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Spatial and temporal characterization of endometrial mesenchymal stem-like cells activity during the menstrual cycle

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ABSTRACT

The human endometrium is a highly dynamic tissue with the ability to cyclically regenerate during the reproductive life. Endometrial mesenchymal stem-like cells (eMSCs) located throughout the endometrium have shown to functionally contribute to endometrial regeneration. In this study we examine whether the menstrual cycle stage and the location in the endometrial bilayer (superficial and deep portions of the endometrium) has an effect on stem cell activities of eMSCs ($CD140b^+CD146^+$ cells). Here we show the percentage and clonogenic ability of eMSCs were constant in the various stages of the menstrual cycle (menstrual, proliferative and secretory). However, eMSCs from the menstrual endometrium underwent significantly more rounds of self-renewal and enabled a greater total cell output than those from the secretory phase. Significantly more eMSCs were detected in the deeper portion of the endometrium compared to the superficial layer but their clonogenic and self-renewal activities remained similar. Our findings suggest that eMSCs are activated in the menstrual phase for the cyclical regeneration of the endometrium.

1. Introduction

In response to the cyclical changes in ovarian sex steroids, estrogen and progesterone, the human endometrium displays cyclic and rapid changes in proliferation and differentiation. The inner mucosal lining of the uterus is composed of the lumen epithelium supported by stroma and mature glands. The endometrium is comprised of two layers: the basalis which persists into the next cycle to give rise to a new functionalis that is shed during menses [1]. Approximately 400 cycles of shedding and renewing take place during the lifespan of a woman's reproductive years. The menstrual cycle is divided into 3 phases: proliferative, secretory and menstrual [2,3]. Endometrial regeneration commences at the beginning of menstrual phase and the growth continues into the proliferative phase. In the estrogen dominating proliferative phase, 5–7 mm of endometrial tissue is generated within 10 days [3]. The secretory phase is characterized by glandular secretion and stromal maturation in response to progesterone from the corpus luteum [4]. In the late secretory phase, luteolysis of the corupus luteum causes withdrawal of estrogen and progesterone that triggers breakdown of the functionalis and shedding of a substantial amount of tissue [5].

In recent years, a distinct adult stem cell population known as endometrial mesenchymal stem-like cells (eMSCs) have been shown to be responsible for endometrial remodeling [6]. Endometrial stromal cells co-expressing two surface markers: CD140b and CD146 are enriched with eMSCs and are localized to perivascular regions in the functionalis and basalis layers [7]. These cells are clonogenic, have broad differentiation capacity, display properties and phenotype similar to other mesenchymal stem cells [8–10]. Although eMSCs share a core genetic profile with bone-marrow mesenchymal stem cells in stemness, several genes mainly related to endometrial functions such as, vasculogenesis, angiogenesis, inflammation, immunomodulation and cell communication are specifically upregulated in eMSCs [11].

EMSC expressing CD140b and CD146 have been identified in proliferative and secretory endometria and can be isolated from hysterectomy or endometrial biopsy tissues [7,8,11]. However, the characterization of eMSCs at menstruation is limited and no studies have compared the properties of eMSCs in the different layers of the endometrium (superficial vs. deep portion of the endometrium). We hypothesize that (1) more eMSCs reside in the basalis but their stem cells activities will be similar between the endometrial layers. (2) These eMSCs will exhibit unique properties at menstruation for the repair and regeneration of the endometrium. Therefore, this study aims to investigate the changes of eMSCs during the menstrual phases (proliferative, secretory and menstrual) and in the endometrial layers.

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2. Material and methods

2.1. Human tissues

Full thickness endometrial tissue was collected from 30 ovulating women, 35- to 50-years old undergoing total abdominal hysterectomy (TAH) for benign non-endometrial pathologies (Supplementary data Table S1). They had not taken hormonal therapy for three months before surgery. The phase of the menstrual cycle was categorized into proliferative (n=14; range: 40–48 years old; median: 45 yr; mean: 44 yr) and secretory (n=16, range: 35–50 years old; median: 44 yr; mean: 42 yr) by experienced histopathologists based on hematoxylin-eosin-stained endometrial sections. Menstrual endometrial tissues were obtained from 11 ovulating women aged from 31- to 40-years old attending the infertility clinic on day 2–3 of their menstrual cycle (median: 38 yr; mean: 36 yr, Supplementary data Table S2). Ethic approval was obtained from the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Written consents were signed by recruited subjects after detailed counseling prior to participation of the study. All the samples were processed within 24 h after collection.

