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RESEARCH ARTICLE

Mechanism of E2F1 in the proliferation, migration, and invasion of endometrial carcinoma cells via the regulation of BMI1 transcription

Yanyang Lu[1] · Ying Wei[1] · Xiaoqin Shen[1] · Yixi Tong[1] · Jin Lu[1] · Yahui Zhang[1] · Yun Ma[1] · Rong Zhang[1]

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Abstract

Background Endometrial carcinoma (EC) is the most prevalent gynecological cancer. Transcription factor (TF) regulates a large number of downstream target genes and is a key determinant of all physiological activities, including cell proliferation, differentiation, apoptosis, and cell cycle. The transcription factor E2F1 shows prominent roles in EC. BMI1 is a member of Polycomb suppressor Complex 1 (PRC1) and has been shown to be associated with EC invasiveness. It is currently unclear whether E2F1 can participate in the proliferation, migration, and invasion processes of EC cells by regulating BMI1 transcription.

Objective We investigated whether E2F1 could participate in the proliferation, migration, and invasion processes of EC cells by regulating BMI1 transcription, in order to further clarify the pathogenesis and etiology of EC, and provide reference for identifying potential therapeutic targets and developing effective prevention and treatment strategies for this disease.

Methods Human endometrial epithelial cells (hEECs) and human EC cell lines were selected. E2F1 expression was assessed by Western blot. E2F1 was silenced in AN3CA or overexpressed in HEC-1 by transfections, or E2F1 was silenced and BMI1 was overexpressed in AN3CA by cotransfection. Cell proliferation, migration, and invasion were detected by MTT, wound healing, and Transwell assays. The binding sites between E2F1 and BMI1 promoters were predicted through JASPAR website, and the targeted binding was verified by dual-luciferase report and ChIP assays.

Results E2F1 was up-regulated in human EC cell lines, with its expression highest in AN3CA, and lowest in HEC-1. AN3CA invasion, migration, and proliferation were repressed by E2F1 knockdown, while those of HEC-1 cells were promoted by E2F1 overexpression. E2F1 overexpression increased the activity of wild type BMI1 reporter vector promoter, while this promotion was weakened after mutation of the predicted binding site in the BMI1 promoter. In the precipitated E2F1, BMI1 promoter site level was higher than that of IgG immunoprecipitant. BMI1 silencing suppressed AN3CA cell growth. BMI1 overexpression partially abrogated E2F1 silencing-inhibited EC cell growth.

Conclusion E2F1 promoted EC cell proliferation, invasion, and migration by promoting the transcription of BMI1.

Keywords Endometrial carcinoma · E2F1 · BMI1 · Transcription · Proliferation · Migration · Invasion · AN3CA

Introduction

Endometrial carcinoma (EC) is the most prevalent gynecological malignant disease, and the 4th most prevalent cancer in North American and European women with steadily increasing incidence, which accounts for about 6% of new cancer cases and about 3% of the cancer deaths per year (Murali et al. 2014). Most EC patients have an excess of estrogen and typically manifest a characteristic clinical profile: high body mass index that is considered obese (BMI 30) or overweight (BMI 25–30), usually with other metabolic syndrome components (diabetes, hypertension) (Trojano et al. 2019). Although many patients with EC are cured by surgery alone, there are still significant numbers of patients with more aggressive variants of EC for whom the prognosis remains poor, and the major clinical challenges include risk predictive/stratification biomarker assessment post-hysterectomy for the determination of the type of and the need for adjuvant treatment and risk stratification based

✉ Rong Zhang
ZhangRongg1123@163.com

[1] Department of Gynecology, The Second Affiliated Hospital of Soochow University, N0.1055, Sanxiang Road, 215000 Suzhou, China

on the diagnostic biopsy for the guidance of the extent of surgery (Huvila et al. 2021). Therefore, it is particularly principal to accurately understand EC and formulate effective preventive measures and treatment strategies by clarifying its pathogenesis and etiology.

EC is widely believed to be caused by genomic instability, accompanied by abnormal expression of many cancer-related genes, and the reasons for abnormal expression levels of these genes in cancer include transcription factor imbalance, small RNA interference, gene copy number changes, and DNA promoter methylation level (Chang et al. 2019). The activity of transcription factors is changed in various cancer types through a variety of direct mechanisms such as gene deletion or amplification, chromosomal translocations, expression alteration, and point mutations, and indirectly through non-coding DNA mutations, which affect the binding of transcription factors (Bushweller 2019). The 3 promising transcription factors, E2F1, PGR, and HMGA1 are identified to be closely correlated with EC, which may be useful biomarkers for EC diagnosis and prognosis (Song et al. 2019). E2F1-initiated PRSS22 transcription facilitates the metastasis of breast cancer by cleaving ANXA1 and activating the FPR2/ERK pathway (Song et al. 2022). E2F1-activated NRSN2 promotes the progression of esophageal squamous cell carcinoma via the AKT/mTOR pathway (Yan et al. 2022). The E2F family of transcriptional factors shows prominent roles in the apoptosis, differentiation, and inhibition of DNA damage response, thus influencing the invasion and growth of EC cells (Zhang et al. 2020).

