Comparative study on the biological characteristics of menstrual blood- and endometrium-derived endometrial cells

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Abstract. During a woman's reproductive period, the endometrial tissue is shed and regenerated every month to prepare for pregnancy or for the next cycle. The aim of the present study was to isolate, culture and characterize human endometrial cells (ECs) derived from menstrual blood (MB) and the endometrium (E). MB-derived ECs (MB-ECs) were isolated from women's MB. E-derived ECs (E-ECs) were isolated from women's endometrial tissues. The present study investigated the epithelial cell marker cytokeratin 18 (CK18) in MB-ECs and E-ECs. Cell proliferation analyses indicated that E-ECs (population doubling time, 20.85 h) grew faster than MB-ECs (population doubling time, 22.05 h; P<0.05). Cell migration ability was found to be significantly greater for MB-ECs than for E-ECs at 48 h (P<0.01). MB-ECs incubated with TGF-β1 (3 ng/ml) exhibited significantly decreased CK18 mRNA expression (P<0.01), and significantly increased vimentin (Vim) mRNA (P<0.05) and protein (P<0.01) expression at 6 and 12 h, respectively. E-EC incubation with TGF-β1 (3 ng/ml) significantly decreased CK18 mRNA expression (P<0.01) at 12 h and significantly increased Vim mRNA (P<0.01) and protein expression (P<0.05) at 6 h. The present results indicated that MB-ECs and E-ECs were biologically different, and that epithelial-mesenchymal transdifferentiation could be induced by TGF-β1 treatment.

Introduction

The human endometrium (E) is a dynamic remodeling tissue, which in response to the prevailing steroid environment of sequential ovarian estrogen and progesterone exposure, undergoes >400 cycles of regeneration, differentiation and shedding during a woman's reproductive years (1,2). Menstruation is the endometrial response to progesterone and estrogen withdrawal that occurs with the decay of the corpus luteum in the absence of pregnancy (1). Chan *et al* (3) first identified the endometrial epithelial and stromal cell clone formation ability, suggesting that there are three types of cells in the E: Epithelial, mesenchymal and endothelial cells. Smalley and Clarke (4) observed that endometrial epithelial cells had enhanced cloning activity during the proliferative phase, and stromal cells had stronger activity during the secretory phase.

Both epithelial and stromal cells are present in the basal layer of the human E, and menstruation is formed after the top two-thirds of the functional layer of the E (2). Therefore, there may be some differences in biological characteristics between menstrual blood-derived endometrial cells (MB-ECs) and E-derived ECs (E-ECs). Ethical and practical considerations often limit the use of primary human E-ECs for research purposes (5). The acquisition of MB is not invasive and does not cause harm to the donor. MB-ECs isolated and cultured *in vitro* demonstrated high proliferation potential and the ability to differentiate into a variety of cells (6). Considering the easier access to MB, ECs have become a convenient source of adult cells and are a relevant cell source for the study of endometrial diseases (7,8).

The present study compared MB-ECs and E-ECs to investigate the isolation, identification, culture, expansion and differentiation of ECs from two provenances, in order to identify entry points and lay a foundation for the treatment of gynecological diseases. The present study also provided important experimental and theoretical guidance to obtain different ECs by different means.

Materials and methods

Experimental materials. All samples were collected between March 2017 and April 2017 in under a protocol approved by the institutional review board of the Gynecology Center of The First Affiliated Hospital of Xinjiang Medical University (Urumqi, China), and written informed consent was obtained from each donor. Human endometrial tissue was obtained from patients (Table I) undergoing hysterectomy, and MB was obtained from healthy women. The age range of the patients

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and healthy controls was 26-43 years. The inclusion criteria were as follows: Childbearing age (30-45 years), benign gynecological diseases, no fertility requirements, hysterectomy, no hormone treatment within 6 months, no or mild anemia, and no liver, kidney, heart, brain or blood system disorders. The exclusion criteria were as follows: Systemic or reproductive malignant disease, endometrial or intrauterine lesions, a history of hormone therapy within 3 months, endometriosis and adenomyosis. The patients were matched according to their age, menstrual history, fertility history, contraceptive methods, uterine size, lack of hormone treatment within 3 months and anemia. MB and endometrial tissues obtained from the resected uterus were used as the research objects.

Isolation and culture of MB-ECs and E-ECs. For MB-EC isolation, 5 ml of MB samples from healthy women were collected, and 0.2 ml amphotericin B (cat. no. 15290026), 0.2 ml penicillin-streptomycin (cat. no. 15140122), 0.1 ml Na_2EDTA (cat. no. 15576028) and 9.5 ml PBS (cat. no. 20012050; all from Gibco; Thermo Fisher Scientific, Inc.) were added; this mixture was transferred to a laboratory at 4˚C. The cells were separated via centrifugation with 1.077 g/ml Ficoll (cat. no. P8900; Beijing Solarbio Science & Technology Co., Ltd.) at 500 x g for 30 min at room temperature, and the middle white membrane layer was collected. The separated cells were washed twice with PBS and cultured in a 60-mm culture dish (cat. no. 430166; Corning Life Sciences) using complete DMEM/F12 (cat. no. 11320082; Gibco; Thermo Fisher Scientific, Inc.) + 10% FBS (cat. no. FND500, Shanghai ExCell Biology, Inc.) + 1% penicillin-streptomycin, followed by incubation at 37˚C with 5% CO_2. Cells were washed twice with PBS to remove non-adherent cells after 3 days.

