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Expression profiles of human somatic mesenchymal stem cells derived from fresh endometrium, ectopic-endometrium and umbilical cord

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ABSTRACT

Objectives: The study investigated the stem cell expression profiles and differentiation capacities of mesenchymal stem cells (MSCs) from different tissues, specifically human eutopic endometrium MSCs (eut-MSCs), ectopic endometrium MSCs (ect-MSCs), and umbilical cord MSCs (UC-MSCs). Our aim was to identify any similarities in subpopulations among these MSCs and lay a foundation for MSCs repair.

Material and methods: MSCs were isolated from endometrial tissue (n = 5), endometriosis tissue (n = 6), and umbilical cords (n = 7). Flow cytometry was used to examine cell phenotype, and three lineage tests were conducted to evaluate the differentiation capacity of the MSCs.

Results: Eut-MSCs expressed CD44 (98.00 ± 0.96%), CD73 (99.54 ± 0.02%), CD140b (99.16 ± 0.50%), CD146 (93.87 ± 2.27%), SUSD2 (50.76 ± 8.15%), and CD271 (2.1 ± 1.22%). Ect-MSCs expressed CD44 (98.23 ± 1.60%), CD73 (99.63 ± 0.04%), CD140b (98.13 ± 0.53%), CD146 (93.88 ± 3.19%), SUSD2 (49.33 ± 6.36%), and CD271 (2.85 ± 1.17%). UC-MSCs expressed CD44 (99.11 ± ± 0.42%), CD73 (99.65 ± 0.12%), CD140b (99.84 ± 0.42%), CD146 (88.09 ± 4.20%), SUSD2 (72.87 ± 7.13%), and CD271 (6.19 ± ± 2.08%). The expression of SUSD2 and CD271 in UC-MSCs was slightly but not significantly higher than that in ect-MSCs and eut-MSCs. However, CD44, CD73, CD140b, and CD146 showed similar expression levels in UC-MSCs, ect-MSCs, and eut-MSCs. All three types of MSCs demonstrated the capacity to differentiate into osteoblasts, adipocytes, and chondrocytes.

Conclusions: Our findings indicate that ect-MSCs, eut-MSCs, and UC-MSCs have similar stem cell phenotypes and the ability to differentiate into three lineages.

Keywords: endometrium; umbilical cord; mesenchymal stem cells; phenotypic expression; differentiation potential

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INTRODUCTION

Mesenchymal stem cells (MSCs) are primitive cells that possess the ability to self-renew and differentiate in multiple directions. They can be found in various tissues and organs, including bone marrow [1, 2], human adipose tissue, endometrium, umbilical cord (UC), amniotic fluid, deciduous pulp, and skeletal muscle [3–5]. Due to their capacity for self-renewal and multi-lineage differentiation, MSCs are highly regarded as attractive candidates for regenerative medicine and tissue engineering [6, 7].

In recent years, bone marrow-derived MSCs (BM-MSCs) in disease treatment has declined due to potential complications and limited availability of bone tissue [8]. As a result, researchers have focused on other somatic materials for tissue repair considering their easier accessibility. One such material is the endometrium of the uterus, known for its regenerative capacity. Studies have demonstrated the presence of a small group of highly proliferative MSCs with multi-directional differentiation potential in the human endometrium. These endometrial MSCs (eMSCs) are considered promising for endometrial repair since they may contribute to the partial repair of the functional layer during shedding cycles. Additionally, Sampson's hypothesis suggests that eMSCs may aberrantly migrate to the peritoneal or pelvic cavity, leading to the formation of endometriosis [9]. Gargett et al. [10] have identified the existence of stem cells in ectopic endometrial lesions. Similar to eutopic endometrial stem cell colony-forming units (CFUs), endometriotic stromal cell CFUs have displayed multipotency and undergone over 25 passages before reaching senescence.

Furthermore, UC-derived MSCs (UC-MSCs) have emerged as therapeutically efficient alternatives for several diseases. They are isolated from Wharton's jelly, which not only provides access to raw materials from medical waste tissue but also exhibits stable biological properties, rapid proliferation, and low immunogenicity [11]. *In vitro* experiments have demonstrated the ability of UC-MSCs to differentiate into fat, bone, cartilage, heart, and neuronal cells [12]. Based on these advantages, UC-MSCs may serve as another potential material for repairing endometrial lesions. However, it remains unclear whether UC-MSCs share similar phenotypic characteristics with MSCs derived from the endometrium.

This study hypothesized that eMSCs located in the basal layer lose their regenerative potential when the endometrium is severely damaged. Consequently, our objective was to explore the possibility of increasing the number of eMSCs for endometrial repair. For this purpose, UC-MSCs and ect-MSCs were considered as suitable options due to their easy accessibility and fewer associated ethical concerns. As a first step towards mimicking eMSCs for the repair of severely damaged endometrium, we sought to determine if UC-MSCs and ect-MSCs exhibit similar phenotype and characteristics.

