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Purification and Characterization of Adipose-Derived Stem Cells From Patients With Lipoaspirate Transplant

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Techniques for medical tissue regeneration require an abundant source of human adult stem cells. There is increasing evidence that adipose stem cells contribute to restoration of tissue vascularization and organ function. The object of our study was to isolate and characterize adult adipose-derived stem cells from patients undergoing on lipoaspirate transplant with the aim to improve tissue regeneration. Adipose-derived stem cells were isolated and purified from the lipoaspirate of 15 patients and characterized for CD markers and the ability to differentiate toward the adipogenic lineage. We found that purified adipose stem cells express high level of CD49d, CD44, CD90, CD105, CD13, and CD71 and these markers of staminality were maintained at high level for at least 3 months and seven passages of in vitro culture. As expected, these cells resulted negative for the endothelial and hematopoietic-specific markers CD31, CD106, CD34, and CD45. Differentiation towards adipogenic lineage demonstrated that purified adipose-derived stem cells are still able to become adipocytes at least 3 months after in vitro culture. The analysis of Akt and MAPK phosphorylation confirmed a modulation of their activity during differentiation. Interestingly, we established for the first time that, among the p53 family members, a strong upregulation of p63 expression occurs in adipocytic differentiation, indicating a role for this transcription factor in adipocytic differentiation. Taken together, these data indicate that purified lipoaspirate-derived stem cells maintain their characteristic of staminality for a long period of in vitro culture, suggesting that they could be applied for cell-based therapy to improve autologous lipoaspirate transplant.

Key words: Lipoaspirate transplant; Tissue regeneration; Stem cells; Adipocyte differentiation; p53 family

INTRODUCTION

The use of lipoaspirate as filling material is a powerful technique for tissue repair in plastic surgery (6,8, 10,11). This technique is often employed for reconstitution of tissues damaged by injuries, burns, radiolesions, ulcers, and general surgery. Of relevance, its use is increasing in oncology to repair tissue damaged by either conservative or nonconservative surgical treatments, such as mastectomy (3,7,29). In the US, at least 400,000 people per year are transplanted by autologous lipoaspirate (13). At present, this technique is considered in plastic surgery, the most qualified for simplicity, safety, and reproducibility. However, the use of purified adipose-derived stem cells might improve this surgical procedure by shortening the time to achieve the esthetical results, increasing patients' life quality (24).

Several in vitro studies and animal models support autologous transplant of bone marrow-derived stem cells in different types of lesion. These data demonstrate the potentiality of bone marrow in reconstituting tissue vascularization and restoring function (14,28,34). Adipose tissue shares several biological properties with bone marrow because adipose adult stem cells can be induced to differentiate toward different lineages such as myogenic (20), epithelial (2), endothelial (4,27), and neurogenic (25,31).

Adipose tissue is a potential source of adult and so-

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matic stem cells and hold promise for a range of therapeutic applications. In addition, adipose tissue is a reservoir of growth factors and cytokines that stimulate the growth of adipose-derived stem cells to improve cell proliferation and differentiation (1,26). Even though autologous transplant of adipose tissue is an old therapeutic procedure still used to repair a wide range of tissue damages, the recent biological studies performed on stem cells suggest the possibility to significantly improve the potentiality of tissue regeneration by making use of adipose-derived stem cells (30). In particular, adipose-derived stem cells can be found in abundant quantities, can be obtained from lipoaspirate with a noninvasive procedure, and can be transplanted to autologous hosts (12).

For this purpose, we speculated on the possibility to purify and characterize adipose-derived stem cells from lipoaspirate of patients undergoing autologous transplant, to evaluate the possibility of improving tissue regeneration. In particular, the object of this study was to purify and characterize adipose-derived stem cells to verify whether these cell cultures are able to maintain the characteristic of stemness after long-term of in vitro culture. The stemness characterization was evaluated by the capability to differentiate toward the adipogenic lineage after 2 weeks and 3 months of in vitro culture. Overall, the data we obtained strongly suggest that the adipose-derived stem cells we purified can be applied in the near future for cell therapy using the cell-assisted lipotransfer technique, previously described in mice (16).

