

HEPATOCYTES AND EPITHELIAL CELLS OF DONOR ORIGIN IN RECIPIENTS OF PERIPHERAL-BLOOD STEM CELLS

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ABSTRACT

Background Bone marrow contains stem cells with the potential to differentiate into mature cells of various organs. We determined whether circulating stem cells have a similar potential.

Methods Biopsy specimens from the liver, gastrointestinal tract, and skin were obtained from 12 patients who had undergone transplantation of hematopoietic stem cells from peripheral blood (11 patients) or bone marrow (1 patient). Six female patients had received transplants from a male donor. Five had received a sex-matched transplant, and one had received an autologous transplant. Hematopoietic stem-cell engraftment was verified by cytogenetic analysis or restriction-fragment-length polymorphism analysis. The biopsies were studied for the presence of donor-derived epithelial cells or hepatocytes with the use of fluorescence in situ hybridization of interphase nuclei and immunohistochemical staining for cytokeratin, CD45 (leukocyte common antigen), and a hepatocyte-specific antigen.

Results All six recipients of sex-mismatched transplants showed evidence of complete hematopoietic donor chimerism. XY-positive epithelial cells or hepatocytes accounted for 0 to 7 percent of the cells in histologic sections of the biopsy specimens. These cells were detected in liver tissue as early as day 13 and in skin tissue as late as day 354 after the transplantation of peripheral-blood stem cells. The presence of donor cells in the biopsy specimens did not seem to depend on the intensity of tissue damage induced by graft-versus-host disease.

Conclusions Circulating stem cells can differentiate into mature hepatocytes and epithelial cells of the skin and gastrointestinal tract. (N Engl J Med 2002; 346:738-46.)

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PLURIPOTENT bone marrow stem cells have the capacity for self-renewal and can differentiate into hematopoietic or mesenchymal¹ cell lineages. Studies in laboratory animals and humans indicate that bone marrow stem cells can give rise to hepatic oval cells, hepatocytes, cholangiocytes,^{2,4} skeletal-muscle cells,^{5,6} astrocytes, and neurons.⁷⁻⁹ To investigate whether such progenitor cells circulate in the blood, we studied biopsy specimens of skin, liver, and gastrointestinal tract from recipients of peripheral-blood stem cells from HLA-matched, sex-mismatched siblings for the presence of donor-derived epithelial cells and hepatocytes.

METHODS

Characteristics of the Donors and Recipients

Eleven patients received high-dose chemotherapy alone or chemotherapy combined with radiotherapy, followed by a transplant of allogeneic peripheral-blood stem cells (in 10 patients) or autologous peripheral-blood stem cells (in 1 patient) for the treatment of hematologic cancers or breast cancer. One patient underwent allogeneic bone marrow transplantation. Both myeloablative and nonmyeloablative¹⁰ regimens were used before transplantation. All allogeneic grafts were derived from HLA-matched siblings. The peripheral-blood stem cells were obtained by apheresis after the donor had been treated for four days with recombinant human granulocyte colony-stimulating factor at a dose of 12 μ g per kilogram of body weight per day. The total number of CD34+ cells transplanted ranged from 3.9×10^6 per kilogram of the recipient's body weight to 14.8×10^6 per kilogram.

Six women received stem cells from a brother; five patients received sex-matched stem cells, and one woman received autologous stem cells. The latter six patients served as controls. Hematopoietic stem-cell engraftment was verified by cytogenetic analysis or restriction-fragment-length polymorphism (RFLP) analysis.¹¹ In three control patients, engraftment was documented on the basis of the recovery of peripheral-blood cells alone.

Tissue Specimens

After stem-cell transplantation, tissue specimens were obtained by a needle or punch biopsy that was performed for diagnostic purposes. All 12 patients gave informed consent for biopsies to be performed for diagnostic purposes. By the time our study began, all patients had died. The retrospective analysis of biopsy specimens was approved by the internal review board of the M.D. Anderson Cancer Center. All biopsies were performed for the purpose of establishing the diagnosis of graft-versus-host disease.

A total of five consecutive sections were obtained from each biopsy specimen. Each section was 4 μ m thick, which is approximately half the thickness of a nucleus, with neighboring sections cut 4 μ m apart. The sections closest to the center section that was used for fluorescence in situ hybridization were those stained for cytokeratin and CD45 (leukocyte common antigen), followed by those stained with hematoxylin and eosin and with a hepatocyte-specific antigen. This procedure allowed matching fields to be as close to each other as possible.

Immunohistochemistry

Pretreatment of Slides

After removal of paraffin with xylene, tissue sections were rehydrated with graded alcohols (100 percent, 90 percent, and 70 percent ethanol in distilled water) and washed with water and phosphate-buffered saline. Endogenous peroxidase activity was blocked

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by the application of 0.3 percent hydrogen peroxide in methanol for 15 minutes at room temperature, and the slides were washed in phosphate-buffered saline again. Tissues were then digested with 0.2 percent ficin (Sigma, St. Louis) in distilled water for 15 minutes at room temperature and washed in phosphate-buffered saline.