MATERIAL AND METHODS

Collection tissues

Following the guidelines outlined in the declaration of Helsinki and adhering to ethical standards, approval was obtained from the ethical committee at Henan Province People's Hospital. Prior to enrollment in the study, patients provided informed consent and approved the use of their tissue samples. Five healthy women with no prior medical interventions (mean age: 30.05 ± 5.67 years, during the follicular phase), six patients diagnosed with endometriosis (mean age: 29.15 ± 6.55 years), and seven healthy pregnant women (mean age: 28.85 ± 6.55 years) were recruited. Normal endometrial tissues were collected through uterine curettage. Ectopic ovarian cyst tissues were obtained from patients diagnosed with endometriosis during surgery. Confirmation of the endometrial and endometriosis tissues was conducted by at least two pathologists. Umbilical cord samples were collected from healthy women undergoing cesarean section. Within 30 minutes of surgery, all samples were transferred from the hospital to the laboratory on ice, using a sterilized container containing Dulbecco's Modified Eagle Media supplemented with Nutrient Mixture F-12 (DMEM/F-12, Hyclone, USA). The tissues were immediately washed multiple times with phosphate buffer saline (PBS, Hyclone, USA). The UC was stripped of blood vessels, and the remaining UC tissues, endometrial tissues, and endometriosis tissues were retained in a sterile culture dish containing DMEM/F12.

Hematoxylin-eosin (HE) staining & Immunohistochemical (IHC) analysis

Eutopic/ectopic endometrial, and UC tissues were fixed in 4% formalin for paraffin section preparation. Each segment was sliced to a thickness of 3 microns. Hematoxylin-eosin staining was performed to identify the morphological structure of the segments. Additionally, these slides were stained with vimentin or cytokeratin7 (CK7) antibodies using immunohistochemistry to determine the cell types. The rabbit SP detection kit (ZSGB, Beijing, China) was utilized for IHC analysis following the manufacturer's instructions. Antigen retrieval was conducted using sodium citrate buffer (pH 6.0) in a pressure cooker. The slides were incubated overnight at 4°C with primary antibodies against vimentin (1:100 dilution; BS1491; Bio-world Technology) or CK7 (1:100 dilution; 22208-1-AP; Protein tech). PBS was used as a negative control. Chromogenic reaction was achieved using the DAB kit (ZSGB, Beijing, China).

Mesenchymal stem cell isolation and primary culture

Fresh tissues were subjected to enzymatic digestion to isolate stromal cells. After washing the samples three

times with sterile PBS, they were cut into 1 mm^3 fragments. Fragments from each sample were placed in a 15 mL tube containing 400 U/mL collagenase II (Sigma) and digested in a YKW-303 shaker incubator (Yong le kang, Hunan, China) at 37°C for approximately 45 minutes with a speed of 120 rpm. The cell suspension was filtered through a 100 μm cell strainer (Corning, New York, USA), followed by a 40 μm cell strainer (Corning, New York, USA) to remove excess tissue debris. The final filtered liquid was centrifuged at 1500 rpm for 8 minutes. The supernatant was discarded, and the cell pellets were resuspended in 2 mL complete culture medium DMEM/F-12 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin mixture (Gibco, Grand Island, NY). The cells were seeded in a flask (25 cm^2) in the complete culture medium and cultured at 37°C in a humidified incubator with 5% CO_2. A similar isolation and culture protocol was employed for stromal cells from UC as for endometriosis samples. The culture medium was refreshed every 3 days. When reaching 90% confluence, the cells were passaged using 0.25% Trypsin-Ethylene Diamine Tetraacetic Acid (Trypsin-EDTA, Gibco, Grand Island, USA) at a ratio of 1:2. At each passage, a small portion of cells were frozen in FBS supplemented with 20% dimethyl sulfoxide (DMSO, Solarbio, Beijing, China) for future evaluation. Differentiation studies were conducted on cells from passages 3 and 4.

Flow cytometry analysis

For flow cytometry analysis, cells from passages 3 and 4 were harvested using 0.25% Trypsin-EDTA into flow tubes, then centrifuged at 1000 rpm for 5 minutes. The cells were washed with PBS, diluted to a density of 1×10^6/mL, and incubated in the dark at 4°C for 30 minutes with 5μL of antibodies (CD31-PE cy7, CD45-PerCP, SUSD2-PE, CD73-APC/cy7, CD140b-APC, CD146-FITC, CD271-FITC, CD146-Alexa647, and CD44-Alexa488) (Table S1). Afterward, the cells were washed with 1 mL of PBS and centrifuged at 1000 rpm for 4 minutes. The supernatant was discarded, and the cells were resuspended in PBS. One tube without antibody served as a blank control. The antibody-labeled cells were analyzed using FACS Calibur flow cytometry (BD Canto, San Jose, USA), and the resulting data were analyzed using Flowjo 10 software (Leonard Herzenberg, USA).

Three lineages differentiation

In this study, three lineages of differentiation were investigated. MSCs were seeded in a 24 well plate (Corning, New York, USA) at a density of 5×10^4 cells/well using 500 μL of complete culture medium.

