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# Characterization of Transplanted Green Fluorescent Protein$^+$ Bone Marrow Cells into Adipose Tissue

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**Key Words.** Adipose • Chimera • Stem cell • Green fluorescent protein

## ABSTRACT

**Following transplantation of green fluorescent protein (GFP)-labeled bone marrow (BM) into irradiated, wild-type Sprague-Dawley rats, propagated GFP$^+$ cells migrate to adipose tissue compartments. To determine the relationship between GFP$^+$ BM-derived cells and tissue-resident GFP$^-$ cells on the stem cell population of adipose tissue, we conducted detailed immunohistochemical analysis of chimeric whole fat compartments and subsequently isolated and characterized adipose-derived stem cells (ASCs) from GFP$^+$ BM chimeras. In immunohistochemistry, a large fraction of GFP$^+$ cells in adipose tissue were strongly positive for CD45 and smooth muscle actin and were evenly scattered around the adipocytes and blood vessels, whereas all CD45$^+$ cells within the blood vessels were GFP$^+$. A small fraction of GFP$^+$ cells with the mesenchymal marker CD90 also existed in the perivascular area. Flow cytometric and immunocytochemical analyses showed that cultured ASCs were CD45$^-$/CD90$^+$/CD29$^+$. There was a significant difference in both the cell number and phenotype of the GFP$^+$ ASCs in two different adipose compartments, the omental (abdominal) and the inguinal (subcutaneous) fat pads; a significantly higher number of GFP$^-$/CD90$^+$ cells were isolated from the subcutaneous depot as compared with the abdominal depot. The in vitro adipogenic differentiation of the ASCs was achieved; however, all cells that had differentiated were GFP$^-$. Based on phenotypical analysis, GFP$^+$ cells in adipose tissue in this rat model appear to be of both hematopoietic and mesenchymal origin; however, infrequent isolation of GFP$^+$ ASCs and their lack of adipogenic differentiation suggest that the contribution of BM to ASC generation might be minor.** STEM CELLS *2008;26:330–338*

Disclosure of potential conflicts of interest is found at the end of this article.

## INTRODUCTION

The distribution of cells within adipose tissue has been investigated, with the primary cell type present in adipose tissue being adipocytes. However, as fat is a vascularized tissue, other cell types that reside in the adipose tissue compartment include endothelial cells, macrophages, smooth muscle cells, and fibroblasts. The determination of a population of cells other than adipocytes was first made in the mid-1970s. In 1975, Stiles et al. documented a morphologically different phenotype of cells in human adipose tissue [1]. In 1976, Dardick et al. determined that these cells can differentiate into adipocytes [2]. Several groups began studying these cells, termed "preadipocytes " [3–9]. It was in 2001 that the plasticity of these cells was reported by Zuk et al. [10]. Initial reports demonstrated the mesenchymal plasticity of the cells, followed by several reports on the transdifferentiation of these seemingly mesenchymal stem cells into both ectodermal and endodermal phenotypes [11–16]. The identification of a stem cell population in adipose tissue has led to an extensive and rapid growth of research in the fields of regenerative medicine and adipose biology.

Although the plasticity and potential of the stem cells derived from the adipose tissue compartment remain under intense scrutiny, the origin of the stem cells is unclear. One possibility is that the cells are derived from the bone marrow. Recently, Crossno et al. reported that progenitor cells originating from the bone marrow can contribute to an increase in adipocyte number [17]. Their in vivo mouse study demonstrated that exposure to a high-fat diet or treatment with the thiazolidinedione rosiglitazone for 3 weeks promoted the trafficking of circulating bone marrow-derived progenitor cells into adipose tissue. This was evidenced by the appearance of green fluorescent protein (GFP)$^+$ multilocular adipocytes.

To test the hypothesis that adipose-derived stem cells (ASCs) are derived from the bone marrow, we identified a population of GFP-positive cells, as well as GFP$^-$ cells, from the adipose compartments of bone marrow (BM) chimeric rats. There was migration of the GFP$^+$ cells to two different adipose compartments (the omental and the inguinal fat pad), as identified by flow cytometry and immunohistochemistry. In addition to whole adipose tissue analysis, ASCs were isolated from the two different adipose compartments and cultured to passage 3. Our results indicate that GFP$^+$ cells in adipose tissue in this rat model appear to be of both hematopoietic and mesenchymal

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**Table 1.** Antibodies and counterstains used in this study

| Cells | Epitope | Primary antibody, clone, source, and dilution | Secondary antibody, source, and dilution |
|---|---|---|---|
| Endothelial cells | CD31/platelet-endothelial cell adhesion molecule | IF: Mouse anti-rat CD31 (Serotec), 1:100; FACS: Mouse anti-rat CD31 (Chemicon), 1:100 | IF: Goat anti-mouse Cy3 (Jackson Immunoresearch Laboratories), 1:1,000; FACS: Goat anti-mouse PE (Caltag), 1:100 |
| Endothelial cells/BM stromal cells | CD106/VCAM | IF and FACS: PE-conjugated mouse anti-rat CD106 | IF: Goat anti-mouse Cy3 (Jackson Immunoresearch Laboratories), 1:1,000 |
| Mesenchymal stem cells | CD29/$\beta$1 integrin | IF and FACS: Biotin-conjugated CD29 (BD Pharmingen), 1:100 | IF: Streptavidin Cy3 (Jackson Immunoresearch Laboratories), 1:1,000; FACS: Streptavidin PE (Cedar Lane), 1:500 |
| Mesenchymal stem cells | CD90/Thy-1 | IF and FACS: PE-conjugated CD90 (BD Pharmingen), 1:100 | IF: Streptavidin Cy3 (Jackson Immunoresearch Laboratories), 1:1,000 |
| Endothelial cell precursors/mesenchymal stem cells | CD133 | IF and FACS: Rabbit anti-CD133 (GeneTex, Inc.), 1:100 | IF: Goat ant-rabbit Cy3 (Jackson Immunoresearch Laboratories), 1:1,000; FACS: Mouse ant-rabbit PE (SouthernBiotech), 1:100 |
| Leukocytes/hematopoietic cells | CD45/common leukocyte antigen | IF: Mouse anti-rat CD45, OX1 (Serotec), 1:100; FACS: Mouse anti-rat CD45 conjugated to PE (BD Pharmingen), 1:100 | IF: Goat anti-mouse Cy3 or Cy5 (Jackson Immunoresearch Laboratories), 1:1,000 |
| Smooth muscle cells/pericytes | Smooth muscle actin | IF: Mouse anti-SMA conjugated to Cy3 (Sigma), 1:250. | |
| GFP | GFP | TEM ImmunoGold: rabbit anti-GFP (Abcam) 1:100 | TEM ImmunoGold: Goat anti-rabbit 5 nm gold (Amersham), 1:25 |
| Counterstain | Nucleus | Hoechst 33258: Bis-benzimidizole (Sigma), 10 $\mu$g/ml | |
| Counterstain | F-actin | Phalloidin-Alexa 647 or rhodamine-phalloidin (Invitrogen), 1:250 | |