Staining for Cytokeratin

For staining for cytokeratin, antigen retrieval was performed by incubating the tissue for eight minutes in 0.01 M citrate buffer in a microwave oven. Blocking serum (bovine serum albumin) was applied to the slides for 30 minutes at room temperature, and the slides were then incubated for 60 minutes at room temperature with monoclonal mouse antihuman cytokeratin antibodies (CAM 5.2 [25 μ g per milliliter; Becton Dickinson, San Jose, Calif.] at a dilution of 1:5 plus AE1/AE3 [1 mg per milliliter; Boehringer Mannheim, Indianapolis] at a dilution of 1:480).

Staining for CD45

For staining for CD45, antigen retrieval was performed by incubating the tissue for 45 minutes in TRIS-EDTA buffer in a steamer. Blocking serum (bovine serum albumin) was applied to the slides for 30 minutes at room temperature, and the slides were then incubated with monoclonal mouse antibodies against CD45 (clones PD7/26 and 2B11, Dako, Carpinteria, Calif.) at a dilution of 1:300 for 45 minutes. To detect the antigen-antibody reaction, a streptavidin-biotin detection system (Super Sensitive Immunodetection System, Biogenex, San Ramon, Calif.) was used according to the manufacturer's instructions. Sections from tonsils and peripheral-blood smears were used as positive controls.

Staining for Hepatocytes

For staining for hepatocytes, antigen retrieval was performed by incubating the tissue for 45 minutes in TRIS-EDTA buffer in the steamer. Blocking serum (bovine serum albumin) was applied to the slides for 30 minutes at room temperature, and then slides were incubated with a monoclonal mouse IgG antihuman hepatocyte antibody (clone OCH1E5, Dako) at a dilution of 1:50 for 60 minutes. To detect the antigen-antibody reaction, we used a streptavidin-biotin detection system (Super Sensitive Immunodetection System, Biogenex) according to the manufacturer's instructions.

Fluorescence in Situ Hybridization

Paraffin-embedded slides were deparaffinized by baking in an oven overnight at 56°C and then clearing in xylene three times, for 10 minutes each, for a total of 30 minutes; they were then dehydrated and air-dried. Slides were pretreated in 0.2 N hydrochloric acid for 20 minutes, washed with water, and rinsed in 2 \times saline sodium citrate (SSC) (1 \times SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) for 3 minutes at room temperature. Slides were then incubated in 1 M sodium thiocyanate in distilled water at 80°C for 30 minutes, washed with water, washed with 2 \times SSC for 3 minutes, and air-dried. Tissue was digested with 1.5 μ g of proteinase K (Sigma) per milliliter in 0.2 N hydrochloric acid, pH 2.0, at 37°C for 1 hour, washed with water, and then rinsed in 2 \times SSC for 3 minutes, air-dried, and fixed in Carnoy's solution (methanol and acetic acid in a 3:1 ratio) for 10 minutes. Slides were then denatured with 70 percent formamide in 2 \times SSC at 73°C for five minutes and rinsed with 70 percent ethanol for three minutes, dehydrated, and air-dried. The mixture of probes for the X and Y chromosomes (Vysis, Downers Grove, Ill.) was denatured at 74°C for five minutes and applied to the denatured tissue. The slides were covered with a coverslip, sealed with rubber cement, and incubated in a humid chamber overnight at 37°C for hybridization. After 16 hours of hybridization, slides were washed in 0.4 \times SSC containing 0.3 percent Nonidet P-40 for two minutes at 73°C, transferred to 2 \times SSC containing 0.1 percent Nonidet P-40 for one minute at room temperature, and drained. Slides were then coun-

terstained with 10 μ l of 4',6-diamidino-2-phenylidole dihydrochloride (DAPI, Boehringer Mannheim) at a concentration of 14 μ g per milliliter of VectaShield mounting medium (Vector Laboratories, Burlingame, Calif.), and a coverslip was applied.

Quantification of XY-Positive, Donor-Derived Nonlymphohematopoietic Cells

The slides were scanned at a magnification of 100 under a fluorescence microscope (Leica, Wetzlar, Germany) equipped with an epi-illumination system, a 100-W mercury lamp, and a set of filters, including DAPI single-bandpass (DAPI counterstain), Spectrum Orange single-bandpass, Spectrum Green single-bandpass, and Red/Green dual-bandpass filters (all from Vysis). A total of 200 nonoverlapping cells and nuclei with distinct cells were counted, and the Y-positive (red) and X-positive (green) signals were identified. The percentage of cells that were XY-positive or XX-positive was less than 100 percent because of the truncation of nuclei during sectioning and incomplete hybridization. The stringent criteria used in counting positive signals led to an underestimation of the percentages of XX- or XY-positive cells in cases of female-to-female or male-to-male transplantation. Fields were matched to the corresponding fields in photomicrographs of the variously stained slides according to the location and architecture of the tissue on the slide. Matching microscopic fields were either 4 μ m apart (in the slides stained with antibodies against cytokeratin or CD45) or 8 μ m apart (in the slides stained with hematoxylin and eosin or the anti-hepatocyte antibody). The slides stained with hematoxylin and eosin and with antibody against CD45 were carefully evaluated to exclude the presence of lymphocytes, monocytes, and granulocytes, and XY-positive epithelial cells were identified with the use of only those cells that could reliably be classified on the basis of their staining properties.

