ORIGINAL RESEARCH ARTICLE

Paraneoplastic Ma antigen 5 promotes cellular processes of primary cells in ovarian endometriosis

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Abstract

This study investigates the role of paraneoplastic Ma antigen 5 (PNMA 5), a member of the PNMA family of proteins, in ovarian endometriosis (EM). These proteins are known to play a significant role in various physiological activities and are highly expressed in cells undergoing carcinogenesis, where they influence cell cycle, apoptosis, and other cellular processes. However, the involvement of PNMA 5 in ovarian EM has not been fully understood. To explore this, the study analyzed PNMA 5 levels in both normal and ectopic endometrial tissues from ovarian EM patients using immunohistochemistry. Small interfering RNA (siRNA) was used to knock down PNMA 5 in ectopic tissue cells, and the effects of this intervention were assessed through qRT-PCR and western blot to evaluate the efficiency of knockdown. Additionally, CCK-8 assays, flow cytometry, transwell, and wound healing assays were employed to examine the impact of PNMA 5 on cell proliferation, apoptosis, migration, and invasion. The results demonstrated that PNMA 5 expression was significantly higher in ectopic tissues compared to normal endometrium. Moreover, the protein inhibited cell proliferation and invasion while promoting apoptosis in primary cells from ectopic tissues. These findings suggest that PNMA 5 could serve as a potential therapeutic target for EM, though further research is needed to fully understand its mechanisms. *(Afr J Reprod Health 2025; 29 [4]: 37-48).*

Keywords: Endometriosis; Paraneoplastic Ma antigen 5 (PNMA5); Progression; Apoptosis

Résumé

Cette étude examine le rôle de l'antigène paranéoplasique Ma 5 (PNMA 5), un membre de la famille de protéines PNMA, dans l'endométriose ovarienne (EM). Ces protéines sont connues pour jouer un rôle important dans diverses activités physiologiques et sont fortement exprimées dans les cellules en cours de carcinogenèse, où elles influencent le cycle cellulaire, l'apoptose et d'autres processus cellulaires. Cependant, l'implication du PNMA 5 dans l'EM ovarienne n'a pas été entièrement comprise. Pour explorer cela, l'étude a analysé les niveaux de PNMA 5 dans les tissus endométriaux normaux et ectopiques de patientes EM ovariennes en utilisant l'immunohistochimie. Un petit ARN interférent (siRNA) a été utilisé pour inhiber le PNMA 5 dans les cellules des tissus ectopiques, et les effets de cette intervention ont été évalués par qRT-PCR et Western blot pour évaluer l'efficacité de l'inactivation. De plus, des tests CCK-8, des tests de cytométrie en flux, de transwell et de cicatrisation des plaies ont été utilisés pour examiner l'impact du PNMA 5 sur la prolifération cellulaire, l'apoptose, la migration et l'invasion. Les résultats ont démontré que l'expression de PNMA 5 était significativement plus élevée dans les tissus ectopiques que dans l'endomètre normal. De plus, la protéine a inhibé la prolifération et l'invasion cellulaires tout en favorisant l'apoptose des cellules primaires des tissus ectopiques. Ces résultats suggèrent que PNMA 5 pourrait servir de cible thérapeutique potentielle pour l'EM, bien que des recherches supplémentaires soient nécessaires pour bien comprendre ses mécanismes. *(Afr J Reprod Health 2025; 29 [4]: 37-48).*

Mots-clés: Endométriose, Antigène paranéoplasique Ma 5 (PNMA5), Progression, Apoptose

Introduction

Endometriosis (EM) represent the abnormal position of glandular epithelial cells and stromal cells in the endometrial tissue, which are distributed in areas such as the pelvis, ovaries, and lungs[1-3]. Essentially, it is an estrogen dependent disorder, with women of childbearing age being the main affected group[4,5]. According to clinical experience, the body is affected by sex hormones, leading to periodic ischemia, bleeding, and other conditions in diseased tissues. The ovaries and pelvic peritoneum are common sites of onset. Ovarian EM is particularly common, and patients with this disease

often exhibit symptoms of pain, pelvic masses, and even affect fertility, bringing enormous physical and mental burden to women[6,7].

EM is a benign disease, but often exhibits a series of malignant biological behaviours such as rapid division, spread, and involvement of other tissues[8,9]. So far, the medical community has not fully elucidated its pathogenesis. With the gradual deepening of related research, humans have expressed different views on its pathogenesis, but the most authoritative one is undoubtedly the "theory of ectopic implantation". This conclusion was drawn by Sampson in the 1920s[10-12], who suggested that female menstrual blood flows into the pelvic cavity along the fallopian tubes. During this process, endometrial glandular epithelial cells and stromal cells are carried together into the pelvic cavity and slowly implant, ultimately developing into EM. After the theory was introduced, most scholars conducted further exploration and reached consistent conclusions[13,14]. In other studies, it has been found that the vast majority of women experience menstrual blood reflux during their menstrual period, but only a few women may develop Ems[15,16]. Therefore, it is believed that the formation of this disease is not only related to the presence of endometrial tissue in the abdominal cavity.

