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Chemoattraction of bone marrow-derived stem cells towards human endometrial stromal cells is mediated by estradiol regulated CXCL12 and CXCR4 expression

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Abstract
Bone marrow derived cells engraft to the uterine endometrium and contribute to endometriosis. The mechanism by which these cells are mobilized and directed to the endometrium has not been previously characterized. We demonstrate that human endometrial stromal cells (hESCs) produce the chemokine CXCL12 and that bone marrow cells (BMCs) express the CXCL12 receptor, CXCR4. Treatment with physiological levels of estradiol (E2) induced both CXCL12 and CXCR4 expression in hESCs and BMCs, respectively. BMCs migrated towards hESCs conditioned media; a CXCR4 antagonist blocked migration indicating that CXCL12 acting through its receptor, CXCR4, is necessary for chemoattraction of BM cells to human endometrial cells. E2 increased both CXCL12 expression in endometrial cells and CXCR4 expression in BM cells, further enhancing chemoattraction. E2 induced CXCL12/CXCR4 expression in endometrium and BM, respectively, drives migration of stem cells to the endometrium. The E2-CXCL12/CXCR4 signaling pathway may be useful in determining treatments for endometrial disorders, and may be antagonized to block stem cell migration to endometriosis.

Abbreviations: hESCs, human endometrial stromal cells; BMCs, bone marrow cells; CXCR4, chemokine receptor type 4; E2, estradiol; CXCL12 or SDF-1α, stromal derived factor; BM, bone marrow; HSCs, hematopoietic stem cells; ESCs, endometrial stromal cells; EPCs, endothelial progenitor cells; MSCs, mesenchymal stromal cells; mBMCs, mouse bone marrow cells; ER, estrogen receptor.

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Introduction

CXCR4 belongs to the CXC family of chemokine receptors. Interaction of CXCR4 with its ligand, stromal derived factor (SDF-1α, CXCL12) plays a key role in the mobilization and homing of stem cells (Hopman and DiPersio, 2014). CXCR4, expressed on the surface of stem cells, serves as a target for modulating migration (Lai et al., 2014). CXCL12 is produced by the stromal cells and endothelial cells of many organs including bone marrow (BM), endometrium, skeletal muscle,

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liver and brain (Sharma et al., 2011). In human endometrium, CXCL12 is expressed by stromal cells. Estradiol (E2) stimulates CXCL12 production from endometrial stromal cells (ESCs) (Ruiz et al., 2010; Tsutsumi et al., 2011) suggesting a role in stem cell recruitment to the uterus.

BM-derived cells including hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), and endothelial progenitor cells (EPCs), significantly contribute to peripheral tissue repair and angiogenesis (Beauséjour, 2007). Therefore, factors influencing BM-derived cell migration and function are likely to have a broad impact. Overexpression of CXCR4 in stem cells (by cytokine induction or gene transfection) enhances MSCs homing *in vivo* to bone marrow as well as migration *in vitro* towards CXCL12 (Shi et al., 2007; Liu et al., 2013a; Marquez-Curtis et al., 2013; Hu et al., 2013). Recently it has been demonstrated that estrogen receptor (ER) is expressed in EPCs *in vivo* and *in vitro* (Baruscotti et al., 2010). EPCs proliferation is induced during the menstrual phase and the proliferation can be affected by estrogen through ERα activation (Foresta et al., 2010). These studies suggested the potential regulation of stem cells by sex steroids. Previous studies from our laboratory showed that BM-derived stem cells can engraft in the murine endometrium (Du and Taylor, 2007). We have shown that ischemia–reperfusion injury, toxicant exposure, and medications can alter the migration of BM-derived stem cells to the uterus, however the molecular mechanism responsible for the recruitment and engraftment of these cells is unknown (Zhou et al., 2011; Sakr et al., 2014; Lapidot, 2001). Here we report the effects of female sex hormones estradiol and progesterone on CXCR4 and CXCL12 expression, and the role of this chemokine and its receptor in migration of BMCs towards hESCs.

