)* 2016;70: 186-193.

Dasen B, Vlajnic T, Mengus C, Ruiz C, Bubendorf L, Spagnoli G, Wyler S, Erne P, Resink TJ, Philippova M. T-cadherin in prostate cancer: relationship with cancer progression, differentiation and drug resistance. *The journal of pathology Clinical research* 2017;3: 44-57.

Frismantiene A, Kyriakakis E, Dasen B, Erne P, Resink TJ, Philippova M. Actin cytoskeleton regulates functional anchorage-migration switch during T-cadherin-induced phenotype modulation of vascular smooth muscle cells. *Cell adhesion & migration* 2018;12: 69-85.

Fulga V, Rudico L, Balica AR, Cimpean AM, Saptefrati L, Margan MM, Raica M. Differential expression of e-cadherin in primary breast cancer and corresponding lymph node metastases. *Anticancer research* 2015;35: 759-765.

Guan YT, Huang YQ, Wu JB, Deng ZQ, Wang Y, Lai ZY, Wang HB, Sun XX, Zhu YL, Du MM *et al.* Overexpression of chloride channel-3 is associated with the increased migration and invasion ability of ectopic endometrial cells from patients with endometriosis. *Human reproduction* 2016;31: 986-998.

Guan YT, Xie Y, Zhou H, Shi HY, Zhu YY, Zhang XL, Luan Y, Shen XM, Chen YP, Xu LJ *et al.* Overexpression of chloride channel-3 (ClC-3) is associated with human cervical carcinoma development and prognosis. *Cancer cell international* 2019;19: 8.

Herington JL, Bruner-Tran KL, Lucas JA, Osteen KG. Immune interactions in endometriosis. *Expert Rev Clin Immunol* 2011;7: 611-626.

Hu T, Yao M, Fu X, Chen C, Wu R. Polychlorinated biphenyl 104 promotes migration of endometrial stromal cells in endometriosis. *Toxicol Lett* 2018;290: 19-28.

Jiang J, Yu K, Jiang Z, Xue M. IL-37 affects the occurrence and development of endometriosis by regulating the biological behavior of endometrial stromal cells through multiple signaling pathways. *Biological chemistry* 2018;399: 1325-1337.

Jin A, Chen H, Wang C, Tsang LL, Jiang X, Cai Z, Chan HC, Zhou X. Elevated expression of CD147 in patients with endometriosis and its role in regulating apoptosis and migration of human endometrial cells. *Fertility and sterility* 2014;101: 1681-1687 e1681.

Kim TH, Yu Y, Luo L, Lydon JP, Jeong JW, Kim JJ. Activated AKT pathway promotes establishment of endometriosis. *Endocrinology* 2014;155: 1921-1930.

Kong DD, Wang MH, Yang J, Li L, Wang W, Wang SB, Zhou YZ. T-cadherin is associated with prognosis in triple-negative breast cancer. *Oncology letters* 2017;14: 2975-2981.

Lagana AS, Vitale SG, Salmeri FM, Triolo O, Ban Frangez H, Vrtacnik-Bokal E, Stojanovska L, Apostolopoulos V, Granese R, Sofo V. Unus pro omnibus, omnes pro uno: A novel, evidence-based, unifying theory for the pathogenesis of endometriosis. *Medical hypotheses* 2017;103: 10-20.

Li Y, Wu T, Wang Y, Yang L, Hu C, Chen L, Wu S. gamma-Glutamyl cyclotransferase contributes to tumor progression in high grade serous ovarian cancer by regulating epithelial-mesenchymal transition via activating PI3K/AKT/mTOR pathway. *Gynecologic oncology* 2018;149: 163-172.

Lin J, Chen Z, Huang Z, Chen F, Ye Z, Lin S, Wang W. Upregulation of T-cadherin suppresses cell proliferation, migration and invasion of gastric cancer in vitro. *Experimental and therapeutic medicine* 2017;14: 4194-4200.

