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Silencing of Forkhead Box M1 Reverses Transforming Growth Factor-β1-Induced Invasion and Epithelial-Mesenchymal Transition of Endometriotic Epithelial Cells

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Keywords
Forkhead box protein M1 · Epithelial-mesenchymal transition · Endometriosis · Snail

Abstract
Aim: The aim of this study was to investigate the expression of Forkhead box M1 (FoxM1) in endometriosis and determine FoxM1's possible effects on endometriotic epithelial cells (EECs) invasion and epithelial-mesenchymal transition (EMT). ***Methods:*** The expression of FoxM1 and E-cadherin in endometrium and ectopic tissues was analyzed by immunohistochemistry. The transforming growth factor-β1 (TGF-β1) was added to induce EMT of EECs, which were purified from ectopic tissues. The Short hairpin RNA (ShRNA) intervention technique was used to silence FoxM1. The morphological changes of EECs were observed by microscope. The invasion ability of EECs was determined by transwell invasion assay. The expression of FoxM1 and EMT-related gene (E-cadherin, N-cadherin, vimentin, and Snail) in EECs was detected by quantitative reverse transcription-polymerase chain reaction and western blot. ***Results:*** FoxM1 expression was significantly increased, while E-cadherin expression was significantly decreased in ectopic tissues than that in endometrium tissues. After TGF-β1 treatment, EECs showed a transformation from an epithelial sheet-like structure to a mesenchymal fibroblastic spindle shape; EECs invasion ability was enhanced; the level changes of EMT-related molecule also indicated an EMT phenotype of EECs. After FoxM1-shRNA intervention, TGF-β1-induced changes of EECs in morphology, invasion ability and EMT-related molecule expressions were partially reversed. ***Conclusions:*** Silencing of FoxM1 could reverse TGF-β1-induced invasion and EMT of EECs.

Introduction

Endometriosis is a chronic disease characterized by the presence of ectopic endometrial stroma and epithelium within a position other than the endometrium [1]. The symptoms of endometriosis mainly include pelvic pain, dysmenorrhea, dyspareunia, and infertility, which

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affect approximately 6–10% females in their reproductive age and at least one-third of women with infertility [2]. Currently, the treatment modalities for endometriosis include surgical, medical, or a combination of both [2]. Although the recurrence risk of surgery is quite high, there is still no radical cure other than surgery for endometriosis treatment due to its unclear pathogenesis. Hence, it is necessary to investigate the possible biological mechanism that may trigger endometrium metastasis from eutopic to ectopic, and then promote endometriotic lesions formation.

Endometriotic lesions are recognized to be benign inflammatory lesions, whereas its biological behavior has cancer-like features such as local invasion, implantation, and resistance to apoptosis [1]. For the past few years, the epithelial-mesenchymal transition (EMT) has been attracting attentions as one of the molecular mechanisms of migration and invasion in metastatic diseases and human cancers [3]. During EMT, cells transform from an epithelial phenotype to a mesenchymal phenotype. The most significant characteristic of EMT is that its epithelial marker E-cadherin appears downregulated, while its mesenchymal markers such as N-cadherin appear upregulated [4]. Since EMT endows cells with invasive and metastatic properties, it seems a prerequisite for the formation of endometriotic lesions in endometriosis [5]. A previous study has demonstrated that E-cadherin expression is reduced, while N-cadherin expression is increased in endometriosis when compared to that in endometrium, suggesting that EMT may contribute to endometriotic lesions formation [6].

The Forkhead Box M1 (FoxM1) is a member of Forkhead box transcription factors [7]. Accumulated studies have proved that FoxM1 plays crucial roles in cell cycle regulation, cell proliferation, differentiation, transformation, angiogenesis, metastasis, and invasion [7, 8]. For instance, FoxM1 promotes cell cycle progression by regulating growth factor-induced expression of kinase-interacting stathmin [9]. FoxM1 mediates growth inhibition during the terminal differentiation of human metastatic melanoma cells [10]. In addition, ERK/FoxM1 signaling cascade aberrant activation triggers cell invasion and migration in ovarian cancer cells [11]. It is well known that endometrium is a highly dynamic tissue that experiences repeated cycles of proliferation, differentiation, and regeneration during every menstrual cycle [12]. Previously, a study has discovered that FoxM1 expresses in human endometrium with a dynamical manner during menstrual cycle [13], which indicates that FoxM1 may participate in the endometrium remodeling. However, a few studies have focused on FoxM1 expression in endometriosis. Therefore, it is very meaningful and interesting to investigate whether FoxM1 expression level is different between that in endometrium tissue and endometrium ectopic tissue.

In recent years, studies have revealed that FoxM1 is associated with the EMT progress in multiple human diseases [10, 14]. In lung fibrosis, FoxM1 can induce EMT by directly activating the promoter of EMT-associated transcriptional factors such as Snail [14], which inhibits E-cadherin expression and activates mesenchymal transcriptomes. In human gastric cancer, the downregulation of FoxM1 can reverse EMT phenotype characterizing by increased E-cadherin expression, and decreased mesenchymal markers expression such as vimentin (VIM), Snail Family Transcriptional Repressor 2 (SNAI2), also named SLUG, and Snail Family Transcriptional Repressor 1 (SNAI1) [15]. Whether the regulation of FoxM1 on EMT still exists in endometriosis remains unknown and indistinct; it needs to be explored and revealed. In this study, we detected the expressions of FoxM1 and E-cadherin in endometrium and ectopic tissues by immunohistochemical analyses. By utilizing purified endometriotic epithelial cells (EECs), we further investigated the influences of mediated FoxM1 silencing on EECs morphologic change, invasion, and EMT in vitro.

