ORIGINAL RESEARCH

# Effects of EZH2 on Invasion and Migration of Endometrial Stromal Cells in Endometriosis Patients by Regulating PCDH10 Gene H3K27 Methylation

Tian Xiaolei, MSc; Man Jiang, MSc; Nan Yang, MSc; Zhang Jing, MSc


## ABSTRACT

**Context** • Endometriosis refers to the appearance of ectopic endometrioid tissue outside the uterus. Low PCDH10 expression has been associated with enhancer of zeste homolog 2 (EZH2), which catalyzes histone 3 (H3K27me3). H3K27me3 is an epigenetic marker associated with endometriosis.

**Objective** • The study intended to explore the influence of protocadherin 10 (PCDH10) on the invasion and migration of endometrial stromal cells in endometriosis as well as its mechanism.

**Design** • The research team designed a laboratory study using endometrial tissue.

**Setting** • The study took place in Department of Obstetrics and Gynecology at South University of Science and Technology Hospital in Shenzhen, Guangdong Province, China.

**Participants** • Participants were 10 patients with ovarian endometriosis (ovarian chocolate cysts) who were undergoing surgical treatment at the hospital between January and December 2019. The endometrial tissue of those participants became the endometriosis group. Other participants with normal endometrial tissue became the controls (n=10).

**Outcome Measures** • The research team collected tissues from participants and used immunofluorescence, real-time quantitative polymerase chain reaction (qPCR), and Western blot assay to determine the expression levels of PCDH10, enhancer of zeste homolog 2 (EZH2), and histone H3 (H3K27me3). The team cultured endometrial stromal cells from participants primarily to detect the effects of silencing EZH2 on PCDH10 and H3K27me3 expression. The team used a Transwell assay and scratch test to examine the influence of silencing EZH2 on invasion and migration of endometrial stromal cells and applied chromatin immunoprecipitation to determine H3K27me3 enrichment in the PCDH10 gene promoter region.

**Results** • PCDH10 in heterotopic endometrial tissues of endometriosis patients had low expression, while EZH2 and H3K27me3 were highly expressed. Silencing EZH2 inhibited EZH2 protein expression, increased PCDH10 expression, and inhibited invasion and migration of endometrial stromal cells by increasing PCDH10 expression. Silencing EZH2 also reduced H3K27me3 enrichment in PCDH10 promoter region.

**Conclusions** • Low PCDH10 expression may be associated with high EZH2 expression and H3K27me3 enrichment in endometriosis patients, which promotes the migration and invasion of endometrial stromal cells. This connection provides a theoretical basis for the treatment of endometriosis. (*Altern Ther Health Med.* 2023;29(2):42-49)


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Endometriosis refers to the appearance of ectopic endometrioid tissue outside the uterus. As an estrogen-dependent disease, it's the principal reason for pelvic pain and infertility.[1] The incidence of endometriosis is high, and approximately 10% of women of childbearing age are deeply troubled by it.[2] The real incidence may be higher because of the limitations of diagnostic methods. Furthermore, the symptoms are highly heterogeneous, including dysmenorrhea, pelvic pain, dyspareunia, dyschezia, dysuria, infertility, and fatigue.

Lesions consist of superficial peritoneal lesions of different colors—transparent, yellow, red, brown, blue, and

black—and at different locations in the abdominal cavity, endometrial tumors, and even extrapelvic symptoms. Coexisting events can easily occur in endometriosis patients, such as uterine fibroids, adenomyosis, fibromyalgia, rheumatoid arthritis, and ulcerative colitis.

Endometriosis' characteristics include the deposition and growth of functional, endometrial-like tissue outside the uterus due to molecular aberrations during hormone production, which can result in an increase in local estrogen production.[3] The exact pathogenesis and pathophysiology of endometriosis remain unclear, so it's very important to study its pathogenesis.

Endometriosis has multifactor genetic potential.[4] The current hypotheses addressing the origin of ectopic endometrium include theories implicating menstrual blood reflux, lymphatic vascular metastasis, and body-cavity epithelial metaplasia. Usually, endometriosis patients have a low immune function, and their bodies can't remove the intimal debris that shouldn't appear in the pelvic or abdominal cavity. Intimal-debris adhesion, invasion, and angiogenesis can occur, thereby forming lesions.[5] The ectopic lesions can shrink and degenerate after menopause.