Staining for Cytokeratin and Fluorescence in Situ Hybridization

Slides were prepared for staining for cytokeratin as described above. They were then washed in 1 \times phosphate-buffered saline for 5 minutes, and Texas Red-conjugated donkey antimouse IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa.) at a dilution of 1:200 was applied for 60 minutes. After they had been washed in phosphate-buffered saline for five minutes, the slides were counterstained with 10 μ l of DAPI at a concentration of 14 μ g per milliliter of VectaShield antifade solution (Vector Laboratories). After they were stained for cytokeratin, the sections were systematically scanned and photographed at a magnification of 63 with the use of a calibrated and automated motorized stage. Next, the slides were washed in phosphate-buffered saline for five minutes and prepared for fluorescence in situ hybridization as outlined above. The slides were then scanned for XY-positive cells; pictures were taken at a magnification of 63 and were matched with the stored cytokeratin images.

RESULTS

Characteristics of Patients and Transplantations

The characteristics of the donors and recipients, the type of regimen used before transplantation, and the quantity of CD34+ cells transfused are presented in Table 1.

Hematopoietic Chimerism after Allogeneic Stem-Cell Transplantation

Complete hematopoietic chimerism was demonstrated by RFLP analysis in four of the six recipients of sex-mismatched stem cells (Patients 7, 8, 10, and 12) and by cytogenetic analysis of bone marrow cells

TABLE 1. CHARACTERISTICS OF THE DONORS AND THE RECIPIENTS.*

RECIPIENT NO.	SEX OF DONOR	SEX OF RECIPIENT	AGE OF RECIPIENT yr	DIAGNOSIS	CONDITIONING REGIMEN	CD34+ CELLS TRANSFUSED ×10 ⁻⁶ /kg
Sex-matched						
1	F	F	58	Chronic lymphocytic leukemia	Thiotepa, busulfan, cyclophosphamide (myeloablative)	5.5
2	F	F	24	Large-cell lymphoma	Carmustine, etoposide, cytarabine, melphalan (myeloablative)	14.4
3	—	F	50	Multiple myeloma	Thiotepa, busulfan, cyclophosphamide (myeloablative)	4.4
4	M	M	39	Acute myelogenous leukemia; previous failed bone marrow transplantation	Cyclophosphamide, total-body irradiation (myeloablative)	5.1
5	M	M	55	Chronic myelogenous leukemia	Thiotepa, busulfan, cyclophosphamide (myeloablative)	5.7
6	M	M	43	Follicular large-cell lymphoma	Carmustine, etoposide, cytarabine, melphalan (myeloablative)	4.8
Sex-mismatched						
7	M	F	54	Follicular small-cell lymphoma	Thiotepa, busulfan, cyclophosphamide (myeloablative)	14.8
8	M	F	32	Diffuse large-cell lymphoma	Thiotepa, busulfan, cyclophosphamide (myeloablative)	6.2
9	M	F	44	Chronic myelogenous leukemia	Thiotepa, cyclophosphamide, total-body irradiation (myeloablative)	Not performed
10	M	F	52	Breast cancer, stage IV	Cyclophosphamide, carmustine, thiotepa (myeloablative)	7.0
11	M	F	30	Acute myelogenous leukemia secondary to treatment of stage III Hodgkin's disease	Busulfan, cyclophosphamide (myeloablative)	3.9
12	M	F	45	Follicular small-cell lymphoma	Cyclophosphamide, fludarabine (nonmyeloablative)	5.9

*The stem cells were obtained from peripheral blood in all cases except that of Patient 9, who received bone marrow. Each donor was an HLA-matched sibling, except in the case of Patient 3, who received autologous cells.

TABLE 2. INCIDENCE OF DONOR CHIMERISM AND BIOPSY REPORTS.*

RECIPIENT NO.	DONOR CHIMERISM		BIOPSY REPORT
	RESTRICTION-FRAGMENT-LENGTH POLYMORPHISM ANALYSIS	CYTOGENETIC ANALYSIS	
Sex-matched			
1	NP	NA	
2	Inconclusive, day 24; complete donor chimerism, day 84	NA	
3	NP	NA	
4	Mixed chimerism, days 196 and 227	NA	
5	Complete donor chimerism, days 27, 89, 238, 298, and 365	NA	
6	NP	NA	
Sex-mismatched			
7	Complete donor chimerism, days 27, 91, and 209	Complete male karyotype, days 27, 90, and 209	Liver: consistent with the presence of acute viral hepatitis GI tract: normal, no evidence of GVHD Skin: consistent with the presence of GVHD
8	Complete donor chimerism, day 29	Complete male karyotype, day 29	Liver: drug-induced hepatotoxicity Skin: perivascular lymphocytic infiltrate
9	NP	Complete male karyotype, day 94; two pseudo-diploid clones of female origin, day 865	Skin: perivascular lymphocytic infiltrate
10	Complete donor chimerism, days 37, 99, and 350	Complete male karyotype, days 37, 99, 350, and 512	GI tract: esophagitis with ulceration Skin: consistent with the presence of GVHD
11	NP	Complete donor chimerism, days 30 and 95; mixed chimerism, day 319; recipient-only cells, days 521 and 625; mixed chimerism, day 779	Liver: consistent with the presence of GVHD Skin: perivascular lymphocytic infiltrate
12	Complete donor chimerism, day 34	Complete male karyotype, day 34	Liver: consistent with the presence of GVHD Skin: perivascular lymphocytic infiltrate

*Day 0 was the day of transplantation. A complete male karyotype was defined as 100 percent male cells. NP denotes not performed, NA not applicable, GI gastrointestinal, and GVHD graft-versus-host disease.

in all six (Table 2). In three of the six control patients, donor chimerism was documented by RFLP analysis.