Materials and Methods

Sample Collection

This study was approved by the Ethics Committee of The Affiliated Hospital of Qingdao University. Informed consent was obtained from each participant. Totally, 50 subjects (control) who underwent dilation and curettage for benign indications and 85 patients with endometriosis (ovarian endometriosis) were recruited. Among those 85 endometriosis patients, we acquired matched samples of eutopic and ectopic endometrium with 40 cases, singled eutopic endometrium with 20 cases, and singled ectopic tissue with 25 cases. Hence, a total of 60 eutopic tissues and 65 ectopic tissues were obtained. For those 50 control patients, normal endometrium tissues were obtained. All the sample tissues were collected during surgical procedures. All participants were of reproductive age, with menstrual regularity, and received no hormonal therapy for at least 3 months before this study. The cycle phase of the study population is shown in Table 1.

Cell Isolation and Culture

The EECs were isolated and cultured according to a previous study [16]. In all, 65 ectopic tissue specimens were minced and immersed in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 media containing 0.5% collagenase and 0.02% DNAase (Wako Junyaku Co., Ltd., Osaka, Japan), then incubated at 37 °C for 30 min. By aspirating through a siliconized pasteur pipette, cell clumps were

Table 1. Cycle phase of the study population

Cycle phase	Total	Control endometrium (n = 50)	Endometriosis patients (n = 85)	
			eutopic endometrium (n = 60)	ectopic tissue (n = 65)
Proliferative	87	24	30	33
Secretory	88	26	30	32

dispersed. After filtrating with 106 μm monofilament nylon mesh, the epithelial-enriched cell clumps were retained and further digested by an enzyme mixture (0.5% collagenase, 0.1% pronase, and 0.02% DNAase) at 37 °C for 20 min. Then, an 88-μm monofilament nylon mesh membrane filter, which permitted most stromal and endothelial cells to pass, was used to filtrate the above mixture solution to obtain the EECs. The EECs were re-suspended and cultured in DMEM/Ham's F12 media containing 5% fetal bovine serum (FBS; Gibco, NY, USA), 100 U/mL Penicillin G (Gibco), 100 μg/mL streptomycin (Gibco), and 25 μg/mL amphotericin B (Gibco) under 37 °C in 5% CO_2. The purity was determined by immunocytochemical staining with monoclonal anti-cytokeratin 56 kDa (reacts with cytokeratin polypeptides 17; Serotec Ltd., Oxford, UK) and the purity was more than 92%. For the induction of EMT, 5 ng/mL transforming growth factor-β1 (TGF-β1; R&D Systems, inc., Minneapolis, MN, USA) which has been identified as the most potent factor to induce EMT, was added to EECs [17] for 48 h treatment.

Immunohistochemistry Analysis

In graded ethanol, the paraffin tissue sections were deparaffinized and then rehydrated. After antigen retrieval, sections were dealt with hydrogen peroxide (3%) to inhibit the activity of endogenous peroxidase. After blocking, sections were incubated with the primary antibodies at 4 °C overnight, and further incubated with peroxidase-labeled anti-rabbit IgG for 30 min. The reaction was visualized by using DAB-Substrate (Beyotime, China). The rabbit monoclonal antibodies directed against FoxM1 (1:100; Abcam, UK) and E-cadherin (1:150; Cell Signaling Technology, MA, USA) were used as primary antibodies. A semi-quantitative subjective scoring system was employed to evaluate the intensity, quantity, and localization of immunoreactivity. For each sample, the staining of stromal cells and epithelial cells was scored separately. The scores were calculated basing on the following criteria [18]: (i) the percentage of positive cells: 0 (0%), 1 (1–10%), 2 (11–50%), 3 (51–70%) and 4 (71–100%); (ii) staining intensity: 0 (none), 1 (weak), 2 (moderate) and 3 (strong). The scores from the above 2 aspects were combined by multiplication to generate a final immunohistochemistry score.

Short Hairpin RNA Transfection of EECs

For *FoxM1* silencing, human FoxM1 Short hairpin RNA (shRNA) sequence and the corresponding normal control (NC) shRNA sequence were cloned into the retroviral transfer vector pSuper-retro-puro (Addgene, Cambridge, MA, USA). Then, the acquired vectors were transfected into EECs by using the calcium phosphate method [19]. Human FoxM1 shRNA (5′-GGA CCA CUU UCC CUA CUU U-3′) and control-shRNA (5′-GGA CCU GUA UGC GUA CAU U-3′) were synthesized by GenePharma (Shanghai, China). Then FoxM1 silencing efficacy was verified by western blot and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). To analyze the influences of FoxM1-silencing on TGF-β1 treated EECs, EECs were transfected with FoxM1-shRNA or its NC-shRNA in the presence of TGF-β1.

Western Blot Analysis

The whole protein extracts were lysed in the RIPA lysis buffer (Beyotime Inc., Nantong, China) as per the manufacturer's protocols. For each sample, 50 μg total proteins were separated by 10% SDS-PAGE and then electro-transferred onto PVDF membranes (Millipore, Burlington, MA, USA). The primary antibodies were used as follows: FoxM1 (1:500), E-cadherin (1:2,000), N-cadherin (1:1,000, Cell Signaling Technology), Vimentin (1:500, Cell Signaling Technology), Snail (1:500, Cell Signaling Technology), and then secondary antibodies (1:4,000, Zhongshan, China). The visualizations of target proteins were realized by Enhanced Chemiluminescence Plus kit (Beyotime,China). After quantification, data were normalized to GAPDH.