Material and methods

Cell culture

Mouse bone marrow cells (mBMCs) were prepared from 8–10 weeks old female C57 BL/6 mice (Charles River Laboratories, Wilmington, USA) by flushing bone marrow from the tibia and femur, and filtering the marrow through sterile 70-μm nylon mesh. The filtered mBMCs were grown at a density of 2.5×10^6 cells/ml in DMEM/F-12 medium supplemented with 15% fetal bovine serum, containing penicillin (100 μg/ml) and streptomycin (100 μg/ml) (GIBCO-BRL, Rockville, USA). After 48 h the cells were gently washed with PBS and fresh medium added; the medium was subsequently changed for every 3–4 days until two weeks when the cells were used for experiments described below. Mouse uterine cells (mUCs) were prepared from 6–8 weeks old female C57 BL/6 mice by enzymatic digestion of the uterus in 0.125% type IA collagenase (Sigma, USA) for 1 h at 37 °C, and then filtered through a 70-μm filter. Human endometrial stromal cells (hESCs) were obtained from human endometria in the proliferative phase as described by Ryan et al. (1994). Both mUCs and hESCs were cultured in DMEM/F12 medium supplemented with 10% FBS and penicillin/streptomycin (100 μg/ml) for one week. The cells were then washed with PBS, trypsinized, plated and cultured for an additional 48 h before carry out the experiments. Experiments used to obtain the mouse and human cells were conducted under approved Yale Institutional Animal Care and Use Committee and Human Investigations Committee protocols, respectively.

ABC-immunocytochemistry (ICC) and fluorescent ICC

Cells grown (80% confluent) on glass microscope slides were fixed with freshly prepared 4% formaldehyde for 10 min and rinsed three times for 5 min each with PBS. The cells were blocked with 4% BSA in PBS for 30 min and incubated with the primary antibody (diluted in 1% BSA in PBS) in a humidified chamber overnight at 4 °C. For ABC-ICC, the cells were incubated with the secondary antibody in 1% BSA for 30 min at room temperature. The ABC staining and 3, 3′diaminobenzidine (DAB) kits (Vector Laboratories, USA) were used to visualize the immunocytochemical reaction under light microscope (Olympus BX41). For fluorescence-ICC, the cells were incubated with the secondary antibody in the dark for 30 min at room temperature and 4′, 6-diamidino-2-phenylindole (DAPI) (Vector laboratories, UK) was added on to the cells. The slides were examined under inverted fluorescence microscope (Axiovert 200 M, Zeiss Company, Germany).

Flow cytometry

After two weeks of culture, mBMCs were analyzed for mesenchymal stromal cells (MSCs), and endothelial progenitor cells (EPCs) by flow cytometry. The cells were incubated with the fluorescent-labeled antibodies against CD90, CD105, CD34, Flk-1 (BioLegend, San Diego, USA) and CD31 (eBiosciences, USA), or with isotype-matched irrelevant antibody (1 μg for 10^6 cells) for 30 min on ice in dark. The cells were then washed with flow cytometry staining buffer 3 times for 5 min at 3,000 rpm and the cell pellet was resuspended in 1 ml ice cold staining buffer for cell sorting. Flow acquisition was performed on LSRII Fortessa, LSRII, or FACSCalibur analyzers (BD Biosciences), and data were analyzed using Diva software (BD Biosciences, USA).

Detection of CXCL12 by ELISA

CXCL12α was assayed from the supernatants of cell cultures using ELISA kit (R & D Systems, USA) according to the manufacturer's instructions. mBMC, hESCs and mUC were cultured in DMEM/F12 supplemented with 10% FBS and 1% penicillin and streptomycin in a 6-well plate (1×10^5 cells/well). The supernatants were collected from 48 h old cell cultures. For steroid treatment, the 48 h old mBMC and hESCs cells were serum starved overnight and treated for 24 h with E2 or progesterone (P4) (Sigma, USA) at concentrations of 1×10^{-8}, 1×10^{-7}, 1×10^{-6} M. The supernatants were then collected.

Migration assay

The migration assay for mBMC and hESC cells was carried out using 8-μm pore size polycarbonate membrane (Millipore, USA). The serum free conditioned medium (600 μl) collected from 48 h old cultures from both cell types was added into the lower chamber and 200 μl of cells (5×10^4 cells) was placed into the upper insert. The cells in the upper