Donor-Derived Epithelial Cells and Hepatocytes in Recipients of Sex-Mismatched Stem Cells

We studied biopsy specimens of skin, liver, and gastrointestinal tract for the presence of donor-derived epithelial cells and hepatocytes. Whereas a DNA probe specific for the centromeres detected X and Y chromosomes in 35 to 75 percent of cells in biopsy specimens from the three male patients who received an allograft from a male donor (Patients 4, 5, and 6) (Table 3), they did not detect XY-positive cells in any biopsy specimens from women who received a stem-cell transplant from a sister (Patients 1 and 2) or the woman who received her own cells (Patient 3). By contrast, XY-positive cells were present in biopsy specimens of the skin, liver, or gastrointestinal tract from the five female recipients of peripheral-blood stem cells from male donors (Patients 7, 8, 10, 11, and 12) and in the female recipient of a bone marrow allograft from her brother (Patient 9).

In epidermal tissue of the skin, donor-derived cells were located in the deep layer of Malpighi (the stratum spinosum of the stratum germinativum), close to the dermal-epidermal junction and the stratum granulosum (Fig. 1). In the liver, XY-positive hepatocytes were distinguished by large, round nuclei and abundant granular cytoplasm (Fig. 2). In the glandular epithelium of the gastric cardia, cells containing the Y chromosome were found in the foveolae or tubular pits of the superficial glandular layer, which is composed of mucus-containing cells lining the foveolae (Fig. 3). The organ specificity of these cells was indicated by their location, staining for cytokeratins and hepatocytes (in the liver), and the absence of CD45.

We also analyzed slides from biopsy specimens of all three organs after they had been stained with anti-cytokeratin antibodies and examined by fluorescence in situ hybridization with probes for the X and Y chromosomes. XY-positive signals in cytokeratin-positive cells would indicate the epithelial character of donor-derived cells. As demonstrated in epidermal cells in skin (Fig. 1E and 1F), hepatocytes (Fig. 2E and 2F) and mucosal cells of the gastrointestinal tract (Fig. 3E and 3F), XY-positive signals were detectable in cytokeratin-positive cells.

The frequency of XY-positive cells in biopsy specimens from female recipients of grafts from male donors ranged from 0 to 7 percent (Table 3). XY-positive cells were detected in liver tissue as early as 13 days after transplantation (in Patient 8) and were seen in skin tissue 354 days after transplantation of peripheral-blood stem cells (in Patient 10) and 867 days after transplantation of bone marrow (in Patient

TABLE 3. PERCENTAGE OF XY-POSITIVE CELLS IN VARIOUS TISSUE BIOPSY SPECIMENS FROM SIX CONTROL RECIPIENTS OF SEX-MATCHED STEM-CELL ALLOGRAFTS OR AUTOGRAFT AND SIX RECIPIENTS OF SEX-MISMATCHED STEM-CELL ALLOGRAFTS.*

RECIPIENT NO.	TYPE OF TISSUE SPECIMEN	DAY OBTAINED†	PERCENTAGE OF XY-POSITIVE CELLS
Sex-matched			
1	Liver	23	0
	GI tract	39	0
2	GI tract	212	0
	Skin	28	0
4	Skin	91	52
	Liver	230	35
5	Skin	229	72
	Liver	22	72
6	Skin	35	75
	Sex-mismatched		
7	Liver	217	5
	GI tract	251	4
	Skin	11	0
8	Liver	13	5
	Skin	62	3
9	Skin	867	6
	GI tract	60	6
10	Skin	354	7
	Liver	191	7
11	Skin	88	5
	Liver	41	4
12	Skin	19	2

*The percentage of XY-positive cells was determined by fluorescence in situ hybridization and immunohistochemical staining of consecutive sections. GI denotes gastrointestinal.

†Day 0 was the day of transplantation.

9). The biopsy reports and the patients' clinical status at the time of biopsy did not suggest that donor-cell engraftment was more likely in tissues injured by graft-versus-host disease than in other tissues (as shown by the results for Patients 7, 10, 11, and 12 in Table 2).

