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Surface Protein Characterization of Human Adipose Tissue-Derived Stromal Cells

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Human bone marrow stromal cells are a multipotent population of cells capable of differentiating into a number of mesodermal lineages as well as supporting hematopoeisis. Their distinct protein and gene expression phenotype is well characterized in the literature. Human adipose tissue presents an alternative source of multipotent stromal cells. In this study, we have defined the phenotype of the human adipose tissue-derived stromal cells in both the differentiated and undifferentiated states. Flow cytometry and immunohistochemistry show that human adipose tissue-derived stromal cells have a protein expression phenotype that is similar to that of human bone marrow stromal cells. Expressed proteins include CD9, CD10, CD13, CD29, CD34, CD44, CD 49_d, CD 49_e, CD54, CD55, CD59, CD105, CD106, CD146, and CD166. Expression of some of these proteins was further confirmed by PCR and immunoblot detection. Unlike human bone marrow-derived stromal cells, we did not detect the STRO-1 antigen on human adipose tissue-derived stromal cells. Cells cultured under adipogenic conditions uniquely expressed C/EBPα and PPARδ, two transcriptional regulators of adipogenesis. Cells cultured under osteogenic conditions were more likely to be in the proliferative phases of the cell cycle based on flow cytometric analysis of PCNA and Ki67. The similarities between the phenotypes of human adipose tissue-derived and human bone marrow-derived stromal cells could have broad implications for human tissue engineering. J. Cell. Physiol. 189: 54–63, 2001.

The bone marrow microenvironment contains a unique population of multipotent stromal cells; these can differentiate along the adipocyte, osteoblast, and other mesodermal pathways (Owen, 1988; Gimble, 1990; Gimble et al., 1996; Bianco and Robey, 2000; Nuttall and Gimble, 2000). Bone marrow stromal cells also support the proliferation and differentiation of hematopoietic stem cells (Kincade et al., 1988). This support is apparently dependent upon cell-cell interactions mediated by specific adhesion molecules expressed on the stromal cells (Kincade et al., 1988). The gene and protein expression profile of both murine and human bone marrow stromal cells has been defined. Significant antigens include VLA-4, VCAM-1, CD44, among others (Thomas et al., 1988; Gimble et al., 1989; Miyake et al., 1990, 1991a,b; Simmons and Torok-Storb, 1991a,b; Haynesworth et al., 1992; Gronthos et al., 1994, 1997, 1999; Simmons et al., 1994; Bruder et al., 1997, Bruder et al., 1998; Barry et al., 1999; Pittenger et al., 1999; Conget and Minguell, 1999, 2000; Deschaseaux and Charbord, 2000; Shin et al., 2000).

Bone marrow-derived mesenchymal stromal cells have clinical importance in applications for tissue engineering; however, they may only be obtained by bone marrow biopsy, a potentially painful procedure. It is hypothesized that similar multipotent stromal cells exist in tissue sites outside the bone marrow microenvironment. Support for this concept comes from a rare pathological condition known as progressive osseous heteroplasia (Kaplan et al., 1994). The subcutaneous adipose tissue in these patients forms ectopic bone for unknown reasons, suggesting that adipocytes or adipocyte progenitor cells may differentiate into osteoblasts (Kaplan et al., 1994). Improved methods have been

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developed to isolate and culture human subcutaneous adipose tissue-derived stromal cells (Hauner et al., 1989; Halvorsen et al., 2001). Under appropriate culture conditions, these cells differentiate along both the adipocyte and osteoblast pathways. The current work set out to define the gene and protein expression phenotype of the human adipose tissue-derived stromal cells in their undifferentiated and differentiated states. These results are compared to those of bone marrow stromal cells as defined in the literature.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher (Norcross, GA) unless otherwise noted. Type I collagenase was obtained from Worthington Biochemical (Lakewood, NJ). Bovine serum albumin (BSA), dexamethasone, biotin, pantothenate, Krebs–Ringer buffer, isoproterenol, and isobutyl methylxanthine (IBMX) were purchased from Sigma (St. Louis, MO). Human recombinant insulin was obtained from Boehringer Mannheim (Indianapolis, IN). The fetal bovine serum (FBS) was from HyClone (Logan, Utah). The Dulbecco modified Eagle's medium, phosphate buffer saline (PBS), and Ham's F-10 nutrient broth were obtained from Biologos (Naperville, IL) and the BGJ_b medium (Fitton–Jackson modification) from Gibco/BRL (Gaithersburg, MD). The thiazolidinedione BRL49653 was a gift from Coelacanth (New Brunswick, NJ). Dihydroxy (1,25) vitamin D_3 was purchased from BioMol (Plymouth Meeting, PA). All tissue culture flasks and plates were obtained from Corning (Corning, NY); 8-well chamber slides were obtained from Nalge Nunc International (Naperville, IL). Antibodies and their sources are listed in Table 1. NuPage™ polyacrylamide gels and immunoblot transfer reagents were purchased from Invitrogen (Carlsbad, CA).