For E-EC isolation, the surgically removed human endometrial tissues were washed in PBS to remove excess tissue and blood. The endometrial tissues were cut into 1-mm³ pieces, then dissociated into single-cell suspensions using 200 μg/ml collagenase type IV (cat. no. C5138; Sigma-Aldrich; Merck KGaA) in DMEM/F12 with 10% FBS and mechanical methods for 1-2 h at 37˚C. The undigested endometrial tissues were incubated with 0.25% trypsin-EDTA (cat. no. 25200056, Gibco; Thermo Fisher Scientific, Inc.) for 15 min at 37˚C. Single-cell suspensions were collected using a cell strainer, then centrifuged at 200 x g for 8 min at room temperature. The bottom cells were resuspended in complete medium, plated into a 60-mm culture dish and incubated at 37˚C in 5% CO_2. Cells were washed twice with PBS to remove non-adherent cells after 24 h.

When cultures reached 90% confluence, cells were digested with warmed 0.25% trypsin-EDTA at 37˚C for 3 min, then subcultured onto fresh culture plates. To purify the cells, the isolated cells were screened using different time digestion methods. The cells were digested with warmed 0.25% trypsin-EDTA at 37˚C for 1-2 min, then digestion was stopped and the digested cells were removed. Subsequently, the remaining cells in the plate were digested again and cultured. This step was repeated between three and five times.

Proliferation kinetics. Cells from passage 5 were used to analyze proliferation kinetics. MB-ECs and E-ECs were seeded in 24-well plates (cat. no. 3524; Corning Life Sciences)

at a density of $1x10^4$ cells/well. The cells from three random wells were counted each day for 7 days. Proliferation curves were plotted according to mean values. The population doubling time (PDT) was calculated as follows: $PDT=(t-t_0)$ $lg2/(lgN_t-lgN_0)$, where t_0 is the start time of the logarithmic proliferation period, t is the termination time of the logarithmic proliferation period, N_0 is the initial number of cells in the logarithmic proliferation period and N_t the final number of cells in the logarithmic proliferation period.

In vitro scratch assay. MB-ECs and E-ECs were seeded and grown to confluence in a 6-well plate (cat. no. 3516; Corning Life Sciences). Both cell types were starved for 12 h in DMEM/F12. The scratch was performed with a pipette tip running through the dish. The plates were washed twice with fresh medium to remove non-adherent cells, and the cells were subsequently cultured in DMEM/F12 at 37˚C for 48 h. Cell migration was evaluated by measuring the wound area under a light microscope.

Epithelial-mesenchymal transdifferentiation (EMT) assay in vitro. MB-ECs and E-ECs were seeded and grown to confluence in a six-well plate. All cells were cultured for 24 h in DMEM/F12 + 10% FBS. The medium was changed to DMEM/F12 + 10% FBS + 3 ng/ml TGF-β1 (dissolved in ddH₂O; cat. no. 96-100-21; PeproTech, Inc.). The cells were cultured at 37˚C for 6, 12 or 24 h. As a control group, cells were cultured in DMEM/F12 + 10% FBS.

RNA extraction and reverse transcription-quantitative PCR assay (qPCR). Total cellular RNA was isolated using TRIzol® Reagent (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.) with genomic DNA removed. RNA quality was assessed by spectrophotometry. RNA (1-μg aliquots) was reverse-transcribed into complementary DNA (cDNA) using a RevertAid First Strand cDNA Synthesis kit (cat. no. K1621; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. cDNA was amplified using a Taq PCR Master Mix kit (cat. no. KT201; Tiangen Biotech Co., Ltd.) in a PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for PCR (total volume per reaction, 10 μl): Initial denaturation at 95˚C for 5 min; 30 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec; and final extension at 72˚C for 10 min. The reaction products were analyzed using 2.0% agarose gel electrophoresis and GelRed (cat. no. 41003; Biotium, Inc.). The sequence of each product was confirmed using DNA marker I ladder (cat. no. MD101; Tiangen Biotech Co., Ltd.).

qPCR was performed in duplicates using SYBR® Premix Ex Taq™ (cat. no. RR420A; Takara Biotechnology Co., Ltd.) and performed using a 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). qPCR reactions of 20 μl for each sample consisted of cDNA (200 μg cDNA after dilution), 2X SYBR Premix Ex Taq, 50X ROX Reference Dye, dH₂O and 10 μM of each gene-specific primer. Primer sequences are shown in Table II. The following thermocycling conditions were used for qPCR: Initial denaturation at 95˚C for 30 sec; 40 cycles of 95˚C for 5 sec, 60˚C for 30 sec and 72˚C for 30 sec; and final extension at 72˚C for

Table I. Information of samples of human endometrium.