Based on the above theory, scholars such as Lang Jinghe further proposed the "in situ endometrial determinism", which believes that the formation of this disease is mainly related to changes in certain genes in the endometrium. In addition, the medical community has proposed theories such as "coelomic metaplasia" and “induction theory”[17,18], which provide different perspectives on the causes of EM. With the gradual deepening of medical research, humans have proposed various therapies for EM, such as surgery and drug therapy. At present, the commonly used drugs in clinical practice can be divided into two categories: steroid drugs and non-steroid drugs. Overall, the problem of EM recurrence has not been effectively solved by which therapy is used[19-22]. Humanity still needs to further explore the influencing factors of EM and find more reliable and safe therapies to benefit more patients

The PNMA family is a class of proteins encoded by the human genome that can act on the nervous system. According to existing research data, the family has at least 15 members, most of whom share an amino acid sequence located near the amino terminus. Medical research has shown that the carboxyl region mainly consists of two parts, namely the nuclear localization signal region and the DNA binding region, but the two exhibit significant sequence differences, which result in members of this family having different physiological functions and a higher content in cancerous cells, thereby affecting the cell's ability to divide, spread, and to undergo apoptosis[23]. PNMA5 is one of the members of this family, and it has been reported to contain tumour neuron antigens, but further evidence is currently lacking[24]. According to relevant research results, Pnma5 can encode a protein composed of 448 amino acid residues, and in general, the body does not exhibit corresponding immune responses. In cancerous tissues, the body will exhibit corresponding immune responses. Some scholars believe that cancer patients have no obvious symptoms in the early stages of onset, and at this time, disease prediction and assessment can be carried out through relevant neuroma syndromes[25,26]。

To date, the correlation between PNMA 5 and tumors has not been elucidated in humans. Some scholars have tested 14 kinds of normal human tissues and found that they only exist in testis and brain tissues, and can regulate the division, spread, apoptosis of cancer cells[27,28]. In the future, in-depth research in this area could provide new ideas for cancer treatment. Some scholars believe that the level of PNMA 5 in cancerous tissues may predict the poor prognosis of this type of disease[23], and this study has attracted wide attention in the medical community. According to Lee[25] *et al*., such proteins can coordinate with PNMA 4 (MOAP-1) through the N-terminal region, and activate the apoptotic signals in cancer cells, thus affecting the apoptosis of diseased cells and the sensitivity of body tissue to chemotherapy. For this class of proteins, the corresponding carboxy-terminal region is very critical in nuclear localization, and both the carboxyl and amino termini play a critical role in apoptosis. We previously conducted animal experiments and found that in mouse oocytes, this protein mainly regulates the proliferation capacity of cells[29] through a series of phosphorylation.

Meanwhile, the results showed that PNMA 5 was significantly higher in epithelial ovarian cancer (EOC) than in healthy ovarian tissue, and its knockout from the diseased cell line HO8910 decreased[30]. At present, the medical community has not formed an authoritative theory about the expression of this protein in ovarian EM. According to the results of previous studies, it is believed that it can promote the development of this disease.

For EM cases, PNMA 5 levels in the ectopic endometrium as well as in healthy tissue were determined by immunohistochemistry. By conducting a series of in vitro experiments, we have analyzed the effects of such proteins on the division, spread, and apoptosis of ectopic endometrial primary cells. The results obtained in this paper can make up for the gap in the field of ovarian EM etiology research to some extent, so as to provide new ideas for the treatment of this disease, which has certain practical significance.

Methods

Human Specimens

In this study, ovarian EM samples from the Second Affiliated Hospital of Nantong University were collected from March 2020 to April 2022. Among them, there were 12 cases of normal endometrial tissue and 12 cases of ectopic endometrium tissue. All cases included in the study underwent tissue biopsy and were diagnosed with ectopic endometrium by experienced clinical physicians. Meanwhile, no ectopic tissue was observed in the control group after laparoscopic surgery. The cases included in the study were all women of childbearing age, with the youngest being 23 years old and the oldest being 52 years old. These cases did not receive hormone or antibody therapy in the six months prior to surgery and did not exhibit liver or kidney dysfunction. Patients with severe heart disease, hypertension, and other related conditions were excluded. All cases were aware of the content and purpose of this study and provided informed consent. After surgical resection of the included cases, the obtained tissue samples were immediately washed with Phosphate Buffered Saline (PBS). After the above operation was completed, the sample was fixed with 10% paraformaldehyde, which dehydrated it. It was embedded cut it into slices with a thickness of 4 μm. Immunohistochemical techniques was used to determine the levels of PNMA5 in different tissues.

Isolation and culture of primary cells in ectopic endometrium

After collecting ectopic tissue samples, they were carefully placed in 1.5-ml tubes containing PBS with the aid of ice handling. Repeated washing with PBS, with the purpose of fully removing the red blood cells was carried out. The samples were processed into a paste with clean ophthalmic scissors, and tryptase (without EDTA) was added in the established proportion to mix them. The mixture was placed in a 37℃ incubator for 0.5h and blown every 10 minutes during the operation. Thereafter, complete medium was used to promote the cessation of trypsin digestion in the samples, and finally the samples were filtered.

After the above treatment, the resulting cell filtrate was centrifuged at low speed. The supernatant was removedafter centrifugation, and the remaining sediment was used in F12 medium containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin to maintain the activity and growth status of the cell samples; the incubation conditions here were: 37 ℃, 5% CO_2; the incubation time was 24h. The fresh medium was replaced with the aim of removing all the cells in suspension. Finally, the sample was observed under a high-precision microscope and recorded.

siRNA production and transfection

In this study, transient transfection experiments were mainly performed with *Pnma*5 short interfering RNA (*Pnma*5-siRNA) and scrambled siRNA (NC) purchased from RiboBio Company in Guangzhou. In this process, the sequences used specifically include :
Scrambled siRNA: 5′-UUCUGACTCCAGACTCCATGUUU-3′; Pnma5 siRNA-1#: 5′-GGCGACTCCGACTCCATCATA-3′; *Pnma5* siRNA-2#: 5′-GGATGACTCCATGACTTTAGA-3′; *Pnma5* siRNA-3#: 5′-GAAGACTCCATCGACTCCATA-3′.