Lin YL, He ZK, Li ZG, Guan TY. Downregulation of CDH13 expression promotes invasiveness of bladder transitional cell carcinoma. *Urol Int* 2013;90: 225-232.

Mai H, Wei Y, Yin Y, Huang S, Lin H, Liao Y, Liu X, Chen X, Shi H, Liu C *et al.* LINC01541 overexpression attenuates the 17beta-Estradiol-induced migration and invasion capabilities of endometrial stromal cells. *Systems biology in reproductive medicine* 2019: 1-9.

Mate G, Bernstein LR, Torok AL. Endometriosis Is a Cause of Infertility. Does Reactive Oxygen Damage to Gametes and Embryos Play a Key Role in the Pathogenesis of Infertility Caused by Endometriosis? *Front Endocrinol (Lausanne)* 2018;9: 725.

McKinnon BD, Kocbek V, Nirgianakis K, Bersinger NA, Mueller MD. Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics. *Human reproduction update* 2016;22: 382-403.

Nguyen HPT, Xiao L, Deane JA, Tan KS, Cousins FL, Masuda H, Sprung CN, Rosamilia A, Gargett CE. N-cadherin identifies human endometrial epithelial progenitor cells by in vitro stem cell assays. *Human reproduction* 2017;32: 2254-2268.

Nicolas A, Mohammedi K, Bastard JP, Fellahi S, Bellili-Munoz N, Roussel R, Hadjadj S, Marre M, Velho G, Fumeron F. T-cadherin gene variants are associated with nephropathy in subjects with type 1 diabetes. *Nephrol Dial Transplant* 2017;32: 1987-1993.

Pavlidou A, Vlahos NF. Endometriosis and ovarian cancer: clinical and molecular aspects. *Minerva endocrinologica* 2014;39: 155-165.

Pfaff D, Philippova M, Kyriakakis E, Maslova K, Rupp K, Buechner SA, Iezzi G, Spagnoli GC, Erne P, Resink TJ. Paradoxical effects of T-cadherin on squamous cell carcinoma: up- and down-regulation increase xenograft growth by distinct mechanisms. *The Journal of pathology* 2011;225: 512-524.

Philippova M, Pfaff D, Kyriakakis E, Buechner SA, Iezzi G, Spagnoli GC, Schoenenberger AW, Erne P, Resink TJ. T-cadherin loss promotes experimental metastasis of squamous cell carcinoma. *European journal of cancer* 2013;49: 2048-2058.

Russell L, Swanner J, Jaime-Ramirez AC, Wang Y, Sprague A, Banasavadi-Siddegowda Y, Yoo JY, Sizemore GM, Kladney R, Zhang J *et al.* PTEN expression by an oncolytic herpesvirus directs T-cell mediated tumor clearance. *Nature communications* 2018;9: 5006.

Tang Y, Dai Y, Huo J. Decreased expression of T-cadherin is associated with gastric cancer prognosis. *Hepato-gastroenterology* 2012;59: 1294-1298.