Tissue preparation

The subcutaneous adipose was acquired from elective surgeries with the patient's consent as approved by the Institution Review Boards. The tissues used were from female patients of ages between 23 and 58 with a mean (±SEM) of 39.4 ± 3.1 years. The patients displayed a mean body mass index (kg/m^2) (± SEM) of 32.2 ± 4.6 (summarized in Table 2). Liposuction tissues were transported to the laboratory in saline solution within 2 h post-surgery. The tissue was washed at least three times with 2 volumes of Krebs–Ringer-bicarbonate (KRB; Sigma) to remove blood. The tissue was then digested with one volume of collagenase type I (1 g/liter of KRB with 1% BSA) for 60 min at 37°C with intermittent shaking. The floating adipocytes were separated from the stromal–vascular fraction by centrifugal force (300*g*) for 5 min. The preadipocytes in the stromal–vascular fraction were plated in tissue culture

TABLE 1. Monoclonal antibodies

Antigen	Clone	Application	Source
CD9, Tetraspan	B2C11	H	DSHB
CD9, Tetraspan	ALB6	F	IT
CD10, CALLA	ALB1	F	IT
CD11a, Integrin α_L /LFA-1	G-25.2	F	BD
CD11b, Integrin α_M /Mac-1	D12	F	BD
CD11c, Integrin α_X	3.9	F	BS
CD13, Aminopeptidase N	L138	F	BD
CD14	MΦP9	F	BD
CD18, Integrin β_2	L130	F	BD
CD29, Integrin β_1	4B4	F	C
CD31, PECAM-1	L133.1	F	BD
CD34	8G12(HPCA-2)	F	BD
CD44, Pgp-1	L178	F	BD
CD44, Pgp-1	H4C4, H9H11	H, W	DSHB
CD45, LCA	J.33	F	IT
CD49d, Integrin α_4	L25	F	BD
CD49e, Integrin α_5	SAM1	F	IT
CD49e, Integrin α_5	BIIG2	H	DSHB
CD50, ICAM-3	152-2D11	F	BS
CD54, ICAM-1	LB-2	F	BD
CD55, DAF	IA10	F	P
CD56, NCAM	MY31	F	BD
CD56, NCAM	AG1, B58	H	DSHB
CD59, Complement protectin	P282(H19)	F	P
CD62E, E-selectin	P2H3	F	DSHB
CD105, Endoglin	P4A4	F, H	DSHB
CD106, VCAM-1	P8B1	F, H	DSHB
CD146, Muc-18	CC9	H, W	S. Gronthos
CD166, ALCAM	RD1-CD166-3FT	F	RD
HLA-ABC	G46-2.6	F	P
HLA-DR	L243	F	BD

Abbreviations: ALCAM, Activated Leukocyte Adhesion Molecule; BD, Becton-Dickinson; BS, BioSource; C, Coulter; CALLA, Common Acute Lymphocytic Leukemia Antigen; CD, cluster of differentiation; DAF, Decay Accelerating Factor; DSHB, Developmental Studies Hybridoma Bank, Iowa City, IA; F, fluorescence activated cell sorting; H, Immunohistochemistry; ICAM, Intercellular Adhesion Molecule; IT, Immunotech; LCA, Leukocyte Common Antigen; NCAM, Neural Cell Adhesion Molecule; P, Pharmingen; PECAM-1, Platelet Endothelial Cell Adhesion Molecule; Pgp-1, Phagocytic glycoprotein-1/Hyaluronate Receptor; RD, Research Diagnostics; VCAM, Vascular Cell Adhesion Molecule; W, Western Immunoblot.

TABLE 2. Tissue donor information

Lot number	Age (years)	BMI (kg/m^2)	Experimental use
1	42	27.34	H
2	39	55.10	H
3	37	34.64	H
4	42	24.10	H
5	27	73.33	H
6	57	27.55	H
7	23	22.55	F,W
8	32	23.81	F
9	41	20.29	F
10	58	27.48	W
11	45	25.11	F
12	30	22.71	F,W
Mean (±SEM)	39.4 ± 3.1	32.6 ± 4.6	

All lots were obtained from female patients. There was no significant difference in age or BMI between lots used for immunohistochemistry (H), fluorescence activated cell sorting (F), or Western immunoblot analysis (W) (ANOVA, $P = 0.66$ for age and $P = 0.11$ for BMI).

flasks at 3,500 cells/cm^2 in Dulbecco's modified Eagle's–Ham's F-10 medium (vol/vol, 1:1) supplemented with 10% FBS, 15 mM HEPES (pH 7.4), 100 U/ml penicillin, 100 μg/ml streptomycin, and 25 ng/ml amphotericin B (pre-adipocyte medium). The primary cells were cultured for 4–5 days until they reached confluence and were defined as "Passage 0". The cells were then harvested by digestion with 0.5 mM EDTA/ 0.05% trypsin (Biologos), centrifuged at 1200 rpm for 5 min, resuspended in pre-adipocyte medium, and plated at a density of approximately 10,000 cells/cm^2. The cells were passaged twice and were then used for assays or cyropreserved in liquid nitrogen in media supplemented with 7% dimethyl sulfoxide (DMSO) prior to subsequent experimentation (Halvorsen et al., (in press)).

Osteogenic conditions

To induce mineralization, the stromal cells were harvested using trypsin/EDTA and re-plated in multiple-well plates at 30,000 cells/cm^2 for 16 h to allow attachment in pre-adipocyte medium (Day 0). On Day 1, the medium was changed to BGJ_b medium (Fitton–Jackson Modification) supplemented with 10% FBS, 100 U penicillin/ml, 100 μg streptomycin/ml, 10 mM β-glycerophosphate, and 50 μg/ml 2-phosphate ascorbate. Additional supplements included 10 nM 1,25 vitamin D_3 and/or 10 nM dexamethasone. Cultures were fed every 3rd day throughout the study.

Adipogenic conditions

To induce adipogenesis, the stromal cells were harvested using trypsin/EDTA and plated in multiple-well plates at 30,000 cells/cm^2 for 16 h to allow attachment in pre-adipocyte medium (Day 0) (Halvorsen et al., 2001). On Day 1, the medium was then changed to Dulbecco's modified Eagle's –Ham's F-10 medium (vol/vol, 1:1) supplemented with 3% FBS, 15mM HEPES (pH 7.4), biotin (33 μM), pantothenate (17 μM, Sigma), human recombinant insulin (100 nM, Boerhinger Mannheim), dexamethasone (1 μM), 1-methyl-3-isobutylxanthine (IBMX) (0.25 mM), and BRL49653 (1 μM) (differentiation medium) for a 3-day period. From Day 4 onwards, the cells were fed every 3rd day with the same medium without IBMX and BRL49653 supplementation (adipocyte medium).