Quantitative Reverse Transcription-Polymerase Chain Reaction

The RNA extraction and qRT-PCR were performed by using a commercial kit (Promega, Madison, WI, USA) as per the manufacturer's introduction. Oligonucleotide primers and length of PCR products are listed in Table 2. A total of 30 cycles were carried out. Each cycle comprised of 1 min at 94 °C, 1 min at 50–60 °C depending on different genes, 1.5 min at 72 °C, and a final extension of 8 min at 72 °C. The relative level of each objective gene was calculated according to the following formula: $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct[\text{objective gene}]-\Delta Ct[GAPDH])}$.

Observation of Morphological Changes

Morphological changes of EECs were observed by using an inverted microscope. The images were photographed using a Leica microscope image system (Leica, Germany).

Cell Invasion Assays

Cell invasion ability was detected by matrigel-coated trans-well invasion assays. The 24-well chambers/microfilters (8-μm pore size polycarbonate filters, BD Bioscience, Bedford, MA, USA) were coated by matrigel (Sigma-Aldrich, St. Louis, MO, USA). Then, the EECs (5×10^4 cells) in 500 μL serum-free DMEM/F12 were added into the upper chamber. And then, 500 μL DMEM/F12 containing 10% FBS was added into the lower chamber. The invasion ability of EECs was evaluated by measuring the number of invaded cells in 24 h. After fixing, cells were stained with hematoxylin. The cells

Table 2. Sequences of PCR primers and length of PCR products

Genes	PCR primers	PCR primers
FoxM1	5′-GCT TGC CAG AGT CCT TTT TGC-3′ 5′-CCA CCT GAG TTC TCG TCA ATG C-3′	123bp
E-cadherin	5′-ATTCTGATTCTGCTGCTCTTG-3′ 5′-AGTAGTCATAGTCCTGGTCTT-3′	420bp
N-cadherin	5′-GATGTTGAGGTACAGAATCGT-3′ 5′-GGTCGGTATGGATGGCGA-3′	403bp
Vimentin	5′-CCAGGCAAAGCAGGAGTCCAC-3′ 5′-GGCCATCTTAACATTGAGCAGGT-3′	247bp
Snail	5′-CAGACCCACTCAGATGTCAA-3′ 5′-CATAGTTAGTCACACCTCGT-3′	557bp
GAPDH	5′-TGA TGA CAT CAA GAA GGT GGT GAA G-3′ 5′-TCC TTG GAG GCC ATG TGG GCC AT-3′	240bp

PCR, polymerase chain reaction; FoxM1, Forkhead box M1.

on the top surface of the filter were removed, and then the filter was mounted on the glass slide. The number of infiltrating cells was counted under a microscope at ×200 magnification from 5 regions selected at random.

Statistical Analysis

The data were expressed as mean ± SD and analyzed by SPSS 17.0 software (IBM, New York, NY, USA). The comparisons between the 2 groups were performed using *t* test. And the comparisons among 3 or more groups were performed by one-way analysis followed by Bonferroni's test post hoc. $p < 0.05$ was considered a statistically significant difference.

Results

FoxM1 Is Significantly Increased in Ectopic Lesions of Endometriosis Patients

Through immunohistochemistry assay, we observed that FoxM1 was expressed both in nucleus and cytoplasm of epithelial and stromal cells (Fig. 1a). For unpaired tissues, the expressions of FoxM1 were markedly higher in ectopic tissues than that in control and eutopic endometrium tissues; there was no significant difference of FoxM1 expression between control and eutopic endometrium tissues. For paired tissues, the expressions of FoxM1 were markedly higher in ectopic tissues than that in eutopic endometrium tissues (Fig. 1b, epithelium and stroma, $p < 0.05$). No significant difference in epithelial or stromal FoxM1 expression was observed between the proliferative and the secretory phase irrespective of the control endometrium, eutopic endometrium, or ectopic tissue (data not shown). These results suggested that the expression of FoxM1 was significantly increased in ectopic lesions of endometriosis patients.

E-cadherin Is Significantly Decreased in Ectopic Lesions of Endometriosis Patients

In order to evaluate the correlation between EMT and FoxM1 expression, we further detected E-cadherin expression by immunohistochemistry in endometrium and ectopic tissues. As shown in Figure 2a, we discovered that E-cadherin was mainly expressed along the membranes of epithelial cells. The stromal cells showed negative staining of E-cadherin (Fig. 2a). For unpaired tissues, E-cadherin expression in epithelium was dramatically lower in ectopic tissues than that in control and eutopic endometrium tissues. There was no significant difference of E-cadherin expression between control and eutopic endometrium tissues. For paired tissues, the expressions of E-cadherin were dramatically lower in ectopic tissues than that in eutopic endometrium tissues (Fig. 2b, $p < 0.05$). These results indicated that E-cadherin expression was significantly decreased in epithelium of ectopic lesions in endometriosis patients, which was contrary to the results obtained with FoxM1 expression. FoxM1 expression was negatively correlated with E-cadherin expression in epithelial cells of 65 ectopic lesions ($r = -0.318$, $p = 0.01$).