For adipogenic differentiation, cells were cultured until they reached 80% to 90% confluence. The culture medium was then replaced with an adipocyte-genic medium containing Human MSC Adipocyte-genic Basal Medium A and B, FBS, Penicillin-Streptomycin, Rosiglitazone, Glutamine, Insulin, Dexamethasone, and IBMX (Cyagen, California, USA), according to the manufacturer's instructions. The medium was changed every 3 days for 21 days. The adipocyte capacity of MSCs was evaluated using oil red staining. Cells were fixed with 4% paraformaldehyde for 35 minutes, washed thrice with PBS, and then stained with 60% Oil Red solution for 25 minutes (Cyagen, California, USA). After gently washing the plates twice with PBS, adipocyte-like cells were observed under a microscope (Olympus, Japan).

For osteogenic differentiation, to prevent MSCs from floating during induction, gelatin coating (Cyagen, California, USA) was applied to the surface of osteoblast-induced culture plates for 30 minutes. Afterwards, cells were seeded and cultured until they reached 60–70% confluence. The medium was replaced with an osteoblast-genic medium consisting of Human MSC Osteoblasts-genic Basal Medium, FBS, Glutamine, Penicillin-Streptomycin, Ascorbate, β-Glycerophosphate, and Dexamethasone (Cyagen, California, USA), as per the manufacturer's instructions. Cells were then cultured in this medium for 2 weeks. The osteoblast-genic capacity was assessed using Alizarin red dye solution (Cyagen, California, USA). Cells were fixed with 4% paraformaldehyde for 30 minutes, washed twice with PBS, and stained with Alizarin red dye solution for 3–5 minutes. Following three washes with PBS, the cells were imaged using a microscope (Olympus, Japan).

For chondrogenic differentiation, once cells adhered to the surface of the well, the medium was replaced with a chondrocyte-genic medium containing Human Stem Cell Chondrocyte-genic Basal Medium, Ascorbate, Dexamethasone, ITS + Supplement, Proline, Sodium Pyruvate, and TGF-β3 (Cyagen, California, USA), following the manufacturer's instructions. Cells were cultured for 21 days, with the medium changed every 2–3 days. To evaluate chondrogenic capacity, cells were stained with Alcian Blue. After fixing the cells with 4% paraformaldehyde for 30 minutes, they were washed twice with PBS and then stained with Alcian Blue (Cyagen, California, USA) for 30 minutes. Following three washes with PBS, images were captured using a microscope (Olympus, Japan). Positive staining with Alcian Blue indicated the presence of acid mucopolysaccharides in the chondrocytes.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Software Inc, version 5.00). Data were presented as mean ± standard error of the mean (SEM). Statistical comparisons between groups were conducted using the t-test or non-parametric Mann-Whitney test. A p-value of less than 0.05 was considered to indicate a statistically significant difference.

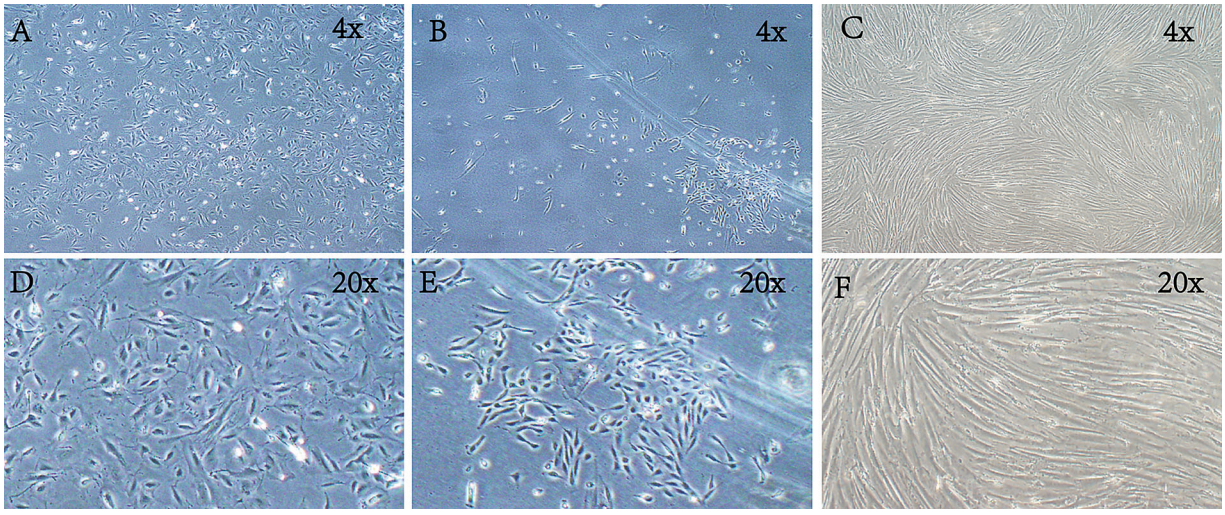

Figure 1. Morphology of primary eut-stromal cells, ect-stromal cells and umbilical cord-stromal cells — eut-stromal cells (**A, D**), ect-stromal cells (**B, E**), umbilical cord-stromal cells (**C** and **F**)

