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Inhibition of IGF2BP1 attenuates the progression of endometriosis through PTBP1

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Abstract

Introduction. Endometriosis (EMs), manifested by pain and infertility, is a chronic inflammatory disease. The precise pathophysiology of this disease remains uncertain. Insulin-like growth factor-2 mRNA-binding protein 1 (IGF2BP1) and polypyrimidine tract-binding protein 1 (PTBP1) have both been found to regulate proliferation, apoptosis, and invasion. This study aimed to investigate the effects of IGF2BP1/PTBP1 in treating EMs.

Materials and methods. qRT-PCR and western blotting were employed to quantify IGF2BP1 and PTBP1 expression in six patients with EMs (mean age 33.83 years). The correlation analysis, STRING database prediction, and RNA immunoprecipitation were utilized to identify the relationship between IGF2BP1 and PTBP1. Ectopic endometrial volume, weight, HE staining, and IGF2BP1 silencing were utilized to estimate the effects of IGF2BP1 in EMs model rats. qRT-PCR, CCK-8, 5-ethynyl-2'-deoxyuridine (EDU) labeling, Transwell assay, and flow cytometry were utilized to assess the effects of IGF2BP1/PTBP1 on the proliferation, migration, invasion, and apoptosis of ectopic endometrial stromal cells (eESCs). Furthermore, western blotting was employed to evaluate expressions of PCNA, VEGF, and E-cadherin in EMs rats and eESCs.

Results. The mRNA and protein levels of IGF2BP1 and PTBP1 in the ectopic and eutopic endometrium of EMs patients were significantly increased. RNA immunoprecipitation revealed a close interaction of IGF2BP1 with PTBP1. Additionally, the endometrial volume, weight, and histopathologic scores in rats were significantly reduced after IGF2BP1 silencing. IGF2BP1 silencing also decreased the expression of PCNA and VEGF, and increased E-cadherin expression in endometrial tissues of EMs rats. Moreover, IGF2BP1 silencing inhibited proliferation, migration, and invasion and promoted apoptosis through PTBP1 in eESCs.

Conclusions. IGF2BP1 exhibits potential beneficial properties in the management of EMs by interacting with PTBP1, thereby highlighting IGF2BP1 as a promising therapeutic target for EMs. *(Folia Histochemica et Cytobiologica 2024, Vol. 62, No. 1, 25–36)*

Keywords: endometriosis; IGF2BP1; PTBP1; interaction

Introduction

Endometriosis (EMs) is a chronic, inflammatory gynecologic disease characterized by the presence and growth of tissue resembling the endometrium outside the confines of the uterus, particularly in the pelvic cavity. This condition is associated with symptoms such as pelvic pain, dysmenorrhea, dyspareunia, and infertility [1]. EMs is known to impact around 10% of women during their reproductive years [2]. One of the most frequently utilized therapies for managing EMs involves the use of dienogest, a progestin with anti-ovulatory properties. However, it is important to note that prolonged exposure to dienogest may potentially exacerbate infertility [3]. Given the constraints of current therapeutic methods in addressing EMs, there exists a need to explore innovative strategies for the prevention or treatment of EMs.

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The insulin-like growth factor-2 mRNA-binding protein 1 (IGF2BP1), classified as an m6A reader, is known to have a significant impact on the regulation of specific mRNAs by influencing their stability, translatability, or localization [4, 5]. Previous research has provided evidence that IGF2BP1 functions as an RNA-binding protein (RBP) that plays a role in the regulation of proliferation, apoptosis, and invasion [6]. Additionally, it has been discovered that METTL3, which is an m6A writer, plays a role in impeding the advancement of EMs [7]. Polypyrimidine tract-binding protein 1 (PTBP1) is a well-established regulator of posttranscriptional gene expression, exerting control over various aspects of mRNA splicing, translation, stability, and localization [8]. It assumes a pivotal role in the regulation of numerous cellular processes in cancer cells, including glycolysis, apoptosis, proliferation, tumorigenesis, invasion, and migration [9]. A prior study showed that PTBP1 negatively regulated endometrial decidualization, likely contributing to recurrent implantation failure [10]. However, the effects of IGF2BP1 and PTBP1 against EMs are yet to be explored.

Our initial investigation revealed a notable upregulation of IGF2BP1 and PTBP1 expressions in ectopic endometrial tissues obtained from patients diagnosed with EMs. In the current study, we postulated that IGF2BP1 might have a role in the process of EMs by modulating PTBP1. This study aimed to first detect the expression and correlation of IGF2BP1 and PTBP1 in the endometrial tissues of EMs patients, assess the impact of IGF2BP1 in rats with EMs, and investigate the effects of IGF2BP1/PTBP1 on endometrial receptivity in endometrial mesenchymal stromal cells (eESCs). This study may thereby establish a theoretical foundation for the targeted therapy of EMs.

Materials and methods

Clinical samples. We recruited samples of 17 female participants, ranging in age from 22 to 49 years, who were scheduled to undergo either laparoscopic surgery or hysterectomy at Changsha Central Hospital in 2023. Ethical approval for this project was obtained from Medical Ethics Committee of Changsha Central Hospital (2022-S0206). All participants exhibited regular menstrual cycles ranging from 21 to 35 days. They were not subjected to any hormonal treatment, were not pregnant, not breastfeeding, and had not utilized an intrauterine device in the preceding six months. Patients diagnosed with pelvic inflammatory disease, adenomyosis, and dysfunctional uterine bleeding were not included in the study. The clinical samples included three groups: Normal, Eutopic, and Ectopic. Sufficient samples of paired ectopic and eutopic endometrial tissues in the Ectopic and Eutopic groups were obtained from six cases diagnosed with EMs. Control eutopic endometrial samples in the Normal group were obtained from 11 patients who did not have EMs, as confirmed by laparoscopic surgery. Shortly after collection, the tissue samples were rapidly frozen in liquid nitrogen and subsequently stored at –80°C to facilitate the extraction of RNA and proteins.