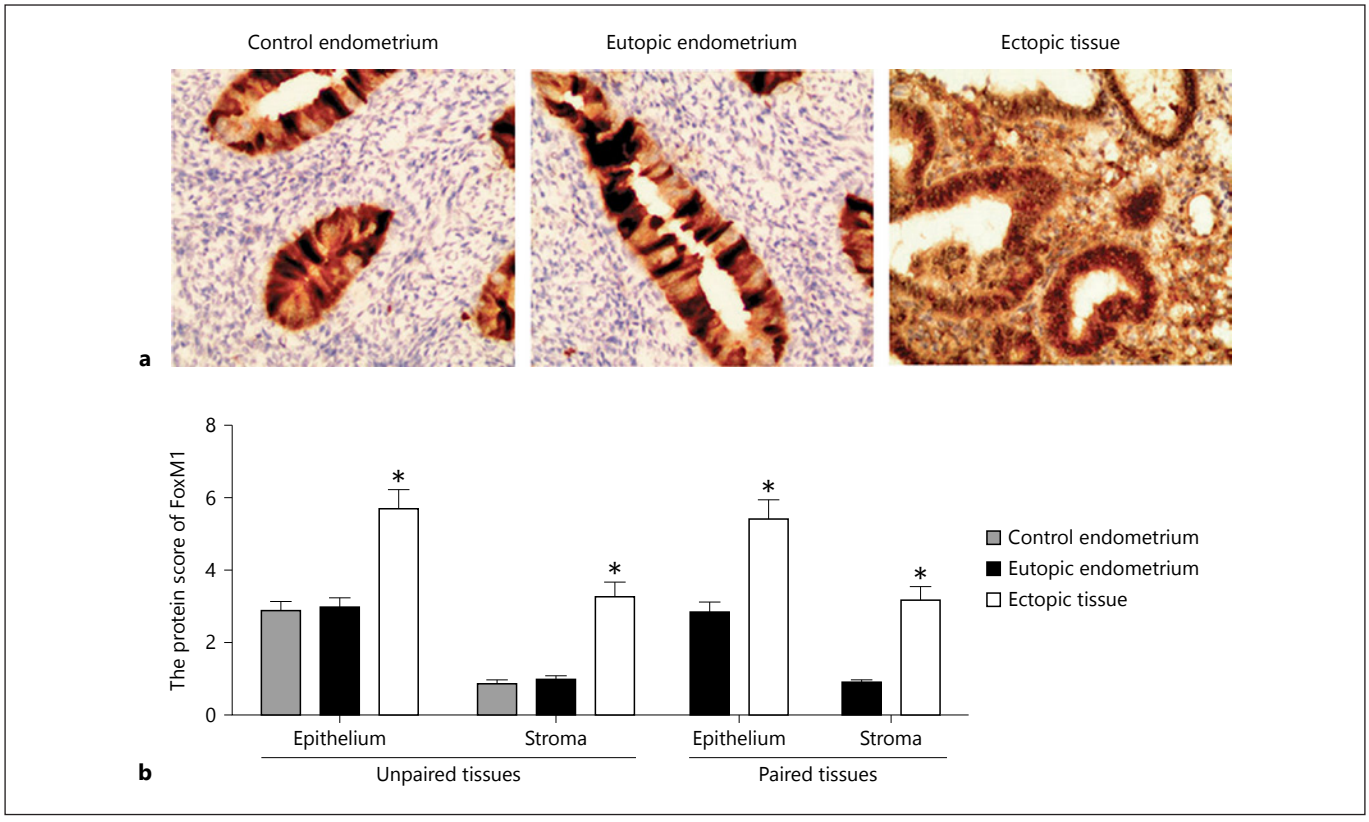

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Fig. 1. Immunohistochemical analyses of FoxM1 expressions in endometrium and ectopic tissues. **a** Immnohistochemical staining of FoxM1 in control endometrium, eutopic endometrium, and ectopic tissue. Magnification = 200×. The brown color represents FoxM1 positive staining. **b** The protein score of FoxM1 in unpaired tissues (50 control cases, 60 eutopic cases, and 65 ectopic cases). **c** The protein score of FoxM1 in paired tissues (40 eutopic cases and 40 ectopic cases). * $p < 0.05$ vs. control and eutopic endometrium tissues. FoxM1, Forkhead box M1.

Silencing of FoxM1 in EECs

In order to explore the influences of FoxM1 on EECs metastasis and its EMT, FoxM1 gene was silenced by using FoxM1-shRNA as descried in the Methods section. From Figure 3a, we observed that FoxM1 mRNA level was significantly decreased in the FoxM1-shRNA group than that in the control group ($p < 0.05$) and NC-shRNA group ($p < 0.05$). Similar results could be found on the FoxM1 protein level ($p < 0.05$ vs. control and NC-shRNA group). These results demonstrated that FoxM1 gene was successfully silenced in EECs, which could satisfy our following experiments.

Silencing of FoxM1 Reverses TGF-β1-Induced Morphological Changes and Invasion of EECs

As shown in Figure 4a, the morphology of EECs transformed from an epithelial sheet-like structure to a mesenchymal fibroblastic spindle shape after TGF-β1 treatment, which could be partially reversed with FoxM1 silencing (Fig. 4a). The data of invasion (Fig. 4b, c) assays suggested that the number of invaded EECs was markedly increased in TGF-β1 treatment group ($p < 0.05$), which emerged to be a significant decrease with FoxM1 silencing ($p < 0.05$). Taken together, these data demonstrated that FoxM1 silencing could reverse TGF-β1-induced morphological changes and invasion of EECs.