Strictly following the procedure, primary cells from ectopic tissue were carefully laid in six-well plates at a concentration of 1.0×10^5, and after

Pnma5 siRNA was introduced into cells, transfection was completed with Lipo8000™ transfection reagent. After 72h, the corresponding transfection efficiency was determined with the help of protein blotting and other techniques, and the results were recorded.

qRT-PCR

Total RNA from the cells of cases was extracted by TRIzol reagent. To determine the concentration and purity of RNA, the model OneDrop 1000 spectrophotometer is used, and the remaining part is cryopreserved for subsequent experiments. The resulting cDNA was obtained by treating 1ug RNA with a reverse transcription kit. The qRT-PCR experiment was completed with the ABI 7500 real-time PCR system. The reagent used in this process is SYBR Green (Roche, Basel, Switzerland), and the specific thermal cycle conditions are : 95 ˚C for 10 min, 60 ˚C for 15 s and 72 ˚C for 20 s, 40 cycles. The relative expression was calculated via the 2-△△CT method and with reference of Gapdh. The primer sequences: Gapdh: 5'-GACGTCGCTGCGTCTA-3' (Forward) and 5'-AGGAGTGGGTGTCGCTGT-3' (Reverse). Pnma5: 5'-CGTGTCGCTGCACTACAG-3' (Forward) and 5'- AGGTTTCACACAGCCACTC-3' (Reverse).

Western blot analysis

In this experiment, EM samples and cells were processed by RIPA solution to extract total protein; the material was quantified by BCA kit and brought to the established concentration. The 5 loading buffer was mixed well with the protein supernatant (1:4), and boiled for 5 minutes. After completion, 20ul of protein was accurately used to unfold further SDS-PAGE, and the separated protein was carefully transferred to the PVDF membrane. The membranes weretreated with 5% skimmed milk and sealed for 60min at room temperature. Thereafter, the primary antibodies that could specifically recognize and bind PNMA 5, BCL-2 and BAX were incubated with the corresponding target proteins and removed the next day. During this time, β -actin was used as a control. Next, the membrane was fully washed by using TBST and then co-incubated with anti-rabbit and anti-mouse secondary HRP-conjugated antibodies for 60 min at room temperature. The target protein was processed by ECL reagent, and then imaged by the chemiluminescence gel imaging system. The results were input into ImageJ (NIH) software for quantitative analysis, from which the expression difference of the target protein was obtained.

CCK-8 Assay

In this experiment, primary cell samples from the ectopic tissue were first carefully seeded into 96-well plates at a concentration of 3×10^3. After transfection, sample viability was determined by CCK-8 technology at four time points: 24, 48, 72, 96, and 120 hours. The detailed process is: first, add 10ul CCK-8 solution to each well and culture for a period of time at room temperature. Then, the OD value was measured at 450nm. On this basis, the cell growth curve was further drawn to analyze the change of sample survival rate at different time points.

Wound healing assay

This experiment was mainly used to examine the migratory ability of the cells. The specific process is as follows: First, the drawing tool was used to draw several horizontal lines on the back of the six-hole plate. The ectopic tissue cells were inoculated to the corresponding wells at 1.5×10^5, and then incubated in an incubator and removed the next day; here, the culture conditions were set as 37℃, 5%CO_2. Cell scratches were applied for well-growing cell samples. After the above operation, the samples were washed repeatedly with PBS and the prepared serum-free medium (F12) was added to the culture under equivalent conditions. During this process, photographs were taken at three time points: 0, 24 and 48 hours. Based on the purpose of gene interference and control, they were divided into *Pnma*5-siRNA-3 # and NC groups. Data obtained from the experiments were processed using the Image J tool. Finally, the wound healing rate was calculated as the difference between the wound area at 24h (or 48h) and the 0h wound area, divided by the 0h wound area as a percentage.

Transwell assay

The experiments in this subsection were mainly used to examine the cell migration and invasion

capacity. To test the migration ability of the cell samples, the primary cells of the ectopic tissue were digested after 48 hours of transfection; then the samples were resuspended to complete the cell count and adjusted to a density of 1×10^5 / ml. After the above procedure, 500 ul of medium containing 10% FBS was poured into a 24-well plate and the transwell insert was placed into it. Use a pipette to absorb the sample suspension and carefully inoculate 200 ul into the upper chamber to see no air bubbles. After 2d of culture, cells in the upper chamber were slowly removed with cotton swabs, and the remaining part was fixed in 4% paraformaldehyde and stained with 0.1% crystal violet for 0.5h at room temperature, observed under a high-precision microscope and recorded. Next, the cell count was completed, and then the number of migrating cells in the two groups was determined.

The test of cell invasion ability was not significantly different from the above tests. All steps were consistent except that 60 μl Matrigel of matrix was added to the incubator under room temperature conditions before inoculation of the cells. To ensure the reliability of the study conclusions, the experiment was repeated more than twice.