Vendors were as follows: Abcam, Cambridge, MA, http://www.abcam.com; Amersham Biosciences, Piscataway, NJ, http://www.amersham.com; BD Pharmingen, San Diego, http://www.bdbiosciences.com/index_us.shtml; Caltag Laboratories, Burlingame, CA, http://www.caltag.com; Cedar Lane, Hornby, ON, Canada, http://www.cedarlanelabs.com; Chemicon, Temecula, CA, http://www.chemicon.com; GeneTex, San Antonio, http://www.genetex.com; Invitrogen, Carlsbad, CA, http://www.invitrogen.com; Jackson Immunoresearch Laboratories, West Grove, PA, http://www.jacksonimmuno.com; AbD Serotec, Raleigh, NC, http://www.ab-direct.com; Sigma-Aldrich, St. Louis, http://www.sigmaaldrich.com; SouthernBiotech, Birmingham, AL, http://www.southernbiotech.com.
Abbreviations: BM, bone marrow; FACS, fluorescence-activated cell sorting; GFP, green fluorescent protein; IF, immunofluorescence; PE, phycoerythrin; VCAM, vascular cell adhesion molecule.

origin; however, infrequent isolation of $GFP^+$ ASCs and their lack of adipogenic differentiation suggest that the contribution of BM to ASC generation may be a rare event.

## Materials and Methods

### In Vivo Model

GFP-transgenic and wild-type (WT) Sprague-Dawley rats were obtained from Japan SLC, Inc. (Hamamatsu, Japan, http://www.jslc.co.jp). The expression of GFP was under the control of the cytomegalovirus enhancer and the chicken $\beta$-actin promoter derived from an expression vector, pCAGGS [18, 19]. Animals were maintained in laminar flow cages in a specific pathogen-free animal facility at the University of Pittsburgh. All procedures in this experiment were performed according to the guidelines of the Council on Animal Care at the University of Pittsburgh and the National Research Council's Guide for the Humane Care and Use of Laboratory Animals.

Bone marrow cells (BMCs) were obtained from 8–12-week-old GFP rats by flushing the tibias and femurs with RPMI 1640 (Invitrogen, Carlsbad, CA, http://www.invitrogen.com) supplemented with 25 mM HEPES buffer, 2 mM L-glutamine, and 10 $\mu$g/ml gentamicin (all from Life Technologies, Grand Island, NY, http://www.lifetech.com). Flow cytometric analysis showed that 69.7% $\pm$ 5.1% of GFP BMCs were GFP-positive, whereas ~30% did not express GFP. A similar observation was reported in the BMCs from the GFP mouse [20]. Furthermore, the majority of $GFP^+$ BMCs were $CD45^+$, whereas the $GFP^-$ BMCs were $CD45^-$.

Unfractionated BMCs ($2 \times 10^8$ cells per animal) with >95% viability in a trypan blue exclusion test were intravenously injected into WT rat recipients after 9.5 Gy of whole body irradiation ($^{137}Cs$ source). Tacrolimus (0.5 mg/kg/day; a gift from Astellas Pharma Inc., Tokyo, http://www.astellas.com) was given for 7 days after BMC infusion. The successful creation of a GFP chimera was confirmed by detecting $GFP^+$ peripheral blood mononuclear cells (PBMCs) with flow cytometry in the blood. The percentage of $GFP^+$ PBMCs in WT recipients quickly increased and reached >95% by 100 days [21]. At 116–402 days after transplantation, subcutaneous and intra-abdominal fat tissues were obtained from 26 GFP radiation chimeras and used in this study.

### Cell Characterization Using Immunofluorescence Microscopy

For immunocytochemistry analysis, cultured ASCs were fixed and processed for immunofluorescence as described [22] using the antibodies listed in Table 1. For immunohistochemistry, adipose tissue was removed from $GFP^+$ bone marrow chimeric rats and immersion-fixed in 2% paraformaldehyde in phosphate-buffered saline