DISCUSSION

Circulating blood is known to contain stem cells that can completely restore hematopoiesis after ablation of the bone marrow.^{12,13} Recently, mesenchymal stem cells with a capacity for self-renewal and the potential to differentiate into bone, cartilage, fat, tendon, muscle, or marrow stroma have been identified in human bone marrow.^{1,14} Whether such stem cells circulate in the blood is unsettled.¹⁵⁻¹⁷ A stem cell in rat bone marrow has been found to differentiate into the epithelial lineage that generates hepatic oval cells,² and in mice with a metabolic defect that impairs liver function, the infusion of purified hematopoietic stem cells can restore both hematopoiesis and liver function.¹⁸ Progenitors in mouse bone marrow have also been shown to be myogenic and can induce muscle regeneration.^{5,6}

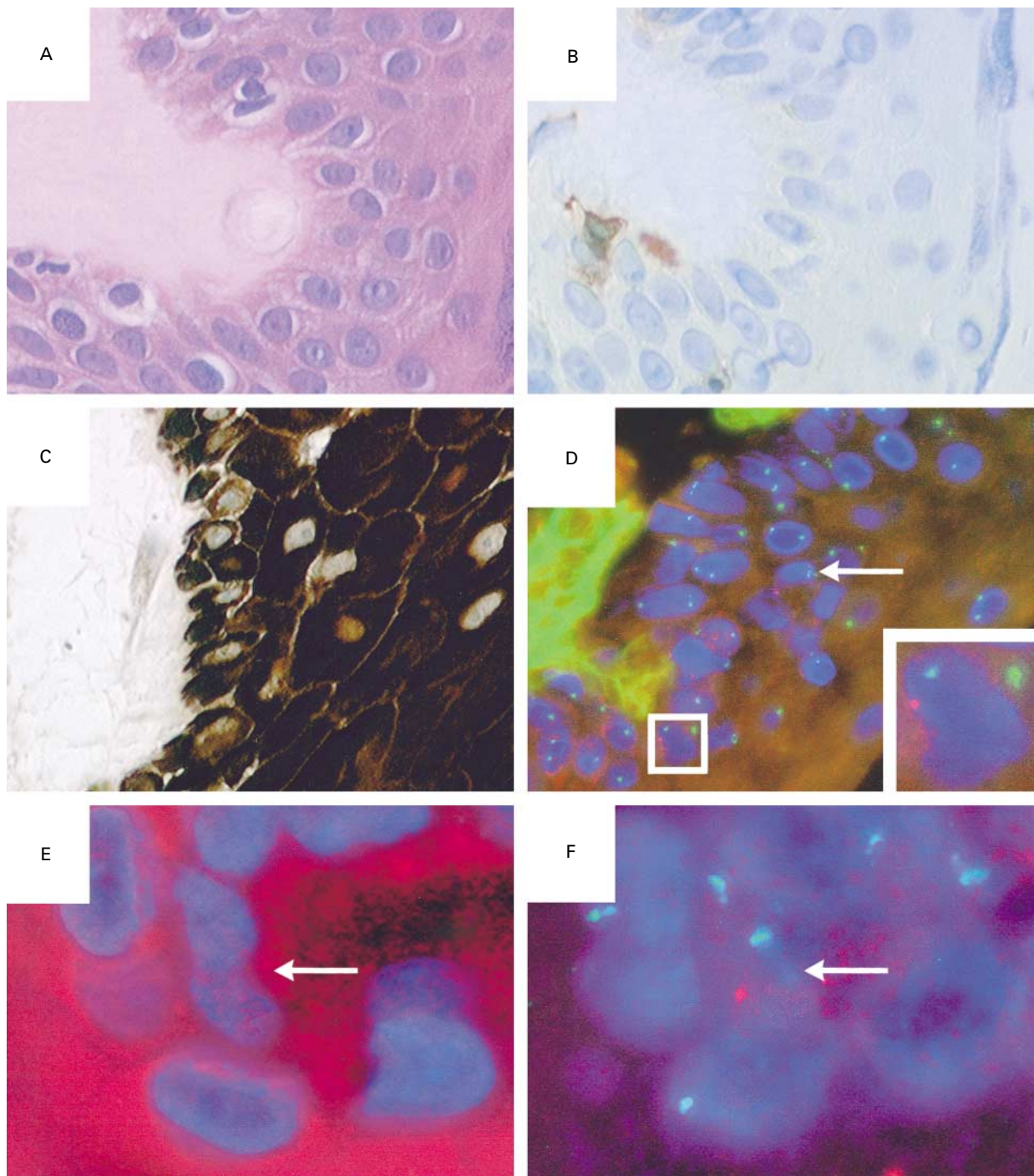


Figure 1. Donor-Derived Epidermal Cells in the Skin of a Female Recipient (Patient 10) of Peripheral-Blood Stem Cells from a Male Donor.

Neighboring tissue sections were stained with hematoxylin and eosin (Panel A), CD45 (Panel B), and cytokeratin (Panel C) and were examined by interphase fluorescence in situ hybridization for centromeres of X (green) and Y (red) chromosomes (Panel D). Panels E and F show a single tissue section that was first stained with fluorescent cytokeratin (Panel E) and then examined by fluorescence in situ hybridization (Panel F). The cells are predominantly epithelial in nature (cytokeratin-positive and CD45-negative) and of female origin (XX-positive). A smaller population of XY-positive epithelial cells (inset and arrow in Panel D) is also present. Cells shown in Panels E and F are both cytokeratin-positive and XY-positive (arrows). (Panels A through F, $\times 63$; inset in Panel D, $\times 160$.)