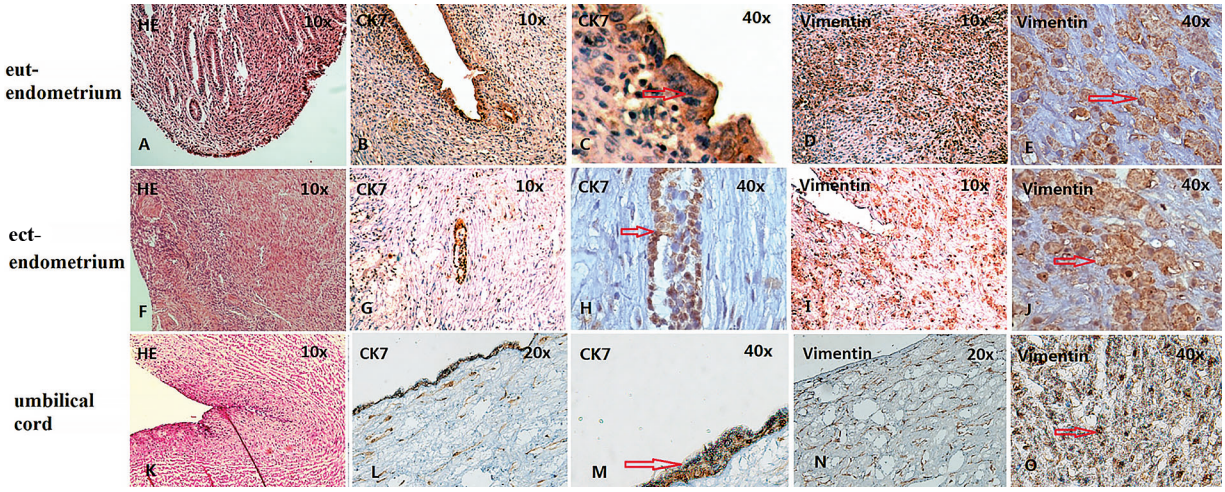

Figure 2. Identification of eut-endometrial and ect-endometrial tissues and umbilical cord; Hematoxylin-eosin (HE) staining identified the types of tissues (**A, F and K**); Expression of vimentin and CK7 in eut-endometrial (**B–E**), ect-endometrial (**G–J**), and umbilical cord **(L–O**) tissues were confirmed. Positive cells were presented with brown-yellow staining (red arrow); Eut-endometrial tissues (n = 5), ect-endometrial tissues (n = 6), and umbilical cord (n = 7)

RESULTS

Isolation and culture of eut-stromal cells, ect-stromal cells and UC-stromal cells

Figure 1 (A–F) presents the morphological characteristics of freshly isolated primary stromal cells from eutopic, ectopic endometrium, and UC, respectively. In Figure 1A and D, primary stromal cells from the healthy endometrium were cultured for five days, showing a predominantly triangular morphology. Figure 1B and E display the cell morphology after changing the culture medium on the third day, where small clusters of cells are visible. In Figure 1C and F, the morphology of primary stromal cells from the UC is shown on the fifth day after isolation, with approximately 90% of the cells exhibiting a long spindle-shaped appearance.

Identification of UC, ectopic–endometrium and eutopic-endometrium

Through HE staining, the cellular cytoplasm was stained pink, while the nuclei appeared blue. IHC results demonstrated that vimentin was primarily expressed in stromal cells of all three tissues, whereas CK7 was mainly expressed in the glandular epithelial cells (Fig. 2).

Cell surface antigen expression

Phenotypic analysis (Fig. 3) using flow cytometry with CD45- and CD31-gating (to exclude peripheral blood mononuclear cells and endothelial cells) revealed that primary stromal cells from UC expressed CD44 (99.11 ± 0.42%), CD73 (99.65 ± 0.12%), CD140b (99.84 ± 0.42%), CD146 (88.09 ±