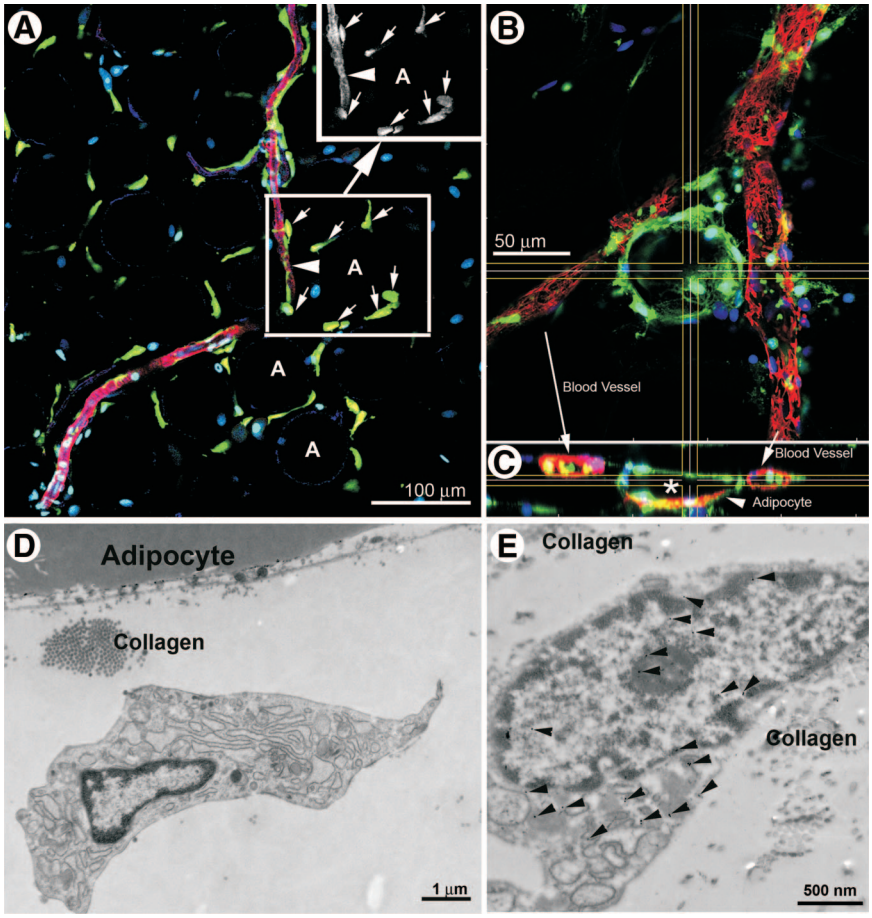


**Figure 1.** Evaluation of the distribution and ultrastructure of bone marrow-derived green fluorescent protein (GFP)$^+$ cells within the fat of a radiation chimera rat 165 days following bone marrow transplantation. **(A):** Tissue was stained for smooth muscle actin (SMA) (red), F-actin (blue), and the nucleus (Hoechst dye, cyan), in addition to collecting the endogenous fluorescence of GFP (green). Single slice confocal microscopy of the tissue shows that GFP$^+$ cells were evenly scattered around the 70–100-$\mu$m adipocytes (some labeled as reference). Most GFP$^+$ cells were observed to be SMA-positive (arrows) as more clearly shown in the black and white rendition of the red channel in the inset **(A)**. The SMA signal in the GFP$^+$ cells was equal to that observed in the pericytes surrounding the blood vessels (arrowhead). **(B):** Partial confocal stack showing a GFP$^+$ adipocyte in the bone marrow chimera. This is a rare event and was observed only once in six chimeric animals examined (tissue counterstained for F-actin [rhodamine phalloidin, red] and nucleus [Hoechst dye, blue]). GFP signal is cytoplasmic and does not partition into the lipidic inclusion of the adipocyte. **(C):** Expanded *x-y* projection from **(B)** at axes delineated by the horizontal and vertical lines, indicating the GFP$^+$ blood cells within the F-actin$^+$ blood vessels (arrows). The GFP$^+$ cytoplasmic labeling of the positive adipocyte surrounds the lipidic portion of the cell (*). **(D):** Transmission electron micrograph of the typical GFP$^+$ cell within the adipose tissue, showing these cells, integrated between adipocytes, possess large quantities of rough endoplasmic reticulum. **(E):** Immuno-transmission electron microscopy analysis of LRWhite acrylic-embedded chimeric adipose tissue indicated that these cells were GFP$^+$ when stained for the GFP protein (arrows indicate secondary antibody 5-nm gold particles). Abbreviation: A, adipocyte.

(PBS) for several hours. Fixed tissue was stored in PBS at 4°C until it was processed. Tissue was then cut into approximately 1-mm$^3$ pieces and stained in situ. Tissue was mixed on a Nutator instrument (Becton, Dickinson and Company, Franklin Lakes, NJ, http://www.bd.com) during all procedures, and rinses were 15 minutes each. Tissue pieces were rinsed three times in PBS and then rinsed three times in PBS containing 0.5% BSA and 0.15% glycine (PBG buffer). Tissue was permeabilized in PBGT buffer containing 0.1% Triton X-100 (PBG) for 2 hours at room temperature and then blocked in 5% nonimmune goat serum in PBGT buffer (PBG with 0.05% Triton X-100) for 2 hours at room temperature. Primary antibodies (Table 1) diluted in PBGT buffer were added to tissue overnight at 4°C. Tissue was rinsed four times in PBGT buffer, and then fluorescently tagged after secondary and phalloidin (Table 1), diluted in PBGT buffer, were added to the sections overnight at 4°C. Tissue was rinsed three times in PBGT buffer and three times in PBS, and then nuclei were stained using 0.01% Hoechst dye (bis benzimide) in PBS for 10 minutes. Following a wash in PBS, the tissue was coverslipped using gelvatol (23 g of poly[vinyl alcohol] average molecular weight 30,000–70,000), 50 ml of glycerol, 0.1% sodium azide to 100 ml of PBS) and viewed on a Fluoview 1000 confocal microscope (Olympus, Center Valley, PA, http://www.olympus-global.com). Confocal stacks were processed using Imaris image analysis software (Bitplane, St. Paul, MN, http://www.bitplane.com).

## Cell Characterization Using Electron Microscopy

Adipose tissue pieces (1 mm$^3$) were immersion-fixed in 2.5% glutaraldehyde in PBS overnight and then processed as described [23]. For immunoelectron microscopy, tissue pieces were fixed in 2% paraformaldehyde in PBS for 2 hours and then dehydrated, embedded in gelatin capsules with LRWhite acrylic resin (SPI Supplies, West Chester, PA, http://www.2spi.com), and cured at 60°C following the manufacturer's directions. Ultrathin sections (70 nm) were labeled with anti-GFP antibody (Table 1) as described [22]. Sections were viewed on a JEM 1210 or 1011 transmission electron microscope (JEOL, Peabody, MA).