Currently, the diagnosis of endometriosis still relies on laparoscopic surgery as the gold standard. Drug treatments can relieve only the symptoms but can't cure the disease. Moreover, relapse can easily occur after drug withdrawal. Compared with women without endometriosis at childbearing age, endometriosis patients have an increased risk for epithelial ovarian cancer and cardiovascular disease.

Protocadherin 10 (PCDH10), a non-clustered protein, belongs to the cadherin superfamily and specifically includes the six-fold, cadherin extracellular domain that mediates cell adhesion and plays a role in tumor suppression. In tumors, PCDH10 usually has low expression, which is associated with tumor invasion and migration.[6-9] Moreover, PCDH10 participates in signal transduction and growth.

Zhang et al found that PCDH10 can decrease β-catenin expression in lymphoma cells, thereby inhibiting their invasion.[6] Liu et al found that the expression level of PCDH10 is downregulated by abnormal methylation at its promoter region.[10] Another study showed that PCDH10 inactivation by promoter methylation is a common pathogenic event in multiple myelomas.[11]

Bing et al found that outcomes for liver-cancer patients with low PCDH10 expression are poor.[12] Ye et al demonstrated that PCDH10 can suppress the phosphatidylinositol-3-kinase (PI3K)/ protein kinase B (Akt) signaling pathway, can inhibit the proliferation of liver-cancer cells, and can induce apoptosis.[13]

Qi et al found that low PCDH10 expression in gastric cancer is associated with a catalytic subunit, enhancer of zeste homolog 2 (EZH2), of the polycomb repressive complexes 2 (PRC2).[9] EZH2 catalyzes histone 3 (H3K27me3) that the PRC2, which is involved in transcription initiation, contains.[14] H3K27me3 is an epigenetic marker associated with endometriosis.[15] Duan et al found that the methyltransferase EZH2 can catalyze the trimethylation of lysine 27 on H3K27me3 to regulate the expression of related genes.[16]

A previous study has shown abnormal EZH2 expression and H3K27me3 enrichment in endometriosis patients.[17] Qi et al found that EZH2 recruitment to the PCDH10 promoter region can increase its promoter H3K27me3 to inhibit its transcription and promote gastric cancer metastasis[18]

The current study intended to explore the influence of protocadherin 10 (PCDH10) on the invasion and migration of endometrial stromal cells in endometriosis as well as its mechanism.

## METHODS

### Participants

The research team designed a laboratory study using endometrial tissue. The study took place in Department of Obstetrics and Gynecology at South University of Science and Technology Hospital in Shenzhen, Guangdong Province, China. Potential participants were patients with ovarian endometriosis (ovarian chocolate cysts) who were undergoing surgical treatment at the hospital between January and December 2019. Other participants with a normal endometrial tissue became the controls. The participants are all patients in our department, endometrial tissue source these patients removed discarded endometrial tissue.

For the endometriosis group, potential participants were included in the study if they: (1) had no other medical and surgical diseases and (2) hadn't received hormone therapy within the 3 months prior to their operation. Combined with pelvic mass ≥4 cm in diameter or infertility or ineffective drug treatment]

Not the same as 20 people, but 20 normal endometrial samples as controls. The normal endometrial portion is sufficient, not the ectopic area.

Health Science and Technology Plan Project of Nanshan District Shennanke, 2020, (2020128).

### Procedures

**Tissue collection.** For patients undergoing surgery in our department, the discarded tissue removed by surgery is used for experimental research. The research team stored one part of each participant's sample at -80°C and the other part in 4% paraformaldehyde, because it can better protect the morphological structure and nucleic acid of tissues and cells.