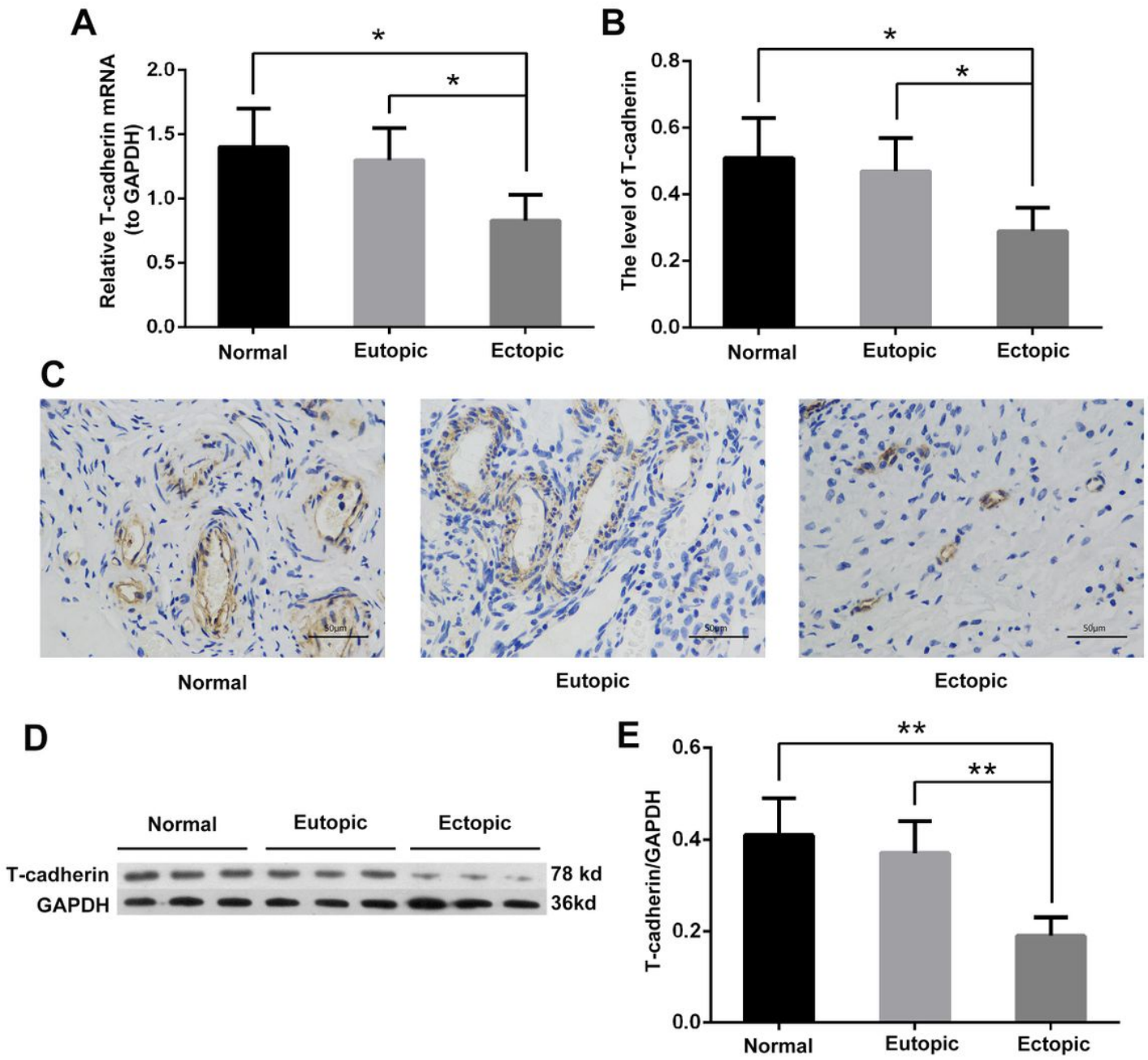
Wang L, Mou X, Xiao L, Tang L. T-cadherin expression in uterine leiomyoma. *Archives of gynecology and obstetrics* 2013;288: 607-614.

Yan Q, Zhang ZF, Chen XP, Gutmann DH, Xiong M, Xiao ZY, Huang ZY. Reduced T-cadherin expression and promoter methylation are associated with the development and progression of hepatocellular carcinoma. *International journal of oncology* 2008;32: 1057-1063.

Yu Y, Lv F, Liang D, Yang Q, Zhang B, Lin H, Wang X, Qian G, Xu J, You W. HOTAIR may regulate proliferation, apoptosis, migration and invasion of MCF-7 cells through regulating the P53/Akt/JNK signaling pathway. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2017;90: 555-561.