Silencing of FoxM1 Reverses TGF-β1-Induced EMT Progress of EECs

Since that EMT is closely associated with cell metastasis, we next detected the influences of FoxM1 silencing on the levels of EMT-related molecules. Through qRT-PCR assays (Fig. 5a), we discovered that the mRNA levels of FoxM1, N-cadherin, Vimentin, and Sail were prominently increased in TGF-β1-treated EECs and NC-shRNA + TGF-β1-treated EECs ($p < 0.05$), which could be partially reversed in FoxM1-shRNA + TGF-β1-treated EECs ($p < 0.05$). On the contrary, the change tendency of E-cad-

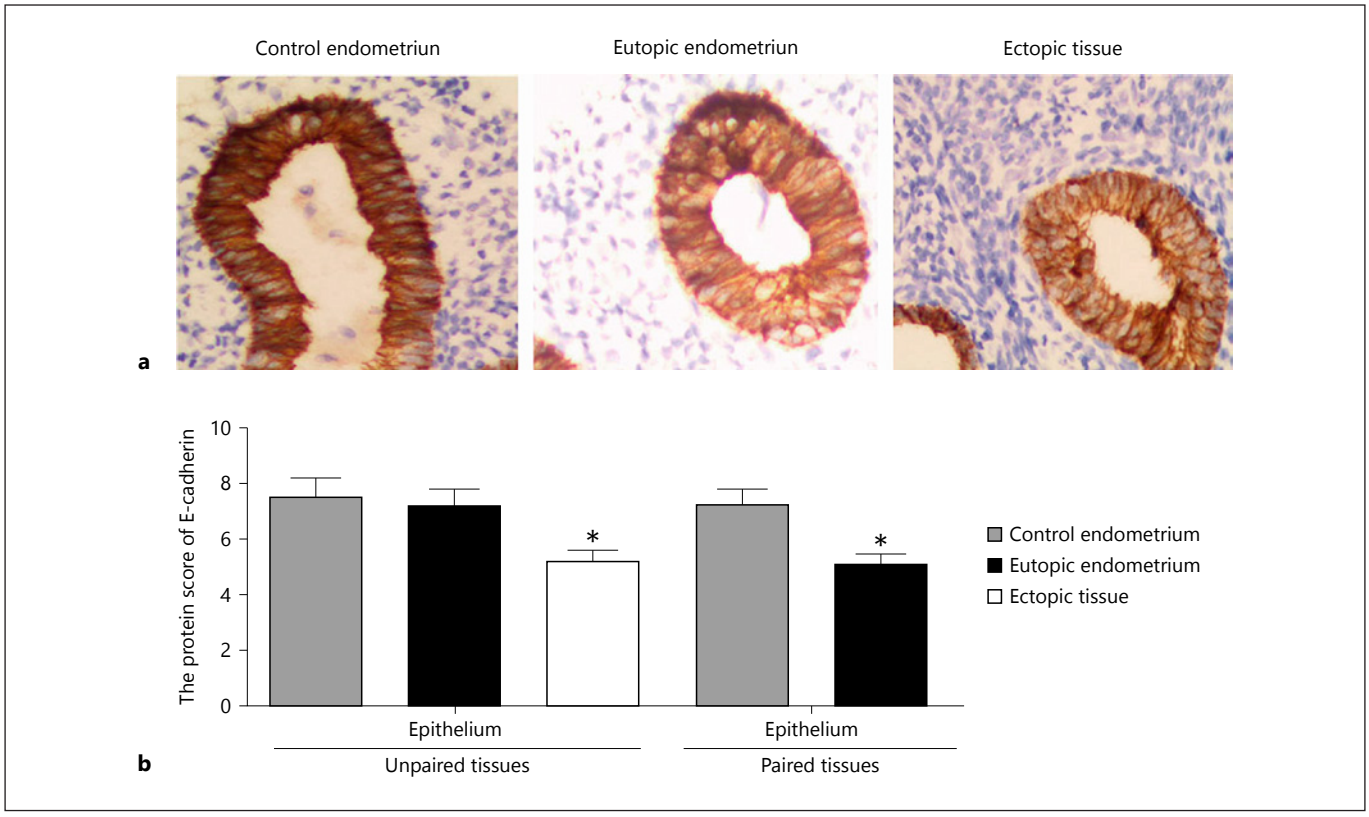

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Fig. 2. Immunohistochemical analyses of E-cadherin expressions in endometrium and ectopic tissues. **a** Immuohistochemical staining of E-cadherin in control endometrium, eutopic endometrium, and ectopic tissue. Magnification = 200×. The brown color represents E-cadherin positive staining. **b** The protein score of E-cadherin in unpaired tissues (50 control cases, 60 eutopic cases, and 65 ectopic cases). **c** The protein score of E-cadherin in paired tissues (40 eutopic cases and 40 ectopic cases). * $p < 0.05$ vs. control and eutopic endometrium tissues.