Animals and study design. All animal experiments conducted were granted approval by Medical Ethics Committee of Changsha Central Hospital (2022-S0206). Adult female specific-pathogen-free Sprague Dawley rats, weighing between 200–250 g, were obtained from Hunan SJA Laboratory Animal Co., LTD (Changsha, Hunan, China). The rats were housed in a pathogen-free facility with controlled environmental conditions, including a 12-h light/dark cycle, a temperature of 22 ± 1°C, and a humidity level of approximately 50%. They had access to water and sterile food ad libitum. Prior to the surgical procedure, a comprehensive health assessment was conducted on all rats over seven days. The rats were randomly assigned to four groups of six rats per group. The groups were labeled as Sham, EMs, EMs+IGF2BP1 silencing negative control (si-NC), and EMs+IGF2BP1 silencing (si-IGF2BP1). Sham stands for sham operated rats, EMs stands for EMs modeling of rats, EMs+si-NC stands for injection of lentiviral vectors with IGF2BP1 silencing negative control in rats following EMs modeling, and EMs+si-IGF2BP1 stands for injection of lentiviral vectors with IGF2BP1 silencing in rats following EMs modeling.

Induction of experimental EMs in rats. The rat model of EMs was established following the previously described methodology [11]. When the rats reached the age of three months, they were subjected to anesthesia using isoflurane. Following this, a midventral incision was made to expose the uterus, and a distal segment measuring 1.0 cm in length was surgically removed from the left uterine horn. The segment was immersed in phosphate-buffered saline (PBS) at a temperature of 37°C and subsequently divided longitudinally. From this, a section measuring 5 × 5 mm was obtained. This uterine tissue specimen was transplanted onto the inner surface of the right abdominal wall while retaining the myometrium and positioning the epithelial lining instead of the peritoneal surface. After 24 h of modeling, lentiviral vectors with IGF2BP1 silencing or the appropriate controls (dissolved in 0.9% NaCl, 100 μL, 1 × 108 TU/mL; Abiowell, Changsha, Hunan, China) were injected into the transplant site of each mouse. In contrast, the Sham and EMs groups received an equivalent volume (100 μL) of PBS. All groups received lentiviral or saline injections repeated once daily for four weeks. Rats were euthanized by administering an overdose of sodium pentobarbital (150 mg/kg; Nembutal, Diamondback Drugs, Scottsdale, AZ, USA) 24 h after the final lentiviral or 0.9% NaCl injection, and lesions of EMs were collected for subsequent experiments.

Isolation and culture of eESCs. The eESCs were obtained by mincing ectopic endometrial tissues into small fragments. The fragments were then washed with DMEM (11965092, Gibco,

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Grand Island, NY, USA) and digested using 0.5% collagenase II (AWH0565a, Abiowell) for 1 h at 37°C. The dispersed cells underwent filtration using a 100-μm filter in order to eliminate any remaining undigested debris. The eESCs were obtained through centrifugation at 1500 rpm for 10 min followed by two washings with PBS. Subsequently, the eESCs were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; 10099141, Gibco) at 37°C. When the cells became 90% confluent, they were used for experiments. Vimentin and cytokeratin 19 (CK19), biomarkers of eESCs, were detected using flow cytometry with vimentin antibody (11-9897-80, Thermo Fisher Scientific, Waltham, MA, USA) and CK19 antibody (11-9897-80, Thermo Fisher Scientific).

RNA isolation and quantitative reverse transcription-PCR (qRT-PCR). Total RNA was extracted from the tissue and cell samples utilizing Trizol reagent (15596026, Thermo Fisher Scientific). Correspondingly, the process of reverse transcription was employed to convert mRNA into cDNA, utilizing the mRNA reverse transcription kit (CW2569, CWBIO, Beijing, China). The qRT-PCR was conducted using the SYBR method and the Ultra SYBR Mixture (CW2601, CWBIO). The time course and temperature in cycles are shown in Table 1. The experiments were conducted using a fluorescence quantitative PCR instrument (PikoReal 96, TCR0096) manufactured by Thermo Fisher Scientific. Primers were designed utilizing Primer 5 software (PREMIER Biosoft, San Francisco, CA, USA) (Table 2). β-actin was employed as a reference gene. Data analysis was conducted using the 2-ΔΔCt method.

Western blotting. The tissue and cell samples were collected and subsequently lysed using RIPA lysate (AWB0136, Abiowell). Following centrifugation at a speed of 12000 rpm for 15 min at 4°C, the liquid portion (supernatant) was carefully transferred into a 1.5 mL centrifuge tube. Proteins were separated using sodium dodecyl sulfate (SDS) gel electrophoresis and subsequently transferred onto nitrocellulose membranes. The membranes were enclosed within a blocking buffer solution comprising 5% skim milk. The membranes were subsequently incubated with primary antibodies overnight at a temperature of 4°C. The primary antibodies utilized in this research were directed against IGF2BP1 (1:20000, 22803-1-AP, Proteintech, Rosemont, IL, USA), PTBP1 (1:10000, 67462-1-Ig, Proteintech), proliferating cell nuclear antigen (1:5000, PCNA, 10205-2-AP, Proteintech), vascular endothelial growth factor (1:3000, VEGF, ab32152, Abcam, Cambridge, MA, USA), and epithelial cadherin (1:50000, E-cadherin, 20874-1-AP, Proteintech). Subsequently, the membranes underwent incubation with the secondary antibodies HRP goat anti-mouse IgG (1:5000, AWS0001, Abiowell) and HRP goat anti-rabbit IgG (1:6000, AWS0002, Abiowell) for 90 min at ambient temperature. Subsequently, the membranes underwent incubation with ECL chemiluminescence solution (AWB0005, Abiowell) for 1 min, followed by observation using the chemiluminescence imaging system (ChemiScope6100, CLiNX, Shanghai, China). The internal reference protein used in this study was β-actin (1:5000, 66009-1-Ig, Proteintech). ImageJ software (version 1.8, US National Institutes of Health, Bethesda, MD, USA) was employed to assess and compare the intensity of individual bands in western blotting experiments.