Patient no.	Age, years	Disease	Surgery or operation check	Material method	Sample location
1	36	Uterine fibroids	TCRM	Hysteroscope	Endometrium
2	32	Endometrial polyps	TCRP	Hysteroscope	Endometrium
3	43	Abnormal uterine bleeding associated with ovulatory dysfunction	TCRE	Hysteroscope	Endometrium
4	42	Ovarian cyst	Hysteroscopy	Hysteroscope	Endometrium
5	33	Ovarian serous cystadenoma	Hysteroscopy	Hysteroscope	Endometrium
6	26	Endometrial polyps	TCRP	Hysteroscope	Endometrium
7	39	Ovarian cyst	Hysteroscopy	Hysteroscope	Endometrium
8	31	Endometrial polyps	TCRP	Hysteroscope	Endometrium
9	38	Pelvic inflammatory disease, hydrosalpinx	Hysteroscopy	Hysteroscope	Endometrium

TCRM, transcervical resection of submucous fibroids; TCRE, transcervical resection of the endometrium; TCRP, transcervical resection of polyps.

10 min. Relative mRNA abundance was calculated using the $2^{-\Delta\Delta Cq}$ method (9) and normalized to the expression levels of GAPDH. The primer pairs used for qPCR are listed in Table II.

Flow cytometry. Following digestion into single cells with warmed 0.25% trypsin-EDTA, MB-ECs and E-ECs were incubated with anti-CD90-PE (1:200; cat. no. bs-10430R-PE; BIOSS), anti-CD34-PE (1:200; cat. no. bs-0646R-PE; BIOSS), unconjugated antibodies or matched-isotype control IgG (1:200; cat. no. bs-0295P-PE; BIOSS) for 1 h at room temperature and analyzed via flow cytometry using a BD Accuri™ C6 flow cytometer (BD Biosciences). Data were analyzed using CFlow Plus software (v1.0.264.15; Accuri Cytometers, Inc.). Events with forward scatter (FSC) <12,500,000 and side scatter (SSC) <5,000,000 were gated in P1, and the events in P1 with FSC >2,000,000 and SSC >100,000 were gated in P2 to remove large cell adhesion bodies and small cell debris.

Cellular immunofluorescence assay. Cultures of MB-ECs and E-ECs at passage 3 were seeded ($0.5x10^5$ cells/1-cm^2 glass coverslip) on glass coverslips coated with poly-L-lysine. For immunofluorescent assay analysis, the MB-ECs and E-ECs were fixed with 4% paraformaldehyde for 15 min at room temperature and washed three times (5 min/wash) with PBS. For vimentin (Vim) staining, cells were permeabilized with 0.25% Triton X-100 (cat. no. X100; Sigma-Aldrich; Merck KGaA) for 10 min. Cells were washed three times (5 min/wash) with PBS, then blocked with 10% goat serum (cat. no. ZLI-9021; OriGene Technologies, Inc.) for 1 h at room temperature prior to incubation with primary antibodies. The cells were incubated with the following primary antibodies: Anti-Vim (1:250; cat. no. ab92547; Abcam) and anti-cytokeratin 18 (CK18; 1:250; cat. no. ab133263; Abcam)

at 4˚C overnight. Cells were washed three times (5 min per wash) with PBS, followed by incubation with Alexa Fluor® 594-conjugated goat anti-rabbit secondary antibody (1:100; cat. no. ZF-0516; OriGene Technologies, Inc.) in the dark for 1 h at room temperature. Finally, 10 µg/ml of DAPI (cat. no. D9542; Sigma-Aldrich; Merck KGaA) were used to label cell nuclei for 15 min, and images were captured using a fluorescence microscope (TE-2000-E; Nikon Corporation). PBS was used as a substitute for primary antibodies as a technical control.

Western blotting. Cells were prepared and lysed in ice-cold RIPA buffer (cat. no. 89900; Thermo Fisher Scientific, Inc.) containing freshly added Halt Phosphatase Inhibitor Cocktail and Protease Inhibitor Cocktail, and protein concentrations were determined using the Bradford protein assay (Bio-Rad Laboratories, Inc.). Samples of 20 µg proteins were separated via SDS-PAGE on a 12% gel (Bio-Rad Laboratories, Inc.) and proteins were immunoblotted onto nitrocellulose membranes. The membranes were blocked in 5% non-fat milk for 1 h at room temperature and probed with primary antibodies [anti-β-actin (1:1,000; cat. no. 60008-1-Ig; ProteinTech Group, Inc.), anti-Vim (1:1,000; cat. no. ab92547; Abcam) or anti-CK18 (1:1,000; cat. no. ab133263; Abcam)] at 4˚C overnight, followed by HRP-conjugated secondary antibodies [HRP-conjugated Affinipure Goat Anti-Mouse IgG (H + L) (cat. no. SA00001-1; 1:2,500; ProteinTech Group, Inc.) and HRP-conjugated Affinipure Goat Anti-Rabbit IgG (H + L) (cat. no. SA00001-2; 1:2,500; ProteinTech Group, Inc.)] for 2 h at room temperature. The blots were visualized using Immobilon Western Chemiluminescent HRP Substrate (cat. no. WBKLS0500; MilliporeSigma), and images were captured using the ECL Tanon 5500 system (Tanon Science and Technology Co., Ltd.). Protein expression was normalized to the expression levels of β-actin.