**Cell isolation, culture, and transfection.** The research team: (1) isolated the endometrial stromal cells from the endometrial tissue of the patients with endometriosis, the endometriosis group, and the control group[8]; (2) washed the tissue with sterile phosphate buffered saline (PBS) from Nanjing City, Jiangsu Province, Nanjing Jiangyuan Biotechnology Co., Ltd. China, to separate blood clots and then cut it into three pieces of one mm each; (3) afterward used one mg/mL of type IV collagenase containing 100 U/mL each of penicillin and streptomycin to digest the pieces for 45 minutes; (4) filtered the stromal cells after continuously stirring the mixture at 37°C; (5) centrifuged the cell

suspension at 4°C and 1200 rpm; (6) after discarding the supernatant, cultured the cells with high-glucose Dulbecco's Modified Eagle Medium (DMEM) from Shanghai (Shanghai Yanjin Biotechnology Co., Ltd.China), 10% fetal bovine serum (FBS), and 1% penicillin and streptomycin, at 37°C with 5% CO2 in an incubator (Shanghai Xinyu Biotechnology Co., Ltd., Shanghai, China); (7) performed the transfection of the endometrial stromal cells according to the instructions for the Lipofectamine 3000 reagent (Waltham, Thermo Fisher Scientific, US) when the cells' confluence reached 60%; (8) divided those cells into a transfected silent EZH2 group (siEZH2), a silent EZH2+silent PCDH10 group, and an siNC group, a group with non-coding, double-stranded RNA molecules; (9) diluted the siRNA and different transfection reagents with serum-free medium, mixed them well, and centrifuged them; (10) mixed the diluted siRNA with the diluted transfection reagent thoroughly and then incubated the mixture at room temperature for 15 minutes; and (11) added the mixture to a culture plate for 24-48 hours and harvested the cells for subsequent experiments.

**Immunofluorescence.** The research team: (1) embedded the paraformaldehyde-fixed tissues in paraffin to prepare 4-μm sections; (2) deparaffinized the sections with xylene that was hydrated through a graded alcohol series, retrieved the sections using citric acid under high pressure, blocked with 5% goat serum, and incubated the sections with primary antibody PCDH10 overnight at 4°C; (3) placed these sections in PBS the next day to wash off the primary antibody, incubated them with the fluorescent secondary antibody at room temperature for one hour, and stained them with 4',6-diamidino-2-phenylindole (DAPI) (Waltham, Thermo Fisher Scientific, US]); and (4) mounted the slides with glycerol and captured images under a fluorescent microscope (Shanghai Yuguang Instrument Factory, Shanghai, China).

**Real-time quantitative polymerase chain reaction (qPCR).** The research team: (1) employed Trizol (Waltham, Thermo Fisher Scientific, US]) and added one-fifth volume of chloroform to it to extract total RNA from tissues or cells; (2) after vigorously shaking the solution, maintained each sample in place at room temperature for 10 minutes and then centrifuged it at 4°C and 12 000 rpm for 15 minutes; (3) transferred the supernatant to an additional centrifuge tube and added an equal volume of isopropanol, followed by mixing it upside down; (4) maintained the supernatant in place at room temperature for 10 minutes and centrifuged it at 4°C and 12 000 rpm for 15 minutes; (5) afterward, discarded the supernatant and added 75% ethanol, followed by centrifugation at 4°C and 12 000 rpm for 15 minutes; (6) after discarding the supernatant, air-dried each sample and incubated it with RNA dissolved in 0.1% diethylpyrocarbonate (DEPC) water; (7) removed the appropriate amount of RNA and reverse-transcribed it into complementary DNA (cDNA) with a reverse transcription kit (QIAGEN, Hilden, Germany]); (8) used an SYBR Green PCR Kit (QIAGEN, Hilden, Germany]) for real-time qPCR analysis; and (9) analyzed the relative expression level using the $2-\Delta\Delta Ct$ method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference.

**Western blot assay.** The research team: (1) used a radioimmunoprecipitation assay (RIPA) lysate to extract the total protein of the tissues and cells; (2) added lysate to the tissues or cells, incubated them on ice for 30 minutes, sonicated them, and centrifuged them at 4°C and 12 000 rpm for 20 minutes; (3) removed the supernatant and quantified the protein using a bicinchoninic acid (BCA) assay (Shanghai, Shanghai Enzyme Link Biotechnology Co., Ltd.China); (4) then boiled the samples and loaded them on a polyacrylamide gel, separated the proteins using electrophoresis equipment from Shanghai (Shanghai Enzyme Link Biotechnology Co., Ltd. China]), and transferred them onto a polyvinylidene fluoride (PVDF) membrane (Shanghai, Absin Bioscience Inc. China); (5) blocked the membrane with 5% skimmed-milk powder, incubated it with the primary antibody overnight at 4°C, washed it with PBST, incubated it with the secondary antibody at room temperature for one hour, and washed with it with PBS with Tween detergent (PBST); and (6) used enhanced chemiluminescence (ECL) from Waltham, Thermo Fisher Scientific,US) and ImageJ (Waltham, Thermo Fisher Scientific, US) for grayscale analysis.