BMI1 is a member of the Polycomb repressor complex 1 family, which mediates gene silencing by modulating chromatin structure, and is indispensable for self-renewal of both cancer stem and normal cells (Bhattacharya et al. 2015). BMI1 has been reported to be up-regulated in EC cell lines, EC patient tissues, and non-endometrioid tissues, and is associated with poor overall survival (Buechel et al. 2018). BMI1 is a target gene of E2F-1, which is strongly expressed in primary neuroblastomas (Nowak et al. 2006). However, whether E2F1 participates in EC cell growth by regulating the transcription of BMI 1 has not been reported. This study aims to accurately understand EC and provide references for finding potential therapeutic targets and formulating effective preventive measures and treatment strategies by clarifying the pathogenesis and etiology of EC.

Materials and methods

Cell culture

Human EC cell lines (AN3CA, RL95-2, HEC-1) (ATCC, Manassas, VA, USA) and human endometrial epithelial cells (hEECs) (CP-H058, Procell Life Science, Wuhan, Hubei, China) were cultured in the Dulbecco's modified Eagle's medium (HyClone; Cytiva) supplemented with 10% fetal bovine serum (FBS) (HyClone; Cytiva), 100 U/mL penicillin and 100 μg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C containing 5% CO_2.

Cell transfection and grouping

The transfection experiments were performed using Lipofectamine®2000 (Invitrogen, Thermo Fisher Scientific) following the manufacturer's instructions. Human EC cell lines HEC-1 and AN3CA were seeded in the 6-well plates at a concentration of 2.5×10^5, and incubated overnight at 37°C containing 5% CO_2. Subsequently, cells were transfected with 50 nM E2F1/BMI1 small interfering (si) RNA or 50 nM E2F1/BMI1 overexpression plasmid (pcDNA3.1 vector). The cells transfected for 24 h were used for subsequent experiments. The sequences of siRNA were as follows: E2F1 [guide (5'-3'): AAAUCAAAGUGCAGAUUGGAG, passenger (5'-3'): CCAAUCUGCACUUUGAUUUGC], BMI1 [guide (5'-3'): UCGUUGUUCGAUGCAUUUCUG, passenger (5'-3'): GAAAUGCAUCGAACAACGAGA]. All siRNAs and over-expression plasmids were produced by Sangon Biotech (Shanghai, China).

HEC-1 cells used in this study were allocated into the following 3 groups: (1) HEC-1 (without any treatment); (2) oe-NC [transfected with negative control (NC) plasmid pcDNA3.1-NC]; oe-E2F1 (transfected with pcDNA3.1-E2F1).

AN3CA cells were allocated into the following 5 groups: (1) AN3CA (without any treatment); (2) si-NC (transfected with siRNA NC); (3) si-E2F1 (transfected with E2F1 siRNA); (4) si-BMI1 (transfected with BMI1 siRNA); (5) si-E2F1 + oe-NC (transfected simultaneously with E2F1 siRNA and pcDNA3.1-NC at 1:1 ratio); 5) si-E2F1 + oe-BMI1 (transfected simultaneously with E2F1 siRNA and pcDNA3.1-BMI1 at 1:1 ratio).

Western blot

The collected cells were lysed with frozen sodium dodecyl sulfate (SDS) lysis buffer (Beyotime, Shanghai, China). According to the manufacturer's instructions, the protein concentration in the lysate was determined using the bicinchoninic acid kit (Keygen Biotech, Nanjing, Jiangsu, China). The equivalent amount of denatured protein (20 μg/lane) was separated by 10% SDS-polyacrylamide gel electrophoresis and then transferred to the polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). Then, the samples were blocked with 5% bovine serum albumin (Solarbio, Beijing, China) for 2 h and incubated with

primary antibodies anti-E2F1 (1:2000, ab288369, Abcam, Cambridge, UK) and anti-BMI1 (1:10000, ab126783, Abcam) at°C overnight. On the next day, after full washing with Tris-buffered saline with Tween 20 (TBST), the samples were incubated with horseradish peroxidase-labeled IgG H&L secondary antibody (1:5000, ab6721) at room temperature for 1 h. After full washing with TBST, the samples were developed with enhanced chemiluminescence developer (P0018M, Beyotime) and the images were collected. With β-actin (1:1000, ab8227, Abcam) as the internal reference, the gray value was analyzed using the Image-Pro Plus 6.0 (MEDIA Cybernetics, Bethesda, MD, USA).