RNA immunoprecipitation (RIP) assay. The interaction between IGF2BP1 and PTBP1 was investigated utilizing the RIP kit (Sigma-Aldrich, St. Louis, MO, USA). The tissues underwent lysis, and the resulting tissue lysis solutions were subjected to incubation with an antibody specific to IGF2BP1 (22803-1-AP, Proteintech). The immunoprecipitation of RNA-protein complexes was performed using magnetic beads, followed by RNA extraction using TRIzol. PTBP1 was quantified using qRT-PCR. The negative control employed in this study involved the utilization of anti-IgG (30000-0-AP, Proteintech), which could verify if the discovered RNA-binding protein truly interacts with the PTBP1, instead of non-specific binding caused by other factors in the experimental conditions.

Evaluation of ectopic uterine tissue. The rats were placed on a platform in a supine position immediately after anesthesia was administered by intraperitoneal injection of 3% sodium pentobarbital (50 mg/kg). The abdominal cavity was then opened, and the dimensions of the ectopic uterine tissue were evaluated in situ using a caliper, encompassing all three axes. The prolate ellipsoid formula, V = 0.52 × length × width × height [11], was utilized to calculate the spherical volume of each ectopic uterine

Table 1. Quantitative PCR amplification procedure

Temperature	Time	Cycles
95°C	10 min	1
95°C	15 s	40
60°C	30 s	

Table 2. Sequences of the primers

Gene name	Species	Forward (5'-3')	Reverse (5'-3')
IGF2BP1	Human	AAAATCACCATCTCCTCGTTGC	ATTATTTCCTGCTCGGCCCTG
PTBP1	Human	GCTCAGGATCATCGTGGAGAAC	CTGTGCCGAACTTGGAGAAAA
β-actin	Human	ACCCTGAAGTACCCCATCGAG	AGCACAGCCTGGATAGCAAC
IGF2BP1	Rat	CGTGGAGGCTTTGGGTCT	TGATGTTTCGGATGGTGGC
PTBP1	Rat	AAAGTTACCAACCTCCTTATGC	GATGGGCTGTCCACGAAG
β-actin	Rat	ACATCCGTAAAGACCTCTATGCC	TACTCCTGCTTGCTGATCCAC

tissue. Tissues were captured in photographs using a digital camera, followed by excision and subsequent weighing.

Histological examination. Hematoxylin-eosin (HE) staining was used to assess morphological changes in ectopic endothelial tissues. Briefly, paraffin-embedded tissue sections were baked for 12 h at 60°C, followed by immersion in xylene for 20 min three times. Next, the sections were placed in 100%, 95%, 85%, and 75% ethanol, each concentration for 5 min, and then rinsed with distilled water for 5 min. Sequentially, the sections were successively stained with hematoxylin (AWI0020, Abiowell) and eosin (AWI0020, Abiowell). The sections were then dehydrated by gradient alcohol (95–100%) for 5 min per stage and placed in xylene for 10 min, 2 times. The sections were finally sealed using neutral gum and examined under an optical microscope (BA210T, Motic, Xiamen, Fujian, China) to identify the histological characteristics associated with EMs.

Evaluation of persisting epithelium in endometrial lesions. The evaluation of epithelial cell persistence was conducted using the method determined by Lebovic et *al.* [11]. The scoring system employed in this study classified the preservation of the epithelial layer into four categories. A score of 3 denoted a well--preserved epithelial layer, while a score of 2 indicated a moderately preserved epithelium with leukocyte infiltrate. A score of 1 indicated a poorly preserved epithelium with occasional epithelial cells only, and a score of 0 indicated the complete absence of epithelium. Three sections from each explant were evaluated by an independent evaluator in a blinded manner.

Cell transfection. To inhibit the expression of IGF2BP1, small interfering RNA, human IGF2BP1 silencing plasmid (si-IGF2BP1; HG-Si160423, HonorGene, Changsha, Hunan, China), was utilized. eESCs were cultured in serum-free DMEM medium at 37°C for 6 h in the presence of 2.5% v/v Lip2000 as well as 2.5% v/v si-NC or si-IGF2BP1. To obtain the overexpression of PTBP1, PTBP1 overexpressing plasmid (oe-PTBP1; pcDNA3.1-PTBP1, HG-HO411140, HonorGene) was used. The si-IGF2BP1+oe-NC group was transfected with the empty vector (PTBP1 overexpressing negative control plasmid, oe-NC). The eESCs were cultured in serum-free DMEM medium containing oe-NC or oe-PTBP1 at 37°C for 6 h. The transfected cells were cultured in a medium with 0.5 μg/mL puromycin for seven days to select stable cells.

Cell viability assay. To assess the viability of eESCs, a cell counting kit-8 (CCK-8, NU679, Dojindo, Kumamoto, Japan) assay was conducted. eESCs were seeded at 5 × 103/well in 96-well plates. After 24 h the initial culture medium was removed, and the cells were subsequently resuspended in 100 μL of fresh culture medium supplemented with 10% CCK-8 solution in each well. The plates were subsequently incubated at 37°C with a 5% concentration of CO_2 for 4 h. The absorbance at 450 nm was quantified using a Microplate Reader (Heales, Shenzhen, China).

5-Ethynyl-2-Deoxyuridine (EDU) assay. eESCs were seeded at 5 × 103/well and subjected to incubation with a 50 μL solution containing 50 μM EDU (C10310, RiboBio, Guangzhou, Guangdong, China) overnight. Following this, the cells were treated with 50 μL of 4% paraformaldehyde and fixed for 30 min. Next, the cells underwent treatment with a solution containing 2 mg/ /mL glycine and 0.5% Triton X-100 sequentially. Subsequently, the cells were stained with 300 μL of 1 × Apollo reaction cocktail for 30 min. Finally, the cells were stained with DAPI (C1005, Beyotime, Shanghai, China) and subsequently observed under an inverted microscope (DSZ2000X, Cnmicro, Beijing, China).

Transwell assay. After subjecting the eESCs to 6–8 h of starvation in serum-free DMEM, a total of 1 × 104 cells were introduced into the upper chamber in the migration assay. The upper chamber was filled with 200 μL of serum-free medium. Furthermore, a total of 2 × 10^4 cells were introduced into the upper Transwell chambers coated with Matrigel to conduct the invasion assay. The lower chambers were filled with DMEM containing 10% FBS. After being incubated at 37°C for 24 h, the cells located on the lower surface of the membrane were immobilized using 100% methanol and subsequently subjected to staining with a 0.1% crystal violet dye for 20 min at ambient temperature. After being washed with PBS, the cells were observed in five randomly chosen fields using a microscope (DSZ2000X, Cnmicro).