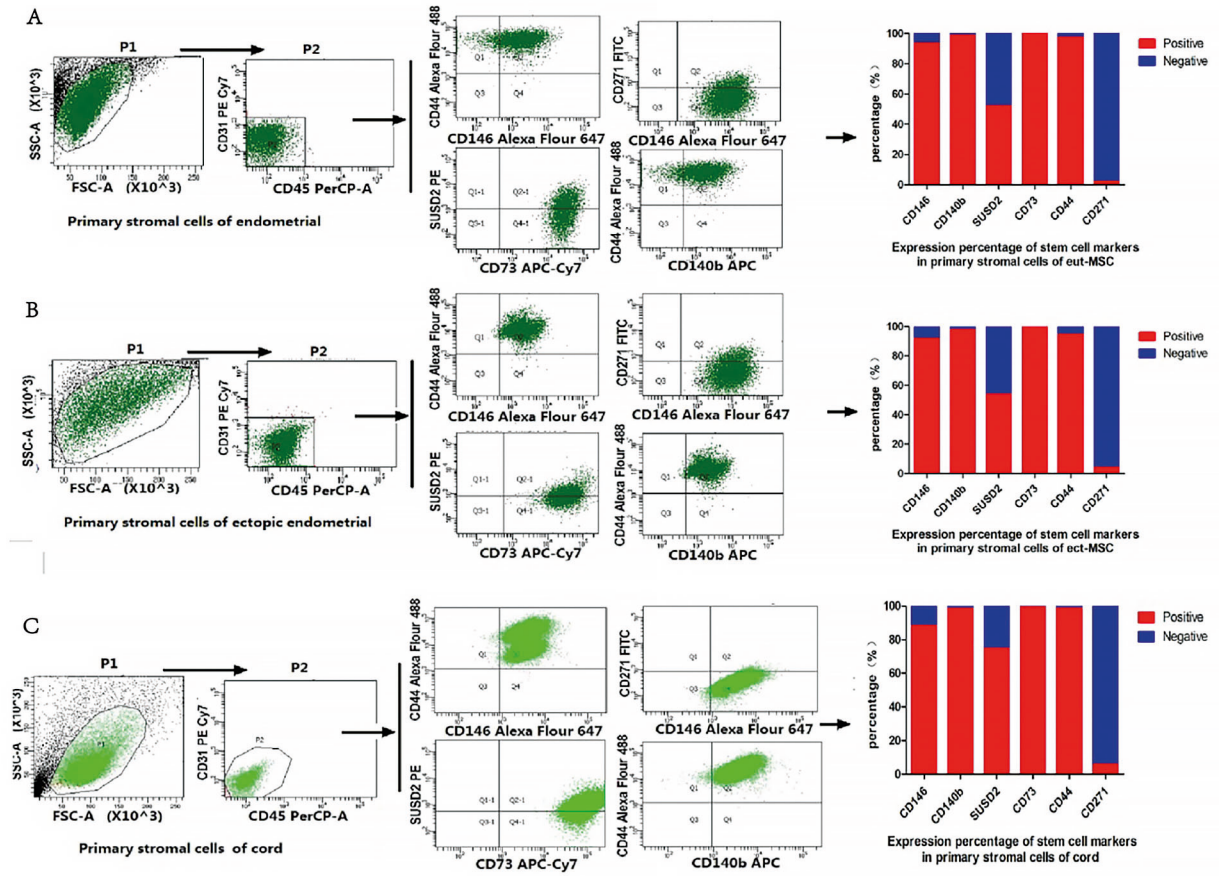

Figure 3. Expression profiles of primary mesenchymal stem cells (MSCs) derived from eut-,ect-endometrium and umbilical cord. Flow cytometry of MSC markers. Cells were gated by CD31-&CD45-. Data were presented with mean expression percentage of stem cell markers; **A.** Eut-endometrial tissues (n = 5); **B.** Ect-endometrial tissues (n = 6); **C.** Umbilical cord (n = 7)

4.20%), Sushi-domain-containing-2 (SUSD2) (72.87 ± 7.13%), and CD271 (6.19 ± 2.08%). Similarly, primary stromal cells from the eutopic endometrium expressed CD44 (98.00 ± 0.96%), CD73 (99.54 ± 0.02%), CD140b (99.16 ± 0.50%), CD146 (93.87 ± 2.27%), SUSD2 (50.76 ± 8.15%), and CD271 (2.1 ± 1.22%). Additionally, primary stromal cells from the ectopic endometrium expressed CD44 (98.23 ± 1.60%), CD73 (99.63 ± 0.04%), CD140b (98.13 ± 0.53%), CD146 (93.88 ± 3.19%), SUSD2 (49.33 ± 6.36%), and CD271 (2.85 ± 1.17%). Notably, UC-MSCs, eutopic endometrial MSCs (eut-MSCs), and ectopic endometrial MSCs (ect-MSCs) strongly expressed CD44, with a percentage exceeding 98% (Fig. 4A). The expression percentages of CD73 and CD140b were over 95% in all three types (Fig. 4B–C). Furthermore, the average expression of SUSD2 in primary MSCs from the UC slightly exceeded that in eut-MSCs and ect-MSCs, with an expression level above 75% in 5 out of 7 cases of primary MSCs from the UC. The percentage spread was more uniform among the five samples (Fig. 4F). There was no significant difference in the expression of CD146 and SUSD2 between eut-MSCs and ect-MSCs, as indicated by similar p-values (1.000 and 0.931, respectively) (Fig. 4D and F). While the expression ratio of CD271 cells was less than 10% in all three MSC types, the percentage of CD271-positive eut-MSCs and ect-MSCs was similar but lower than that in UC-MSCs (Fig. 4E).

Multi-lineage differentiation

Eutopic endometrium MSCs, ect-MSCs, and UC-MSCs differentiated into adipocytes, osteoblasts, and chondrocytes when cultured with the corresponding differentiation medium. Oil drop-like fat particles indicative of adipocyte differentiation was visible on day 10 for eut-MSCs and ect-MSCs, while UC-MSCs displayed these particles on day 12. Formalin terminated the differentiation process once the fat particles reached 80% growth under the microscope (Fig. 5). During osteoblast differentiation, formalin termination occurred when osteocytes fused to 80% on day 18. As a result, the red staining of eut-MSCs and ect-MSCs appeared brighter compared to UC-MSCs (Fig. 5). Similarly, after 21 days of stimulation, chondrocyte differentiation was halted using formalin. The blue stain of UC-MSCs appeared lighter than that of eut-MSCs and ect-MSCs (Fig. 5). However, no