**Transwell assay.** The research team: (1) collected the cells after transfection, prepared a single-cell suspension using serum-free cell-culture medium, and transferred the suspension to the upper Transwell chamber (Corning-Costar, Pittsburg, Pennsylvania, US) on the gel; (2) added a cell-culture medium containing 20% FBS to the lower 12-well plate to induce cell invasion for 24 hours; (3) collected the upper-chamber membrane, washed it with PBS, fixed it with 4% paraformaldehyde, stained it with 0.1% crystal violet at room temperature for 30 minutes, and finally washed it with PBS; and (4) wiped off excess dye with cotton swabs, took images randomly in five fields under an optical microscope (Tokyo, Olympus, Japan), and counted the cells.

**Scratch test.** After transfection, the research team: (1) collected the cells and added them to a six-well plate; (2) when the confluence of the cells reached 90%, scratched the cells vertically with a 200-μl pipette tip and washed away the scratched cells with PBS; and (3) at 24 hours later, observed the cells and photographed them under the optical microscope.

**Chromatin immunoprecipitation.** The research team: (1) cultured the transfected cells in a 10-cm cell-culture dish, added an appropriate amount of formaldehyde to the cell-culture medium, and gently mixed it until the final concentration was 1%; (2) incubated the mixture at 37°C for 10 minutes, added 2.5 M of glycine, mixed it gently, and placed it at room temperature for 5 minutes; (3) collected the cells by scraping them on ice with a precooled PBS containing phenylmethylsulfonyl fluoride (PMSF) from Nanjing City, Jiangsu Province (Nanjing Jiangyuan Biotechnology Co., Ltd. China), and centrifuged them at 3000 rpm for 5 minutes; (4) after discarding the supernatant, added lysate and

sonicated and centrifuged it at 12 000 rpm for 20 minutes; (5) collected the supernatant, treated it with protein A+G agarose beads (Shanghai, Ybscience, China), and then rotated it at 4°C for one hour; (6) added EZH2 and H3K27me3 antibodies, rotated the mixture overnight at 4°C, added protein A+G agarose beads, rotated the mixture at 4°C for 4 hours, and washed it separately with low salt, high salt, lithium chloride, and Tris-EDTA0 (TE) buffer (Shanghai,Kilton Biotechnology (Shanghai) Co., Ltd. China); (7) added a mixture of 1% SDS and 0.1 M of sodium bicarbonate (NaHCO3) in equal proportions and rotated the mixture at room temperature for 15 minutes; (8) collected the supernatant, incubated it with 5 M of sodium chloride (NaCl) at 65°C overnight, and extracted the DNA for real-time qPCR.

**Outcome measures.** The research team collected tissues from participants and used immunofluorescence, real-time qPCR, and Western blot assay to determine the expression levels of PCDH10, EZH2, and H3K27me3.The team cultured endometrial stromal cells from participants primarily to detect the effects of silencing EZH2 on PCDH10 and H3K27me3 expression. The team used a Transwell assay and scratch test to examine the influence of silencing EZH2 on invasion and migration of endometrial stromal cells and applied chromatin immunoprecipitation to determine H3K27me3 enrichment in the PCDH10 gene promoter region.

**Outcome Measures**

**Expression of PCDH10 protein and mRNA.** The research team used immunofluorescent staining to detect the expression of PCDH10 protein and real-time qPCR to detect the expression of PCDH10 mRNA in endometrial tissue. Compared with normal endometrial tissue, the expression of PCDH10 in endometrial tissue of patients with endometriosis was significantly decreased, and the difference was statistically significant ($P < .05$). The results are shown in Figure 1A. Compared with normal endometrial tissue, the expression of PCDH10 mRNA in endometrial tissue of patients with endometriosis was significantly decreased, and the difference was statistically significant (P<0.05). The result is shown in Figure 1B.