Histochemistry

Cells were cultured under each condition in 8-well chamber slides for 9 days. All subsequent steps were carried out at room temperature. Prior to immunostaining, the specimens were washed with PBS and then fixed in 95% ethanol with 5% glacial acetic acid for 3 min. After washing in PBS the specimens were treated with 5% DMSO containing 0.25% Triton-X 100 for 10 min and then washed three times in PBS containing 0.05% Tween 20 (PBS-T). Non-specific antibody binding was blocked by pre-incubating the slides with 5% normal goat serum for 1 h. Subsequently, the normal goat serum was removed and the slides were incubated with saturating levels of primary antibodies (Table 1) for 1 h. Replicate samples were treated with the appropriate negative control isotype-matched mouse antibodies, or with pre-immune rabbit serum under the same conditions. The slides were washed with PBS-T and then incubated with the respective secondary antibodies (biotin-conjugated goat anti-rabbit antibody, Vector Laboratories, Burlingame, CA, or biotin-conjugated goat anti-mouse antibody, Caltag Laboratories, San Francisco, CA) diluted 1/200, for 45 min. After washing three times in PBS-T the slides were incubated with peroxidase/ streptavidin complex (ABC kit, Vector Laboratories) for 30 min. Peroxidase activity was assessed using a peroxidase AEC substrate kit (Vector Laboratories) according to the manufacturer's recommendations. The specimens were washed in water and mounted using Vectormount.

Immunofluorescent analysis of cell cycle

Adherent monolayers for each condition were harvested with trypsin/EDTA to obtain single cell suspensions then washed twice in cold PBS. Approximately, 2×10^5 cells were pelleted into several 4 ml polypropylene tubes (Becton Dickinson, Lincoln Park, NJ). The cells were fixed for 10 min with cold 70% ethanol on ice. The cells were then washed three times with PBS and then incubated with the monoclonal antibodies specific for Ki-67 (FITC-conjugated) and PCNA (PE-conjugated) (DAKO Corp., Carpinteria, CA) for 45 min on ice. Replicate tubes were incubated with FITC- and PE-conjugated isotype-matched control antibodies of irrelevant specificity (Becton Dickinson). After washing twice in PBS the cells were analysed using a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems). Positive expression was defined as the level of fluorescence greater than 99% of the corresponding isotype-matched control antibodies.

Flow cytometry

For flow cytometric analyses, cells cultured under either control or differentiation conditions were harvested using trypsin-EDTA digestion, centrifuged for 5 min at 1200 rpm, and resuspended at a concentration of 10^6 cells/ml in Iscove's modified Dulbecco's medium/ 2% FBS. Aliquots containing 10^5 cells were incubated with individual primary antibodies (Table 1) for 15 min at room temperature. The cells were washed in PBS containing 2% FBS. When necessary, the cells were

TABLE 3. Oligonucleotide primers for polymerase chain reaction

Gene		Primer	Size (bp)	T_m (°C)
Actin	F	AGCCATGTACGTTGCTA	745	60
	R	AGTCCGCCTAGAAGCA		
Collagen type I	F	TGACGAGACCAAGAACTG	599	60
	R	CCATCCAAACCACTGAAACC		
CD 10	F	TTGTAAGCAGCCTCAGCCG	459	60
	R	TTGTCCACCTTTTCTCGGAG		
CD 13	F	GCCGTGTGCACAATCATCGCACT	449	60
	R	CACCAGGGAGCCCTTGAGGTG		
CD 44	F	GATCCACCCCAATTCCATCTGTGC	674	60
	R	AACCGCGAGAATCAAAGCCAAGGCC		
CD 59	F	ACACTCTACTACATGTGACTG	439	58
	R	TGCAAAAGTCAGCCTATGCC		
CD 105	F	TGTCTCACTTCATGCCTCAGCT	377	58
	R	AGGCTGTCCATGTTGAGGAGT		
CD 166	F	AGATACCATTATCATCATACCTTGCCGACT	157	60
	R	TGTCTTTGTATTCGTGTACATCGTCG		

Abbreviations: F, forward primer; R, reverse primer.

incubated with a fluorescent conjugated secondary antibody at room temperature. Finally, the cells were fixed in 10% formalin prepared in PBS containing 2% FBS. Samples were analyzed using a FACSCalibur cytometer (Becton Dickinson Immunocytometry Systems).

Immunoblots

Undifferentiated human adipose tissue-derived stromal cells were rinsed three times in ice cold PBS containing 0.1 mM Na_3VO_4 (PBS-V) and lysed in 100 μl of buffer containing 10% glycerol, 2% sodium dodecyl sulfate, 11.3 μg/ml aprotinin, 6.86 μg/ml leupeptin, 0.686 mM PMSF, 6.86 μM Na_3VO_4, 0.637% NP40, 12.6 mM Tris-HCl (pH 8.0), 95.1 mM NaCl, 0.634 mM EDTA, 0.0634% NaN_3. Aliquots containing μg of boiled protein lysate were loaded onto 4–12% bis-Tris acrylamide gels, electrophoresed, and transferred to nitrocellulose membranes. The immunoblots were blocked in PBS with 5% non-fat powdered milk and 0.1% Tween for 1 h at room temperature and incubated with primary antibodies (murine hybridoma supernatants) diluted 1:20 in PBS with 1% non-fat powdered milk and 0.1% Tween overnight with rocking at 4° C. After four washes with PBS/0.1% Tween, the immunoblots were incubated with horseradish peroxidase coupled goat anti-mouse Ig secondary antibodies diluted 1:10,000 in PBS with 0.2% non-fat powdered milk and 0.1% Tween with rocking for 1 h at room temperature and washed three times with PBS/0.1% Tween. Antibody complexes were visualized using the ECL plus chemiluminescent kit (Amersham Pharmacia, Piscataway, NJ) and autoradiographic exposure.