Table II. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Primer sequence	Melting temperature, ˚C	Product length, bp
Vim	F: 5'-GGACCAGCTAACCAACGACA-3'	60.0	116
	R: 5'-CGGCTTCCTCTCTCTGAAGC-3'	60.2	
CK18	F: 5'-CCTACAAGCCCAGATTGCCA-3'	60.0	115
	R: 5'-CCGAGCCAGCTCGTCATATT-3'	60.0	
GAPDH	F: 5'-TATGACAACAGCCTCAAGAT-3'	54.1	104
	R: 5'-AGTCCTTCCACGATACCA-3'	54.4	

Vim, vimentin; CK18, cytokeratin 18; F, forward; R, reverse.

Figure 1. Morphology of cultured cells *in vitro*. Morphology of (A) MB-ECs and (B) E-ECs at passage 3. Scale bar=100 µm. ECs, endometrial cells; MB, menstrual blood-derived; E, endometrium-derived.

Statistical analysis. Data are presented as the mean ± SEM for n=3 samples. Statistical differences between two groups were analyzed using Student's t-test (two-tailed unpaired). Comparisons between multiple groups were performed using one-way ANOVA followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Isolation, culture and morphology of MB-ECs and E-ECs. MB-ECs were isolated from MB by density gradient centrifugation. MB-ECs demonstrated a flat two-dimensional morphology after three generations of subculture (Fig. 1A). E-ECs were isolated from endometrial tissue by enzymatic digestion. E-ECs demonstrated a round and flattened shape morphology after three generations of subculture (Fig. 1B), distinct from the MB-EC cell morphology of small spindle-like cells (Fig. 1A).

Characterization of MB-ECs and E-ECs. In order to confirm that the isolated and cultured cells were not mesenchymal stem cells or hematopoietic stem cells, the cell expression of CK18 (a marker of epithelial cells), Vim (a marker of mesenchymal stem cells), CD90 (a marker of mesenchymal stem cells) and CD34 (a marker for hematopoietic stem cells) was quantified (10,11). Specific markers of MB-ECs and E-ECs

were detected via immunofluorescence and flow cytometry. Immunofluorescence assays indicated that MB-ECs and E-ECs both expressed CK18 (Fig. 2A), but neither expressed Vim (Fig. 2B). Flow cytometry assays demonstrated that neither MB-ECs nor E-ECs expressed CD90 and CD34 (Fig. 2C).

Cell proliferation and migration capacity assays. The proliferation curves of both MB-ECs and E-ECs appeared as typically sigmoidal, which consisted of a latent phase, a logarithmic phase and a plateau phase (Fig. 3). The PDT of MB-ECs was 22.05±0.44 h and that of E-ECs was 20.85±0.82 h (Fig. 3; P<0.05). The migration capacity assay results indicated that the wounds in confluent MB-ECs and E-ECs healed after 48 h, but the wound area of MB-ECs was significantly smaller than that of E-ECs at 48 h (P<0.01; Fig. 4).