3-(4, 5-Dimethyl-2-thizolyl)-2, 5 diphenyltertazolium bromide (MTT) assay

Cell proliferation was detected using the MTT method. Cells in the logarithmic phase were detached with trypsin for cell suspension preparation. Each well of the 96-well plates was added with 3000 cells and cultured with 100 µL culture medium. Cell proliferation was recorded at 0, 24, 48, and 72 h, respectively. Cells were added with 20 µL (5 mg/mL) prepared MTT reagent (Sigma-Aldrich, St. Louis, MO, USA) at each time point and incubated at 37 °C for 2 h. Then, cell culture medium was collected, added with 150 µL dimethyl sulfoxide, and shaken up at low speed in the dark. The optical density (OD) was read at 490 nm using a microplate, and the OD value of control cells (HEC-1/AN3CA without any treatment) was set to 1 for normalization.

Cell scratch test

Migration was detected by wound healing tests. Cells were seeded into the culture plates and cultured until the confluence reached more than 80%. The bottom of each well was scratched using a 1-mm wide sterile pipette tip (P200 micropipette tip) and the culture solution was collected. After rinsing with 1 × phosphate buffer saline, the samples were added with 2 mL serum-free medium in each well and then incubated at 37 °C for 0 and 48 h. At each time point, cell migration was observed using a phase contrast microscope (Olympus, Melville, NY, USA) and the samples were imaged with an Olympus DP70 digital camera on the Olympus DP controller software (Olympus). The wound area was measured using the ImageJ software. The wound closure percentage was calculated: wound closure (%) = $(A_0 - A_{48})/A_0 \times 100$, where A_0 was the wound area immediately determined after scratching (0 h), and A_{48} was the wound area determined at 48 h after treatment. In each experiment, the relative cell migration was calculated by dividing the percentage of cell wound area changes in the treatment group by the percentage of cell wound area changes in the control group (HEC-1/AN3CA without any treatment).

Transwell

The 8-µm pore size Matrigel-coated Transwell cell culture chamber (BD Biosciences, SanJose, CA, USA) was used for cell invasion experiments. The apical chamber was coated at 37 °C overnight with 0.5% Matrigel (BD Biosciences). Subsequently, cells were seeded in the apical chamber added with serum-free medium at 2×10^4 cells/well, while the basolateral chamber compartment was added with the complete medium supplemented with 10% FBS (Merck, DA, Germany) as a chemoattractant. After 24-h incubation at 37 °C, the non-invasive cells in the apical chamber were discarded using a cotton swab, and the invasive cells in the basolateral chamber were fixed with 4% paraformaldehyde for 10 min, then stained with crystal violet for 10 min, and imaged under an Olympus fluorescence microscope (BX53, Olympus). The relative invasion rate was calculated by dividing the number of invasive cells in the treated group by the number of invasive cells in the untreated group in each experiment.

Dual-luciferase report assay

The binding sites between E2F1 and BMI1 promoter region (2 kb upstream and 100 bp downstream of the gene origin) were predicted through the JASPAR website (https://jaspar.genereg.net/). To detect the interaction between E2F1 and BMI1, the 3'UTR fragment of BMI1 was amplified by PCR and cloned into the downstream of Renilla psiCHECK2 vector (Promega, Madison, WI, USA), named WT-BMI1 3'UTR. To generate E2F1 mutation reporter, the binding region of E2F1 and BMI1 was mutated to eliminate its complementarity with HIF-1α, named MUT-BMI1 3'UTR. AN3CA cells were cotransfected with E2F1 or NC vectors. After 48-h transfection, cell luciferase was detected using the Dual-Luciferase reporter system (Promega). Renilla luciferase activity was normalized to Firefly luciferase activity in each transfected well.

Chromatin immunoprecipitation (ChIP) assay

Referring to the previous studies (Sun et al. 2019; Xiao et al. 2019), whether E2F1 bound to the BMI1 promoter was detected using the ChIP assay kit (Catalog: 17–371, Millipore). Firstly, EC cells transfected with E2F1 were fixed with 1% formaldehyde for covalent crosslinking of protein and DNA, and then chromatin was extracted from EC cells. The cross-linked DNA was cut into 200–1000 base pairs by ultrasound, and then immunoprecipitated with anti-E2F1

(ab245308, Abcam). ChIP-PCR primers were designed to amplify the promoter region of BMI1, which contained possible binding sites of E2F1. The effectiveness of the kit was verified using NC nonimmune IgG. The immunoprecipitated DNA was then washed, released, and eluted, and the eluted DNA was adopted for ChIP-PCR. The fold-enrichment (FE) was calculated as the ratio of ChIP sample amplification efficiency to that of the nonimmune IgG. FE (%) = 2 (IgG CT-Sample CT) × 100%.