**Expression of EZH2 mRNA and of EZH2 and H327Kme3 protein.** The research team used real-time qPCR to determine the expression of EZH2 mRNA and western blot to determine the expression of EZH2 and H327Kme3 protein in endometrial tissue. Real-time qPCR detected the expression level of EZH2 mRNA in endometrial tissue. The results are shown in Figure 2A. Compared with normal endometrial tissue, the expression level of EZH2 mRNA in endometrial tissue of patients with endometriosis was significantly lower, and the difference was statistically significant. ($P < .05$). The expression of EZH2 and H327Kme3 proteins in endometrial tissue was detected by Western blot, and the results were shown in Figure 2B. The expression of EZH2 and H327Kme3 in endometrial tissue of patients with endometriosis was significantly higher than that of normal endometrial tissue, and the difference was statistically significant ($P < .05$).

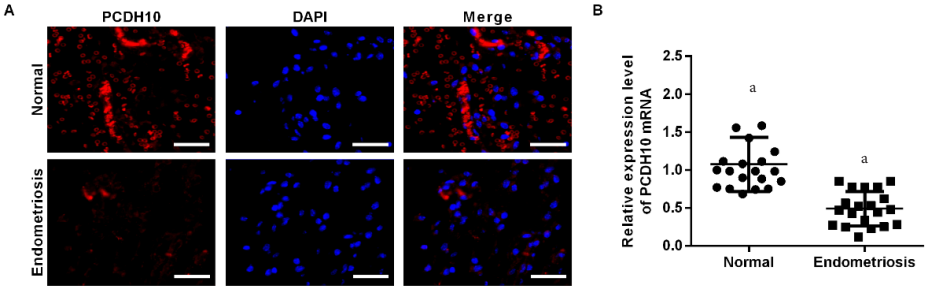
**Effects of silencing EZH2 on PCDH10 expression.** The research team used a Western blot and real-time qPCR to examine the effects of silencing EZH2 on PCDH10 expression. Western blot results showed that compared with the control group and the NC group, the EZH2 protein expression in the silenced EZH2 group was significantly decreased, and the PCDH10 protein expression was significantly increased, and the difference was statistically significant ($P < .05$). The results are shown in Figure 3A. Real-time qPCR results showed that compared with the control group and the NC group, the EZH2 mRNA expression in the silenced EZH2 group was significantly decreased, and the PCDH10 mRNA expression was significantly increased, and the difference was statistically significant ($P < .05$). The results are shown in Figure 3B.

**Invasion ability of endometrial stromal cells.** The research team used the Transwell assay to examine the invasion ability of endometrial stromal cells. The results of Transwell experiment showed that compared with the control group and NC group, the invasive ability of endometrial stromal cells in the silenced EZH2 group was significantly reduced ($P < .05$); compared with the silenced EZH2 group, the silenced EZH2 and PCDH10 were silenced The invasive ability of endometrial stromal cells in the group was significantly increased, and the difference was statistically significant ($P < .05$).

**Migration ability of endometrial stromal cells.** The research team used the scratch test to examine the migration ability of endometrial stromal cells. The scratch test results showed that compared with the control group and the NC group, the migration ability of endometrial stromal cells in the silenced EZH2 group was significantly reduced, and the difference was statistically significant ($P < .05$). The migration ability of endometrial stromal cells in PCDH10 group was significantly increased, and the difference was statistically significant ($P < .05$).

**Effects of silencing EZH2 on the expression of H327Kme3 protein.** The research team used the Western blot assay to detect the effects of silencing EZH2 on the expression of H327Kme3 protein. The team used chromatin immunoprecipitation to detect the enrichment of H3K27Me3 at the transcription start site of the PCDH10 promoter region after silencing EZH2. Compared with the control group and the NC group, the expression of H327Kme3 protein in endometrial stromal cells in the EZH2 silenced group was significantly decreased, and the difference was statistically significant ($P < .05$), as shown in Figure 6A. Chromatin immunoprecipitation was used to detect the enrichment level of H3K27Me3 at the transcription start site of PCDH10 promoter region by silencing EZH2. The results showed that after silencing EZH2, the enrichment level of H3K27Me3 in the PCDH10 promoter region was significantly reduced, and the difference was statistically significant ($P < .05$), as shown in Figure 6B.