RNA isolation and polymerase chain reaction

Human adipose tissue-derived stromal cells were cultured under control conditions or were induced with either the adipocyte differentiation or mineralization protocol for 9 days. Total RNA was isolated from these cells using TriReagent (Molecular Research Center, Cincinnati, OH) (Chomczynski and Sacchi, 1987). Reverse transcriptase reactions were performed with 1 μg of total RNA using the GeneAmp RNA PCR Kit (Perkin Elmer, Branchburg, NJ). Polymerase chain reactions were performed using a 5 min 94°C; a cycle of 30 sec at 94°C, 1 min at a primer set specific annealing temperature, and 1 min at 72°C; and an 8 min extension at 72°C (annealing temperatures are indicated in Table 3 for each oligonucleotide primer pair). Oligonucleotide primer sets (forward, F; reverse, R) specific for the listed human cDNAs (28,29) were synthesized by Gibco-BRL (Gaithersburg, MD).

RESULTS

Surface protein expression by undifferentiated adipose tissue-derived stromal cells

Initial studies used flow cytometry to quantify the surface protein expression on stromal cells derived from human adipose tissue. In these preliminary experiments, the stromal cells were cultured in the absence of any inductive stimuli. Representative histograms are shown in Figure 1. Positive staining was defined as a fluorescent intensity greater than 99% of that obtained with the isotype-matched control antibody ("M1" bracket in histogram panel). The mean results obtained using a panel of 25 antibodies on stromal cells from 2 to 5 individual donors are summarized in Table 4. The undifferentiated stromal cells were consistently positive for the following surface proteins: class I histocompatibility antigen (HLA-ABC), CD9, CD10, CD13, CD29, CD34, CD44, $CD49_e$, CD54, CD55, CD59, CD105, and CD166.

These results were confirmed using polymerase chain reactions performed with reverse transcribed cDNA from the undifferentiated adipose derived stromal (Fig. 2). In addition, the expression of CD44 and CD146 were confirmed by Western immunoblotting (Fig. 3).

Relative protein expression by adipose tissue-derived stromal cells cultured under adipogenic, osteogenic, and undifferentiated conditions

After 14 days or more of induction with appropriate culture media, the human adipose tissue-derived stromal cells display morphologic features consistent with adipogenesis or osteogenesis. Representative photomicrographs in Figure 4 show adipocytes stained positive with Oil Red O and mineralized cultures stained positive with Alizarin Red. To examine whether cell antigen expression changed with differentiation, adipose tissue