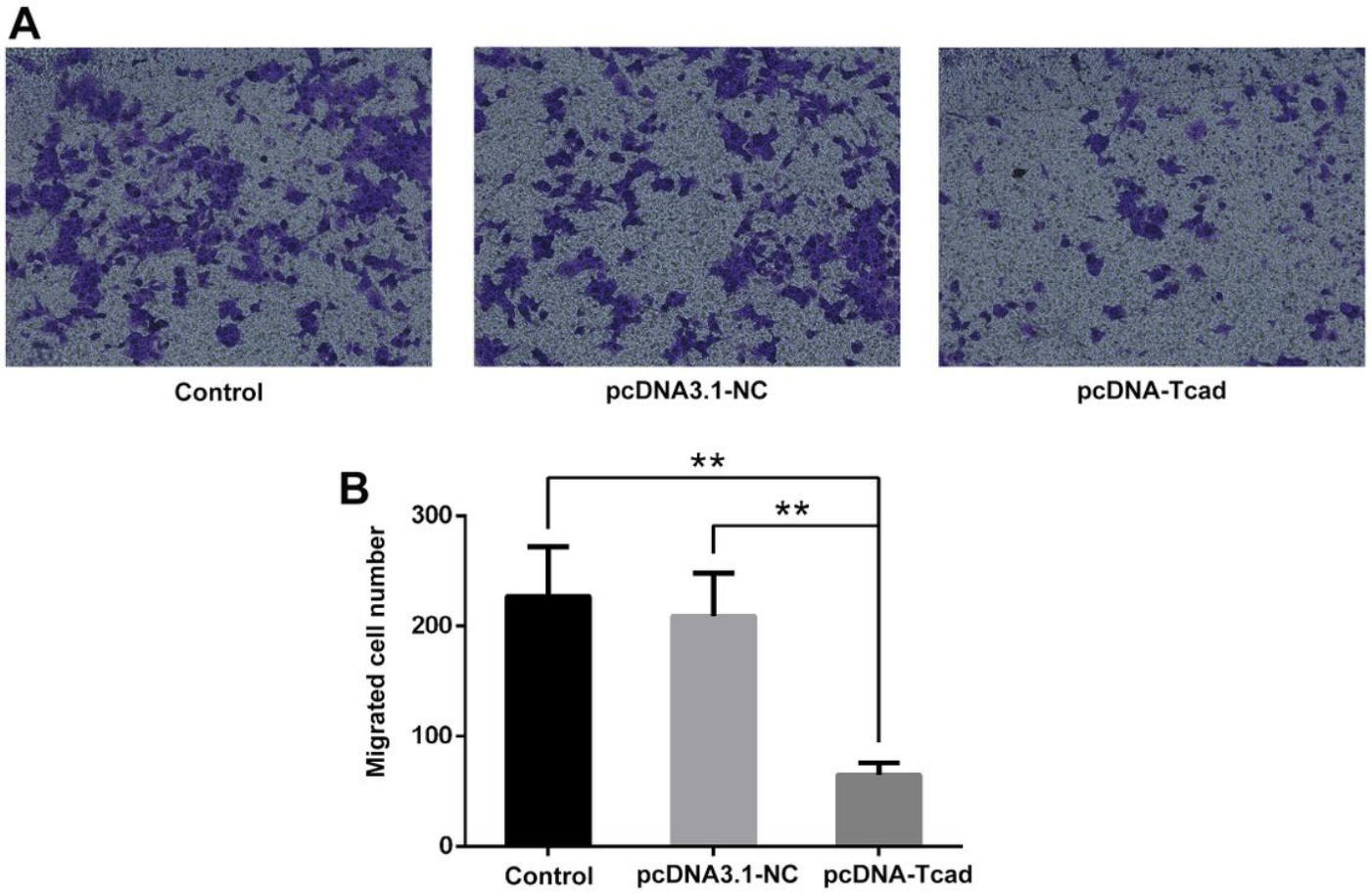
Zhang W, Yin L, Song G, Han X, Yin Z, Luo D. LKB1 loss cooperating with BRAF V600E promotes melanoma cell invasion and migration by up-regulation MMP-2 via PI3K/Akt/mTOR pathway. *Oncotarget* 2017;8: 113847-113857.

