BASIC SCIENCE: GYNECOLOGY

Bone marrow–derived cells from male donors can compose endometrial glands in female transplant recipients

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OBJECTIVE: For continuous regeneration of human endometrium in menstrual cycles, endometrial stem cells are assumed to supply differentiating endometrial glandular cells. To elucidate the origin of endometrial stem cells, we examined the presence of donor-derived cells in endometria from patients who received bone marrow transplantation from male donors.

STUDY DESIGN: Endometrial specimens biopsied after hormone replacement therapy were obtained and examined using fluorescent in situ hybridization analysis targeting X or Y chromosomes.

RESULTS: All recipients had donor-derived Y chromosome–positive endometrial cells, accounting for 0.6-8.4% of glandular epithelial cells and 8.2-9.8% of stromal cells. Most of the endometrial glands were chimeric, consisting of both donor-derived and recipient cells.

CONCLUSION: Donor-derived cells are capable of composing endometrium in recipients, even those of the opposite sex. These results suggest unexpected plasticity of bone marrow stem cells as well as a potential origin of endometrial stem cells.

Key words: bone marrow transplant, endometrium, fluorescent in situ hybridization, stem cell

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The human endometrium is a unique tissue characterized by constant and rapid cell proliferation, differentiation, and breakdown during the menstrual cycle.[1] After shedding by menstruation, the endometrium dramatically proliferates and completely regenerates within 2 weeks.[1] Regeneration of the endometrium incessantly repeats throughout the 35-40 reproductive years. Such a highly regenerative feature suggests the presence of stem cells in endometrial glands; otherwise, the proliferative potential would become exhausted after several dozen menstrual cycles. However, little information has been available about stem cells in endometrium.

Stem cells of bone marrow origin have the ability to differentiate into multiple nonhematopoietic cells.[2] In animal models, it has been reported that bone marrow stem cells can differentiate into hepatocytes,[3-5] skeletal-muscle cells,[6,7] astrocytes,[8] and neurons.[9,10] In human recipients who received bone marrow transplantation, donor-derived cells have been proven to differentiate into various types of cells, such as hepatocytes, neurons, cardiomyocytes, skin, and gastrointestinal cells.[11-14]

The presence or origin of endometrial stem cells remains unclear. Whereas tissue stem cells may be present in endometrium, it is possible that stem cells may also be supplied from bone marrow continuously or transiently. To clearly explore such possibilities, examination of the presence of donor-derived cells in the endometrium of bone marrow transplant recipients is needed.

One previous study[15] examined the presence of donor-derived endometrial cells in endometrial biopsy samples from patients who underwent bone marrow transplantation from female donors and found that donor-derived endometrial cells were engrafted in the endometrium. However, no subsequent study has been reported using human tissue samples.

We are interested in whether similar engraftment can occur in endometrium from sex-mismatched donors. Additionally, we also wanted to clarify whether there is any difference in the efficacy of engraftment between male and female donors in such cases. This question prompted us to examine the presence of donor-derived cells in the endometrium of recipients who received bone marrow transplantation from male donors.

Materials and Methods

Patients and tissue samples

Three patients who received bone marrow transplantation from human leukocyte antigen (HLA)-identical male donors for the treatment of hematologic cancers at the Department of Hematology, Kanazawa University Hospital,

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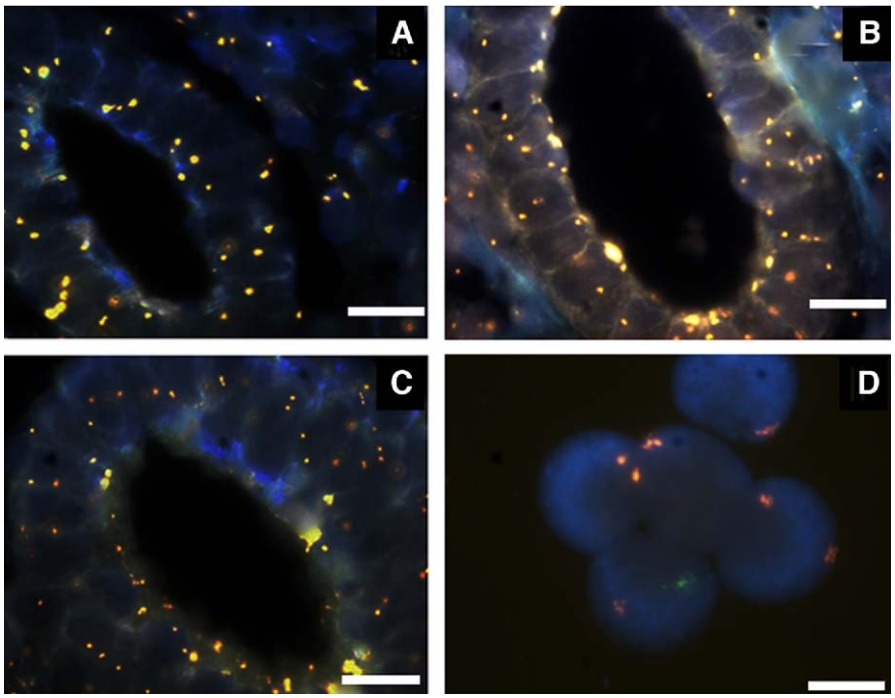
FIGURE 1
Control experiments for FISH analysis for detecting X chromosome-positive or Y chromosome-positive cells