## Isolation and Culture of Rat ASCs

Adipose tissue was excised from both the omentum (i.e., abdominal) and the inguinal (i.e., subcutaneous) fat pads of naïve (e.g.,

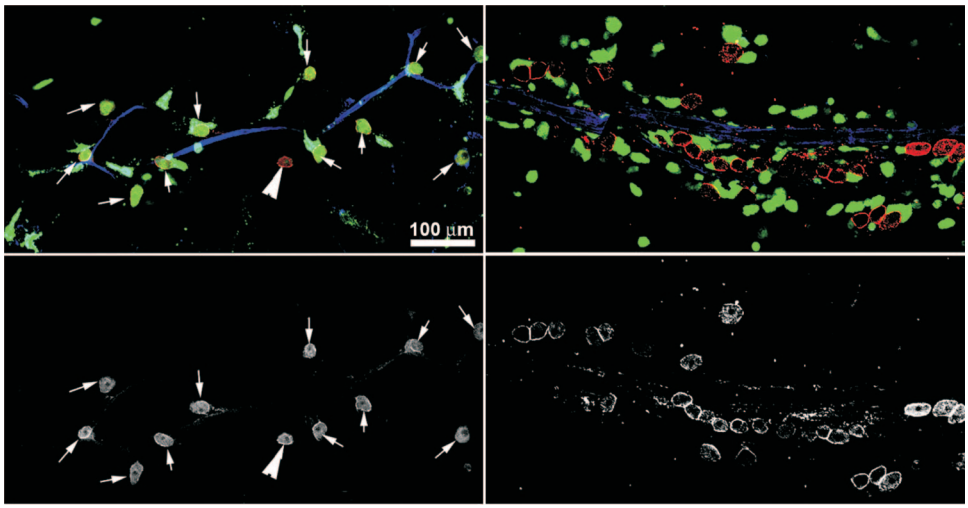


**Figure 2.** Adipose tissue from green fluorescent protein (GFP)$^+$ chimeric rats was stained for CD90 (Cy3, red) and counterstained for F-actin (blue). Areas within the tissue show variation in colocalization of CD90 with the GFP bone marrow marker. Top left panel, arrows indicate dual signal, arrowhead indicates GFP$^-$/CD90$^+$ cell. Bottom panels indicate black and white rendition of red channel. Top right panel shows an area without apparent colocalization of CD90 and GFP signal.

nonchimeric, GFP$^+$) and chimeric rats and underwent enzymatic digestion by 0.075% collagenase II (Worthington Biochemical, Lakewood, NJ, http://www.worthington-biochem.com) in Hanks' balanced saline solution for 60 minutes at 37°C with shaking. Digested tissue was filtered and centrifuged, and erythrocytes were removed by treatment with erythrocyte lysis buffer. The remaining cells were transferred to tissue culture flasks with Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, http://www.invitrogen.com) plus supplemental Ham's F-12 medium (F12) (Gibco), and after an attachment period of 6 hours, nonadherent cells were removed by a PBS (Gibco) wash. Attached cells were cultured in DMEM/F12 supplemented with 10% fetal bovine serum (Gibco), 0.1 μM dexamethasone (Sigma-Aldrich), 1% penicillin-streptomycin (Gibco), and 1.25 mg/l amphotericin B (Gibco) and expanded in vitro until passage 3.

### Adipocytic Differentiation and Histochemical Assays

ASCs (passage 3) were seeded into six-well tissue culture plates ($5 \times 10^5$ cells per well) and grown until subconfluence (1–2 days) in DMEM/F12. Medium was then replaced with adipogenic or control medium. Adipogenic medium (AM) consisted of DMEM/F12 supplemented with 33 μM biotin, 0.5 μM insulin, 17 μM D-pantothenic acid, 0.2 nM dexamethasone, 1 μM ciglitazone, 0.2 nM triiodothyronine, and 10 mg/l transferrin. Control medium was identical to the DMEM/F12 used during the expansion stage. After cells were grown to 100% confluence, adipogenic induction medium with 3-isobutyl-1-methylxanthine (540 μM) was added for 2 days. Then, for 12 days, the medium was changed every 48 hours with adipogenic medium. Cells were washed with PBS with EDTA twice and fixed with 10% buffered formalin for 10 minutes. Cells were then washed with distilled $H_2O$ twice, and stained with oil red O (20 ml of stock solution consisting of 30 mg of oil red O powder in 60 ml of 2-propanol [0.5%]). Next, 13.3 ml of $H_2O$ was added, and the cells were incubated for 30 minutes at room temperature. Cells were washed with $H_2O$ to remove debris. The resultant positive red stain was evaluated via light microscopy.

### Cell Surface Expression Using Flow Cytometry

Cultured ASCs (passage 3) were analyzed by flow cytometry for their surface marker expression. Antibodies used in this study are listed in Table 1. For flow cytometry, cultured ASCs were washed and incubated with monoclonal antibodies at 4°C for 30 minutes. After three washes with PBS, ASCs were further incubated for 30 minutes with secondary antibodies as needed. Stained cells were fixed in 1% paraformaldehyde and analyzed on an LSR II instrument (BD Biosciences, San Jose, CA, http://www.bdbiosciences.com), and data were analyzed using FACSDiva software (BD Biosciences). Isotype-matched nonspecific antibodies were used for the control.

### Statistical Analysis

Unless otherwise specified, the results are reported as mean ± SD. *t* tests were conducted to assess differences among treatment groups. Statistical significance was set at $p \leq .05$.

## RESULTS

### Immunohistochemistry of Rat Adipose Tissue

Whole fat tissue from the chimera was assessed for the markers described in Table 1 using immunofluorescence microscopy. Results indicate GFP$^+$ cells were evenly scattered around the 70–100-μm adipocytes (Fig. 1A). Most GFP$^+$ cells had a mesenchymal morphology and were observed to be smooth muscle actin (SMA)-positive. The SMA signal in the GFP$^+$ cells was equivalent to that observed in the pericytes surrounding the blood vessels. In Figure 1B, partial confocal stack shows a GFP$^+$ adipocyte in the BM chimera. The GFP$^+$ cytoplasmic labeling of the positive adipocyte surrounds the lipidic portion of the cell. This was observed only once in six different chimeric animals examined. GFP$^+$ blood cells were also identified within the F-actin$^+$ blood vessels (Fig. 1C). Transmission electron microscopy revealed that the typical GFP$^+$ cell within the adipose tissue integrated between adipocytes possessed large quantities of rough endoplasmic reticulum (Fig. 1D). Immuno-transmission electron microscopy analysis of LRWhite acrylic-embedded chimeric adipose tissue indicated that these cells were GFP$^+$ when stained for the GFP protein (Fig. 1E).