TGF-β1 effect on EMT gene expression. Following TGF-β1 treatment, CK18 mRNA expression in MB-ECs and E-ECs decreased, and Vim mRNA expression in MB-ECs and E-ECs increased. The CK18 mRNA expression level in MB-ECs decreased significantly after 6 h (P<0.01 vs. 0 h) and in E-ECs after 12 h (P<0.01 vs. 0 h; Fig. 5A). The CK18 mRNA expression level gradually increased but remained significantly lower than its initial level after 24 h (P<0.01 vs. 0 h; Fig. 5A and B). Vim mRNA expression level in MB-ECs and E-ECs significantly increased after 6 h (P<0.05 vs. 0 h; Fig. 5A and C), but was significantly lower
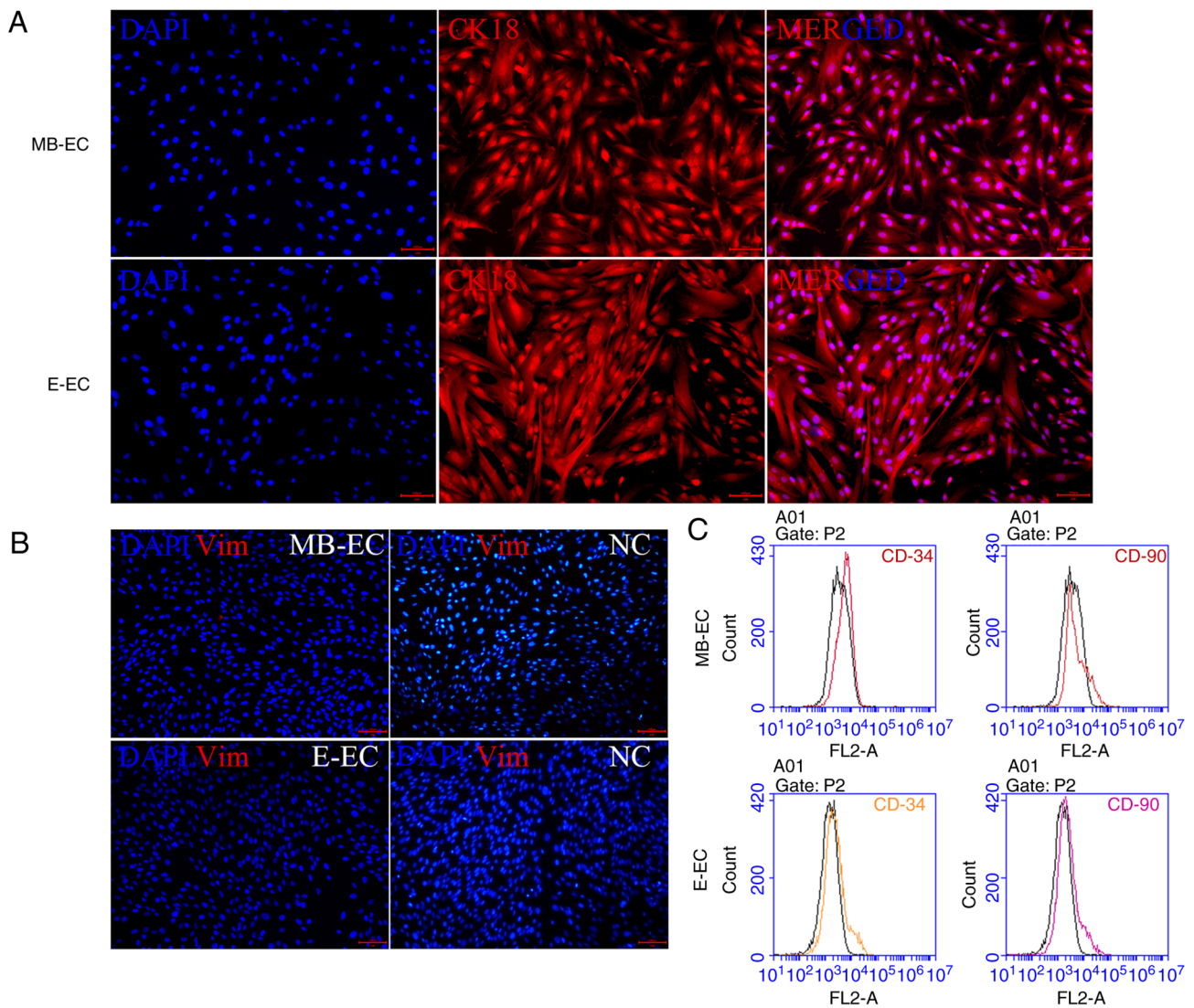
Figure 2. Detection of cell markers by immunofluorescence staining and flow cytometry. (A) MB-ECs and E-ECs expressed CK18. (B) MB-ECs and E-ECs did not express Vim. (C) MB-ECs and E-ECs did not express CD34 and CD90. Scale bar=100 μm. CK18, cytokeratin 18; Vim, vimentin; NC, negative control; ECs, endometrial cells; MB, menstrual blood-derived; E, endometrium-derived.

than its initial level after 24 h (MB-ECs, P<0.05 vs. 0 h; E-ECs, P<0.001 vs. 0 h; Fig. 5A and C).

TGF-β1 effect on EMT protein expression. Following TGF-β1 treatment, CK18 protein expression in E-ECs significantly decreased at 24 h (P<0.01 vs. 0 h), but significantly increased in MB-ECs at 12 h (P<0.001 vs. 0 h) and 24 h (P<0.01 vs. 0 h; Fig. 6A and B). Vim protein expression in MB-ECs was significantly upregulated at 6 h (P<0.01 vs. 0 h), reached its highest level at 12 h (P<0.001 vs. 0 h), and then decreased slightly at 24 h, while significantly higher than that at 0 h (P<0.001 vs. 0 h; Fig. 6A and C). Vim protein expression in E-ECs was significantly upregulated at 6 and 12 h (P<0.05 vs. 0 h), then decreased to initial levels at 24 h (Fig. 6A and C).