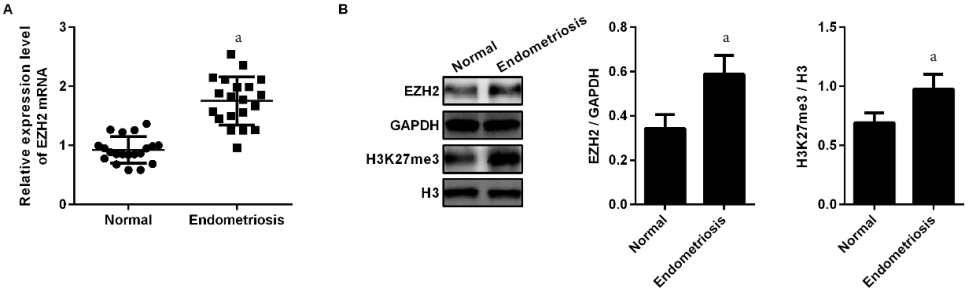
**Figure 1.** PCDH10 Expression in the Endometrial Cells of Patients With Endometriosis and Normal Endometrial Cells. Figure 1A shows the PCDH10 protein expression detected by immunofluorescent staining, with the scale bar = 50 μm, and Figure 1B shows the PCDH10 mRNA expression detected by real-time qPCR.




[a]$P < .05$, indicating that the expression of PCDH10 protein and PCDH10 RNA in the endometriosis group were significantly lower than that in control group

**Abbreviations:** PCDH10, protocadherin 10; qPCR, quantitative polymerase chain reaction.

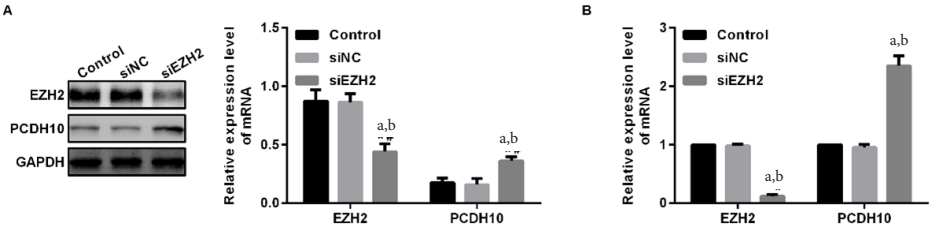
**Figure 2.** EZH2 and H327Kme3 Expression in the Endometrial Cells of Patients with Endometriosis and Normal Endometrial Cells. Figure 2A shows the EZH2 mRNA expression detected by real-time qPCR, and Figure 2B shows the EZH2 and H327Kme3 protein expression detected by Western blot.




[a]$P < .05$, indicating that the expression of EZH2 mRNA, EZH2, and H327Kme3 in the endometriosis group were significantly higher than that in the control group

**Abbreviations:** EZH2, enhancer of zeste homolog 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase (human); H327KMe3, histone H3; qPCR, quantitative polymerase chain reaction.

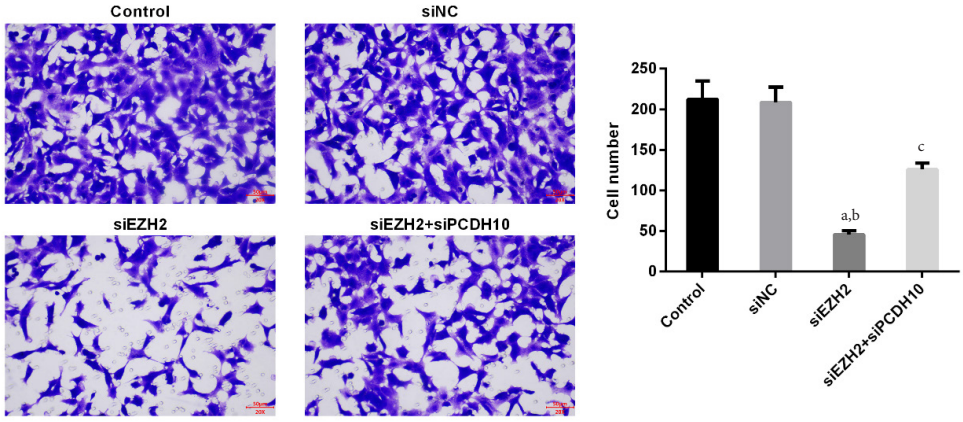
**Figure 3.** Effects of Silencing EZH2 on the Expression of PCDH10 in Endometrial Stromal Cells. Figure 3A shows the results from the Western blot, and Figure 3B shows the results from the Real-time qPCR.