Statistical analysis

GraphPad Prism 8.01 (GraphPad Software Inc., San Diego, CA, USA) and SPSS 21.0 (IBM Corp., Armonk, NY, USA) statistical software were applied for data mapping and statistical analysis. Data were expressed as mean ± standard deviation. The comparisons between 2 groups were performed by independent sample *t*-test. The comparisons among multiple groups were performed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. $P < 0.05$ was indicative of a statistically significant difference.

Results

E2F1 was highly expressed in EC cells

E2F1 expression in human EC cells was first assessed by Western blot. Compared with hEECs, E2F1 was overexpressed in human EC cell lines, with the expression the highest in AN3CA, and the lowest in HEC-1 (all $P < 0.01$) (Fig. 1).

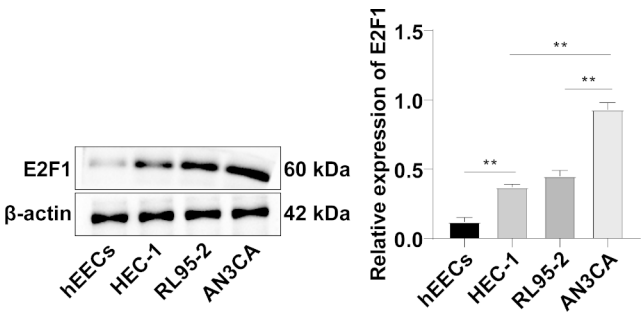

Fig. 1 E2F1 was highly expressed in EC cells. E2F1 expression levels in hEECs and human EC cell lines HEC-1, RL95-2 and AN3CA were determined by Western blot. Cell experiment was repeated 3 times. Data were expressed as mean ± standard deviation. One-way ANOVA was employed for comparisons among groups, followed by Tukey's multiple comparisons test. ** $P < 0.01$

E2F1 promoted EC cell proliferation, invasion, and migration

Furthermore, E2F1 expression was knocked down in AN3CA cells by E2F1 siRNA transfection, or overexpressed in HEC-1 cells by pcDNA3.1-E2F1 transfection. Western blot elicited that compared with the si-NC group (transfected with siRNA negative control), E2F1 expression in AN3CA cells of the si-E2F1 group was repressed, while compared with the oe-NC group (transfected with negative control plasmid pcDNA3.1-NC), E2F1 expression in HEC-1 cells of the oe-E2F1 group was augmented (all $P < 0.01$) (Fig. 2A), indicating successful transfections. Subsequently, cell proliferation was detected by MTT assay. The proliferation ability of AN3CA cells was limited after E2F1 knockdown, while that of HEC-1 cells was facilitated after E2F1 overexpression (all $P < 0.01$) (Fig. 2B). Wound healing test demonstrated that E2F1 knockdown suppressed the migration ability of AN3CA cells, while that of HEC-1 cells was stimulated after E2F1 overexpression (all $P < 0.01$) (Fig. 2C). In addition, Transwell manifested that the invasive ability of AN3CA cells was blocked after E2F1 knockdown, while that of HEC-1 cells was promoted after E2F1 overexpression (all $P < 0.05$) (Fig. 2D).

E2F1 activated BMI1 transcription in EC cells

AN3CA cells were selected to further study whether E2F1 participated in EC cell proliferation, invasion and migration by regulating BMI1 transcription. Western blot showed that in contrast to hEECs, BMI1 was up-regulated in human EC cell line AN3CA while down-regulated after E2F1 silencing (all $P < 0.01$) (Fig. 3A). JASPAR website predicted 5 binding sites between E2F1 and BMI1 promoter region (Supplementary Table 1). The binding site with the highest score (-653~-646) (Score = 9.128) was selected for research, and wild type and mutant BMI1 promoter reporting vectors were constructed. The mutant vector contained mutations that predicted E2F1 binding sites (Fig. 3B). These vectors were cotransfected with E2F1 or NC vectors into AN3CA cells, and the promoter activity was detected by dual-luciferase report assay. E2F1 overexpression led to an increase in the activity of the wild type BMI1 reporter vector promoter, while the promotion of E2F1 was partially weakened after the mutant of the predicted binding site in the BMI1 promoter (all $P < 0.05$) (Fig. 3C). In addition, ChIP assay demonstrated that in the precipitated E2F1, BMI1 promoter site level was higher than that of IgG immunoprecipitant ($P < 0.001$) (Fig. 3D), suggesting that E2F1 bound to the BMI1 promoter to promote BMI1 transcription.