Flow cytometric cell cycle analysis

In the experiments in this subsection, the cell cycle analysis was mainly developed through kits from the Bayyotai Biotechnology Institute in Haimen. The specific method is as follows: The first generation cell samples in the ectopic tissue were collected 72 hours after slicing, and the cycles were measured according to the following steps: First, the samples were fully washed with pre-cooled PBS. Next, 70% ethanol was used to avoid blocking the cells; transfer them to 4℃ and remove them the next day. Treatment was performed by using PBS. The prepared 0.5 ml of PI staining solution was added to each sample, and after centrifugation, the precipitate was resuspended to ensure that the sample had full contact with the staining solution. Red fluorescence at 488 nm was determined by flow cytometry and the instrument model used in this step was FACS Calibur. Finally, the obtained data are input into Modfit software for processing and save the results.

Flow cytometric cell apoptosis analysis

The detection of apoptosis will be strictly followed in this experiment. The kit used in this session is Annexin-V-FITC / PI. 72 h after transfection, the first generation cells of ectopic tissue were collected for unfolding assay. After centrifugation of the samples, the supernatant was collected into a clean, sterile EP tube. Then fully washed with PBS. After the above steps, 195 μl of annexin V-FITC binding solution was refined to slowly resuscitate the sample. Then, an appropriate amount of Annexin V-FITC and PI staining solution are added in order, and mix well. In room temperature for about 15 minutes. Next, the fluorescence of FITC and PI was determined by flow cytometry. The apoptosis data of the obtained samples were accurately input into FlowJo software for processing and preservation.

Statistical analysis

All kinds of data obtained in this study were processed by GraphPad Prism 8.0. All kinds of data are calculated according to the "mean value ± standard deviation". One-way ANOVA was performed when comparing group comparisons, and t-test was expanded. 0.05 was set as the test level, and $P < 0.05$ indicates significant differences between the two data sets.

Ethical consideration

The ethical issues related to this study primarily involve the collection and use of human ovarian tissue samples, the informed consent process, and the protection of participant privacy. All participants were fully informed about the purpose, procedures, and potential risks of the study and provided written informed consent before participation. Ethical considerations were also given to the inclusion and exclusion criteria to ensure that only suitable candidates were included, and vulnerable individuals, such as those with severe heart disease or kidney dysfunction, were excluded. The research adhered to ethical guidelines regarding the handling and storage of human biological samples, ensuring confidentiality and privacy throughout the study. Additionally, the study was approved by the Ethics Committee of the

Second Affiliated Hospital of Nantong University, with approval granted on [2020.3.20] (Approval Number: [2020KT067]).

Results

PNMA5 is increased in ectopic endometrium of ovarian EM

For the cases included in the study, their normal endometrial tissues and ectopic tissues were obtained, in 12 cases each. Figure 1 presents the results of the immunohistochemical staining. It can be clearly seen that PNMA 5 is largely distributed in the ectopic tissues. After staining the treatment, such proteins were observed to cluster mainly around the gland and in the matrix. However, this protein was not detected in the normal tissues, and the difference was significant ($P < 0.05$).

PNMA5 silencing blocks the proliferation of primary cells

In experimental studies, the first generation of cells in ectopic tissues (Figure 2A) were mainly collected for determination to clarify the correlation between the protein and cell proliferation. Its levels in the cells are disturbed by the transfection of the corresponding siRNA. At 72 h after transfection, the transfection efficiency was examined by different methods. The protein was found to be higher in the *Pnma*5-siRNA transfection group compared to the NC group (control group). After testing, *Pnma5*-siRNA-3 # has a more desirable effect (Figure 2B-D) and can be used for the next phase of the study.

In this study, the level of cell proliferation was determined at four time points after transfection with CCK 8 kit. Thus, cell proliferation was significantly weaker in the *Pnma*5-siRNA-3 # group (Figure 3A).

Also in the analysis of the cell cycle, see Figure 3B, after interference with *Pnma*5, the percentage of samples in G1 phase was not significantly different from before. However, in S phase, the two groups showed that the percentage of *Pnma*5-siRNA-3 # group was relatively lower and the difference was obvious ($P < 0.01$). In G2 stage, the data statistics showed a large increase in the percentage of PNMA 5 positive group, and the difference was obvious ($P < 0.05$) (Figure 3B, C). It is concluded that PNMA 5 silencing can greatly delay the proliferation of the first generation of cells in ectopic tissues, with a negative correlation between them.