### Bone Marrow-Derived ASCs in Radiation Chimera

Whole adipose tissue from chimeric rats was stained for CD90 and counterstained for F-actin (Fig. 2). Areas within the tissue show variation in colocalization of CD90 with the GFP BM marker: both GFP$^-$/CD90$^+$ cells. The bottom panels in Figure 2 show black and white rendition of the red channel. The top right panel in Figure 2 shows an area without apparent colocalization of CD90 and GFP signal.

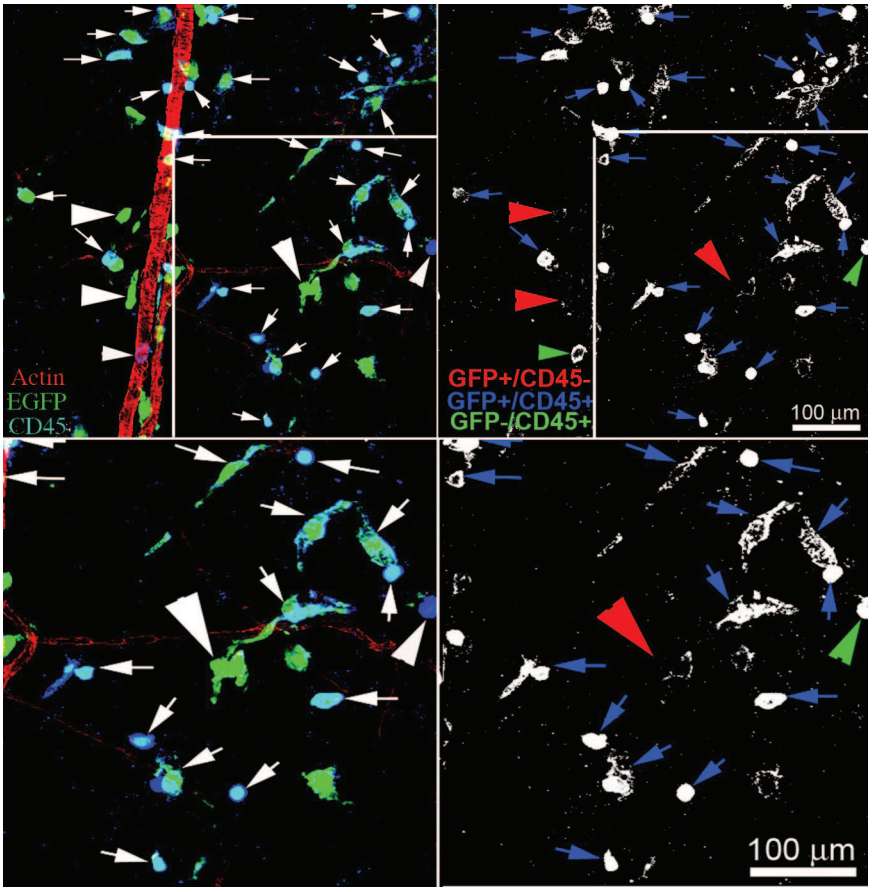


**Figure 3.** Adipose tissue from GFP$^+$ chimeric rats was stained for CD45 (Cy5, blue) and counterstained for F-actin (red). Top left panel shows single optical slice confocal image, and top right panel shows the black and white rendition of the Cy5 channel, accentuating the CD45$^+$ cells. Arrows indicate the cells that are both GFP$^+$/CD45$^+$, and arrowheads indicate the cells that are GFP$^+$/CD45$^-$. In the top right panel, there are also a few GFP$^-$/CD45$^+$ cells in this field with this phenotype. The bottom panels are higher magnification of the top panels. Abbreviations: EGFP, enhanced green fluorescent protein; GFP, green fluorescent protein.

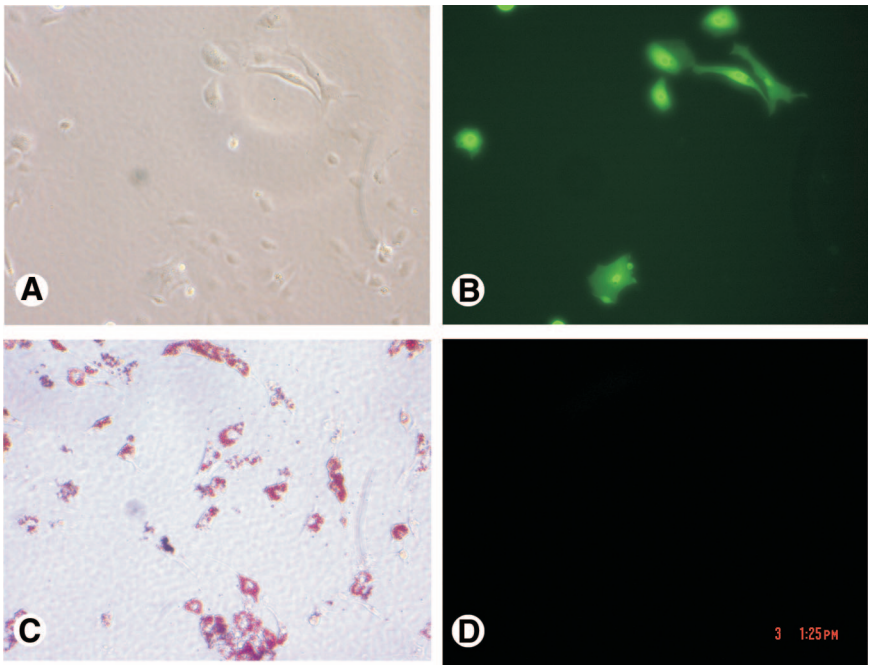


**Figure 4.** Adipose cells derived from tissue. **(A):** Light micrograph of isolated cells from adipose tissue at passage 3. **(B):** Corresponding image with green fluorescent protein (GFP)$^+$. **(C):** Light micrograph of adipose stem cells that had undergone adipogenesis. **(D):** Corresponding image with GFP$^+$.