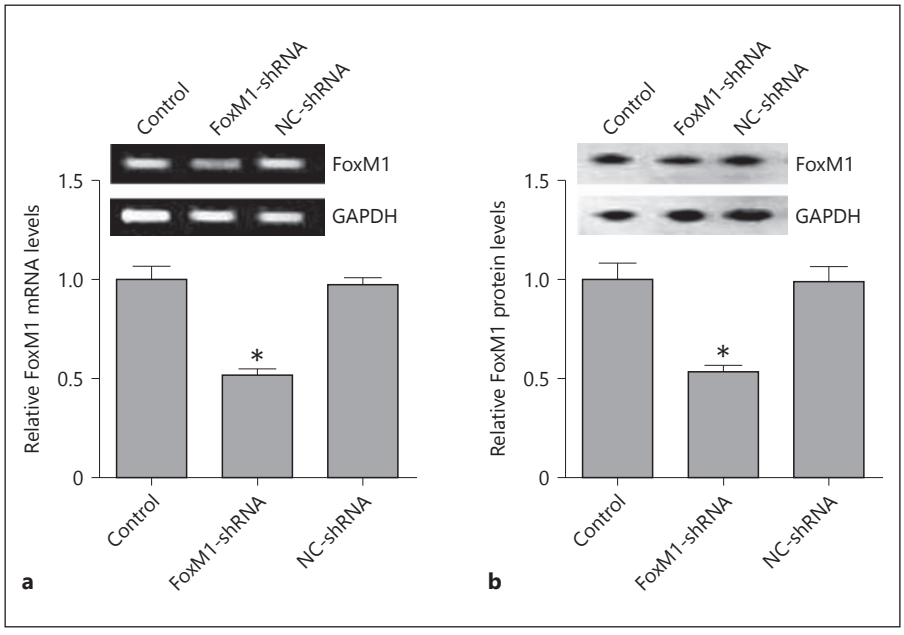

Fig. 3. Levels of FoxM1 mRNA and protein in EECs. The EECs were transfected with FoxM1-shRNA or its NC-shRNA as described in methods. The levels of FoxM1 mRNA and protein were detected by qRT-PCR and western blot respectively. **a** Relative FoxM1 mRNA levels. **b** Relative FoxM1 protein levels. Data were normalized to GADPH and deemed as 1 in control group. * $p < 0.05$ vs. control or NC-shRNA group. FoxM1, Forkhead box M1; ShRNA, short hairpin RNA; NC, normal control.

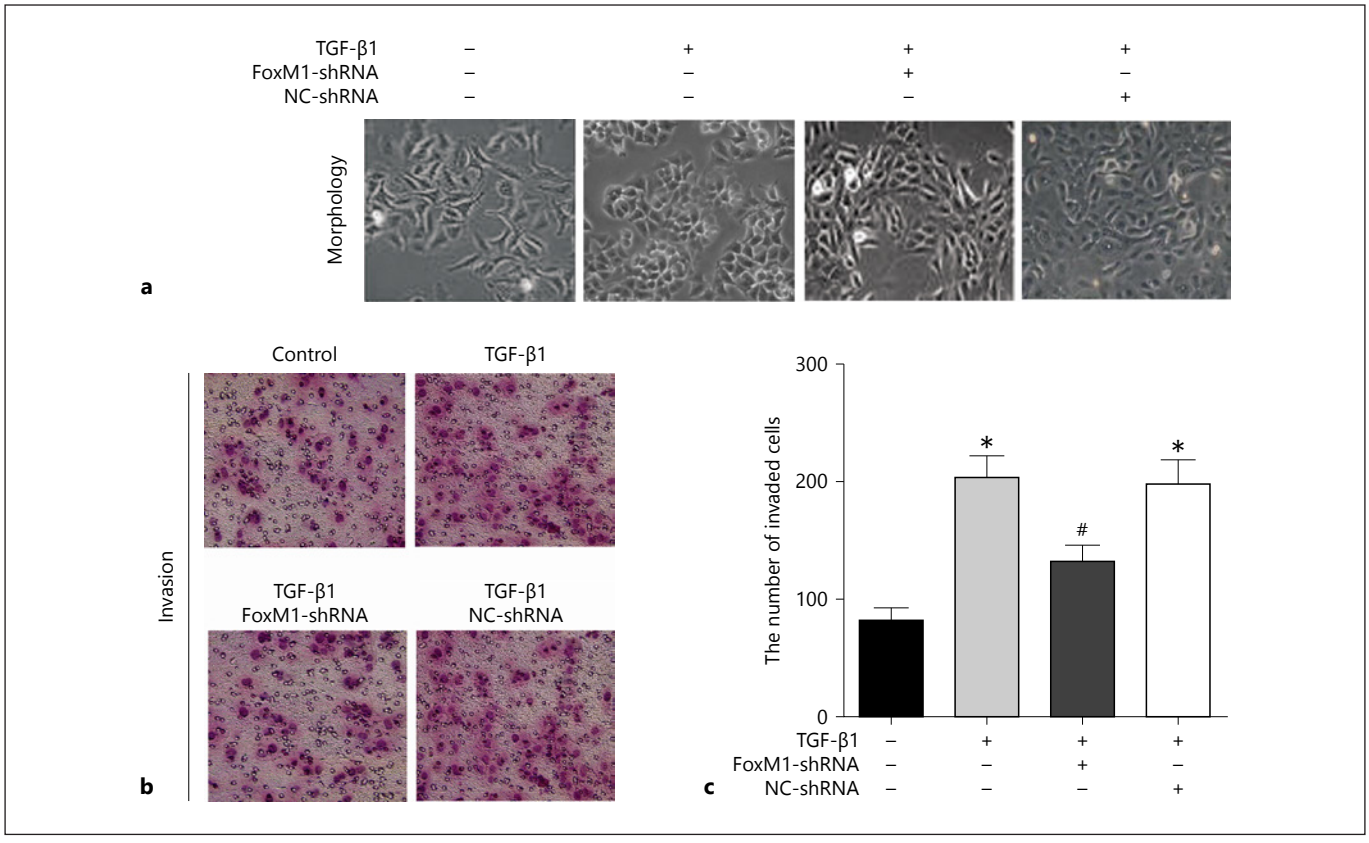

Fig. 4. Influences of FoxM1 silencing on EECs morphology and invasion. The EECs were transfected with FoxM1-shRNA or its NC-shRNA in the presence of TGF-β1 (5 ng/mL) for 48 h as described in methods. **a** Images of EECs morphology. Magnification = 200×. **b** Images of EECs invasion. Magnification = 200×. **c** The number of invaded EECs. * $p < 0.05$ vs. (–,–,–) group; # $p < 0.05$ vs. (+,–,–) or (+,–,+) group. FoxM1, Forkhead box M1; ShRNA, short hairpin RNA; NC, normal control; TGF-β1, transforming growth factor-β1.

herin among these groups was opposite to that of FoxM1, N-cadherin, Vimentin, and Sail. In addition, we also detected the expressions of FoxM1 and EMT-related molecules on protein levels, and the results were similar with that on their mRNA levels (Fig. 5b). These results indicated that FoxM1 silencing could partially reverse TGF-β1-induced EMT progress of EECs.