Cell apoptosis measurement. Flow cytometry was employed to quantify the apoptosis of eESCs using an Annexin V-PI apoptosis detection kit (KGA1030, KeyGEN BioTECH, Nanning, China). Briefly, eESCs were subjected to two washes with PBS and subsequently resuspended in the binding buffer. Subsequently, a mixture of 5 μL Annexin V-APC and 5 μL PI was added to the cells, followed by incubation for 10 min in a light-restricted environment at ambient temperature. Each sample underwent analysis using a flow cytometer (A00-1-1102, Beckman Coulter, Miami, FL, USA).

Statistical analysis. All data were reported in terms of the mean and standard deviation (SD) values. Statistical analyses were conducted utilizing GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). The t-test was employed to compare two groups, while the ANOVA was employed to compare multiple groups. The Pearson correlation analysis was utilized to evaluate the correlation between the mRNA levels of IGF2BP1 and PTBP1. A significance level of $P < 0.05$ was employed to ascertain statistical significance.

Results

The expressions of IGF2BP1 and PTBP1 in the endometrium of patients with EMs

Initially, we collected normal endometrium tissues from healthy humans and eutopic/ectopic endometrium tissues from patients with EMs. According to previous studies, IGF2BP1 and PTBP1 have been investigated as they play crucial roles in the progression of EMs [10, 12]. Therefore, the quantification of IGF2BP1 and PTBP1 expression levels in endometrial

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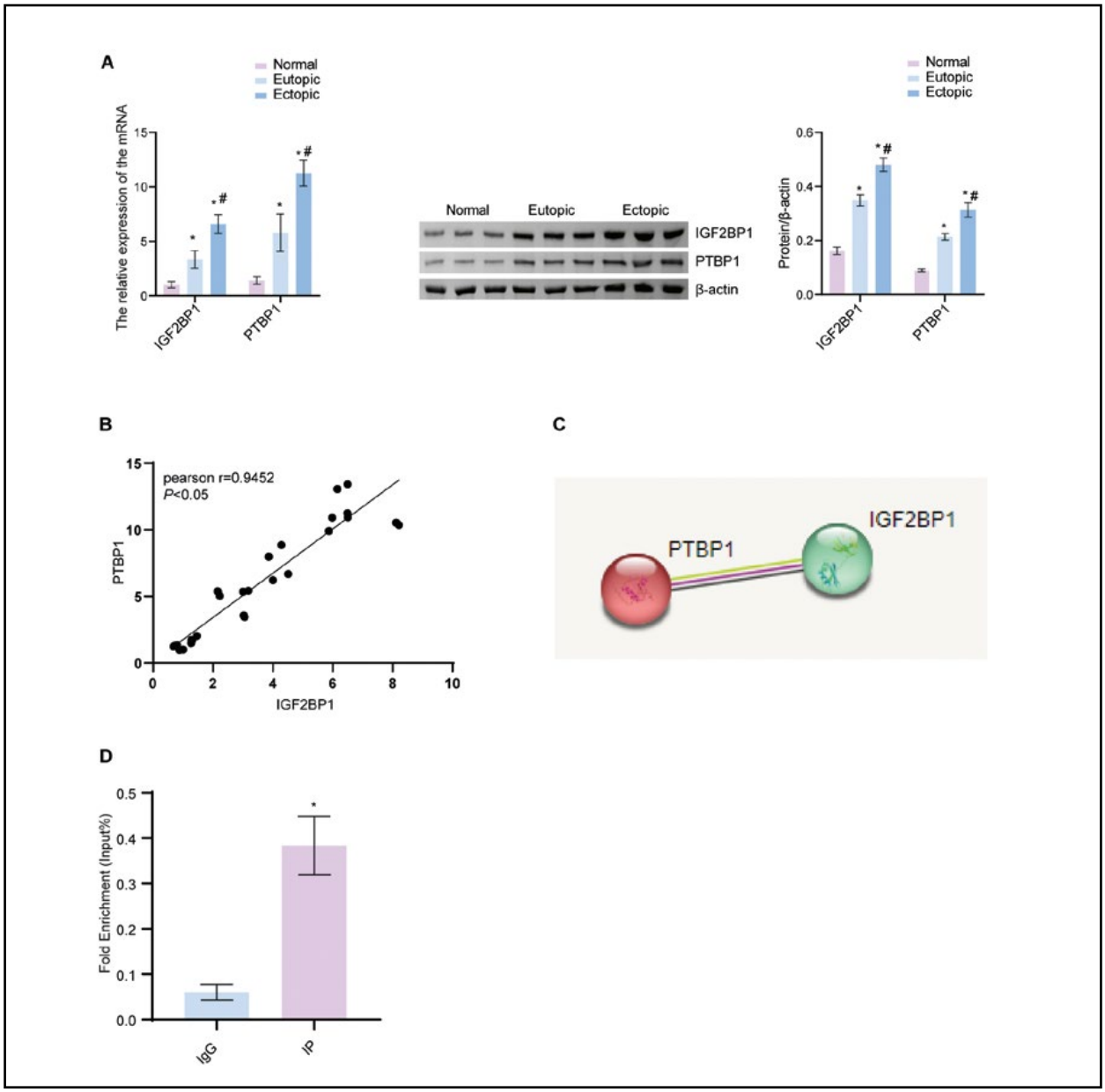

Figure 1. The expressions and relationship of IGF2BP1 and PTBP1 in the endometrium of patients with endometriosis (EMs). **A.** The expression levels of IGF2BP1 and PTBP1 were assessed in the endometrial tissues of women with or without EMs. n = 11 for Normal group, n = 6 for Eutopic and Ectopic groups. *P < 0.05 vs. Normal group, #P < 0.05 vs. Eutopic group. **B.** The strength of the correlation between IGF2BP1 and PTBP1 expression was quantified based on the Pearson correlation coefficient. **C.** The network image shows the protein interaction network between IGF2BP1 and PTBP1. Data were predicted by the STRING database. **D.** RNA immunoprecipitation was performed to analyze the binding of IGF2BP1 and PTBP1. *P < 0.05 *vs.* IgG group. n = 10 *per* group.