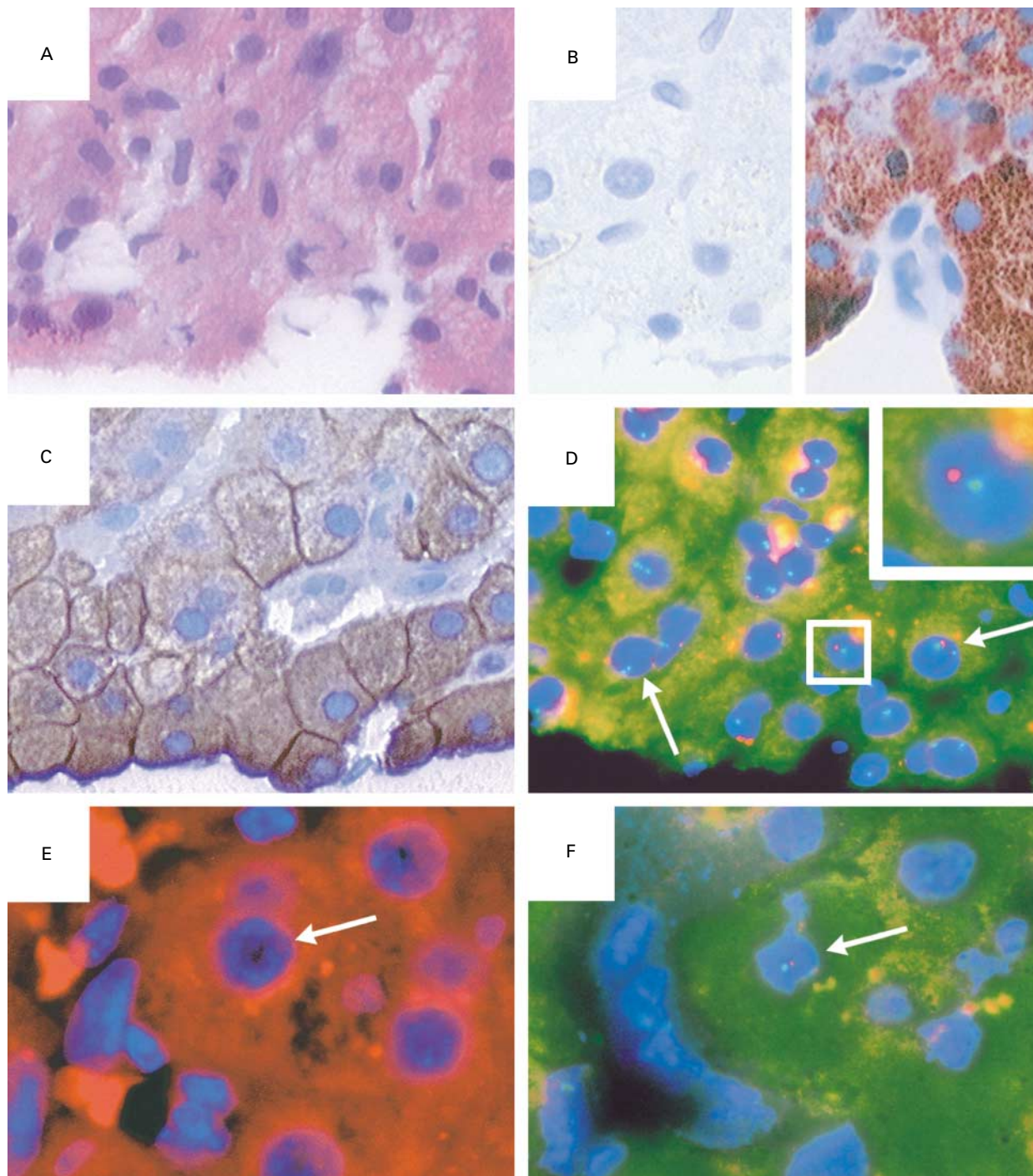


Figure 2. Donor-Derived Hepatocytes in the Liver of a Female Recipient (Patient 7) of Peripheral-Blood Stem Cells from a Male Donor. Neighboring tissue sections were stained with hematoxylin and eosin (Panel A), CD45 (Panel B), and cytokeratin (Panel C) and were examined by interphase fluorescence in situ hybridization for centromeres of X (green) and Y (red) chromosomes. Panels E and F show a single tissue section that was first stained with fluorescent cytokeratin (Panel E) and then examined by fluorescence in situ hybridization (Panel F). Most cells are cytokeratin-positive, CD45-negative, and XX-positive. A small population of XY-positive cells (inset and arrows in Panel D) is also present. Cells shown in Panels E and F are both cytokeratin-positive and XY-positive (arrows). (Panels A through F, $\times 63$; inset in Panel D, $\times 160$.)