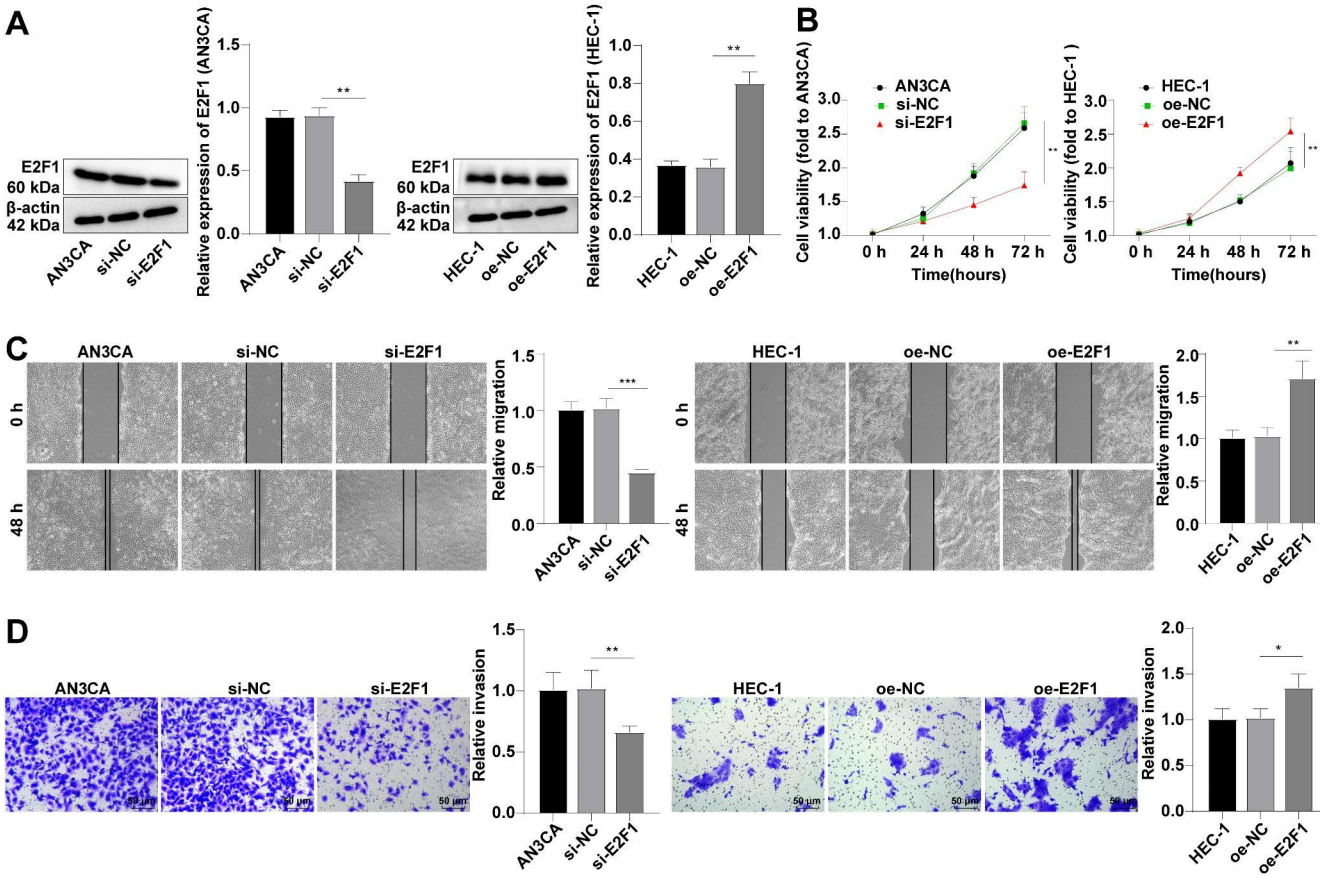

Fig. 2 E2F1 promoted EC cell proliferation, invasion and migration. E2F1 siRNA was introduced into cells to knock down E2F1 in AN3CA cells, or pcDNA3.1-E2F1 was manipulated into HEC-1 cells to overexpress E2F1. (**A**) E2F1 expression was assessed by Western blot; (**B**) Cell proliferation was detected by MTT assay; (**C**) Cell migration was detected by wound healing test; (**D**) Cell invasion was detected by Transwell. Cell experiment was repeated 3 times. Data were expressed as mean ± standard deviation. One-way ANOVA was applied for comparisons among groups, followed by Tukey's multiple comparisons test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

BMI1 silencing inhibited EC cell growth

BMI1 expression was knocked down in AN3CA cells by BMI1 siRNA transfection ($P<0.01$) (Fig. 4A). MTT, wound healing, and Transwell assays manifested that compared with the si-NC group, after knockdown of BMI1 expression, AN3CA proliferation, migration, and invasion were reduced (all $P<0.01$) (Fig. 4B-D).

BMI1 overexpression partially annulled E2F1 knockdown-inhibited EC cell proliferation, migration, and invasion

To further elucidate whether E2F1 participated in the regulation of EC cell behaviors by promoting BMI1 transcription, E2F1 was silenced in AN3CA cells and BMI1 was overexpressed by cotransfection with E2F1 siRNA and pcDNA3.1-BMI1 ($P<0.01$) (Fig. 5A). MTT, wound healing, and Transwell assays elicited that after BMI1 overexpression, E2F1 silencing-inhibited EC cell invasion, migration, and proliferation were partially averted (all $P<0.05$) (Fig. 5B-D).