tissues was conducted using qRT-PCR and western blotting. We observed a significant increase in the expression levels of IGF2BP1 and PTBP1 in the EMs groups compared to the Normal group. Additionally, the expression levels of IGF2BP1 and PTBP1 were found to be significantly elevated in the Ectopic group when compared to the Eutopic group (Fig. 1A). Subsequently, a Pearson correlation analysis was conducted to examine the relationship between the levels of IGF2BP1 and PTBP1. The analysis revealed a statistically significant positive correlation (r = 0.9452, P < 0.05; Fig. 1B). In order to further explore the potential interaction between IGF2BP1 and PTBP1, a bioinformatics analysis was conducted utilizing the STRING database. The protein network obtained from the analysis is depicted in Fig. 1C. The network representation consists of nodes and edges, with different colors indicating diverse types of evidence supporting the predicted interactions. Notably, IGF2BP1 exhibited three edges connected to PTBP1, indicating evidence

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from three perspectives, thus suggesting a potential interaction between IGF2BP1 and PTBP1. Additionally, IGF2BP1 proteins were immunoprecipitated from the tissue lysates, followed by quantification of the bound PTBP1 using qRT-PCR. As depicted in Fig. 1D, the immunoprecipitants of IGF2BP1 exhibited noticeably elevated levels of PTBP1 compared to the IgG control group, thereby confirming the interaction between PTBP1 and IGF2BP1. Collectively, these data provided strong evidence that IGF2BP1 and PTBP1 had close interactions in the endometrial tissues of women with EMs.

IGF2BP1 silencing mitigated the progression of EMs in rats

To further explore the effects of IGF2BP1 on EMs, we established an EMs rat model referring to previous studies [11, 13]. The EMs group exhibited significantly elevated levels of IGF2BP1 and PTBP1 compared to the Sham group. Nevertheless, IGF2BP1 silencing remarkably reduced them, indicating that IGF2BP1 upregulated the expression of PTBP1 (Fig. 2A). Moreover, a notable disparity was observed in the volume and weight of ectopic endometrial tissues after treatment between the EMs+si-NC and EMs+si--IGF2BP1 groups (Fig. 2B). Additionally, neither the EMs group nor the EMs+si-NC group exhibited any adverse effects or organ-specific alterations. However, the uterine autograft in the EMs+si-IGF2BP1 group exhibited significant epithelial alterations and a general decline in the explant's size (Fig. 2C). The persistence of endometrial epithelial cells in the uterine explant was semiquantitatively evaluated. The EMs+si-IGF2BP1 group exhibited a significantly lower score compared to the EMs+si-NC group (Fig. 2D). Furthermore, we evaluated prognostic markers linked to the development of EMs, including PCNA [13], VEGF [8], and E-cadherin [14]. Notably, the levels of PCNA and VEGF were significantly elevated and the E-cadherin level was significantly reduced in the EMs group, while IGF2BP1 silencing reversed them remarkably (Fig. 2E). Thus, as described above, IGF2BP1 downregulation significantly alleviated the progression of EMs in rats.

IGF2BP1 silencing inhibited the proliferation, migration, and invasion of eESCs and promoted apoptosis

To verify the *in vivo* data, we isolated eESCs from ectopic endometrial tissues and then cultured them in vitro. The eESCs, which were recently isolated, initially exhibited a round morphology. Subsequently, they transformed into spindle and polygonal-shaped cells with elongated cytoplasmic processes. Typically, these cells achieved confluence within 5 days of being cultured, forming a monolayer characterized by a single-cell growth pattern (Fig. 3A). A previous study suggested that vimentin and CK19 were the main biomarkers for eESCs [15]. As shown in Fig. 3B, for ESCs in monolayer culture, the percentages of vimentin- and CK19-positive cells were approximately 98.50% and 99.28%, respectively, confirming positive vimentin and CK19 expressions in eESCs. Moreover, to explore the effect of IGF2BP1 on eESCs, we transfected eESCs with si-IGF2BP1 and then validated the mRNA levels of IGF2BP1. As expected, the level of IGF2BP1 was significantly downregulated in the si-IGF2BP1 group (Fig. 3C). Next, the cell function experiments were conducted. As shown in Figs. 3D and 3E, cell proliferation in the eESCs was decreased after IGF2BP1 silencing. Compared with the si-NC group, IGF2BP1 silencing significantly inhibited migration and invasion in eESCs (Fig. 3F). In addition, cell apoptosis of eESCs was significantly enhanced in the si-IGF2BP1 group (Fig. 3G). Consistent with the *in vivo* data, the expression levels of PCNA and VEGF were significantly downregulated and the E-cadherin level was significantly upregulated after IGF2BP1 silencing (Fig. 3H). Collectively, these data confirmed that IGF2BP1 silencing had a significant inhibitory effect on the proliferation, migration, and invasion of eESCs, while also promoting apoptosis.

IGF2BP1 regulated the proliferation, migration, invasion, and apoptosis of eESCs through PTBP1

Next, to further investigate the regulatory role of IGF2BP1/PTBP1 on eESCs, we overexpressed PTBP1 based on silencing IGF2BP1. The mRNA level of PTBP1 was significantly upregulated after PTBP1 overexpression, while there is no significant variation observed in the mRNA expression level of IGF2BP1 among the three groups (Fig. 4A). As shown in Fig. 4B–D, the proliferation, migration, and invasion of eESCs were observed in significant elevations in the si-IGF2BP1+oe-PTBP1 group compared to the si-IGF2BP1+oe-NC group. Moreover, the apoptosis of eESCs was found be significantly reduced after PTBP1 overexpression (Fig. 4E). Furthermore, the levels of PCNA and VEGF were significantly upregulated and the level of E-cadherin was downregulated (Fig. 4F). These comprehensive findings suggested that IGF2BP1 regulated the proliferation, migration, invasion, and apoptosis of eESCs through PTBP1.