In addition, the whole adipose tissue from GFP$^+$ chimeric rats was stained for CD45 and counterstained for F-actin (Fig. 3). It is evident that the majority of cells are both GFP$^+$/CD45$^+$, whereas a much smaller number of cells are GFP$^+$/CD45$^-$. In Figure 3, it is also evident that there are a few GFP$^-$/CD45$^+$ cells in this field.

## Adipose-Derived Stem Cell Isolation

Collagenase digestion of rat adipose tissue yielded three morphologically distinct populations of cells after culturing until passage 3. One population demonstrated a fibroblast-like morphology, similar to the ASCs described by Zuk et al. [10]. These

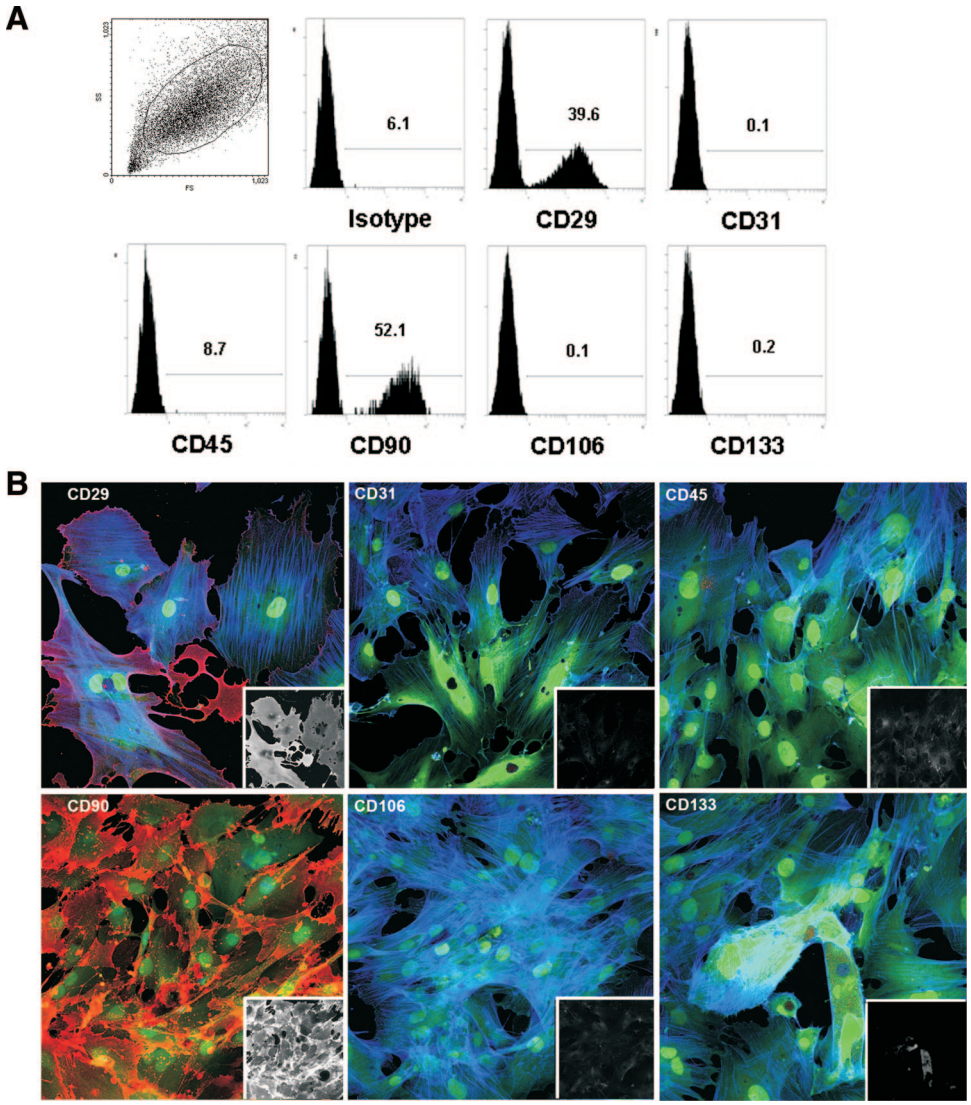


**Figure 5.** Surface marker expression of cultured passage 3 adipose-derived stem cells (ASCs) from naïve green fluorescent protein (GFP)$^+$ rats. **(A):** Representative plots of ASCs obtained by culturing the subcutaneous and abdominal fat tissues obtained from naïve animals. Flow cytometric analysis showed that cultured ASCs expressed CD29 and CD90. **(B):** Cells expressed CD29 and CD90 (red) in vitro, whereas the majority of these cells were negative for CD31, CD45, CD106, and CD133. Blue is F-actin stain and green is endogenous GFP signal. Insets are the black and white rendition of the red channel for each of these markers.

cells exhibited rapid in vitro expansion. A second, smaller population was GFP$^+$. A third population resembled endothelial cells. Figure 4A depicts the three populations in a light micrograph, and Figure 4B is the corresponding GFP$^+$ image, with only one population of the cells labeled positive for GFP.

## Oil Red O Staining

Cells cultured in AM exhibited lipid inclusion after 8 days. Figure 4C depicts a representative positive oil red O lipid stain of ASCs cultured in AM for 2 weeks, and Figure 4D depicts the corresponding GFP$^+$ image. No cells that had differentiated into adipocytes were GFP$^+$.