# Figures



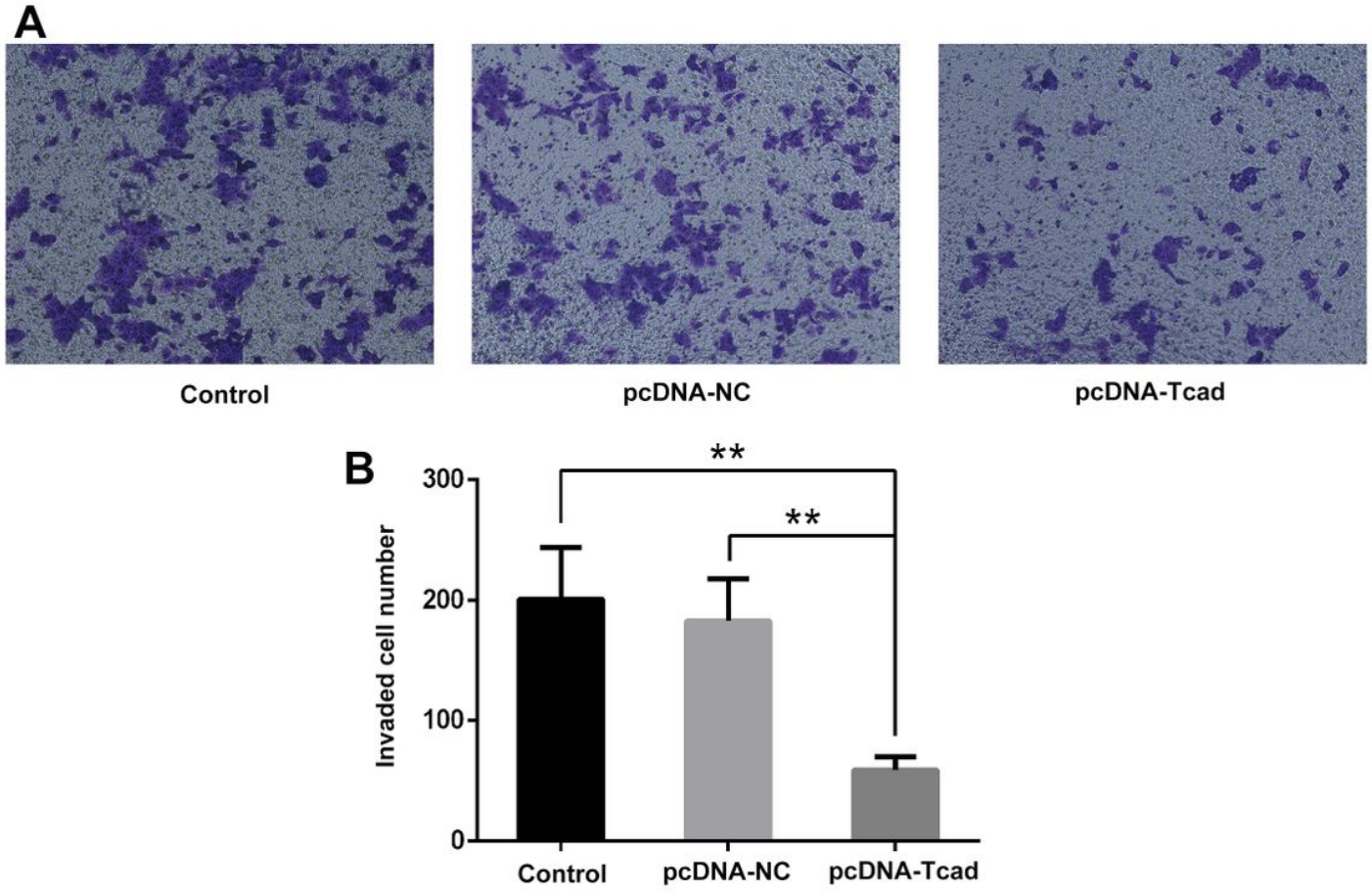

## Figure 1

Decreased expression of T-cadherin mRNA and protein in ectopic tissues from patients with endometriosis. (A) T-cadherin mRNA expression levels detected by quantitative real time RT-PCR in the ectopic and eutopic endometrial tissues from 62 patients with endometriosis and in the endometrial samples (normal) from 51 non-endometriotic patients. (B) Densitometric analysis of T-cadherin protein expression in different tissues by immunohistochemical staining. (C) Images of different tissues showing T-cadherin Immunohistochemical staining. Scale bars, 50 μm. (D) Representative Western blotting of T-cadherin protein and GAPDH from different tissues. (E) Densitometric analysis of T-cadherin protein levels in different tissues detected by Western blot. Data in A, B and E were mean ± SD (51-62 patients). * $P < 0.05$, ** $P < 0.01$.