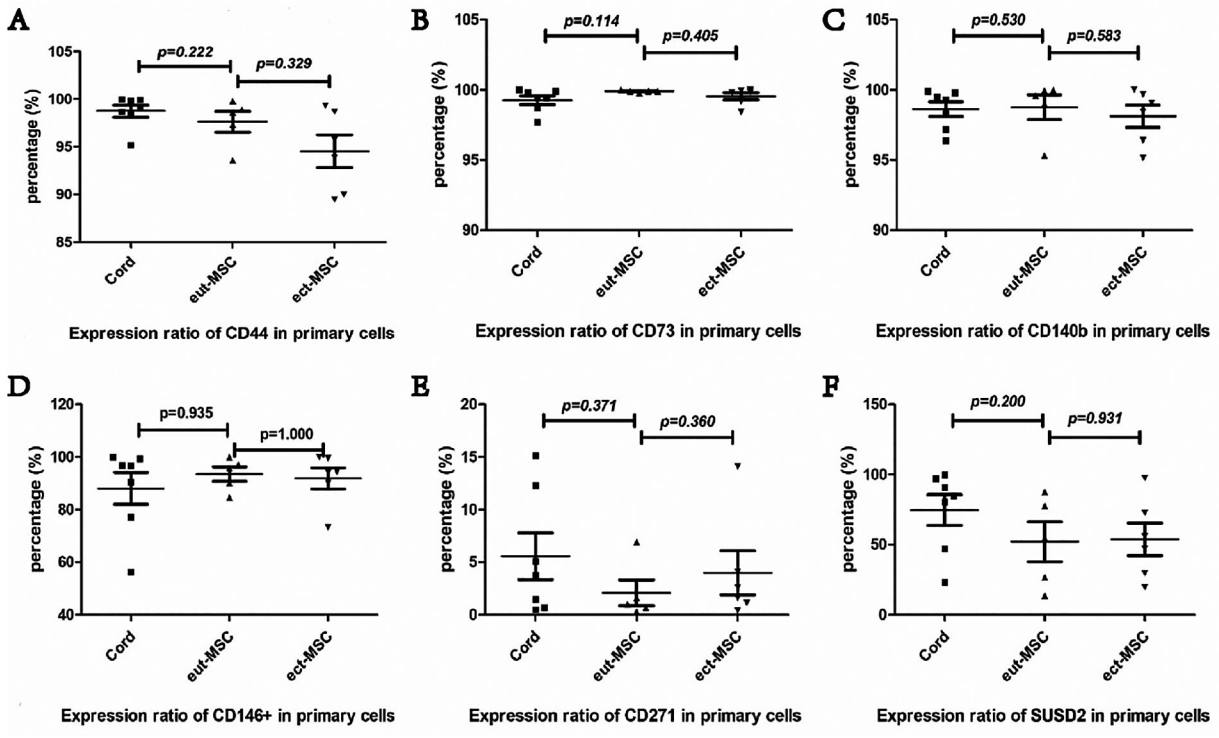

Figure 4. Percentage of the mesenchymal stem cells (MSCs) markers. The expression percentage of MSC markers: CD44 (**A**), CD73 (**B**), CD140b (**C**), CD146 (**D**), CD271 (**E**) and Sushi-domain-containing-2 (SUSD2) (**F**), respectively. Umbical cord (n = 7); Eutopic endometrial MSC (eut-MSC) (n = 5); Ectopic endometrial MSC (ect-MSC) (n = 6)

changes were observed in eut-MSCs, ect-MSCs, and UC-MSCs cultured with complete medium as negative control.

DISCUSSION

Eutopic endometrium MSCs and ect-MSCs share similar surface marker expression. This study was aimed to compare and evaluate the potential enrichment of different components in MSCs derived from the eutopic endometrium, ectopic endometrium, and UC. This is a crucial step in identifying a reliable source of MSCs for endometrial repair.

Single endometrial CFUs have the ability to differentiate into classical mesodermal lineages and express typical MSC surface markers, namely CD44, CD73, CD90, and CD29 [13]. However, they lack the surface markers on hematopoietic stem cells and epithelial cells, such as CD45, CD34, and CD31. The expression profiles of MSCs differ significantly among different tissues [1]. The colocalization of eMSCs with CD140b, CD146, and SUSD2, which are derived from human endometrium, indicates a high enrichment of these markers in eMSCs [3, 5, 14]. These findings suggest the existence of multiple subpopulations of eMSCs with different phenotypes.

Our data demonstrated that the expressions of CD44 and CD73 in UC-MSCs, eut-MSCs and ect-MSCs were all over 98%. Previous studies have consistently shown that CD73+ UC-MSCs comprise more than 98% of the isolated cells [15]. Consistent with our results, Kang et al. found that the expression of CD44 and CD73 in human UC blood was 99.12% and 98.69%, respectively [16]; while Kao et al. demonstrated that the expressions of CD44 in eut-MSCs and ect-MSCs using flow cytometry were 98.6% and 97.6%, respectively [17]. Therefore, we speculated that UC-MSCs, eut-MSCs and ect-MSCs express CD44 and CD73 in significant quantities.