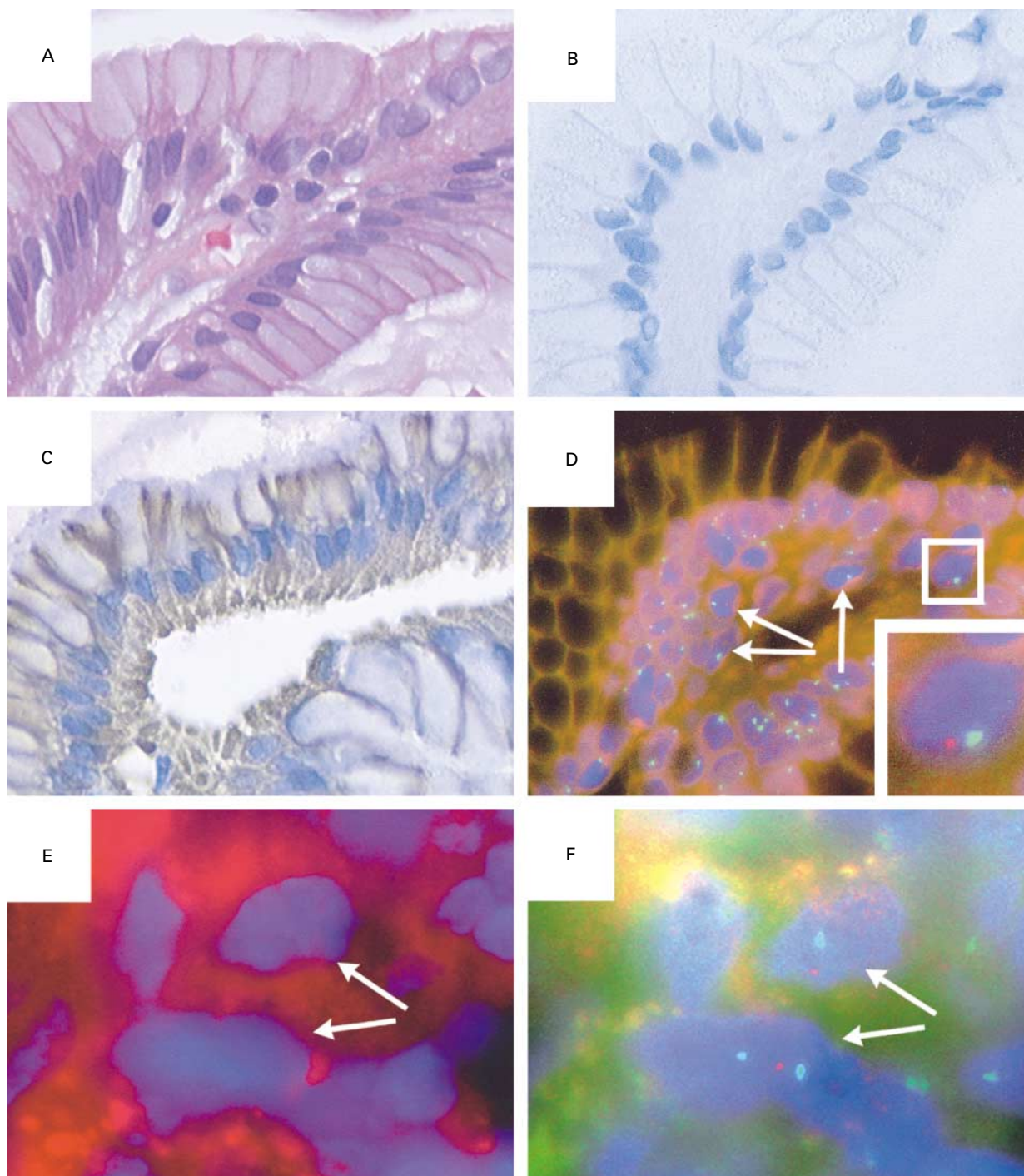


Figure 3. Donor-Derived Mucosal Cells in the Gastric Cardia in a Female Recipient (Patient 7) of Peripheral-Blood Stem Cells from a Male Donor.

Neighboring tissue sections were stained with hematoxylin and eosin (Panel A), CD45 (Panel B), and cytokeratin (Panel C) and were examined by interphase fluorescence in situ hybridization for centromeres of X (green) and Y (red) chromosomes. Panels E and F show a single tissue section that was first stained with fluorescent cytokeratin (Panel E) and then examined by fluorescence in situ hybridization (Panel F). Most cells are cytokeratin-positive, CD45-negative, and XX-positive. Some XY-positive cells (inset and arrows in Panel D) are also present. Cells shown in Panels E and F are both cytokeratin-positive and XY-positive (arrows). (Panels A through F, $\times 63$; inset in Panel D, $\times 160$.)

The existence of stem cells with multiple differentiating capabilities¹⁹ was conclusively demonstrated by Krause et al.,²⁰ who showed that a single bone marrow stem cell not only can restore hematopoiesis in mice that have received otherwise lethal doses of radiation but also can differentiate into mature epithelial cells of the skin, lungs, and gastrointestinal tract. Moreover, human progenitor cells transplanted into fetal sheep have been reported to differentiate into hematopoietic cells and hepatocytes.²¹ There is also evidence that human kidney,²² liver, and muscle cells²³ can transform into blood-forming cells. Moreover, two groups have reported the presence of donor-derived hepatocytes and cholangiocytes in recipients of sex-mismatched bone marrow transplants.^{3,4}