Discussion

Recent studies have indicated that epigenetic modifications have a substantial impact on the regulation of

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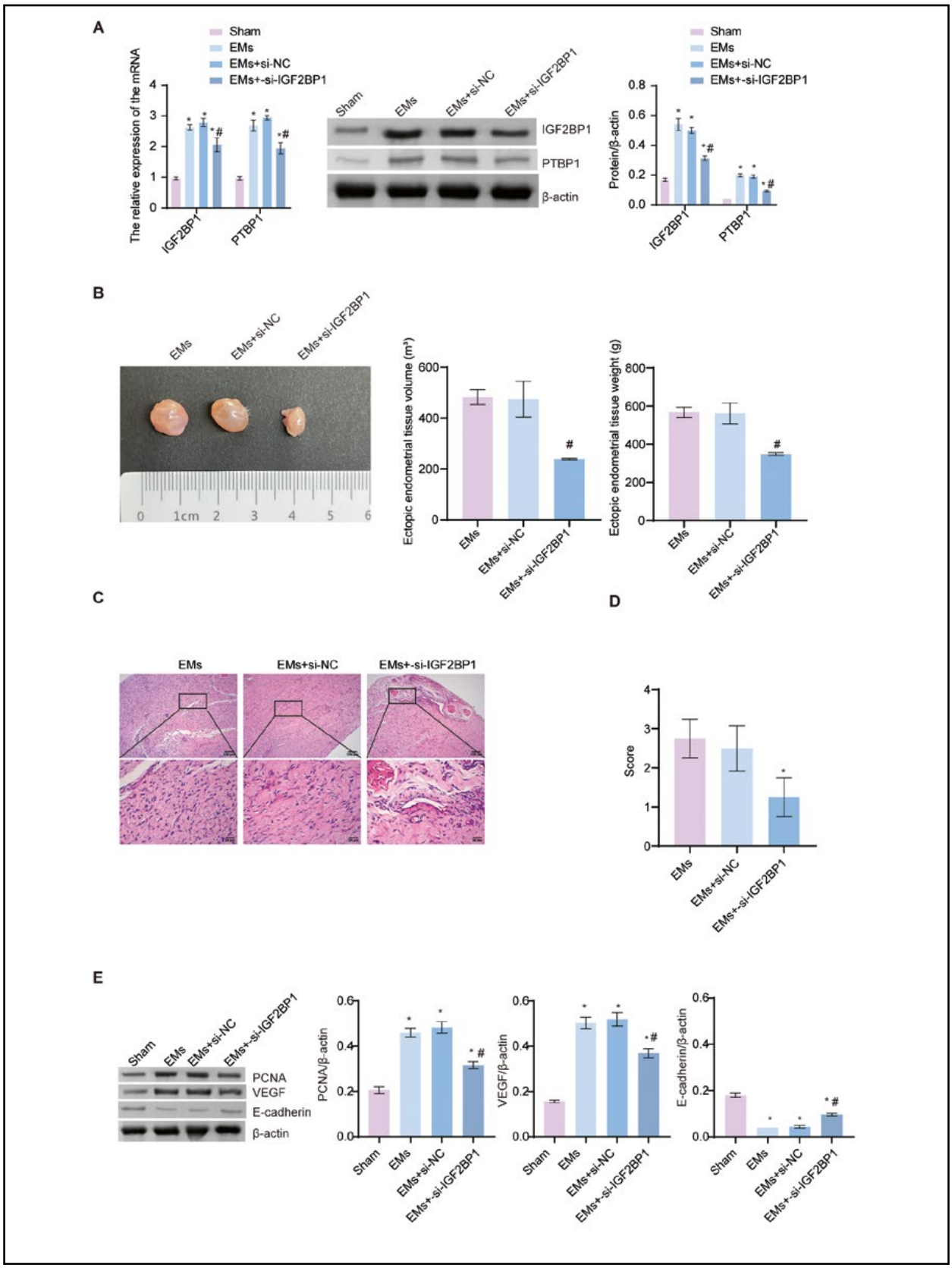

Figure 2. The effect of IGF2BP1 silencing on the progression of EMs in rats. **A.** The levels of IGF2BP1 and PTBP1 were assessed in the endometrial tissues of rats with EMs. **B.** The volume and weight of ectopic endometrium developed in rat groups. **C.** Histopathological observations of endometrial tissues in rats. **D.** Semiquantitative assessment of persistent epithelium in endometrial tissue allografts of rats. **E.** The expressions of PCNA, VEGF, and E-cadherin in ectopic endometrium tissues were assessed. n = 6 *per* group. *P < 0.05 *vs.* Sham group, #P < 0.05 *vs.* EMs+si-NC group.