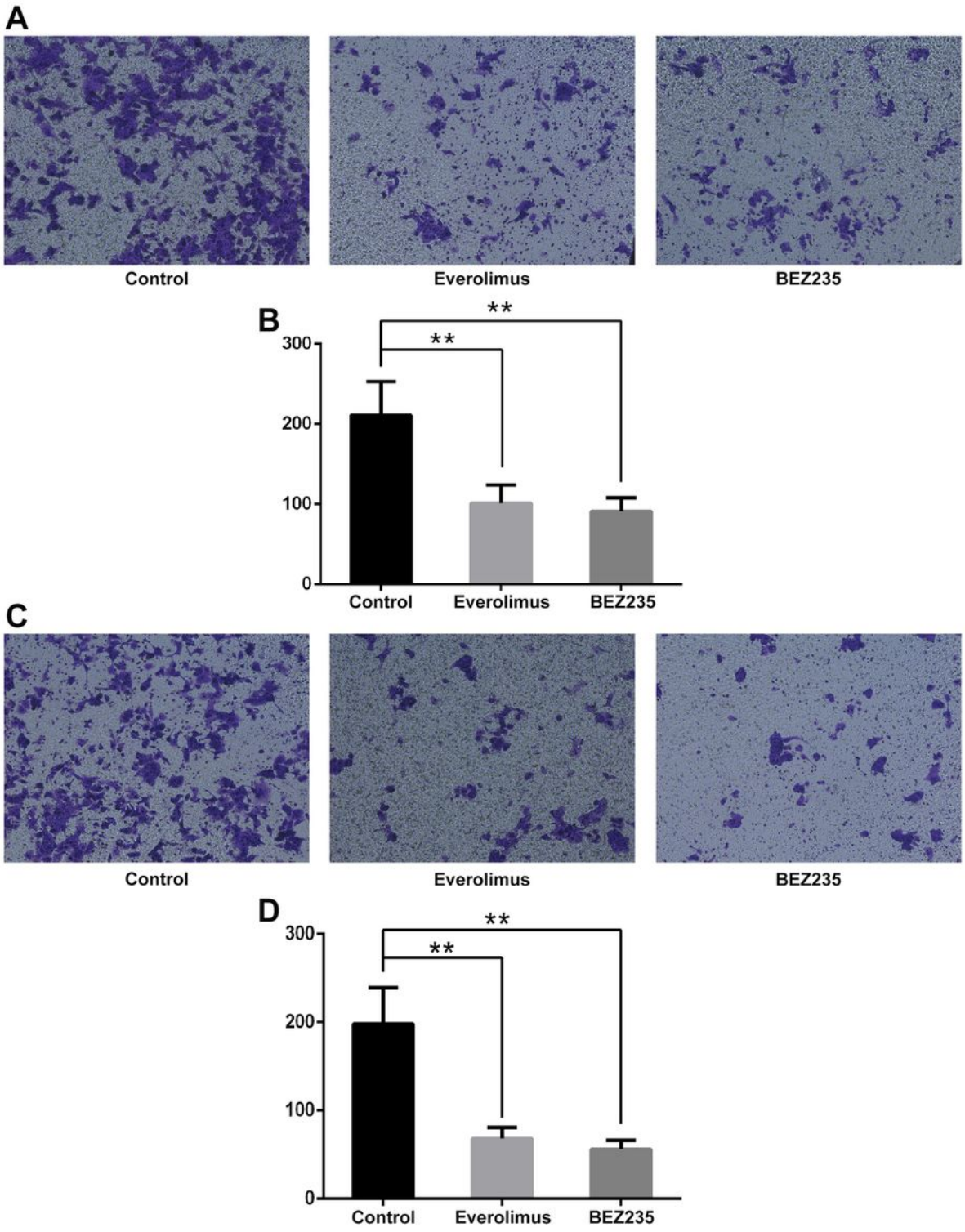

## Figure 2

Increased migration ability of the hEM15A cells with high T-cadherin expression detected by the Transwell migration assay. (A) Images showing the migrated hEM15A cells on the lower surface of the Transwell membranes. (B) Number of migrated hEM15A cells in five random fields under the microscope in different groups (mean ± SD). ** $P < 0.01$.