Our findings indicate that human blood contains stem cells that can differentiate into cells of the liver, gastrointestinal tract, and skin. The origin of these stem cells and the way in which they generate hepatocytes and epithelial cells are unknown. It is possible that multiple lineage-restricted stem cells in the circulating blood can differentiate independently into their corresponding mature tissue. Alternatively, primitive adult multipotent stem cells may give rise to differentiated, lineage-restricted stem cells that can generate mature cells. It is also possible that stem cells that are committed to differentiation primarily along a particular pathway (e.g., hematopoiesis) can switch to another lineage under the influence of signals of the local microenvironment. Beltrami and coworkers²⁴ have postulated that circulating stem cells differentiate into dividing myocytes that repair necrotic myocardium after infarction in humans. It is also conceivable that, in addition to mobilizing hematopoietic stem cells, recombinant human granulocyte colony-stimulating factor mobilizes clonogenic cells of epithelial origin into the peripheral blood.²⁵

Technically, our studies of thin tissue sections are not infallible. Nonhematopoietic tissue may harbor a few lymphohematopoietic cells that standard histologic staining techniques fail to detect. In our study, we used a restricted number of consecutive tissue sections and used stringent criteria in the enumeration of XY-positive cells. Furthermore, to ensure that the X- and Y-chromosome signals on fluorescence in situ hybridization were indeed detected in cytokeratin-positive cells, we used sequential staining of the same tissue sections. This procedure, however, exposed the tissue to rough conditions, which may have led us to underestimate the numbers of donor-derived cells in tissue sections.

Tissue damage caused by radiation, chemotherapy, or graft-versus-host disease, among other causes, is believed to be responsible for the homing of peripheral-blood stem cells and their differentiation into various solid-organ-specific tissues.¹⁹ Our results in-

dicating a rather uniform pattern of engraftment of donor-derived hepatocytes and epithelial cells irrespective of the presence or absence of tissue damage caused by graft-versus-host disease. In conclusion, our findings suggest the existence of a population of circulating stem cells with the capacity to differentiate into epithelial cells and hepatocytes. The physiologic role of these cells is currently unknown.

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REFERENCES

- Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143-7.
- Petersen BE, Bowen WC, Patrene KD, et al. Bone marrow as a potential source of hepatic oval cells. *Science* 1999;284:1168-70.
- Alison MR, Poulosom R, Jeffery R, et al. Hepatocytes from non-hepatic adult stem cells. *Nature* 2000;406:257.
- Theise ND, Nimmakayalu M, Gardner R, et al. Liver from bone marrow in humans. *Hepatology* 2000;32:11-6.
- Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;279:1528-30. [Erratum, *Science* 1998;281:923.]
- Gussoni E, Sonoka Y, Strickland CD, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* 1999;401:390-4.
- Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci U S A* 1999;96:10711-6.
- Li Y, Chen J, Wang L, Lu M, Chopp M. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology* 2001;56:1666-72.
- Fuchs E, Segre JA. Stem cells: a new lease on life. *Cell* 2000;100:143-55.
- Giral S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purin-analog containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997;89:4531-6.
- Knowlton RG, Brown VA, Braman JC, et al. Use of highly polymorphic DNA probes for genotypic analysis following bone marrow transplantation. *Blood* 1986;68:378-85.
- Körbling M, Przepiorka D, Huh YO, et al. Allogeneic blood stem cell transplantation for refractory leukemia and lymphoma: potential advantage of blood over marrow allografts. *Blood* 1995;85:1659-65.
- Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med* 2001;344:175-81.
- Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 1999;5:309-13.
- Fernandez M, Simon V, Herrera G, Cao C, Del Favero H, Minguell JJ. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. *Bone Marrow Transplant* 1997;20:265-71.
- Lazarus HM, Haynesworth SE, Gerson SL, Caplan AI. Human bone marrow-derived mesenchymal (stromal) progenitor cells (MPCs) cannot be recovered from peripheral blood progenitor cell collections. *J Hematother* 1997;6:447-55.
- Huss R, Lange C, Weissinger EM, Kolb HJ, Thalmeier K. Evidence of peripheral blood-derived, plastic-adherent CD34^{low} hematopoietic stem cell clones with mesenchymal stem cell characteristics. *Stem Cells* 2000;18:252-60.
- Lagasse E, Connors H, Al-Dhalimy M, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 2000;6:1229-34.

19. Anderson DJ, Gage FH, Weissman IL. Can stem cells cross lineage boundaries? *Nat Med* 2001;7:393-5.
20. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001;105:369-77.
21. Zanjani ED, Porada CD, Crapnell KB, et al. Production of human hepatocytes by human Lin⁻, CD34^{+/-} cells in vivo. *Blood* 2000;96:494a. abstract.
22. Almeida-Porada G, Porada CD, Zanjani ED. Differentiation potential of human metanephric stem cells: from mesenchyme to blood and liver. *Blood* 2000;96:494a. abstract.
23. Farace F, Guillier M, Elias D, Guinebretiere JM, Vainchenker W, Turhan AG. In vitro and in vivo evaluation of the hematopoietic potential of liver and muscle. *Blood* 2000;96:494a-495a. abstract.
24. Beltrami AP, Urbanek K, Kajstura J, et al. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med* 2001;344:1750-7.
25. Brugger W, Bross KJ, Glatt M, Weber F, Mertelsmann R, Kanz L. Mobilization of tumor cells and hematopoietic progenitor cells into peripheral blood of patients with solid tumors. *Blood* 1994;83:636-40.

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