Sushi-domain-containing-2 was positive in approximately 4.2% endometrial stromal cells [18]. Our study observed SUSD2 expression in 49.33% of ect-MSCs and 50.76% of eut-MSCs, respectively. However, UC-MSCs showed higher enrichment at 72.87% compared to eut-MSCs and ect-MSCs. The percentage of SUSD2 expression varied greatly among individual endometrial samples, ranging from 13.73% to 99.85%, which may be attributed to differences in age and menstrual phase, requiring further confirmation with a larger sample size. Previous reports suggested SUSD2 as a stem cell marker mainly detected in eut-MSCs. However, we found a similar expression percentage of SUSD2 in ect-MSCs and eut-MSCs, suggesting a comparable MSC subpopulation. CD271 expression percentages in our results were 6.19% in UC-MSCs, 2.1% in eut-MSCs, and 2.85% in ect-MSCs. CD271 is known for enriching BM-MSCs [19] but had a low occurrence rate of only 0.71% [20]. Our results indicated that UC-MSCs

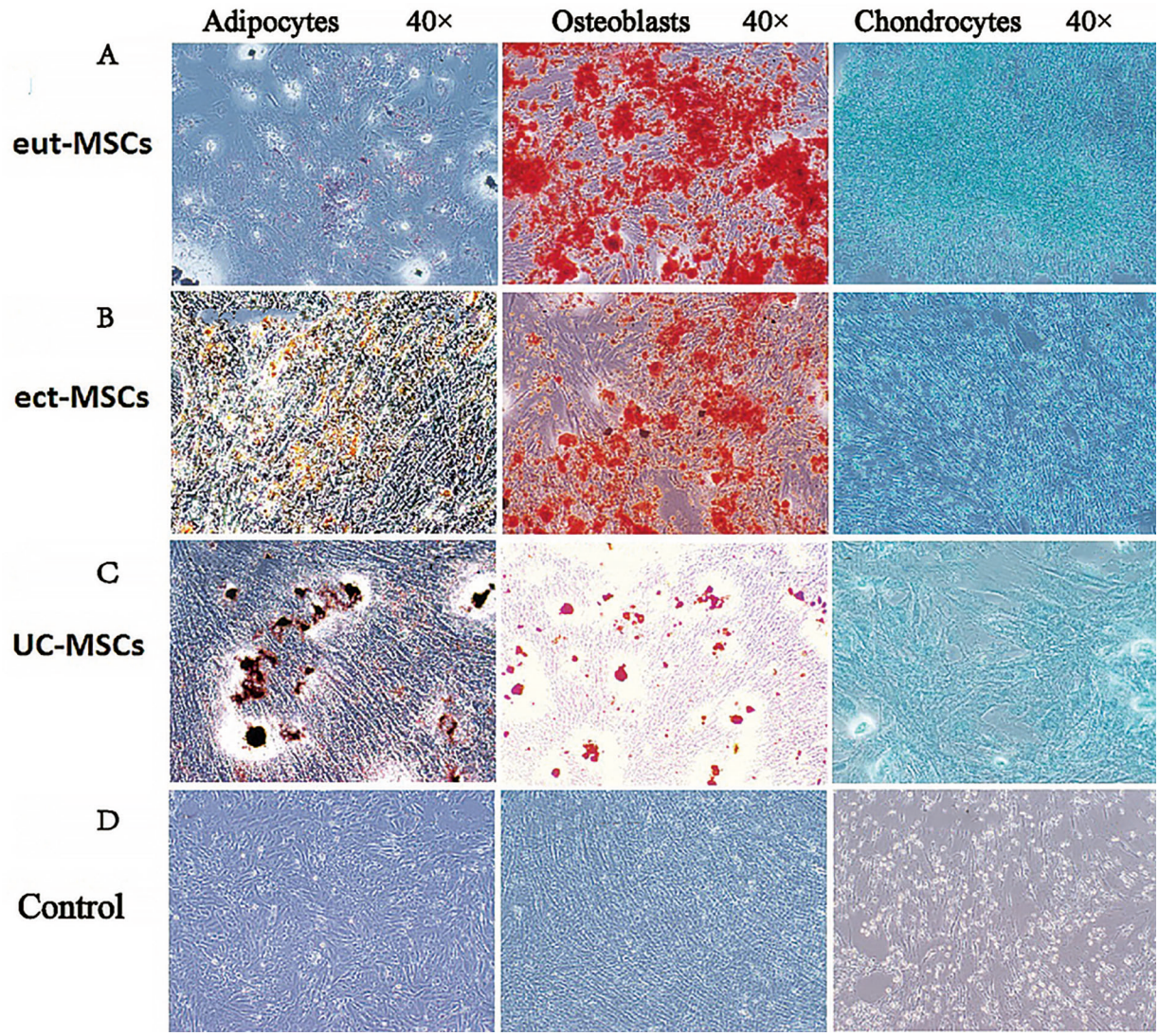

Figure 5. Multi-lineages differentiation of mesenchymal stem cells (MSCs). Adipose differentiation: the red lipid vacuoles stained by oil red; Osteoblastic differentiation: red phosphatases stained by Alizarin red dye solution; Chondrocytic differentiation: blue acid mucopolysaccharide stained by alcian blue.