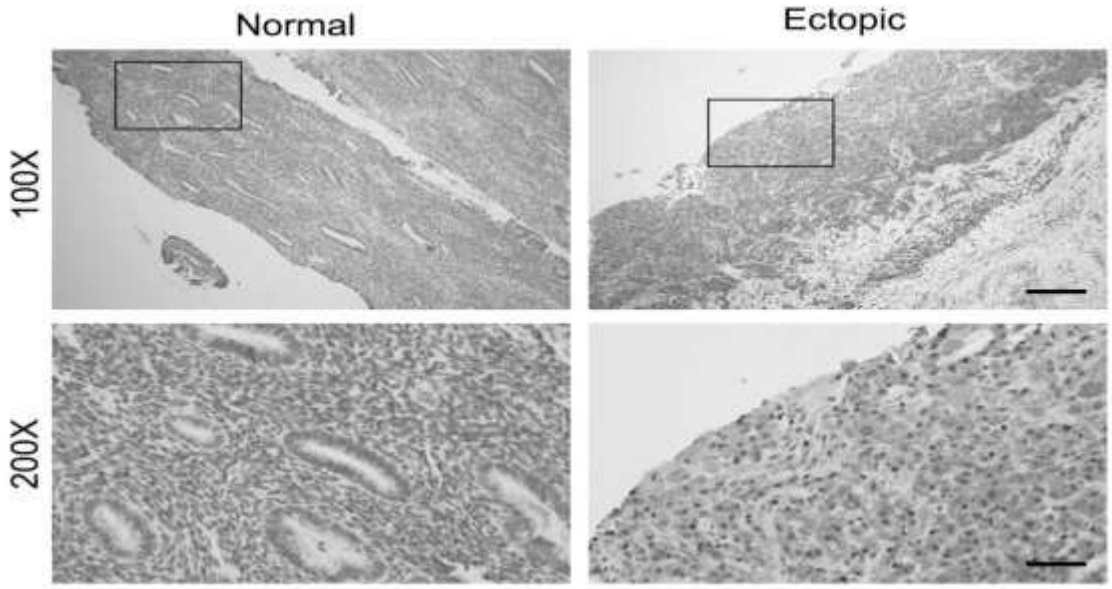
PNMA5 knockdown promotes the apoptosis of primary cells

To fully reveal the role of PNMA5 in EM, this study measured the degree of apoptosis of first generation cells in ectopic tissues after transfection with *Pnma*5-siRNA-3 #. It was found that compared to the control group, the number of apoptotic cells in this group was higher, and the difference between the two groups was very significant ($P < 0.01$) (Figure 4A, B). Meanwhile, this study further examined the levels of "BCL-2" and "BAX" in cells, and the results showed that the ratio of the two was lower in the control group, with a significant difference ($P < 0.01$) (Figure 4C, D). The conclusion drawn from this is that knocking out PNMA5 can accelerate the apoptosis of first generation cells in ectopic tissues, and the two are positively correlated.

PNMA5 silencing inhibits the migration and invasion of primary cells in ectopic endometrium

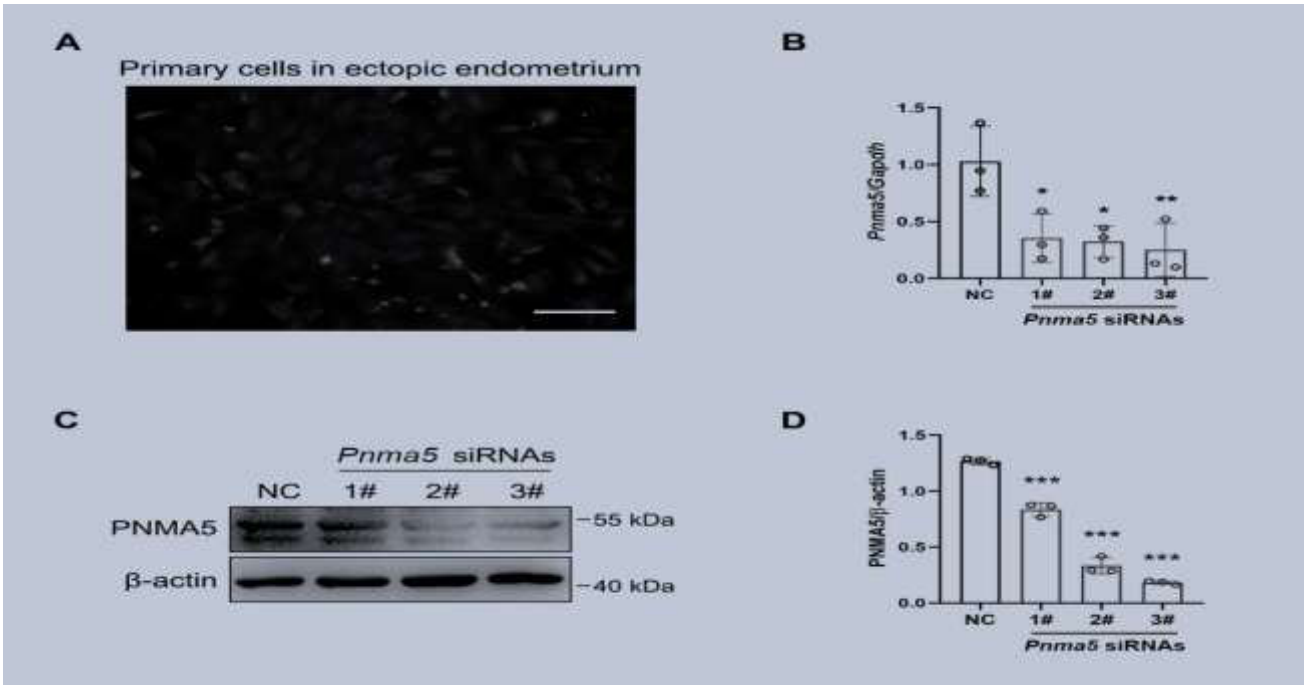
A *Pnma*5-siRNA-3 # transfection was performed on the samples during the experiment. Further transwell migration analysis was unfolded after 72h, aiming to clarify the intrinsic link between this protein and cell migration. The study found that the NC control group had more number of migrated cells, and the difference between the two groups was very obvious. However, in the subsequent detection of cell diffusion ability, it was found that interference with PNMA 5 could greatly weaken the diffusion ability of the first generation of cells in ectopic tissues, and the difference between the two groups was extremely significant（$P < 0.001$ ）（Figure5A, B）.