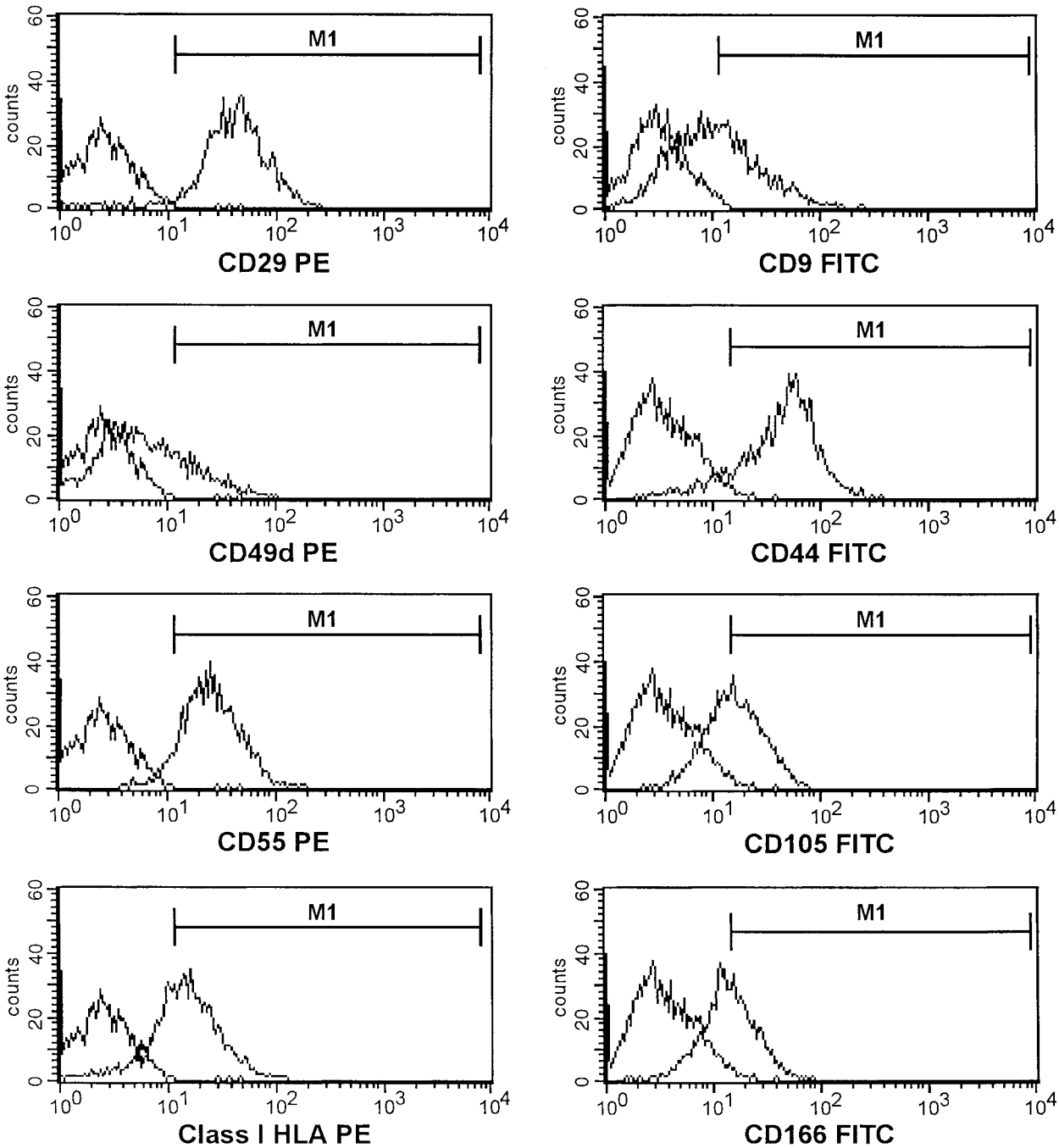

Fig. 1. Flow cytometric analysis of undifferentiated human adipose tissue-derived stromal cells. Undifferentiated stromal cells from an individual donor were stained with monoclonal antibodies directed against either HLA-ABC, CD29, $CD49_e$, or CD55 and coupled to phycoerythrin (PE) or directed against either CD9, CD44, CD105, or CD166 and coupled to fluorescein isothiocyanate (FITC); these signals are indicated as solid lines. An isotype matched monoclonal antibody served as a control; these signals are indicated as dotted lines. The "M1" window represents fluorescent intensity exceeding that of 99% of the antibody control stained cells. Representative of n = 5 donors.

derived stromal cells from five different donors were cultured on chamber slides for 9 days to maintain them as undifferentiated cells or after induction of adipogenesis or osteogenesis.

Immunohistochemical staining was then performed on fixed cultures using a panel of monoclonal or polyclonal antibodies. Representative photomicrographs are shown in Figure 5 the average results from five different donors are summarized in Table 5. The expression profile for the majority of cell surface proteins was similar under undifferentiated, adipogenic, and osteogenic conditions (Table 5). Only the transcriptional regulators of adipogenesis, CCAAT/enhancer binding protein α (C/EBPα) and peroxisome

TABLE 4. Flow cytometric analysis of human adipose derived stromal cells (undifferentiated)

Antigen	Mean % positive cells (±SEM)
HLA- ABC	93 ± 3
HLA- DR	1 ± 0
CD9	28 ± 8
CD11a	0
CD11b	1 ± 0
CD11c	0
CD10	79 ± 5
CD13	99 ± 2
CD14	0
CD18	0
CD 29	98 ± 1
CD 31	1 ± 1
CD34	28 ± 13
CD44	60 ± 15
CD45	0
CD49d	9 ± 2
CD49e	22 ± 7
CD50	0
CD54	42 ± 8
CD55	56 ± 8
CD56	0
CD59	97 ± 1
CD62e	2 ± 1
CD105	36 ± 9
CD166	27 ± 9

Results are the mean ± standard error of the mean (SEM) of flow cytometry of 5,000–10,000 cells obtained from 3–5 donors except for CD10, which is the mean of two donors. Percent positive is defined as fluorescent intensity greater than 99% of isotype matched antibody control.

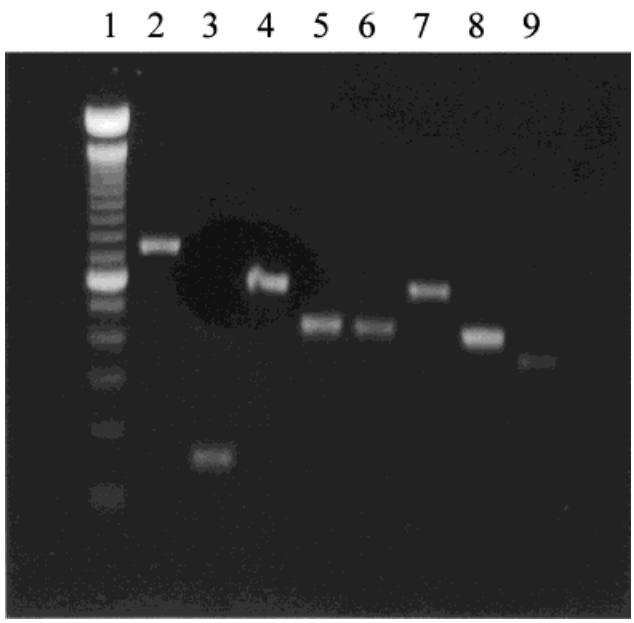

Fig. 2. Gene expression by undifferentiated human adipose tissue-derived stromal cells based on polymerase chain reaction. Total RNA isolated from undifferentiated human adipose tissue-derived stromal cells was reverse transcribed and amplified with primers specific for the indicated cDNAs: (1) 100 bp DNA ladder; (2) actin, 800 bp; (3) CD166, 157 bp; (4) collagen type I, 599 bp; (5) CD10, 459 bp; (6) CD13, 449 bp; (7) CD44, 674 bp; (8) CD59, 434 bp. Representative of n = 3 donors.

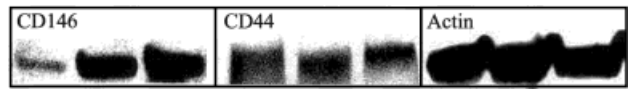

Fig. 3. Immunoblot detection of proteins expressed by undifferentiated human adipose tissue-derived stromal cells. Total cell lysates from three individual donors (18 μg in **lane 1**, 32 μg in **lanes 2** and **3**) were electrophoresed on 4–12% Bis-Tris acrylamide gels, transferred and immunoblotted with antibodies directed against the following antigens (sizes indicated in kDa): CD146 (115 kDa), CD44 (90 kDa), actin (42 kDa).

proliferator activated receptor γ (PPARγ), increased under adipogenic conditions relative to the other culture conditions.