## Figure 3

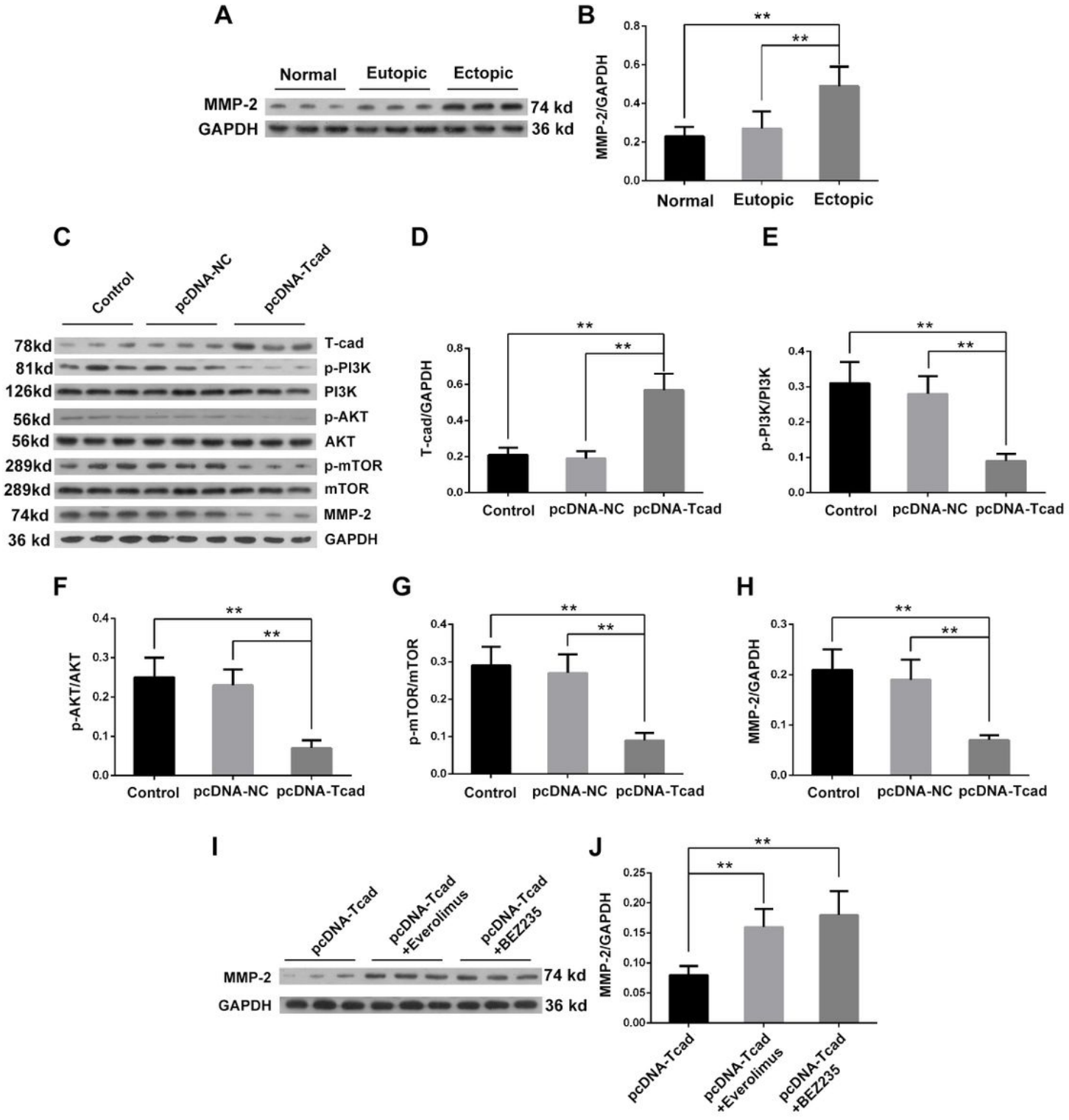
Increased invasion ability of the hEM15A cells with high T-cadherin expression detected by the Matrigel-precoated Transwell invasion assay. (A) Images showing the invaded hEM15A cells on the lower surface of the Transwell membranes. (B) Number of invaded hEM15A cells in five random fields under the microscope in different groups (mean ± SD). ** $P < 0.01$.