Discussion

EC is the most prevalent cancer of the female reproductive organs in developed countries, and the 4th most prevalent cancer in women in the USA, the UK, and Canada, after breast, lung, and colorectal (Huvila et al. 2021). Evidence has shown that knockdown of MELK reduces EC subcutaneous tumorigenesis in vivo and EC cell ability of migration and proliferation in vitro, and high MELK expression can be modulated by the transcription factor E2F1 (Xu et al. 2020). This study found that E2F1 stimulated the invasion, proliferation, and migration of EC cells by amplifying the transcription of BMI1.

Aberrant E2F1 activation is closely related to poor clinical outcomes in a variety of human cancers including prostate cancer (Chun et al. 2020). E2F1-mediated circRNA

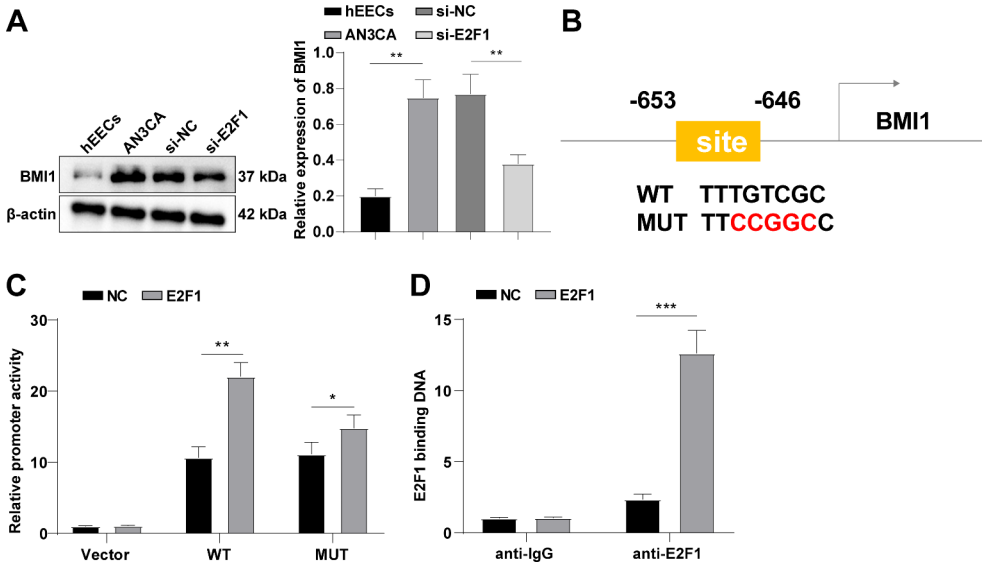

Fig. 3 E2F1 activated BMI1 transcription in EC cells. (**A**) BMI1 expression was assessed by Western blot; (**B**) The binding sites between E2F1 and BMI1 promoter region were predicted using the JASPAR website, and the mutant luciferase reporter vector contained a 5 bp mutation at the binding site; (**C**) These vectors were cotransfected with E2F1 overexpression vector into AN3CA cells to detect their luciferase activity; (**D**) ChIP test was conducted with anti-E2F1 or anti-IgG to confirm the binding of E2F1 and BMI1 promoter. Cell experiment was repeated 3 times. Data were expressed as mean ± standard deviation. The comparisons between C/D groups were performed by independent sample *t*-test. The comparisons among groups in panel A were performed by one-way ANOVA, followed by Tukey's multiple comparisons test. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