[a]$P < .05$, indicating that the expression in the siEZH2 group of EZH2 protein was significantly lower and of PCDH10 protein was significantly higher than the expression in the control group
[b]$P < .05$ indicating that the expression in the siEZH2 group of EZH2 mRNA was significantly lower and of PCDH10 mRNA was significantly higher than the expression in the siNC group

**Abbreviations:** EZH2, enhancer of zeste homolog 2; PCDH10, protocadherin 10; qPCR, quantitative polymerase chain reaction; siEZH2, silent EZH2; siNC, a non-coding, double-stranded RNA molecule.

**Figure 4.** Effects of EZH2 on Endometrial Stromal Cell Invasion by PCDH10, With the Scale Bar = 50 μm




[a]$P < .05$, indicating that the number of invading cells in the siEZH2 group was significantly lower that of the control group
[b]$P < .05$, indicating that the number of invading cells in the siEZH2 group was significantly lower that of the siNC group
[c]$P < .05$ indicating that the number of invading cells in the siEZH2 group was significantly lower that of the siEZH2+siPCDDH10 group

**Abbreviations:** EZH2, enhancer of zeste homolog 2; PCDH10, protocadherin 10.

### Statistical Analysis

The research team analyzed the data using SPSS 22.0 statistical software (IBM, Armonk, New York, US.) and expressed measurement data as means ± standard deviations (SDs). The team analyzed the difference within groups using a t-test and the differences between groups using one-way analysis of variance (ANOVA). $P < .05$ was considered to be statistically significant.

## RESULTS

### Participants

The study included and analyzed the data of 20 participants who were 25-40 years old, including 10 patients with endometriosis and 10 women with normal endometrial tissue.

### PCDH10 Expression

Figure 1A shows that the expression of PCDH10 protein in the endometrial tissue of the endometriosis group was significantly lower than that in the control group ($P < .05$). Figure 1B shows that the expression of PCDH10 mRNA in the endometrial tissue of the endometriosis group was significantly lower than that in the control group ($P < .05$).

### Expression of EZH2 and H327Kme3

Figure 2A shows that the expression level of EZH2 mRNA was significantly higher in the endometrial tissue of the endometriosis group than that of the control group ($P < .05$). Figure 2B shows that the expression of EZH2 and H327Kme3 were significantly higher in endometrial tissue of the endometriosis group than that of the control group ($P < .05$).

### siEZH2 and PCDH10 Expression

Figure 3A shows that the EZH2 protein expression was significantly lower and the PCDH10 protein expression was significantly higher in the siEZH2 group ($P < .05$). Figure 3B shows that the expression of EZH2 mRNA in the siEZH2 group was significantly lower and the expression of PCDH10 mRNA was significantly higher than that of the control and siNC groups ($P < .05$).

### Invasion of Endometrial Stromal Cells

Figure 4 shows that the invasion ability of endometrial stromal cells was significantly lower in the siEZH2 group than that in the control group with normal endometrial cells and the siNC group ($P < .05$). The invasion ability of endometrial stromal cells was significantly higher in the silent EZH2+silent PCDH10 group than in siEZH2 group ($P < .05$).

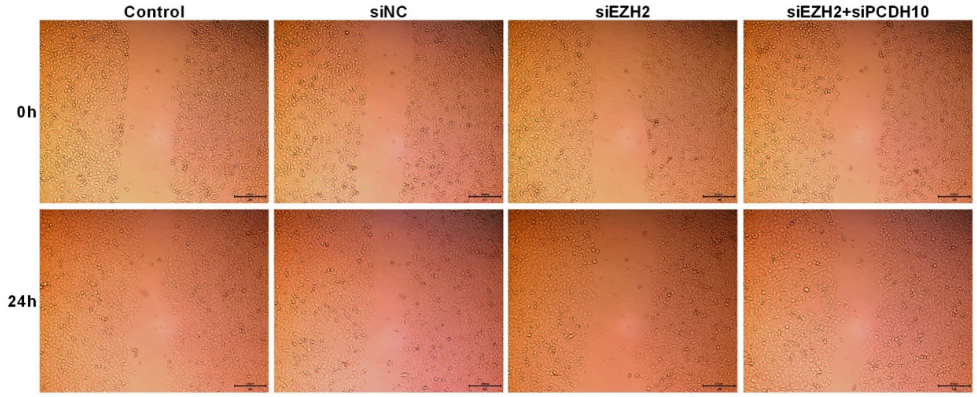
### Migration of Endometrial Stromal Cells