## Figure 4

Everolimus and BEZ235 inhibited of the migration and invasion of the hEM15A cells by up T-cadherin expression with pcDNA-Tcad. (A) Images showing the migrated hEM15A cells on the lower surface of the Transwell membranes in hEM15A cells by up T-cadherin expression with or without Everolimus and BEZ235. (B) Number of migrated hEM15A cells in five random fields under the microscope in different groups. (C) Images showing the invaded hEM15A cells on the lower surface of the Transwell membranes

in hEM15A cells by up T-cadherin expression with or without Everolimus and BEZ235. (D) Number of invaded hEM15A cells in five random fields under the microscope in different groups (mean ± SD). ** P < 0.01.




## Figure 5

T-cadherin mediates MMP-2 expression through the PI3K/AKT/mTOR pathway in hEM15A cells. (A) Representative Western blotting of T-cadherin protein and GAPDH from different tissues. (B)

Densitometric analysis of T-cadherin protein levels in different tissues detected by Western blot. (C) Western blotting analysis of the expressions of PI3K, p-PI3K (Tyr458), AKT, p-AKT (T308), mTOR, p-mTOR (Ser2448) and MMP-2 in hEM15A cells. GAPDH was used as the loading control. The hEM15A cells were incubated in the control medium (control, no additives) or transfected with pcDNA-T-cadherin (pcDNA-Tcad) or negative control pcDNA3.1 (pcDNA-NC) in the presence of the transfection agent lipofectamine (5 μl/ml) for 48 h before Western blotting. (D-H) The intensities of the bands were quantified by densitometry analyses and normalized by the amount of PI3K, AKT, or mTOR. Data are presented as mean± SD of three independent experiments; **P< 0.01 compared with the control group. (I) Representative Western blotting of T-cadherin protein and GAPDH from the hEM15A cells incubated in the presence of the transfection agent pcDNA-Tcad for 48 h before Western blotting with or without Everolimus or BEZ235. (J) Densitometric analysis of T-cadherin protein levels in different groups detected by Western blot. **P< 0.01.