A wound healing assay was also conducted to explore the intrinsic association between PNMA 5 and cell migration. Combining Figure 5C and 5D, we can see that the control group had a higher cell migration rate, and the difference between the two groups was very significant ($P < 0.001$).



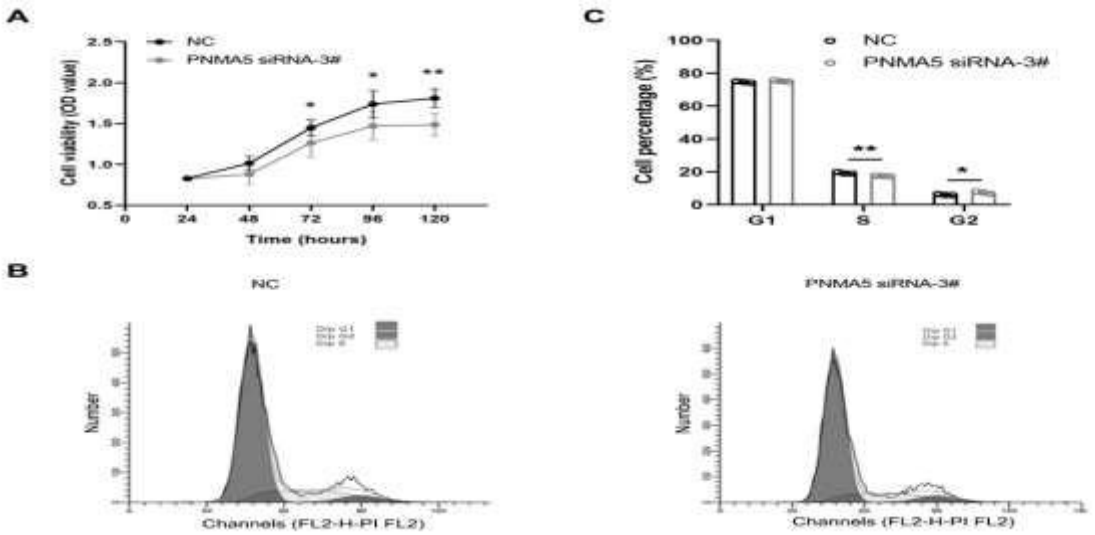
It was detected via immunohistochemical staining method. 100×, Scale bar = 20 μm. 200×, Scale bar = 50 μm.

Figure 1: PNMA5 is obviously enhanced in ectopic endometrium of ovarian EM.



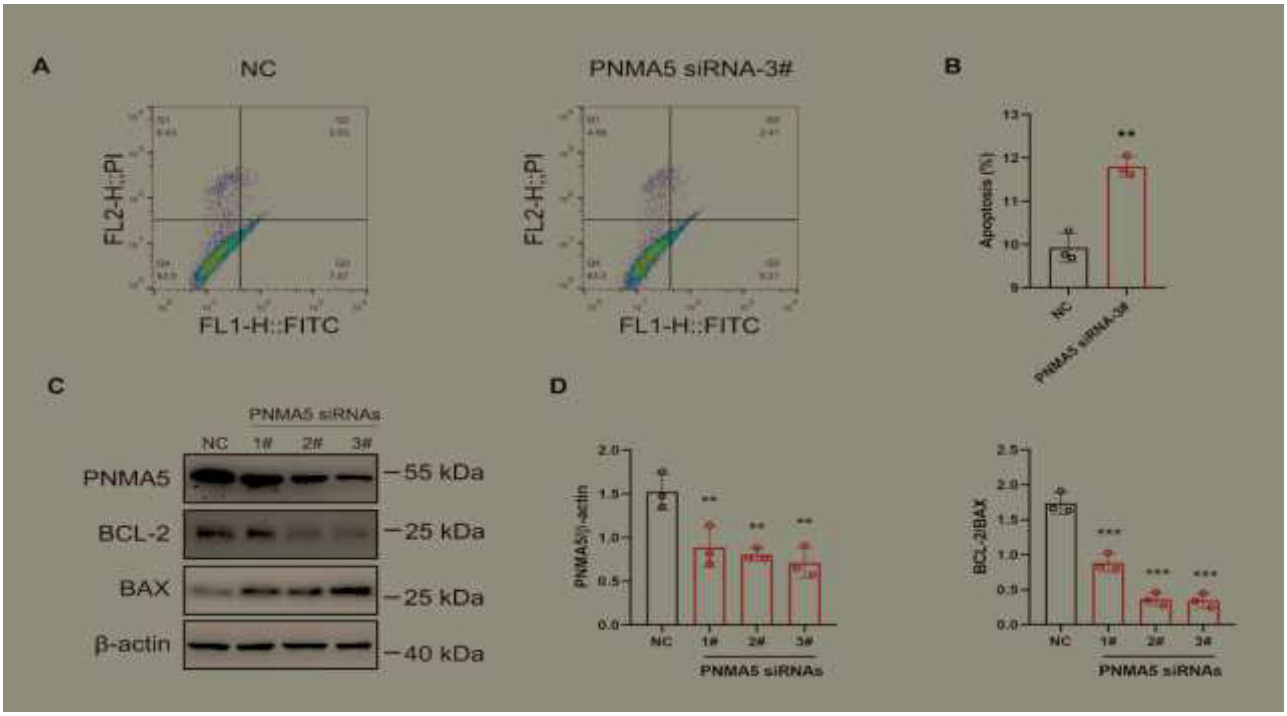
(A) Immunofluorescence staining of the VE-cadherin. 10 μm. (B) The *Pnma5* mRNA levels in the primary cells after transfection was detected via qRT-PCR. (C) The PNMA5 protein levels of that was detected via western blot method. (D) The statistics result of WB data. Data were expressed as mean ± SD, the t-test and ANOVA were applied tocheck the difference level. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001.