Figure 5 shows that the migration ability of endometrial stromal cells between zero and 24 hours was significantly lower in the siEZH2 group than that of the control and the siNC groups ($P < .05$). Compared with the siEZH2 group, the migration ability of endometrial stromal cells was significantly higher in the silent EZH2+silent PCDH10 group ($P < .05$).

### Effect of EZH2 on Modification Level

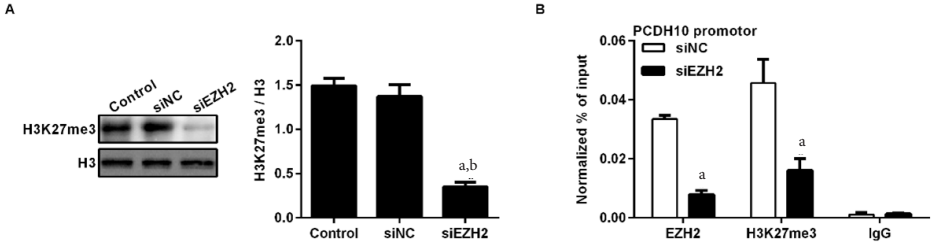
Figure 6A shows that the expression of H327Kme3 protein was significantly lower in the endometrial stromal cells of the siEZH2 group than that of the control group with normal endometrial cells and the siNC group ($P < .05$). Figure 6B shows that after silencing EZH2, the enrichment of H3K27Me3 was significantly lower in the PCDH10 promoter region ($P < .05$).

**Figure 5.** Effect of EZH2 on Endometrial Stromal Cell Migration Through PCDH10, With the Scale Bar = 100 μm




**Abbreviations:** EZH2, enhancer of zeste homolog 2; PCDH10, protocadherin 10.

**Figure 6.** Effect of EZH2 on the Modification Level of PCDH10 Gene H3K27Me3. Figure 6A shows the Western blot, and Figure 6B shows the chip assay.




[a]$P < .05$, indicating that the expression in the siEZH2 group of the PCDH10 gene H3K27Me3 group was significantly lower that of the control group

[b]$P < .05$, indicating that the enrichment after silencing EZH2 of the PCDH10 gene H3K27Me3 was significantly lower in the PCDH10 promoter region

**Abbreviations:** EZH2, enhancer of zeste homolog 2; H3K27Me3, histone H3; PCDH10, protocadherin 10.

## DISCUSSION

The current research mainly focused on the role of PCDH10 as a tumor suppressor gene, but its expression, function, and mechanism in endometriosis hadn't been reported prior to the current study. The present study has provided a theoretical basis for treating endometriosis by analyzing PCDH10 expression and its influence on the migration and invasion of primary, cultured, endometrial stromal cells in the tissues of endometriosis patients.

The current research team examined the enrichment of EZH2 and H3K27me3 in the PCDH10 promoter region of endometrial stromal cells of endometriosis patients and found that silencing EZH2 can reduce H3K27me3 recruitment in the PCDH10 promoter region and inhibit PCDH10 transcription.

The current study has confirmed that PCDH10 expression is low in the tissues of endometriosis patients and that the increased expression of PCDH10 can suppress the migration and invasion of primary, cultured, endometrial stromal cells in the tissues of endometriosis patients, indicating that PCDH10 may have an inhibitory effect on endometriosis.

## CONCLUSIONS

Low PCDH10 expression may be associated with high EZH2 expression and H3K27me3 enrichment in endometriosis patients, which promotes the migration and invasion of endometrial stromal cells. This connection provides a theoretical basis for the treatment of endometriosis.

### FUNDING

Shennanke [2020] Nanshan District Health Science and Technology Project (2020128): Project name: Epigenetic study of PCDH10 gene in endometriosis.

### AUTHOR CONTRIBUTIONS

Tian Xiaolei and Man Jiang contributed equally.

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