2.2. Single cell suspensions of endometrial epithelial and stromal cells

For TAH samples (Fig. 1A), the endometrial layer was scraped off from the underlying myometrium, minced and digested with PBS containing collagenase type III (0.3 mg/ml, Worthington Biochemical Corporation, Freehold, NJ, USA) and deoxyribonuclease type I (40 μg/ml, Worthington Biochemical Corporation) for one hour at 37 °C, as described previously [12]. To separate the superficial layer, a gentle scrape on top of the endometrial layer most distal from the myometrium was performed. The deeper portion of the endometrium was defined as the remnant tissue 1 mm-area from the endo-myometrial junction (Fig. 1B) [13]. Menstrual samples were obtained on day 2-3 of the menstrual cycle by aspiration. They were digested as described above. In brief, red blood cells were removed using Ficoll-Paque (GE Healthcare, Uppsala, Sweden) density-gradient centrifugation. Leukocytes were eliminated using anti-CD45 antibody-coated Dynabeads (Invitrogen, Waltham, MA, USA). Epithelial cells were removed from the stromal cells by using anti-CD326 (EpCAM) antibody-coated microbeads (Miltenyi Biotec Inc., San Diego, CA, USA). The freshly purified stromal cells (6000–8000 cells/cm^2) were plated onto 100 mm dishes (BD Biosciences, San Jose, CA, USA) coated with fibronectin (1 mg/ml, Invitrogen) and cultured in growth medium containing 10% FBS (Invitrogen), 1% antibiotics (Invitrogen) and 1% L-glutamine (Invitrogen) in DMEM/F-12 (Sigma-Aldrich, St Louis, MA, USA). Stromal cells were expand in culture for 7–14 days in a humidified carbon dioxide incubator at 37 °C. Medium was changed every 7 days until it reach 80% confluence.

2.3. Flow cytometry

The expression of eMSC markers (co-expression of CD140b and CD146) on freshly purified endometrial cells were analyzed using multicolour flow cytometry (Supplementary data Fig. S1). Endometrial cells were labeled with phycoerythrin (PE)-conjugated antibody against platelet-derived growth factor receptor beta (PDGFRβ) (CD140b, 2.5 μg/ml, PR7212 clone, mouse IgG_1, R & D Systems, Minneapolis, MN, USA) and fluorescein isothiocyanate (FITC)-conjugated anti-CD146 antibody (1 mg/ml, OJ79c clone, mouse IgG_1, Thermo Fisher Scientific, Waltham, MA, USA) or isotype matched controls. The cells were then labeled with allophycocyanin (APC)-conjugated anti-CD45 antibody (10 μg/ml, Thermo Fisher Scientific) before resuspension in 0.1% BSA/PBS for flow cytometric analysis using BD Fortressa (BD Biosciences, San Jose, CA, USA) in the University of Hong Kong Faculty Core Facility. Flow cytometry data were analyzed using the FlowJo software (Tree Star, Ashland, OR, USA).

2.4. Magnetic bead selection for endometrial mesenchymal stem-like cells

EMSCs isolated by sequential beading with magnetic beads coated with anti-CD140b and anti-CD146 antibodies were used for various functional assessments. In brief, cultured stromal cells were trypsinized, re-suspended in 0.5% BSA/PBS and incubated with PE-conjugated anti-CD140b antibody (10 μl/10^6 cells) for 45 min at 4 °C. The cells were then incubated with anti-mouse IgG1 coated microbeads (Miltenyi Biotec Inc.) for 15 min at 4 °C and the cell suspensions were applied to Miltenyi MS columns with a magnetic field to collect the CD140b$^+$ cells. The stromal CD140b$^+$ population were seeded in fibronectin-coated 100-mm culture dishes (BD Biosciences) containing growth medium and cultured at 37 °C in 5% CO_2 for 7–10 days to allow detachment of the microbeads during cell expansion. The CD140b$^+$

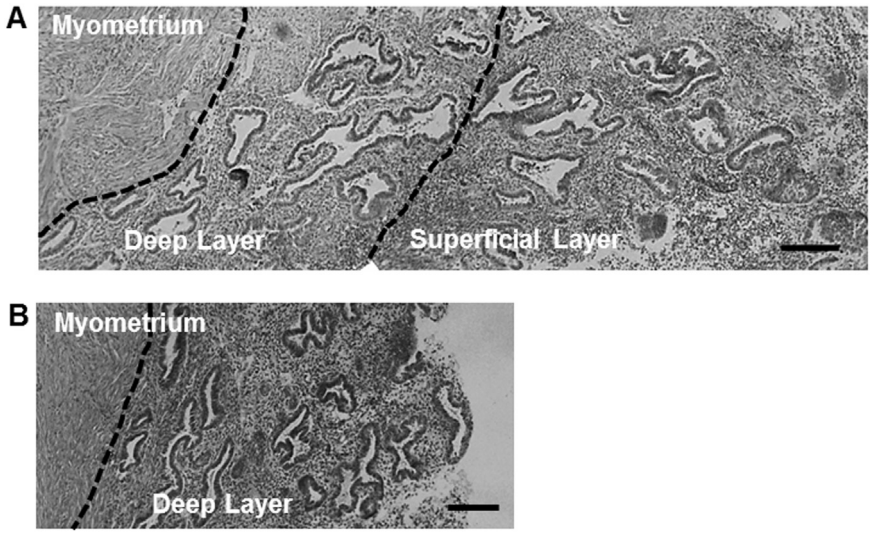

Fig. 1. Hematoxylin and eosin staining of the human endometrium. (A) Full thickness of human endometrium. (B) Depth of the scrape showing the remnant deep endometrial portion after separation from the superficial layer. Superficial layer, deep portion of the endometrium and myometrium are separated by dotted lines. The upper superficial layer contains multiple glands supported by loose stroma, while the lower portion consists of branched glands with dense stroma. Scale bar: 100 μM.