The differentiation assays for adipocytes, osteoblasts and chondrocytes were terminated at day 22, day18 and day21; **A–C.** Showed that eutopic endometrial MSCs (eut-MSCs) (passage 4), ectopic endometrial MSCs (ect-MSCs) (passage 4) and umbilical cord MSCs (UC-MSCs) (passage 4) began to differentiate into adipocytes, osteoblasts and chondrocytes, respectively; **D.** Representatively showed MSCs which were cultured with complete medium as negative control

rarely expressed CD271, and the expression of CD271 in human eut-MSCs and ect-MSCs was lower than that in UC-MSCs. Several studies have demonstrated that MSCs can be isolated from the endometrium using CD140b+CD146+ phenotypes, exhibiting similar differentiation abilities to other MSCs. In our study, CD146+ stromal cells accounted for approximately 90% of UC-MSCs, eut-MSCs, and ect-MSCs, with the average percentage of CD140b+ cells exceeding 98%. Rajaraman et al. [21] reported that eMSCs expressed 69% CD140b and 37% CD146. Masuda et al. [22] showed that freshly isolated human endometrial SUSD2+ cells expressed MSC markers, including CD146 (28.3 ± 4.3%) and CD140b (73.1 ± 11.5%) [13]. To our knowledge, this was the first study to demonstrate CD140b expression in UC-MSCs [23, 24].

Undoubtedly, the use of eut-MSCs for repairing thin endometrium represents an ideal therapeutic strategy for endometrial lesion-associated infertility. However, several obstacles hinder the clinical application of eMSCs, such as their rarity in normal endometrium, donor age [1], and the invasive acquisition method. Therefore, finding a substitute for eMSCs would be advantageous. Since primary eut-MSCs and ect-MSCs express similar percentages of stem cell phenotypes, we speculate that ect-MSCs might serve as an ideal alternative for lesion endometrial therapy.

Moreover, studies have shown that UC-MSCs possess high proliferation ability, multifunctional differentiation capacity, and low immunogenicity, making UC-MSCs efficient alternatives for the treatment of various diseases [25–27], including improving damaged human endometrium. Recently, multiple studies have reported on the potential of UC-MSCs to enhance endometrial repair [28–30]. These results provided a promising source of MSCs for repairing damaged or thin endometrium in women.

CONCLUSIONS

In summary, based on our data, it could be inferred that that: (1) eut-MSCs and ect-MSCs had similar phenotypes, with a high expression percentage for CD44, CD73, CD140b, CD146, and SUSD2; (2) SUSD2-positive expression was slightly higher in UC-MSCs compared to eut-MSCs and ect-MSCs without statistical significance; and (3) MSCs derived from these three tissues had the potential to differentiate into adipogenic, osteogenic, and chondrogenic cells. This study laid the foundation for further research on the application of UC-MSCs and ect-MSCs in repairing damaged endometrium. However, this study had several limitations, including a small sample size and variations in menstrual phase among patients with endometriosis. These limitations should be addressed through future studies with larger sample sizes.

Article information and declarations

Data availability statement

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

Ethics statement

Following the guidelines outlined in the declaration of Helsinki and adhering to ethical standards, approval was obtained from the ethical committee at Henan Province People's Hospital.

Author contributions

Chunmei Li — perform experiments, article writing; Tong Wang — article writing, article revision; Suiyu Luo — study design; You Wu — flow cytometry analysis; Yan Song — collect samples; Ying Su — IHC analysis; Yuhui Zhang — statistical analysis; Yuanyuan Zhang — correspondence, study design, article revision; Guangzhi Liu — article revision, supervision; Lu Wang — collection tissues, concept.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary Table S1. Antibodies used to phenotype human endometrial cells and umbilical cord cells by flow cytometry.

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SUPPLEMENTARY MATERIAL

Table S1. Antibodies for cell phenotypes of human endometrial cells and umbilical cord cells using flow cytometry

Primary antibodies	Isotype	Clone	Concentration	From
CD31	Mouse IgG1	WM59	100 μg/mL/10^6cells	Biolegend, San Diego, USA
CD44	Rat IgG2b	IM7	500 μg/mL/10^6cells	Biolegend, San Diego, USA
CD45	Mouse IgG1	2D1	100 μg/mL/10^6cells	Biolegend, San Diego, USA
CD73	Mouse IgG1	AD2	200 μg/mL/10^6cells	Biolegend, San Diego, USA
CD140b	Mouse IgG1	18A2	300 μg/mL/10^6cells	Biolegend, San Diego, USA
CD146	Mouse IgG1	SHM-57	200 μg/mL/10^6cells	Biolegend, San Diego, USA
CD146	Mouse IgG1κ	541-10B2	100 μg/mL/10^6cells	Miltenyi Biotec, USA
CD271	Mouse IgG1	ME20.4	300 μg/mL/10^6cells	Biolegend, San Diego, USA
SUSD2	Mouse IgG1	W5C5	200 μg/mL/10^6cells	Biolegend, San Diego, USA