Discussion

Endometrial epithelial cells are a functional layer of the uterine surface and play an important role in the implantation of fertilized eggs (12). Wounding or bacterial infection may result in severe uterine diseases and even infertility (8). Therefore, studying endometrial epithelial cell biological function is of great relevance and provides a scientific basis for the potential treatment of endometrial epithelial defects (13). MB-ECs and E-ECs are two types of endometrial cells at different developmental stages. MB, the top two-thirds of the functional layer of the E, is shed during menstruation, and contains cells (a large number of epithelial and stromal cells) and tissues from the functional layer of the E (14,15). This course of events is the process of E-ECs forming MB-ECs. Therefore, it was hypothesized that MB-ECs and E-ECs had different morphological and biological characteristics. As it requires surgery to be sampled, endometrial tissue is difficult to obtain from healthy women. Although endometrial tissues were obtained from patients undergoing hysterectomy and MB from healthy women, the biological characteristics of the two cell types were comparable. Isolated MB-ECs and E-ECs expressed CK18 and not Vim, CD34 (a surface marker of hematopoietic stem cells) (10) nor CD90 (a surface marker of mesenchymal cells) (11). The two cell types demonstrated different cell morphology, proliferation and migration ability.
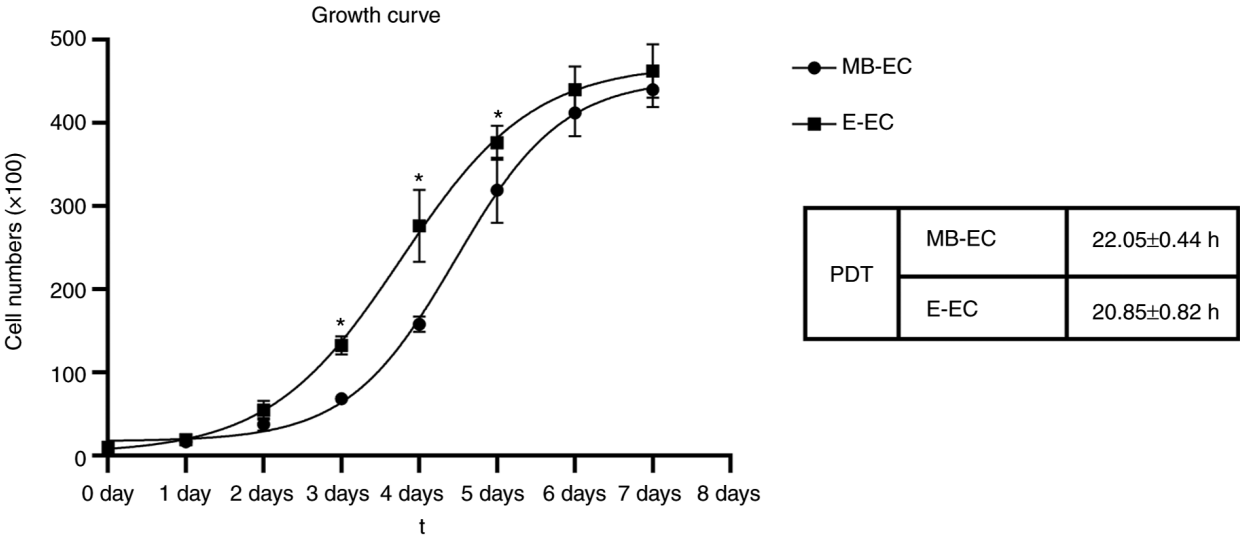
Figure 3. Proliferation curves and PDT of MB-ECs and E-ECs. *P<0.05 vs. E-ECs. PDT, population doubling time; ECs, endometrial cells; MB, menstrual blood-derived; E, endometrium-derived.
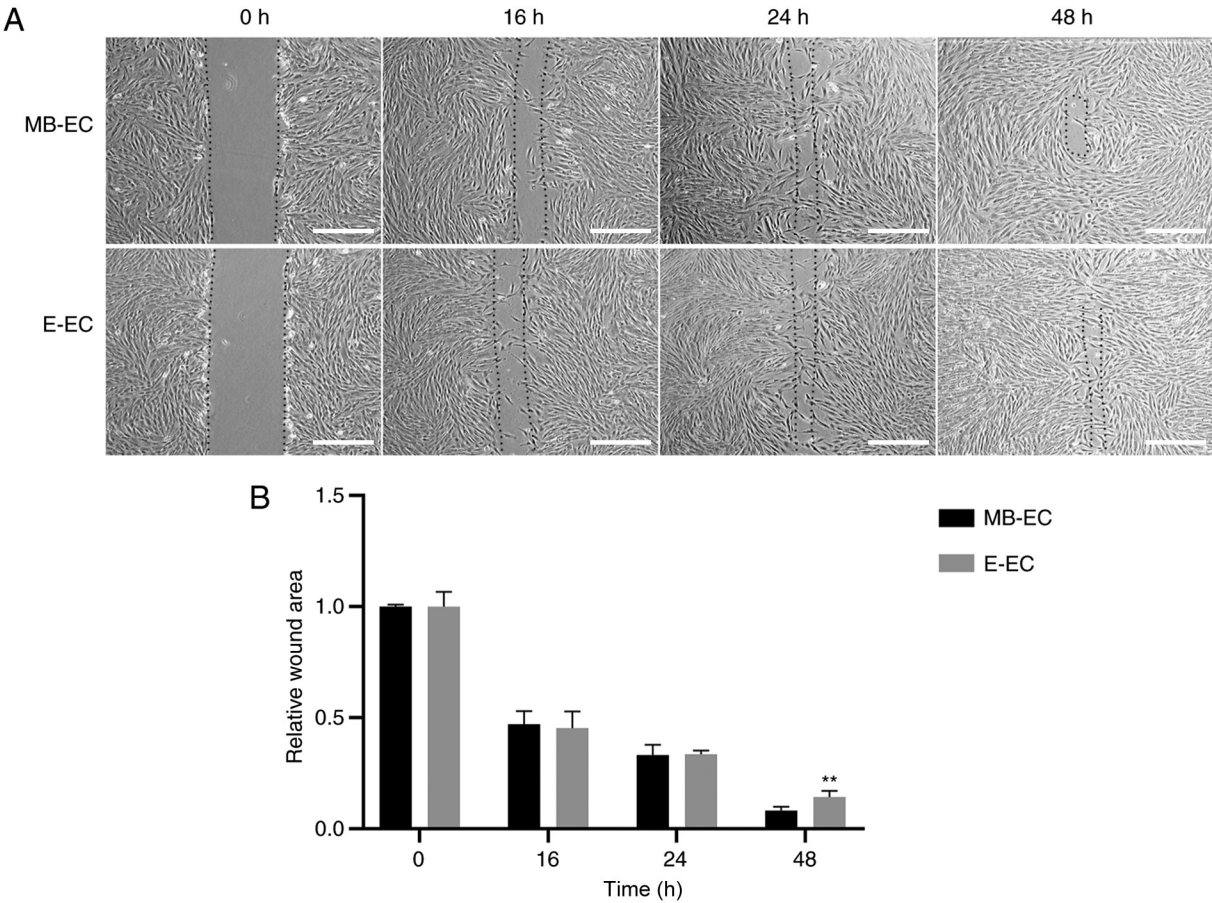