Discussion

In this study, we mainly reported that FoxM1 silencing could partially reverse TGF-β1-induced invasion and EMT of EECs. Apart from human cancers, our findings indicated that the regulation of FoxM1 on EMT also existed in endometriosis. Hence, we inferred that FoxM1 may be a potential target molecule for endometriosis treatment.

FoxM1 is commonly recognized as an oncogenic transcription factor [20], and a promising target for anticancer therapy [21]. Previous studies have proved that FoxM1 is upregulated in multiple human malignancies and its overexpression frequently indicates poor prognosis [18]. Although endometriosis shares many features with tumor metastasis, little is known about the expression of FoxM1 in endometriosis. Our study first discovered that FoxM1 was highly expressed in both epithelial and stromal cells of ectopic tissue compared to that of the eutopic endometrium tissue in endometriosis patients. Interestingly, as the crucial indicator of EMT, E-cadherin was observed to be markedly decreased in the epithelial cells of the ectopic tissue compared to that of the eutopic endometrium tissue. In addition, there was no significant difference of FoxM1 and E-cadherin expression between normal endometrium tissues and endometriosis endometrium tissues. Based on the above findings, we specu-

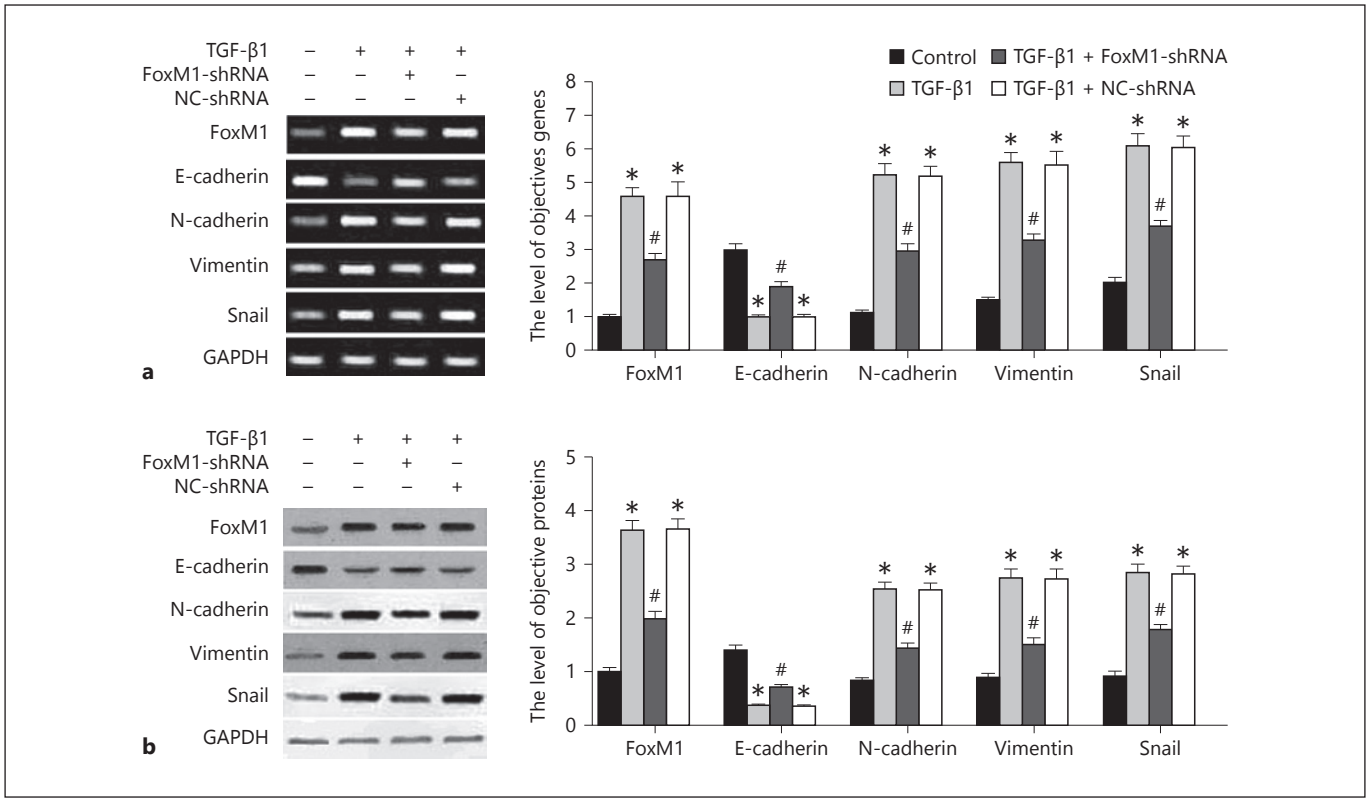

Fig. 5. Levels of FoxM1 and EMT-related molecules in EECs. The EECs were transfected with FoxM1-shRNA or its NC-shRNA in the presence of TGF-β1 (5 ng/mL) for 48 h as described in methods. The mRNA and protein levels of FoxM1 and EMT-related molecules were detected by qRT-PCR and western blot respectively. **a** The image and quantitative level of objective genes in EECs. **b** The image and quantitative level of objective proteins in EECs. Data were normalized to GAPDH. And the data of the FoxM1 level in the control (–,–,–) group was deemed as 1. * $p < 0.05$ vs. control (–,–,–) group; # $p < 0.05$ vs. TGF-β1 (+,–,–) or TGF-β1 +NC-shRNA (+,–,+) group. FoxM1, Forkhead box M1; ShRNA, short hairpin RNA; NC, normal control; TGF-β1, transforming growth factor-β1.

late that FoxM1 may be aberrantly upregulated in disseminated endometrial cells to facilitate their invasion and survival outside the uterus to form endometriotic lesions, which perhaps may be associated with the EMT progress.