A-C, Endometrial samples from 3 female patients who underwent a hysterectomy because of ovarian tumors were examined for FISH to detect X chromosome- (*orange*) or Y chromosome (*green*)-positive cells. Note that all cells composing endometrial glands exhibited an *orange signal*, whereas no *green signal* was detected. **D,** Control slides containing cultured normal male and female lymphoblast cells were also examined by FISH. The expected ratio of XY or XX cells (Table 1) was observed in our assay conditions.

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TABLE 1
FISH control analysis

Patient	Age	% XX-cells	% XY-cells
1[a]	67	100	0
2[a]	56	100	0
3[a]	65	100	0
Control slide[b]		93.6	6.32

[a] Endometrial samples from 3 untransplanted patients who underwent hysterectomy due to ovarian tumors; [b] Control slides contain a mixture of cultured normal female (91.3%) and male (7.8%) lymphoblast cells.

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from January 1996 to December 2004 were enrolled in the study.

Patients received high-dose chemotherapy or chemotherapy combined with irradiation consisting of 4 days of total body irradiation (12.0 Gy) and 2 days of cyclophosphamide 60 mg/kg and 4 days of Ara C 2 g/m^2. Patients 1 and 3 received transplantation of allogeneic peripheral-blood stem cells mobilized with granulocyte colony-stimulating factor (G-CSF), and patient 2 received bone marrow transplants from HLA-matched unrelated donors mobilized with G-CSF. Hemopoietic mononuclear cells including CD34-positive cells were transplanted.

The patients received hormone replacement therapy (HRT) with 0.625 mg of estradiol every day (28 days) and 5 mg of medroxyprogesterone acetate for the latter half (14 days) of the menstrual cycle for secondary amenorrhea after transplantation. Endometrial tissue specimens were obtained by endometrial curettage at the time of cancer screening at least 6 months from the start of HRT. Histological sections of endometrial tissue samples from 3 patients without transplantation who underwent a hysterectomy because of ovarian tumors were used as controls for detecting X-chromosome signals.

These studies were approved by the Medical Ethical Committee of Kanazawa University Graduate School of Medical Science. All patients gave written informed consent.

Fluorescent in situ hybridization

We performed fluorescent in situ hybridization (FISH) analysis with the use of probes that were specific for the X and Y chromosomes (Vysis, Downers Grove, IL). Paraffin-embedded slides were deparaffinized by baking in an oven overnight at 57°C, cleared in xylene 3 times for 10 min each, dehydrated with 100% ethanol, and air dried. Slides were pretreated in 0.2 N hydrochloric acid for 20 minutes, washed with water, rinsed in 2 × saline sodium citrate (SSC) for 5 minutes, and air dried.

Tissue sections were pretreated with a commercial paraffin pretreatment kit (Vysis) in an 83°C water bath for 30 minutes, washed with water, washed with 2 × SSC for 5 minutes, and air dried. Tissue was digested with 1.5 μg of protease (Vysis) per milliliter in 0.2 N hydrochloric acid, pH 2.0, at 38°C for 30 minutes, washed with water, then rinsed in 2 × SSC for 3 minutes, air dried, and fixed in 10% formalin for 10 minutes. Slides were then denatured with 70% formamide in 2 × SSC at 75°C for 5 minutes, dehydrated using an ethanol series (70%, 85%, 100%), and air dried. We added 10 μL of the mixture of probes for the X and Y chromosomes (CEP probe; Vysis) to the sample.

The slides were covered with a coverslip, sealed with rubber cement, and incubated in a humid chamber at 42°C for 72 hours for hybridization. After 72 hours of hybridization, slides were then washed at 47°C in 50% formamide buffer 3 times for 10 minutes each, washed in 2 × SSC for 10 minutes, and washed in 2 × SSC containing 0.1% Nonidet P-40 for 5 minutes. The nuclei were stained with 10 μl of 4′,6-diamidino-2-phenylindole dihydrochloride (Molecular Probes, Eugene, OR), and a coverslip was applied.