Differentiation events are associated with altered cell cycle regulation. Cell cycle events can be distinguished based on expression of proliferating cell nuclear antigen (PCNA) and Ki67; cells in G_0/G_1 are $PCNA^-/Ki67^-$, in S phase are $PCNA^+/Ki67^+$ or $PCNA^+/Ki67^-$, and in G_2/M are $PCNA^-/Ki67^+$ (Landberg et al., 1990). Both PCNA and Ki67 were examined by flow cytometry in stromal cells from two individual donors maintained for 9 days under each differentiation condition (Fig. 6). While the majority of cells under undifferentiated conditions were in $G_0/G1$ phase (69%), a greater percentage of cells under adipogenic or osteogenic conditions were in S phase (47 and 77%).

DISCUSSION

This study has defined the gene and protein expression profile of human adipose tissue-derived stromal cells; these results are summarized in Table 6. The phenotype of the human adipose tissue-derived stromal cells is similar to that of human bone marrow-derived stromal cells (Kuznetsov et al., 1997; Filshie et al., 1998; Nutall et al., 1998; Conget and Minguell, 1999; Gronthos et al., 1999; Park et al., 1999; Pittenger et al., 1999). In general, the two stromal cell populations share the same adhesion and receptor molecules (Simmons and Torok-Storb, 1991a,b; Haynesworth et al., 1992; Galmiche et al., 1993; Simmons et al., 1994; Li et al., 1995; Rickard et al., 1996; Gronthos et al., 1997, 1999; Kuznetsov et al., 1997; Bruder et al., 1997, 1998; Filshie et al., 1998; Hicok et al., 1998; Majumdar et al., 1998; Barry et al., 1999; Conget and Minguell, 1999; Pittenger et al., 1999; Remy-Martin et al., 1999). Two of these common surface markers, CD105 and CD166, have been used to define a bone marrow stromal cell population defined as mesenchymal stem cells or MSCs which are capable of adipogenesis, chondrogenesis, osteogenesis, and hematopoietic support (Haynesworth et al., 1992; Bruder et al., 1998; Majumdar et al., 1998, 2000; Barry et al., 1999; Cortes et al., 1999; Pittenger et al., 1999). A number of the surface markers have been implicated in hematopoietic support. For example, antibodies directed against CD9 (Oritani et al., 1996; Aoyama et al., 1999), CD29 (Jacobsen et al., 1992; Wu et al., 1994), CD44 (Miyake et al., 1990), $CD49_d$ (Miyake et al., 1991a), and CD106 (Miyake et al., 1991b; Funk et al., 1995) interfere with the proliferation and differentiation of hematopoietic stem cells. Preliminary studies indicate that adipose tissue-derived stromal cells are capable of supporting hematopoiesis in vitro (R.W.S.,

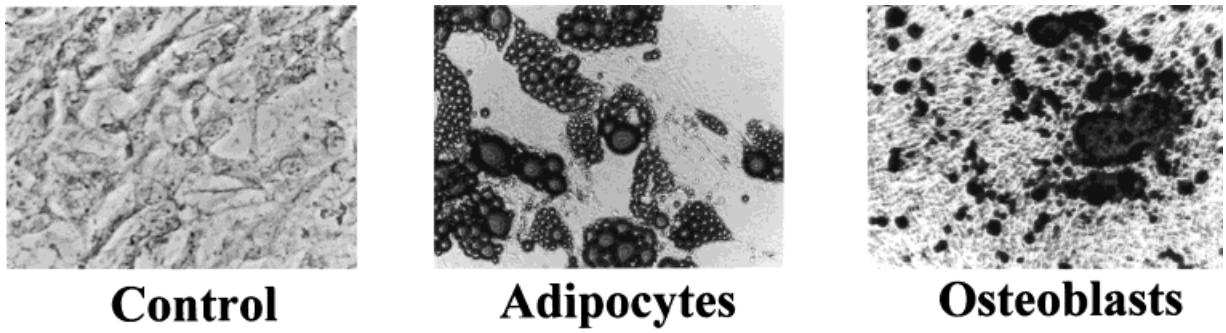

Fig. 4. Representative phase contrast photomicrographs of human adipose tissue-derived stromal cells cultured under control, adipogenic, or osteogenic conditions. Adipogenic cultures were stained with Oil Red O 14 days after induction while osteogenic cultures were stained with Alizarin Red after 21 days of culture.