Figure 4. Migration capacity of MB-ECs and E-ECs. (A) Wound healing migration assay for MB-ECs (top) and E-ECs (bottom) at 0, 16, 24 and 48 h. (B) Relative wound area of MB-ECs and E-ECs. Scale bar=500 μm. **P<0.01 vs. E-ECs. ECs, endometrial cells; MB, menstrual blood-derived; E, endometrium-derived.

MB-ECs exhibited a small, flat, spindle-like cell morphology (15), and E-ECs appeared rounder and flattened cells.

Cell proliferation is closely related to the cell development stage. A previous study indicated that the development of the terminal end of the cells and cell cycle exit substantially reduced

their proliferation ability (8). MB-ECs were derived from the development of the terminal end of E-ECs, and the PDT of MB-ECs was greater than that of E-ECs. Cell migration happens throughout life and is a coordinated physiological process used by normal cells during embryonic morphogenesis, wound
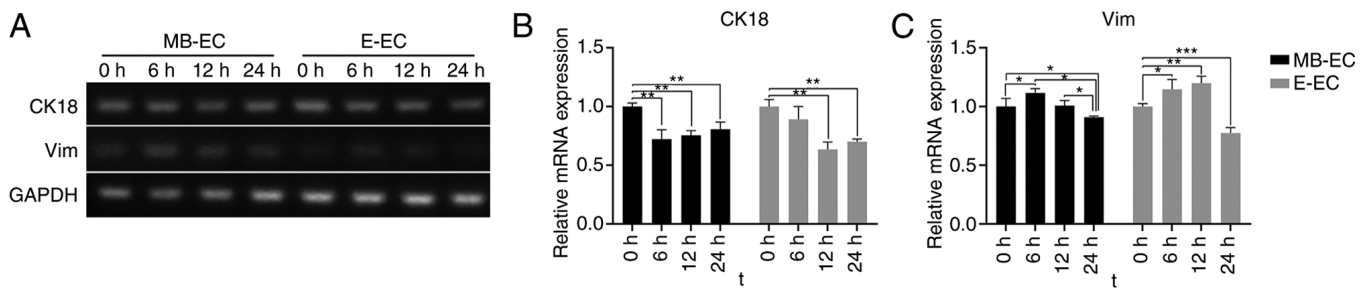
Figure 5. Effect of TGF-β1 on cell epithelial-mesenchymal transdifferentiation. (A) mRNA expression of CK18, Vim and GAPDH detected by PCR. (B) Relative CK18 mRNA expression detected by RT-qPCR. (C) Relative Vim mRNA expression detected by RT-qPCR. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Vim, vimentin; CK18, cytokeratin 18; ECs, endometrial cells; MB, menstrual blood-derived; E, endometrium-derived; RT-qPCR, reverse transcription-quantitative PCR.
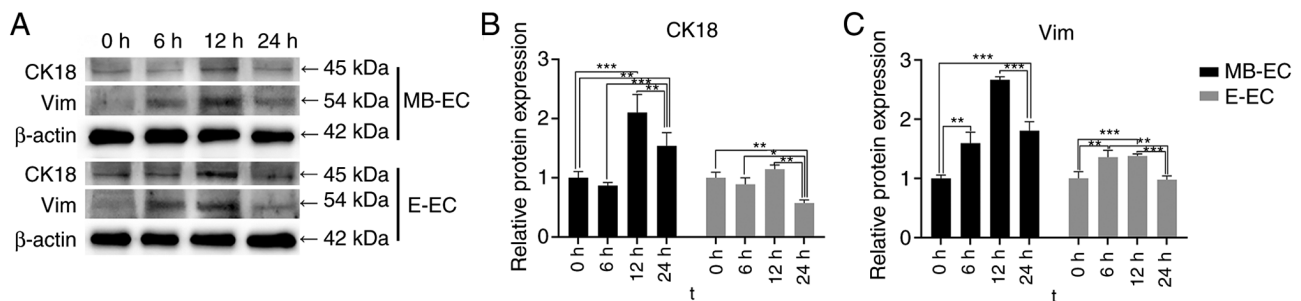