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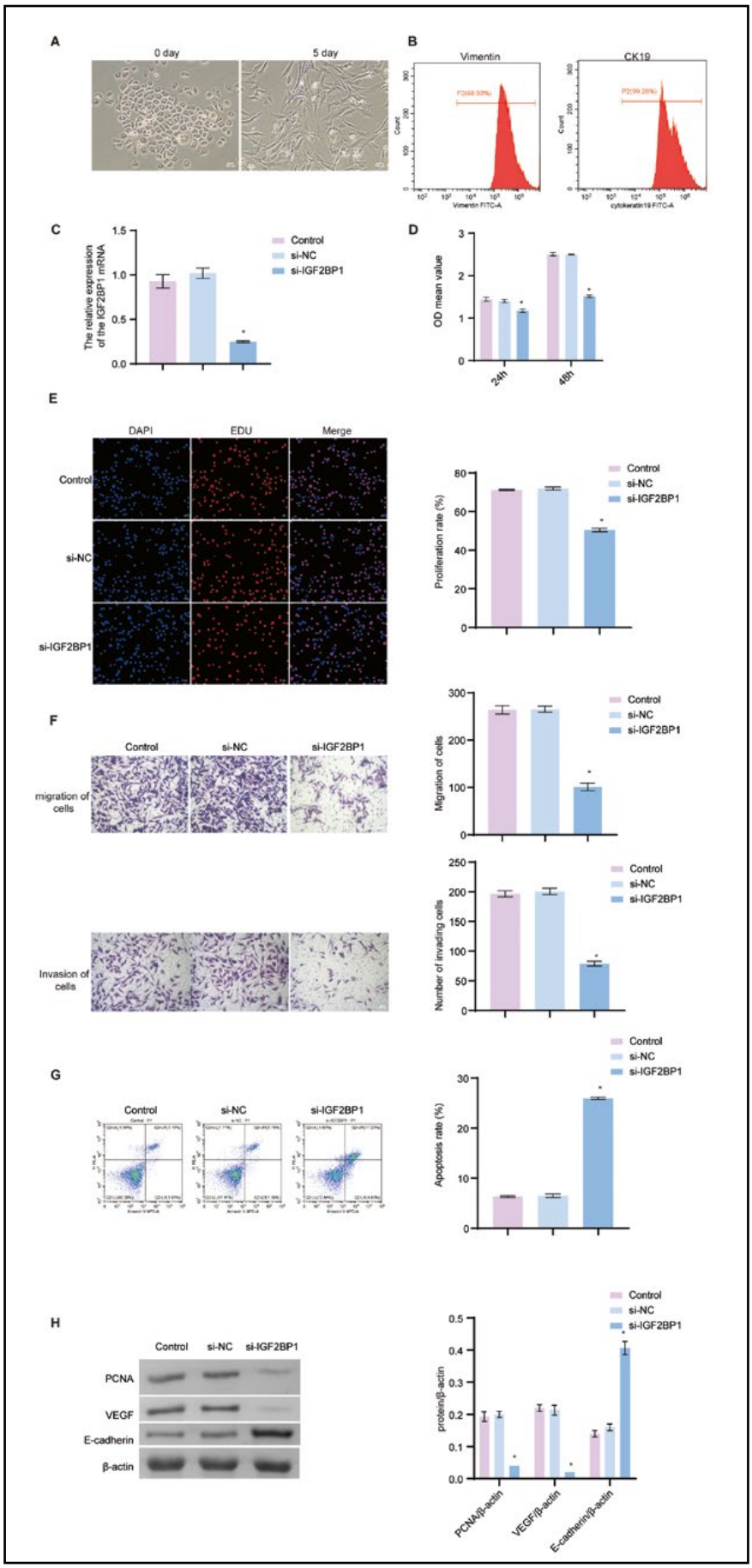

Figure 3. The effects of IGF2BP1 silencing on eESCs. **A.** The observation of eESCs under an inverted microscope. **B.** The expressions of biomarkers of eESCs were detected by flow cytometry (FCM). **C.** The mRNA levels of IGF2BP1 in eESCs. **D.** The cell viability of eESCs was determined. **E.** The cell proliferation of eESCs was detected. **F.** The eESC migration and invasion were detected. **G.** FCM was conducted to identify the apoptotic eESC population. **H.** The expressions of PCNA, VEGF, and E-cadherin in eESCs were assessed. n = 3 *per* group. *P < 0.05 *vs.* si-NC group.

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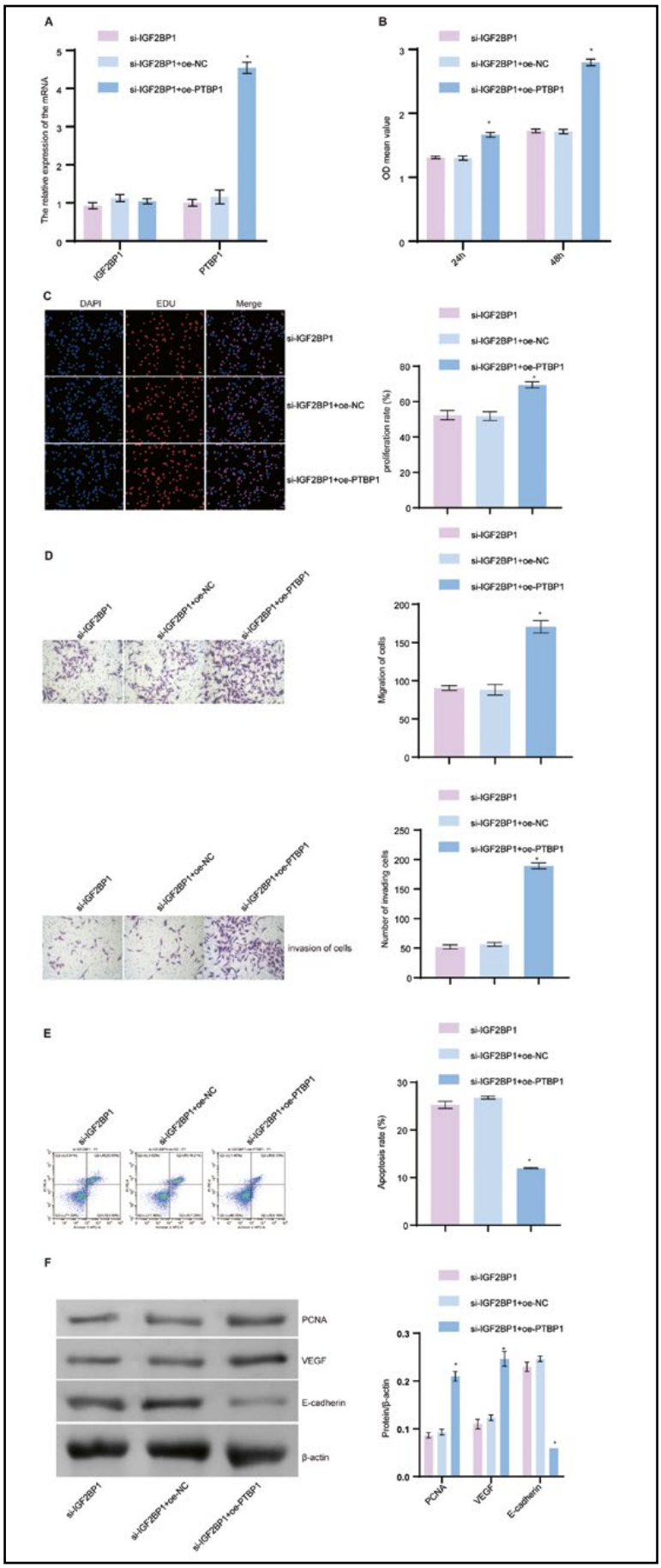

Figure 4. The effects of IGF2BP1/PTBP1 on eESCs. **A.** The mRNA levels of IGF2BP1 and PTBP1 in eESCs. **B.** The cell viability of eESCs was evaluated. **C.** The proliferation was detected using an EDU assay. **D.** eESC migration and invasion were detected using Transwell assay. **E.** Flow cytometry was conducted to identify the apoptotic eESC population. **F.** The expression levels of PCNA, VEGF, and E-cadherin in eESCs were assessed as described in Methods. n = 3 *per* group. *P < 0.05 *vs.* si-IGF2BP1+ +oe-NC group.