Figure 2: PNMA5 knockdown in the primary cells in sample.



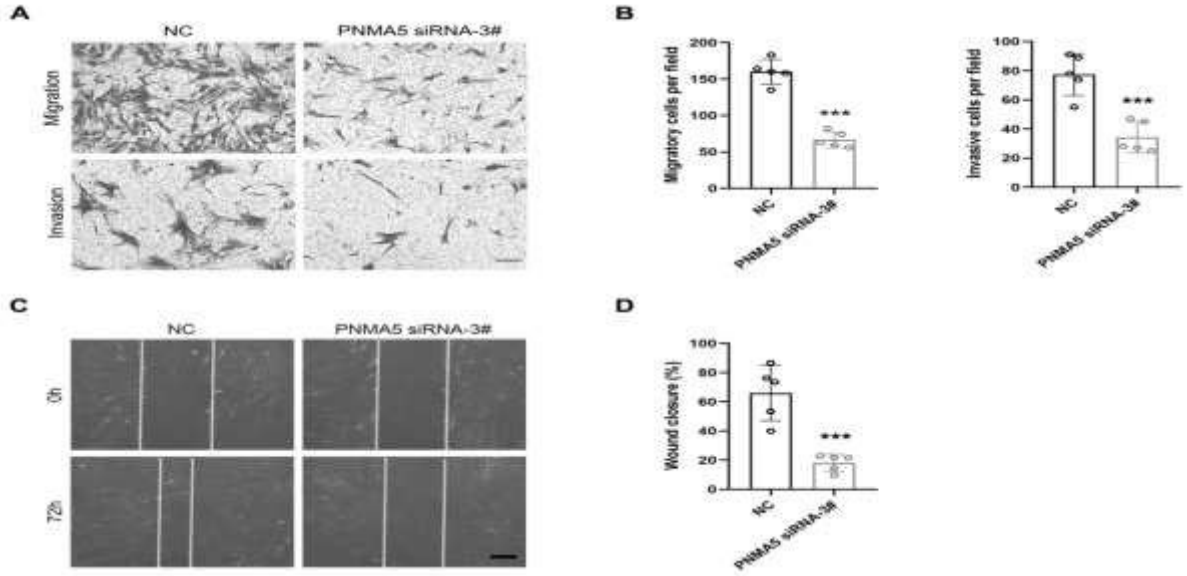
(A) The effect of PNMA5 on proliferation of the primary cells was analyzed via CCK-8 method. (B) The effect of it on cell cycle was analyzed via Flow cytometry. (C) The cell cycle analysis result. Data were expressed as mean ± SD, t-test and ANOVA test were conducted.

Figure 3: PNMA5 silencing inhibits the proliferation of primary cells.



(A) The effect of PNMA5 on primary cells apoptosis was detected via Flow cytometry method. (B) The cell apoptosis analysis result. (C) The effect of PNMA5 on BCL-2 and BAX protein were analyzed via WB method. (D) The statistics result of WB. Data were expressed as mean ± SD, t-test and ANOVA test were conducted.

Figure 4: PNMA5 knockdown promotes the apoptosis of primary cells.



(A) The influence of PNMA5 on the migration and invasion of the primary cells was measured via transwell method. Scale bar =50 μm. (B) The transwell result. (C)The effect of PNMA5 on primary cells migration were measured via wound healing method. Scale bar = 20 μm. (D) The wound healing analysis result. Data were expressed as mean ± SD, t-test and ANOVA test were conducted

Figure 5: PNMA5 silencing inhibits the migration and invasion of primary cell.

Considering the above analysis, it is concluded that such proteins can accelerate the diffusion of the first generation of cells in ectopic tissues, and the two are positively correlated.

Discussion

EM has a higher incidence. Its onset site is usually the ovary, peritoneum, and other positions[6,31]. However, according to previous clinical experience, the incidence of ovarian EM is higher, and one side[7,32] is involved in most cases. In the early stages of the disease, the ectopic lesions present only small lesions, and with the progression of the disease, chocolate-like cysts will appear. At this stage, the medical community has not fully clarified the pathogenesis of this disease. Some scholars believe that it is gradually formed because of the menstrual blood reflux to the ovary. It should be pointed out that, although EM is not a malignant tumor, its occurrence and development process is similar to that of the malignant tumor, which is very harmful to the body[33-35]. Although surgical therapy is widely used, the resection is often not thorough and relapse tends to occur. Therefore, how to effectively treat EM has become the research focus in the medical community. Exploring the etiology and pathogenesis will help to find effective treatments, so that more patients can benefit from it.

Modern medical research has found that *Pnma*5 is located on the X chromosome and it only tested positive for in testis and brain tissue[27,28]. In addition, humans have found that this gene is more abundant in ovarian cancer tissue than in healthy ovarian tissue. Knock-out can delay the division and spread of epithelial cancerous cells[30]. Other scholars noted that PNMA 5 tends to have higher levels in cancerous tissues compared to healthy tissues; specifically, its levels were detected in NSCLC cancer[36]. In addition, cancerous tissues were also highly expressed in colorectal cancer[37] and colon cancer[24]. In general, humans have relatively little research on its gene function, so this study can fill the research gap in this field.