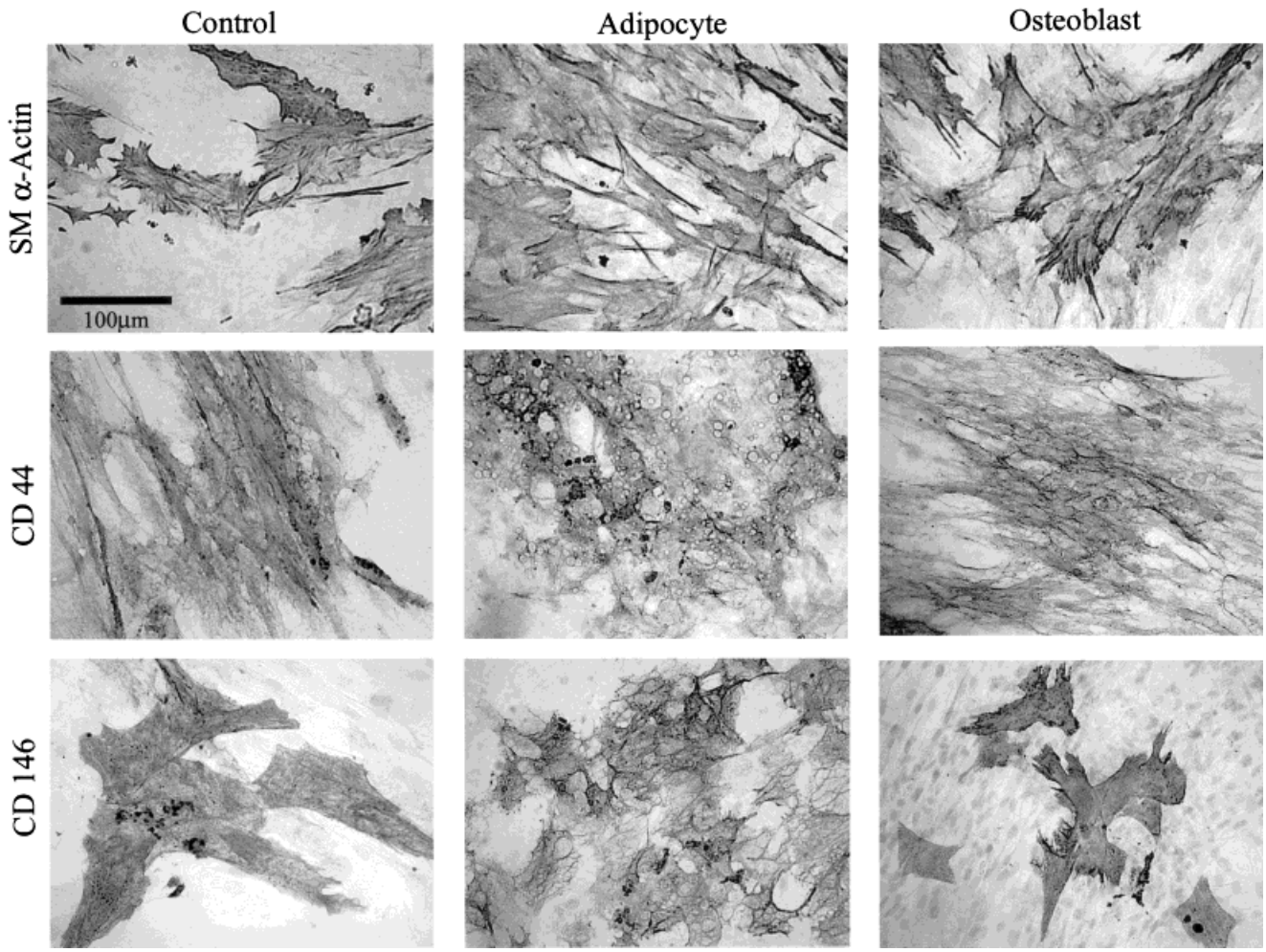

Fig. 5. Histochemical detection of protein expression by human adipose tissue-derived stromal cells cultured under control (undifferentiated), adipogenic, or osteogenic conditions. Adipose tissue-derived stromal cells were maintained for 9 days under each growth condition, fixed, and immunohistochemically stained with antibodies detecting α-smooth muscle actin, CD44, or CD146 and an appropriate peroxidase coupled secondary antibodies. No histochemical staining was obtained on slides stained with the secondary antibody alone. Representative of n = 5 donors.

TABLE 5. Immunohistochemistry of differentiated adipose derived stromal cells

Marker	Pre-adipocyte	Adipocyte	Mineral
CD14	−−	−	−
CD29	++/+	++	++/+
CD31	−	−	−
CD34	−	−	−
CD44	++/+	++/+	++/+
CD45	−	−	−
CD105	+	+	+
CD106	+	+	+
CD146	++/+/−	++/+/−	++/+/−
MyoD	−	−	−
Myoblast	−	−	−
α-SM actin	++/+/−	++/+/−	++/+/−
FIIIV-rag	−	−	−
STRO-1	−	−	−
Collagen-I	++/+	++/+/−	++/+
Collagen-III	++/+	++/+	++/+
Osteonectin	++/+	++/+	++/+
Osteopontin	+/−	+/−	+/−
C/EBPα	+/−	++/+	++/+/−
PPARγ	−	++/+/−	+/−

Strong staining (++); Weak staining (+); Negative (−); Subpopulation (/). Abbreviations: VCAM-1, vascular cell adhesion molecule; α-SM actin, α-smooth muscle actin; FIIIV-rag, factor VIII related antigen; C/EBP α, CAAT/enhancer binding protein α; PPARγ, peroxisome proliferator activated receptor γ.

H.A.L., D.M.F., J.M.G., unpublished observations) in addition to their adipogenic and osteogenic capabilities.

The current study is not an exhaustive examination of the adipose tissue-derived stromal cell's surface protein profile; the bone marrow and other literature suggests that additional markers should be evaluated in the future. Candidate proteins include the integrins α_v β_3 and α_v β_5, which play a role in osteoclast support (McHugh et al., 2000) and adenoviral transduction (Conget and Minguell, 1999, 2000), respectively. The cadherin family also deserves further examination. Recent studies using murine embryonic fibroblasts indicate that the levels of N-cadherin, R-cadherin, and cadherin-11 change in association with adipocyte and ostoblast differentiation (Shin et al., 2000). The behavior of cadherin proteins on differentiating human adipose tissue-and bone marrow-derived stromal cells remains to be determined.