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diverse physiological processes and the development of diseases [16, 17]. As a result, they have gained considerable attention in the realm of bioscience research. [18]. Recently, RNA modification has gained significant attention, with a particular focus on N6-methyladenosine (m6A), which is the predominant internal modification of RNA in the majority of eukaryotes [19]. In the present study, EMs patients have been found to exhibit decreased levels of m6A in the endometrium compared to individuals with normal endometrium. Bioinformatics analysis further corroborates this discovery, as it reveals a decrease in the expression levels of METTL3, YTHDF2, YTHDF3, HNRNPA2B1, HNRNPC, and FTO in ectopic endometrium [20]. HNRNPA2B1 and HNRNPC have been suggested to have a potential association with immune responses and could serve as valuable biomarkers for the diagnosis of EMs [20]. Additionally, Li et *al*. [21] discovered that the m6A modification, facilitated by METTL3 and METTL14, two key enzymes involved in m6A methylation, plays a significant role in enhancing cell proliferation and invasion in human eESCs. Thus, a potentially effective therapeutic strategy would involve targeting crucial components of the m6A modification that are linked to aberrant ectopic proliferation of endometrial cells. Our data revealed that the expression of IGF2BP1 was obviously augmented in eutopic/ectopic endometrium tissues of patients with EMs compared with healthy women. Consequently, in the present study, we focused on IGF2BP1, which is an m6A reader, and explored the target and effect of IGF2BP1 in a rat model of EMs.

IGF2BP1, being an essential protein involved in the recognition of m6A-modified transcripts, assumes a pivotal function in the etiology of diverse diseases [22–24]. Recent data has demonstrated that the upregulation of IGF2BP1 results in the stabilization of PEG10 mRNA through an m6A-dependent mechanism, thereby facilitating the progression of endometrial cancer [25]. Additionally, Mao *et al*. [23] have found that the inhibition of IGF2BP1 has the potential to mitigate renal injury and inflammation. This effect is achieved by alleviating m6A modifications and the E2F1/MIF pathway [23]. In the present investigation, it was observed that the inhibition of IGF2BP1 had a substantial impact on the advancement of EMs in rats. Proliferating cell nuclear antigen (PCNA), a cell proliferation marker, is an important factor in the development of EMs [26]. In addition, it is widely recognized that angiogenesis plays a crucial role in the formation and spread of EMs lesions to abnormal sites [27], and the main regulator of the angiogenic process is VEGF [28]. Meanwhile, EMs is an aggressive condition and E-cadherin expression could reflect its considerable invasiveness [29]. The high expression of PCNA and VEGF and the low expression of E-cadherin in endometrial tissues reported in this study suggests that EMs was successfully induced in rats. Nevertheless, silencing of IGF2BP1 expression significantly attenuated the progression of EMs and the expression levels of these markers. Moreover, IGF2BP1 silencing also had a significant inhibitory effect on the proliferation, migration, and invasion of eESCs, while also promoting apoptosis. Furthermore, IGF2BP1 inhibition was reported to repress inflammation and pyroptosis in human eESCs [12, 30]. Thus, IGF2BP1 was confirmed to be a key contributor to EMs, and its intrinsic mechanism has become the focus of our attention.

PTBP1, a RNA-binding protein (RBP), is known to have a significant impact on the regulation of various posttranscriptional processes and is intricately associated with the pathogenesis of numerous malignancies [31]. In this study, an unexpected finding was observed in which the expression of PTBP1 was found to be upregulated in the ectopic endothelium of patients with EMs. Furthermore, a positive correlation was observed between PTBP1 expression and IGF2BP1 expression. Bioinformatics prediction in our study revealed that IGF2BP1 had a potential binding site with PTBP1 and that they had a reciprocal relationship. Therefore, we hypothesized that IGF2BP1 inhibition mitigated the progression of EMs through the downregulation of PTBP1 and performed *in vitro* experiments to verify this option, accordingly. As anticipated, we found that PTBP1 overexpression restored the reduction of proliferation, migration, and invasion of eESCs induced by IGF2BP1 silencing. To sum up, inhibition of IGF2BP1 expression attenuated EMs through decreasing PTBP1. As far as we know, this study represents the initial investigation of the participation of the IGF2BP1/ /PTBP1 axis in the EMs pathomechanisms. However, it is crucial to recognize the constraints of this study. The precise mechanism by which IGF2BP1 modulates the expression of PTBP1, as well as its potential effects on other tissues, remains unknown. Moreover, in addition to PTBP1, IGF2BP1 may have other regulatory targets in rats with EMs. Further research is imperative in order to acquire a more profound comprehension of the fundamental mechanisms.

Conclusions

In conclusion, we have successfully demonstrated the significant ability of IGF2BP1/PTBP1 to mitigate EMs, which establishes a solid theoretical foundation for the potential application of IGF2BP1 or its analogs in the treatment of EMs.

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Article information and declarations

Data availability statement

Original contributions presented in the study are included in the article and that further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by Medical Ethics Committee of Changsha Central Hospital (2022-S0206). Ethics Committee and that patients/participants provided their written informed consent to participate in this study.

Author contributions

Yanlin Su conducted data collection and analysis and contributed to the writing and revision of the manuscript. Wencai Tian contributed to the study design, data interpretation, and manuscript revision. Li Cheng assisted in data collection and analysis and contributed to the writing and revision of the manuscript. Ling Yin contributed to the study design, data interpretation, and manuscript revision. Xiaoxia He provided critical input in the study design, data interpretation, and manuscript revision. Xin Wei served as the corresponding author, overseeing the entire project, providing guidance in all facets of the study, and finalizing the manuscript. All authors have reviewed and approved the final version of the manuscript.

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Conflict of interest

We declare that we have no conflict of interest related to the submitted manuscript.

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