## Surface Marker Expression of ASCs

ASCs were isolated and cultured from subcutaneous and abdominal fat tissues obtained from GFP$^+$ radiation chimeras at 116–402 days after bone marrow transplantation. Surface marker expression of cultured ASCs was analyzed by flow cytometry. In the naïve (e.g., GFP$^+$, nonchimera) rats, nearly all ASCs obtained from subcutaneous and abdominal fat tissues of naïve animals were CD45$^-$ and CD29$^+$. The majority of the ASCs also expressed CD90, but they did not express CD31, CD106, or CD133 (Fig. 5A). Subsequently, CD90 and CD29 were used to determine the origin of ASCs in experiments of GFP radiation chimera. Mitchell et al. demonstrated that ASCs are positive for both CD90 and CD29, with expression increasing to >90% at passage 4 [24]. The findings were confirmed with immunocytochemistry of ASCs (Fig. 5B).

In the GFP chimeras, flow cytometry showed different levels of CD90 expression depending on the origin of the fat; although ASCs derived from both subcutaneous and abdominal fat tissues expressed CD29, ASCs from the abdominal fat showed significantly lower levels of CD90 expression than those from the subcutaneous fat (Fig. 6A). The majority of cultured ASCs obtained from GFP chimeras were GFP-negative; however, there was a small population (~5%) of GFP-expressing CD90$^+$ and/or CD29$^+$ ASCs (Fig. 6B). Interestingly, cultured GFP$^+$ ASCs tended to express low levels of CD90 and CD29, compared with GFP$^-$ ASCs. The finding was

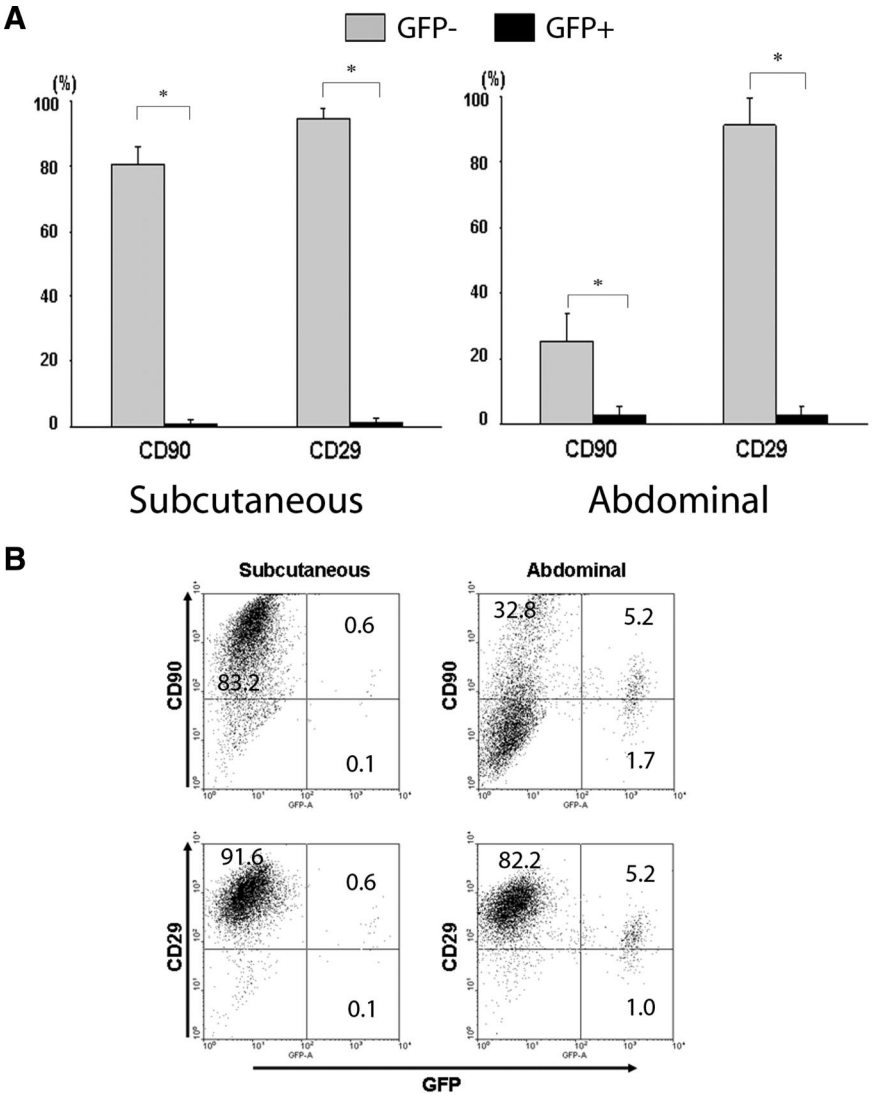


**Figure 6.** Surface marker expression of cultured passage 3 adipose-derived stem cells (ASCs) from a green fluorescent protein (GFP)$^+$ radiation chimera. **(A):** ASCs obtained from fat tissues of a radiation chimera were analyzed by flow cytometry. CD29- and/or CD90-expressing ASCs were mostly GFP-negative; however, small numbers of GFP-positive bone marrow-derived ASCs were detected. Of note, ASCs from the abdominal fat and subcutaneous fat differ in the expression of CD90. Data are mean ± SD of three experiments; $*$, $p \leq .05$. **(B):** Representative scattergrams showing GFP$^+$ CD29$^+$/CD90$^+$ ASCs. The intensity of the expression of CD29 and CD90 on GFP$^+$ ASCs was lower than that expressed on GFP$^-$ ASCs. The abdominal and subcutaneous fats were obtained from a radiation chimera at 161 days after bone marrow transplantation. Abbreviation: GFP, green fluorescent protein.

not different among fat tissues obtained from GFP chimeras at 116–402 days of bone marrow transplantation.