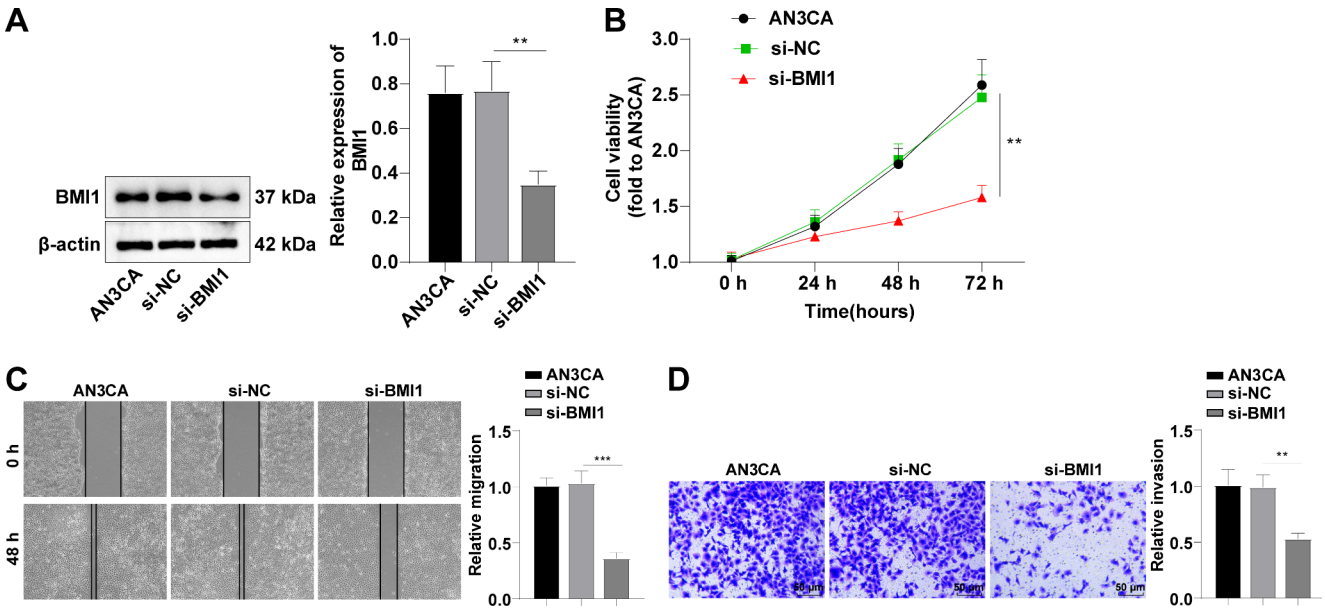

Fig. 4 BMI1 silencing repressed EC cell migration, proliferation and invasion. BMI1 siRNA was introduced into AN3CA cells to knock down BMI1. (**A**) BMI1 expression was determined by Western blot; (**B**) Cell proliferation was detected by MTT; (**C**) Cell migration was detected by wound healing test; (**D**) Cell invasion was detected by Transwell. Cell experiment was repeated 3 times. Data were expressed as mean ± standard deviation. One-way ANOVA was adopted for comparisons among groups, followed by Tukey's test. ** $P<0.01$, *** $P<0.001$

circSEPT9 intensifies the development and carcinogenesis of triple-negative breast cancer (Zheng et al. 2020). CHPF promotes the tumorigenesis of gastric cancer via the activation of E2F1 (Lin et al. 2021). E2F1 is significantly correlated with immune infiltrating cells and the poor prognosis of patients with uterine corpus EC (Liu et al. 2022). Our findings also elicited an up-regulated expression of E2F1 in EC cell lines. Likewise, the expression of E2F1 is stimulated in EC tissues, which predicts a worse relapse-free survival and overall survival in EC patients (Zhang et al.