The protein expression profile of adipose tissue-derived stromal cells is not identical to that of reported for bone marrow-derived stromal cells in the literature. While adipose derived stromal cells are STRO-1 negative, cultured bone marrow stromal cells are reported as STRO-1 positive; however, it should be noted that these STRO-1 positive cells represent less than 3–5% of the total bone marrow stromal population (Simmons et al., 1991; Simmons and Torok-Storb, 1991a,b; Gronthos et al., 1994, 1999; Oyajobi et al., 1999; Stewart et al., 1999; Walsh et al., 2000). Indeed, there are conflicting reports concerning bone marrow stromal cell expression of specific proteins. For example, bone marrow stromal cells are reported as both $CD49_d$ negative (Pittenger

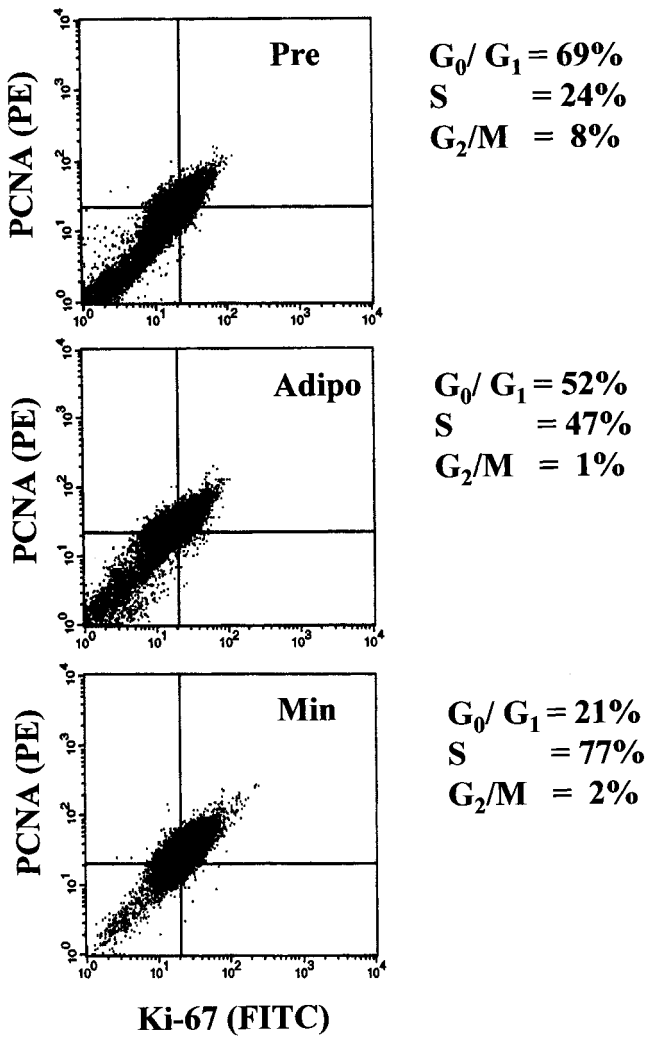

Fig. 6. Flow cytometric analysis of cell proliferation markers PCNA and Ki-67. Human adipose tissue-derived stromal cells were maintained for 9 days under control, adipogenic, or osteogenic growth conditions. Fixed cells were double stained with a FITC-conjugated Ki-67 antibody (X-axis) and a PE-conjugated PCNA antibody (Y-axis) and analyzed by flow cytometry. The percentage of cells in different stages of the cell cycle are indicated. Representative of n = 2 donors.

TABLE 6. Undifferentiated human adipose stromal cell protein summary[a]

	Positive		Negative	
Integrins	β_1	CD29	β_1	CD18
	α_4	$CD49_d$	α_L	CD11a
	a_a	$CD49_e$	α_M	CD11b
			α_X	CD11c
Metalloproteinase	CALLA	CD10		
	Aminopeptidase N	CD13		
Adhesion	Tetraspan	CD9	PECAM	CD31
Receptor molecule	Hyaluronate	CD44	ICAM-3	CD50
	ICAM-1	CD54	NCAM	CD56
	Endoglin	CD105	E Selectin	CD62e
	VCAM	CD106		
	Muc18	CD146		
	ALCAM	CD166		
Extracell matrix	Collagen Type I		STRO-1	
	Collagen Type III		Factor VIII related Ag	
	Osteopontin			
	Osteonectin			
Smooth Muscle	α-smooth muscle actin			
Hematopoietic	CD34[1]			CD14
			LCA	CD45
HLA	A,B,C (Class I)		DR (Class II)	
Other	DAF	CD55		
	Complement protectin	CD59		

[a]Data presented is based on results obtained by flow cytometry or immunohistochemical staining of human adipose tissue-derived stromal cells. Superscript "1" indicates positive staining based on flow cytometry but negative staining based on immunohistochemistry.
Abbreviations: ALCAM, activated lymphocyte cell adhesion molecule; CALLA, common acute lymphocytic leukemia antigen; CD, cluster of differentiation; DAF, decay accelerating factor; E selectin, endothelial selectin; HLA, histocompatibility locus antigens; ICAM, intercellular adhesion molecule; LCA, leukocyte common antigen; NCAM, neural cell adhesion molecule; PECAM, platelet endothelial cell adhesion molecule; VCAM, vascular cell adhesion molecule.

et al., 1999; Conget and Minguell, 1999) and as weakly positive (Simmons et al., 1994). While Pittenger et al. (1999) and Simmons et al. (1994) detected CD106 by flow cytometry, Conget and Minguell (1999) did not. Likewise, human bone marrow stromal cells have been described as both CD34 positive (Simmons and Torok-Storb, 1991a,b) and negative (Pittenger et al., 1999, Conget and Minguell, 1999). Some of these discrepancies may reflect the proliferative stage of the cells in culture. Alternatively, donor heterogeneity may be responsible. Additional studies will be required to fully define the differences between human adipose tissue- and bone marrow-derived stromal cells.

While the bone marrow has been the major source of multipotent stromal cells, other tissues may prove to be alternatives. For example, mesenchymal progenitor cells capable of multiple differentiation pathways were recently isolated from umbilical cord blood (Erices et al., 2000). The current findings now identify the adipose tissue as another site containing stromal cells with phenotypic characteristics resembling those of stromal cells from the bone marrow microenvironment.

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