## DISCUSSION

The mesenchymal stem cell population from adipose tissue has recently been examined in great detail, yet the origin of adipose-derived stem cells is currently unknown. ASCs are easy to obtain and have demonstrated plasticity in differentiating to cells of mesenchymal lineage [11, 25–30]. Cells derived from discarded human fat have many properties that make them ideal for tissue engineering applications, such as immunocompatibility, multipotency, abundance, amenable to gene therapy, and capability of self-renewal. For example, Zuk et al. have reported that processed lipoaspirate contains a heterogeneous population of cells that exhibit a fibroblast-like morphology and have the capacity to differentiate into adipocytes, osteoblasts, and chondrocytes [25]. Gimble and Guilak have also reported the differentiation potential of ASCs [26]. Others have shown the potential of ASCs to differentiate into adipocytes [25], osteoblasts [27], chondrocytes [28], and cardiomyocytes [29].

The migration of stem cells after transplantation has been studied previously, including a study by ten Hove et al., who determined that donor-derived cells (from blood) were found in liver tissue specimens after allogeneic stem cell transplantation in nine female patients [31]. Migration of stem cells into the liver had previously been shown to be a rare event. For example, in a study of sex-mismatched liver transplant recipients, Ng et al. reported that although recipient cells constituted up to 50% of all cells in the liver allograft, most cells demonstrated macrophage/Kupffer cell differentiation, with only 1.6% showing hepatocytic differentiation [32]. In 2002, Körbling et al. reported that recipients of peripheral blood stem cells showed donor-derived hepatocytes up to 7% in liver tissue specimens endothelium by bone-marrow-derived cells [33]. Recently, Nakao et al. reported that infused bone marrow stem cells engraft into both the allogeneic marrow environment and the syngeneic extramarrow environment (intestinal graft) [34].

Bone marrow-derived mesenchymal stem cells have been used to promote engraftment of peripheral mononuclear blood cells in transplanted animals [35–41]. In 2005, Kim et al. reported the examination of adipose-derived mesenchymal stem cells to promote engraftment of hematopoietic stem cell transplantation in NOD/SCID mice, indicating that ASCs could be used as an alternative to bone marrow stem cells [35]. Planat-Benard et al. demonstrated that mouse adipose-derived stem cells, human adipose-derived stem cells, and even dedifferentiated adipocytes all induce significant neovascularization when

injected into the muscles of an ischemic mouse hind limb model (immunodeficient strains were used with human donor cells) [42].

Although the potential of ASCs is exciting, it is important to determine the origin of ASCs, as these cells could potentially cure hematopoietic malignancies that are as yet untreatable. We have observed a $GFP^+$ population of cells residing in the adipose tissue compartments after $GFP^+$ bone marrow transplantation. We conducted detailed immunohistochemical analysis of chimeric whole fat compartments and subsequently isolated, characterized, and cultured ASCs from $GFP^+$ BM chimeras. The immunohistochemistry analysis of fat tissue revealed that a large fraction of $GFP^+$ cells in adipose tissue were strongly positive for CD45 and smooth muscle actin (Fig. 1). The $CD45^+/SMA^+$ cells were evenly scattered around the adipocytes and blood vessels, whereas $CD45^+$ leukocytes within the vessels were $GFP^+$ (Fig. 3). A small fraction of $GFP^+/CD90^+$ cells also existed in the perivascular area (Fig. 2), but their presence was highly variable within the tissue.

In addition to whole fat analysis, ASCs were isolated, characterized, and cultured from two different adipose tissue compartments: the omental (abdominal) and the inguinal (subcutaneous) fat pads. Flow cytometric and immunocytochemical analyses showed that cultured ASCs were $CD45^-/CD90^+/CD29^+$ (Fig. 5). A significantly higher number of $GFP^-/CD90^+$ cells were isolated from the subcutaneous depot as compared with the abdominal depot (Fig. 6). Furthermore, the in vitro adipogenic differentiation of ASCs was achieved; however, all cells that had differentiated were $GFP^-$ (Fig. 4).

Based on phenotypical analysis, $GFP^+$ cells in adipose tissue in this rat model appear to be of both hematopoietic and mesenchymal origin; however, infrequent isolation of $GFP^+$ ASCs and their lack of adipogenic differentiation suggest that the contribution of BM to ASC generation is a rare event. This could also be due to the initial transplantation of a bone marrow cell population with a limited mesenchymal stem cell population or engraftment. Numerous previous studies have used the bone marrow transplantation model to examine the differentiation of BMCs into the parenchyma of various tissues; however, the bone marrow stroma and mesenchymal stem cells have been shown to remain of the host phenotype after experimental and clinical bone marrow transplantation [43–46], suggesting limited engraftment capability of donor BMCs into host bone marrow stroma compartment after transplantation. Nevertheless, both hematopoietic and mesenchymal $GFP^+$ cells were identified in the adipose tissue of radiation chimeras in this study, and the result may indicate the differentiation of hematopoietic stem cells into mesenchymal cell populations. Thus, although improved engraftment of BMCs into mesenchymal compartment might increase BM-derived ASCs, the study demonstrates that in spite of the plasticity of hematopoietic stem cells, they appear to have a limited contribution to ASC generation. Future studies to further understand the cell behavior after migration and the effect of body mass index include treating the rats with a high-fat diet. This study provides further insight into both the frequency of ASCs in two different rat depots and the stem cell migration into the adipose tissue compartments.

## CONCLUSION

These data increase our understanding of the origin of mesenchymal stem cells residing in adipose tissue by providing further insight into the migration of bone marrow cells into adipose tissue compartments. Both hematopoietic and mesenchymal stem cells were identified as migrating from the bone marrow into both adipose compartments; however, infrequent isolation of $GFP^+$ ASCs and their lack of adipogenic differentiation suggest that the contribution of BM to ASC generation might be minor. This could be correlated to the initial population of mesenchymal stem cells in the transplanted bone marrow cells. It was also found that the $GFP^-$ mesenchymal stem cell population was significantly higher in the subcutaneous depot as compared with the abdominal depot, indicating that the inguinal fat pad may be a better source of ASCs. Although in this rat study, the presence of a small population (~5%) of $GFP^+$ ASCs suggests that ASCs could be recruited from the bone marrow, if necessary, a significant $GFP^+$ mesenchymal stem cell population originating from the bone marrow was not identified. Additional studies can be conducted to identify alternative ASC origins, such as pericytes.

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## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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