MATERIALS AND METHODS

Patients

At the First Division of Plastic and Reconstructive Surgery, San Gallicano Hospital, 15 patients were treated with lipoaspirate transplant, in the regimen of Day Hospital or Hospitalization, as reported in Table 1. Among them, 14 patients were affected by mammary carcinoma and one by osteomyelitis in the left leg. Patients with progressive lesions after radiation therapy were screened and assessed according to LENT-SOMA scale (29). Table 1 reports patient classification based on objective clinical evaluation; none of them had a medical history of connective, metabolic, or skin diseases. The study was reviewed and approved by the ethical committee of San Gallicano Institute, and written informed consent was obtained from all patients.

Materials

To purify adipose stem cells, 100–3000 ml of residual adipose tissues obtained from the abdominal area of donors with the biggest body mass was used. In brief, tissue was kept at room temperature for a few hours before stem cells extraction. Hanks balanced salt solution (HBSS), fetal bovine serum (FBS), penicillin-streptomycin mix, fungizone, DMEM F12 were from Invitrogen (Milan, IT). Collagenase A, Histopaque-1077, 1-methyl-3-isobutylxanthine, dexamethasone, indomethacin, insulin, and Oil-Red O were from Sigma-Aldrich (Milan, IT). MidiMACS separation unit and MACS LD columns were from Miltenyi Biotec (Florence, IT). Monoclonal antibodies (mAb) anti-human CD45, CD31, CD49F, CD106, CD13, CD34, CD44, CD71, CD90, and CD105 were from Società Italiana Chimici (Rome, IT). The F(ab′)2 FITC-conjugated secondary antibodies were from Cappel (West Chester, PA, USA). The anti-p53 (FL-393), the mAb to p63 (4A4), p73 (C-20 and H-79), and p21 (C-19) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The rabbit anti-total and phospho-AKT (Ser473) antibodies were purchased from Cell Signaling (Milan, IT). The rabbit anti-total and phospho-MAPK antibodies were purchased from BioLabs (Milan, IT).

Adipose-Derived Stem Cell Isolation and Purification

After informed consent, we obtained liposuction aspirates from donors using an IRB protocol. The patients were transplanted using the Coleman method (7). In brief, fat was harvested during microliposuction under tumescent local anesthesia (injecting Klein's solution) using Coleman's microcannulas. The liposuction aspirates was derived from the abdomen and used as source

Table 1. Summary of Adipose Donor Tissue Patients

No. of Median	Median Age	Area Involved	Radiotherapy	LENT-SOMA Grade*
2	59	breast mastectomy	no	
3	50	breast mastectomy	yes	2 and 3
4	48	breast quadrantectomy	no	
5	55	breast quadrantectomy	yes	1 and 2
1	58	osteomyelitis	no	

*Grade of severity.

for processed lipoaspirate cells (PLA). Fat was centrifuged in closed sterilized systems at 3000 rpm for 3 min and the middle layer of centrifuged tissue was utilized to purify stem cells, whereas the oily upper layer and the red-serum bottom layer were totally discarded. The fatty portion of lipoaspirate was extensively washed with HBSS supplemented with antibiotics and fungizone to remove the majority of the erythrocytes and leucocytes, contaminating debris (bottom layer), and the yellow layer composed of oil from the destruction of fat fragments (top layer).