Figure 6. Effect of TGF-β1 on cell epithelial-mesenchymal transdifferentiation. (A) Protein expression of CK18, Vim and β-actin detected by western blot. (B) Relative CK18 protein expression. (C) Relative Vim protein expression. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Vim, vimentin; CK18, cytokeratin 18; ECs, endometrial cells; MB, menstrual blood-derived; E, endometrium-derived.

healing and cell transport, while tumor cells spread within tissues (16-18). The *in vitro* scratch assay is a simple, economical and well-developed method that mimics the migration of cells *in vivo* to study cell migration *in vitro* (19). The present study demonstrated that MB-ECs had a higher migration ability than E-ECs. Cell movement is a complex process involving a number of steps, including the disruption of cell-cell junctions, cytoskeletal rearrangements and constant remodeling of adhesive contacts with the extracellular matrix (18). A previous study indicated that, at the end of the menstrual cycle, with withdrawal of steroid hormone support, the activity of matrix-degrading enzymes induced endometrial destruction (20), which may lead to enhanced cell migration. Implantation of the human embryo into the uterine wall during the early stages involves embryo apposition and adhesion to the endometrial epithelium, followed by penetration through the epithelium and invasion of the embryonic trophoblast through the endometrial stroma (21). Estradiol administration has been previously demonstrated to have a marked effect on migration kinetics, induced non-dividing gland cells to enter the cell cycle and decreased the loss of epithelial cells (22). Decreased cell death in the mouse uterine epithelium has been observed after repeated estrogen administration (23). However, in intact rabbits with induced ovulation, the uterine epithelium is desensitized to estrogens (22). MB-ECs and E-ECs are two cell types at different developmental stages; MB-ECs are derived from the development of the terminal end of E-ECs (24). In the present study, MB-ECs were isolated from menstrual blood, and E-ECs were isolated from endometrial tissue. The cell proliferation curve and migration ability in MB-ECs and E-ECs were found to be different.

EMT is an organized process in which epithelial cells are induced to differentiate into a mesenchymal phenotype, and has been previously recognized in developmental biology as a means to achieve morphogenetic change (25). The maternal endometrial epithelium undergoes EMT-related changes during the embryo implantation period (26). Molecular signals from within the maternal E regulate the EMT process; a number of these signals have been implicated in embryo implantation failure (26). Human endometrial stromal cells can maintain endometrial homeostasis and play a critical role in repairing endometrial injury, and mesenchymal cells can increase the proliferation of damaged endometrial stromal cells (27). A previous study indicated that TGF-β1 mRNA and protein expression increased around menstruation to contribute to tissue repair following endometrial shedding (28). TGF-β1 is secreted abundantly in the E, specifically in patients with endometriosis (29,30). TGF-β1 not only induces apoptosis but also simultaneously induces EMT (31), and endometrial epithelial cells can induce EMT through TGF-β1 (32). The acquisition of mesenchymal markers (such as N-cadherin and Vim) and the loss of epithelial markers (such as E-cadherin) are hallmarks of EMT in endometriosis (33) and adenomyosis (34). In addition, TGF-β1-induced EMT was previously demonstrated to lead to the migration and invasion of local epithelial cells (31). The present results indicated that MB-ECs and E-ECs may induce EMT through TGF-β1; CK18 gene expression was downregulated and Vim expression upregulated. A previous study indicated that TGF-β1 treatment induced EMT in a dose- and time-dependent manner (31). CK18 and Vim expression returned to their initial levels after 24 h, which may indicate that TGF-β1 was absorbed and removed by the cells.

In conclusion, the present study isolated human MB-ECs and E-ECs, which demonstrated differences in morphology and cell biology; however, both cell types could exhibit EMT via TGF-β1 induction *in vitro*. Therefore, MB-ECs could be used in place of E-ECs for cell biology research and potential bioengineering applications. MB-ECs were isolated from menstrual blood, a convenient source with no harm to the human body. E-ECs were isolated from endometrial tissue, which is an inconvenient source that damages women's uterine tissue. Although some differences were noticed between the two sources of endometrial cells (such as PDT and migration ability), their functions were found to be similar.

Acknowledgements

Funding

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LZ and YD conceived and designed the experiments of the current study. MJ and ZC performed the experiments. LZ and MJ wrote the manuscript. MJ and LY analyzed the data. LZ and MJ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All samples were collected under a protocol approved by the institutional review board of the Gynecology Center of The First Affiliated Hospital of Xinjiang Medical University (China), and written informed consent was obtained from each donor.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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