As the regulation of FoxM1 on EMT in human diseases mentioned in introduction, it is necessary to further explore whether this regulation also happens in endometriosis. Emerging evidences suggest that EMT plays significant roles in the initial formation of endometriosis [5, 6]. Besides, studies have suggested that EMT could be induced by TGF-β1 [22]. In in vitro research, the EECs were isolated and purified from endometriotic lesion tissues of endometriosis patients. We used TGF-β1 to induce EMT, and observed that the morphology of EECs transformed from an epithelial sheet-like structure to a mesenchymal fibroblastic spindle shape, the expression of E-cadherin dramatically decreased, while the expression of N-cadherin and Vimentin dramatically increased, suggesting the EMT introduction was successful. Through FoxM1-shRNA intervention, we discovered that FoxM1 silencing could partially prevent TGF-β1-induced EMT phenotype and cell invasion of EECs. These results enhanced our speculation that the overexpressed FoxM1 in ectopic tissue may contribute to endometrium metastasis and endometriotic lesion formation by regulating the EMT progress, which may play a crucial role in the pathogenesis of endometriosis. Meanwhile, we found that TGF-β1 could also induce the expression of FoxM1 in EECs. Many signal transduction pathways have been found to involve in the interaction between TGF-β1 and FoxM1. For instance, TGF-β1 receptors interact with the MAPK pathway and Raf/MEK/MAPK signaling to stimulate the nuclear accumulation and phosphorylation of FoxM1, thus facilitating the FoxM1-dependent transcriptional program [23]. The transcription of FoxM1 is upregulated by

hypoxia-inducible factor-1α, which is one of the stimulators of breast cancer EMT and can be activated by TGF-β1 [24]. However, how TGF-β1 interacts with FoxM1 in EECs is not clear and needs to be further investigated.

Previous studies have suggested that FoxM1 can promote the EMT process by regulating EMT-associated transcription factors, such as Slug, Snail and Zeb1/2 [3]. Snail is a member of zinc-finger transcription factors and an essential player in EMT aggressive phenotype [25]. Snail transcription factor can inhibit the expression of E-cadherin by binding several E-boxes located in the promoter region, leading to the transcriptional repression of E-cadherin as well as molecular and morphological changes, such as the induction of EMT [26]. Besides, a recent study reported that E-cadherin downregulation and Snail upregulation occurred concordantly in ectopic lesions of endometriosis patients, suggesting that Snail might be important regulators of EMT in endometriosis [27]. Furthermore, Park et al. [28] have indicated that increased FoxM1b levels can enhance Snail expression by activating AKT and subsequently increasing glycogen synthase kinase-3 beta. In addition, FoxM1 has been found to directly bind to and increase the activity of Snail promoter, suggesting that Snail is a direct downstream target of FoxM1 [29]. In accordance with the above findings, our data found that shRNA-mediated the downregulation of FoxM1 inhibited TGF-β1-induced the Snail expression in EECs. Hence, we inferred that the overexpressed FoxM1 may induce EMT progress during endometriosis development by regulating the Snail expression.

It is well known that the epithelial cells will lose their polarity and cell-cell contacts during EMT, which could result in cytoskeleton dramatic remodeling and facilitate cellular movement [30]. The EMT progress commonly receives great attention based on its pivotal roles in metastasis [31]. There have been evidences suggested that FoxM1 promotes the invasion abilities of various cancer cells by facilitating theirs EMT progress [32]. In our study, we observed that FoxM1 silencing could partially inhibit TGF-β1-induced EECs invasion probably by FoxM1 silencing induced inhibition effects on EMT. Because there are other mechanisms that exist to account for the metastasis regulation effects of FoxM1, we cannot exclude the other possibilities that may contribute to the inhibition effect of FoxM1 silencing on EECs invasion. For instance, FoxM1 can regulate the expressions of extracellular matrix degradation factors such as MMP-2, MMP-9, and uPA to modulate cancer cell invasion [33]. Furthermore, FoxM1 can transcriptionally regulate the expression of vascular endothelial growth factor and promote the angiogenesis of nasopharyngeal carcinoma cell [34]. Besides, FoxM1 has also been found to regulate the cell-cycle progression of tumor cells by facilitating S-phase progression and entry into mitosis [35]. Although new views were expounded in our work, there still were some limitations. For instance, the samples were only from ovarian endometriosis, which was one type of endometriotic lesions, and the study of other types of endometriosis, like peritoneal endometriosis, should be performed to comprehensively investigate and analyze endometriosis.

Conclusion

Viewed in totality, our studies have revealed that FoxM1 expression is significantly higher in endometriotic lesions. Meanwhile, FoxM1 silencing could partially reverse the invasion and EMT of EECs. These findings may provide a new insight into understanding the pathogenesis of endometriosis and offer a new biomarker for the treatment of endometriosis.

Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Authors Contribution

Conception and design of the research was done by J.Z.; acquisition of data was the responsibility of H.D. and G.Z.; analysis and interpretation of data were done by Z.X.; statistical analysis was performed by J.Z.; drafting of the manuscript was done by J.Z.; revision of manuscript for important intellectual content was done by T.L. All authors read and approved of the final manuscript.

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