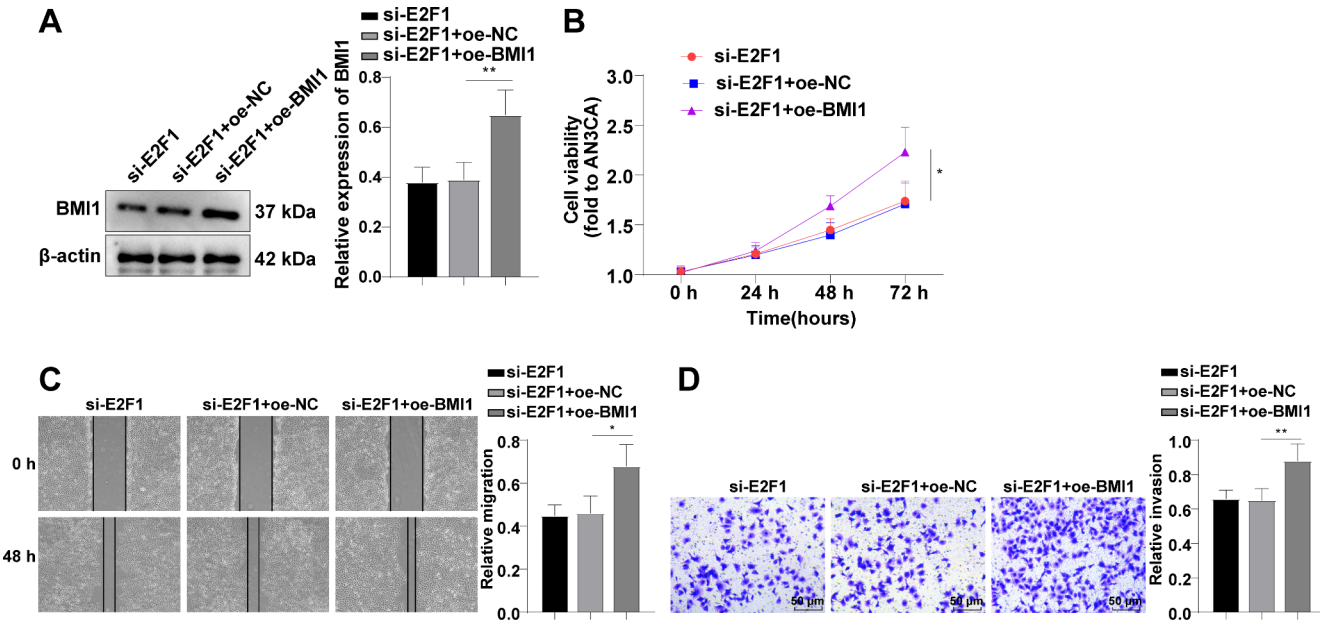

Fig. 5 BMI1 overexpression partially reversed E2F1 knockdown-inhibited EC cell proliferation, invasion and migration. E2F1 was silenced and BMI1 was overexpressed in AN3CA cells through cotransfection. (**A**) BMI1 expression was assessed by Western blot; (**B**) Cell proliferation was detected by MTT assay; (**C**) Cell migration was detected by wound healing test; (**D**) Cell invasion was detected by Transwell. Cell experiment was repeated 3 times. Data were expressed as mean ± standard deviation. One-way ANOVA was employed for comparisons among groups, followed by Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$

2020). Abnormally overexpressed E2F1 and its target genes are involved in the cell cycle, which may promote the occurrence and development of EC (Song et al. 2019). To specify the role of E2F1 in EC cell lines, we knocked down the expression of E2F1 in AN3CA cells or overexpressed the expression in HEC-1 cells, and discovered that knockdown of E2F1 suppressed the migration, proliferation, and invasion abilities of AN3CA cells, while the HEC-1 cells manifested the opposite trends after overexpression of E2F1. Facilitated expression of free E2F1 is well correlated with high S18-2 expression in EC, which leads to an increased proliferation capacity in EC cell line HEC-1-A (Mints et al. 2016). Silencing of E2F1 reduces the capacity for EC cell proliferation (Xu et al. 2020). E2F1 knockdown can repress cell cycle progression, migration, and invasion of prostate cancer cell lines in vitro (Liang et al. 2016). Collectively, E2F1 intensified the malignant biological behaviors of EC cells.

BMI1, as a well-recognised transcriptional suppressor, has the capability of maintaining the proliferation and self-renewal of tissue-specific stem cells, which is highly expressed in various malignant cancers and functions as a key regulator in tumorigenesis (Yang et al. 2021). Our results elaborated an elevated expression level of BMI1, which was suppressed by knockdown of E2F1. Furthermore, overexpression of E2F1 increased the activity of the wild type BMI1 reporter vector promoter, while the effect was weakened by the mutant of the predicted binding site in the BMI1 promoter. Consistently, strong expression of BMI1 has been reported in primary neuroblastomas and the oncogene BMI1 could be activated by E2F1 (Nowak et al. 2006). Disulfiram/copper targets cancer stem cells in differentiated thyroid carcinomas by repressing E2F1-mediated BMI1 expression (Ni et al. 2022). Altogether, E2F1 activated the transcription of BMI1 in EC cells. To investigate the role of BMI1 in EC cells, we silenced BMI1 and discovered that the migration, proliferation and invasion of EC cells were blocked. PTC-028, an inhibitor of BMI1 function, potentiates caspase-dependent apoptosis and represses invasion of EC cells (Buechel et al. 2018). Knockdown of BMI1 expression enhances chemosensitivity and limits cancer stemness in EC cells (Kim et al. 2018). Subsequently, we silenced E2F1 and overexpressed BMI1 in EC cells and found for the first time that the inhibitory effects of E2F1 knockdown on the invasion, migration, and proliferation of EC cells were partially abrogated by overexpression of BMI1. Similarly, the effects of COPZ1 silencing on the autophagy, apoptosis, and proliferation of breast cancer cells are annulled by overexpression of BMI1, implying that BMI1 amplifies the proliferation and blocks the autophagy of breast cancer cells via activating COPZ1 (Chen et al. 2022).

In summary, this study supported that E2F1 enhanced the migration, proliferation, and invasion of EC cells by intensifying the transcription of BMI1. However, in this study, only AN3CA cell line was selected to study the mechanism of E2F1 promoting EC cell growth by enhancing the

transcription of BMI1 without multi-cell line validation in other EC cell lines. The results in this study were only verified in vitro, which lacked animal experiments and clinical data. In addition, other downstream target genes that might be regulated by E2F1 and other transcription regulators upstream of BMI1 remained elusive. These are the direction for our future studies.

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Authors' contributions YL conceived the ideas. YL, RZ and YW designed the experiments. YL, YW, XS, and YT performed the experiments. YL, JL, YZ and YM analyzed the data. YL wrote the manuscript. YL and RZ supervised the study. All the authors have read and approved the final version for publication.

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Data Availability All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Competing interests The authors declare that they have no competing interests.

Consent for publication Not applicable.

Ethics approval This article does not contain any studies with human or animal subjects performed by the any of the authors.

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