Despite the advancements in understanding its pathogenesis, the molecular mechanisms underlying the development and progression of EM remain poorly understood. In this study, we explored the role of PNMA5 in ovarian EM and its

potential impact on disease progression, using a combination of immunohistochemical, cell proliferation, apoptosis, and migration assays. Our results provide novel insights into the molecular processes associated with EM and suggest that PNMA5 could play a crucial role in regulating the proliferation, apoptosis, and migration of ectopic endometrial cells.

One of the major findings of this study was the significant upregulation of PNMA5 in ectopic endometrial tissues compared to normal tissues. Immunohistochemical analysis revealed that PNMA5 was predominantly localized around the glandular and stromal areas in the ectopic tissues, suggesting its involvement in the pathophysiology of EM. This finding aligns with previous studies that have shown a high expression of PNMA family members in various cancers, which are often characterized by abnormal cell proliferation and migration[36,37]. However, the precise role of PNMA5 in ovarian EM had not been previously elucidated, making this an important contribution to the field.

To further investigate the functional significance of PNMA5 in EM, we performed silencing experiments using PNMA5-specific siRNA. The results demonstrated that silencing PNMA5 significantly inhibited the proliferation of primary cells derived from ectopic endometrium. This was further supported by cell cycle analysis, which showed a reduced proportion of cells in the S phase and an increased proportion in the G2 phase after PNMA5 knockdown. These findings suggest that PNMA5 may regulate the cell cycle and promote the proliferation of ectopic endometrial cells, which is a critical feature of endometriotic lesions.

In addition to its role in cell proliferation, PNMA5 silencing also promoted apoptosis in primary ectopic endometrial cells. This was evidenced by an increased number of apoptotic cells and a significant alteration in the BCL-2/BAX ratio, indicating that PNMA5 may influence the apoptosis pathway in EM. Given that one of the hallmarks of endometriosis is the resistance of ectopic endometrial cells to apoptosis, our results suggest that targeting PNMA5 could enhance the apoptosis of these cells, potentially offering a therapeutic strategy to control disease progression.

Moreover, our study demonstrated that PNMA5 is involved in the migration and invasion of ectopic endometrial cells. Transwell migration assays and wound healing assays revealed that silencing PNMA5 significantly impaired the ability of these cells to migrate and invade, suggesting that PNMA5 plays a critical role in the dissemination of ectopic endometrial tissue. This finding is consistent with the malignant-like behavior often observed in endometriotic lesions, which can invade surrounding tissues and contribute to the chronic nature of the disease. Therefore, PNMA5 may serve as a potential target for therapeutic strategies aimed at inhibiting the invasive characteristics of EM. The implications of these findings are substantial, both for understanding the pathophysiology of EM and for developing potential therapeutic strategies. First, the upregulation of PNMA5 in ectopic endometrial tissues suggests that it could serve as a biomarker for diagnosing or monitoring disease progression. Furthermore, given its role in regulating cell proliferation, apoptosis, and migration, PNMA5 represents a promising therapeutic target. Future studies exploring the mechanisms by which PNMA5 influences these cellular processes, and the development of specific inhibitors or RNA-based therapies, could lead to novel treatment options for EM. Importantly, these therapies could be aimed at improving the management of the disease, reducing recurrence rates, and alleviating the physical and psychological burden experienced by patients.

In terms of clinical practice, these findings underline the need for a better understanding of the molecular drivers of EM. Although current treatment options, including surgery and hormone therapy, have been widely used, they do not address the underlying molecular mechanisms and are often associated with recurrence. Targeting proteins like PNMA5, which are involved in crucial aspects of disease progression, could offer a more effective and sustainable approach to managing EM. Additionally, identifying biomarkers such as PNMA5 could aid in the development of more personalized treatment regimens, enabling clinicians to tailor therapies based on the specific molecular profile of the disease in individual patients.

In conclusion, this study provides novel insights into the role of PNMA5 in ovarian EM and highlights its potential as a therapeutic target. By elucidating the molecular mechanisms underlying EM, this research paves the way for the development of more effective and personalized treatments, ultimately improving outcomes for women suffering from this debilitating condition. Future studies should focus on further exploring the functional roles of PNMA5 in other forms of endometriosis and investigating its potential as a therapeutic target in clinical settings

Conclusion

Experimental results show that PNMA 5 is more abundant in ectopic tissue than in normal endometrium in EM cases. The decrease of this protein can delay the division and diffusion of the first generation of cells in ectopic tissues, and play a role in accelerating cell apoptosis, so it is speculated that it is a new target for the treatment of EM. This study result may have an important reference value for the clinical practice.

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Authors contributions

In the process of writing this paper, Qiao Haifeng was mainly responsible for designing the framework of the article; Liu Yinglei and Chen Zhe were responsible for searching and screening the literature and data; Chen Zhe, You Jun and Zheng Yanli were responsible for organizing and summarizing the results; Liu Yinglei and Qiao Hai were responsible for drafting the first draft. All the above authors participated in the examination of the results and the finalization of the paper.

Conflicts of interest

There is no conflict of interest in the writing of this paper or in the experimental study

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