The lipoapirates was digested with 0.075% collagenase in HBSS for 30 min on a shaker at 37°C. The digestion was stopped adding 10% FBS and the digested tissue was separated by centrifugation at $400 \times g$ for 10 min. The pellet containing the stromal vascular fraction (SVF) was resuspended in red blood cell lysis buffer (2.06 g/L Tris base, pH 7.2, and 7.49 g/L NH_4Cl) to remove remaining erythrocytes, incubated at RT for 10 min, and centrifuged at $300 \times g$ for 10 min. Pellets were collected and filtered sequentially through 100- and 40-μm cell strainers to remove undigested tissue. Next, pellets were resuspended and processed for density gradient centrifugation by Histopaque at $300 \times g$ for 30 min. The white band was aspirated, washed, and purified by subtraction using magnetic microbeads anti-CD31 and CD45-conjugated antibodies to eliminate the majority of endothelial cells and leucocytes. Total effluent cells were centrifuged, washed, and resuspended in DMEM/F12 containing 50% of FBS, antibiotics, and fungizone. Primary cells were cultured at the concentration of 10^5 cells/ml at 37°C, 5% CO_2 in humidified air for 7 days. The medium was then removed and replaced with same medium containing 20% of FBS. Cells were expanded until passage 7 and in two cases until passage 11 (more than 3 months) to control their level of staminality and the capability to differentiate toward the adipogenic lineage.

Flow Cytometry Analysis

During expansion, cells at passage 2 and 7 (named AT2 and AT7, respectively) were examined by flow cytometry (FACS) to analyze the expression of stem cell-specific cell surface antigens. To assess the CD expression, cells were harvested using citrate saline buffer (0.134 M KCl, 0.015 M Na citrate), washed twice with cold PBS containing 0.002% EDTA and 10 mM NaN_3. Samples (1×10^5 cells) were incubated for 1 h at 4°C with saturating concentrations of primary Ab diluted in PBS containing 0.5% bovine serum albumin (BSA). Cells were then washed with PBS containing 0.5% BSA and incubated for 1 h at 4°C with 50 μl of FITC-conjugated secondary Ab diluted 1:20 in PBS/BSA. After washing, cell suspensions were analyzed by a flow cytometer (Epics XL analyzer, Coulter Corporation, Miami, FL) after addition of 5 μl of a 1 mg/ml solution of propidium iodide to exclude nonviable cells. At least 1×10^4 cells per sample were analyzed.

Adipose Cell Differentiation

To evaluate the "stemness" of adipose-established stem cell lines, we examined the capability of the cells to differentiate toward the adipogenic lineage. Cells between passage 2 and 7 were plated in complete medium containing 10% FBS and grown until confluence. Then 0.5 μM 1-methyl-3-isobutylxanthine, 1 μM dexamethasone, 10 μg/ml insulin, and 100 μM indomethacin were added to the culture medium for 2 weeks. Complete medium was changed every 4 days until the end of treatment. To visualize lipid droplets, cells were fixed with 4% of formalin and stained with Oil-Red O. Cells were analyzed by deconvolution microscope DM132 from LEICA (Milan, IT).

Western Blot Analysis

To analyze the level of p21, p63, p53, and p73 protein expression, cells were lysed with RIPA buffer [50 mM Tris (pH 8), 150 mM NaCl, 1% Nonidet P40, 0.1% deoxycholate, 0.1% SDS, 1 mM PMSF, 5 mM Na_3VO_4, 50 mM protease inhibitors (Sigma-Aldrich, Milan, IT)] for 30 min at 4°C. Total cell lysates were clarified by centrifugation at 14,000 rpm for 30 min. Aliquots of cell extracts containing equivalent amount of proteins were resolved by SDS-polyacrilamide gel electrophoresis 10% (SDS-PAGE) and transferred to nitrocellulose. To analyze Akt and MAPK activation, stem cells were serum deprived for 12 h before and after differentiation.

Table 2. Number of Purified Adipose-Derived Stem Cells

Patient No.	Age of Patient	Lipoaspirate (ml)	No. of Purified Stem Cells
1	58	200	7.5×10^5
2	50	300	7.5×10^5
3	53	200	2.3×10^6
4	40	200	7.5×10^5
5	50	100	1.5×10^5
6	47	200	3×10^5
7	47	200	3×10^5
8	65	350	2.25×10^6
9	69	300	2.3×10^5
10	67	600	8×10^6
11	45	150	5×10^5
12	48	150	4×10^5
13	70	100	2×10^5
14	45	100	2×10^5
15	60	150	3×10^5

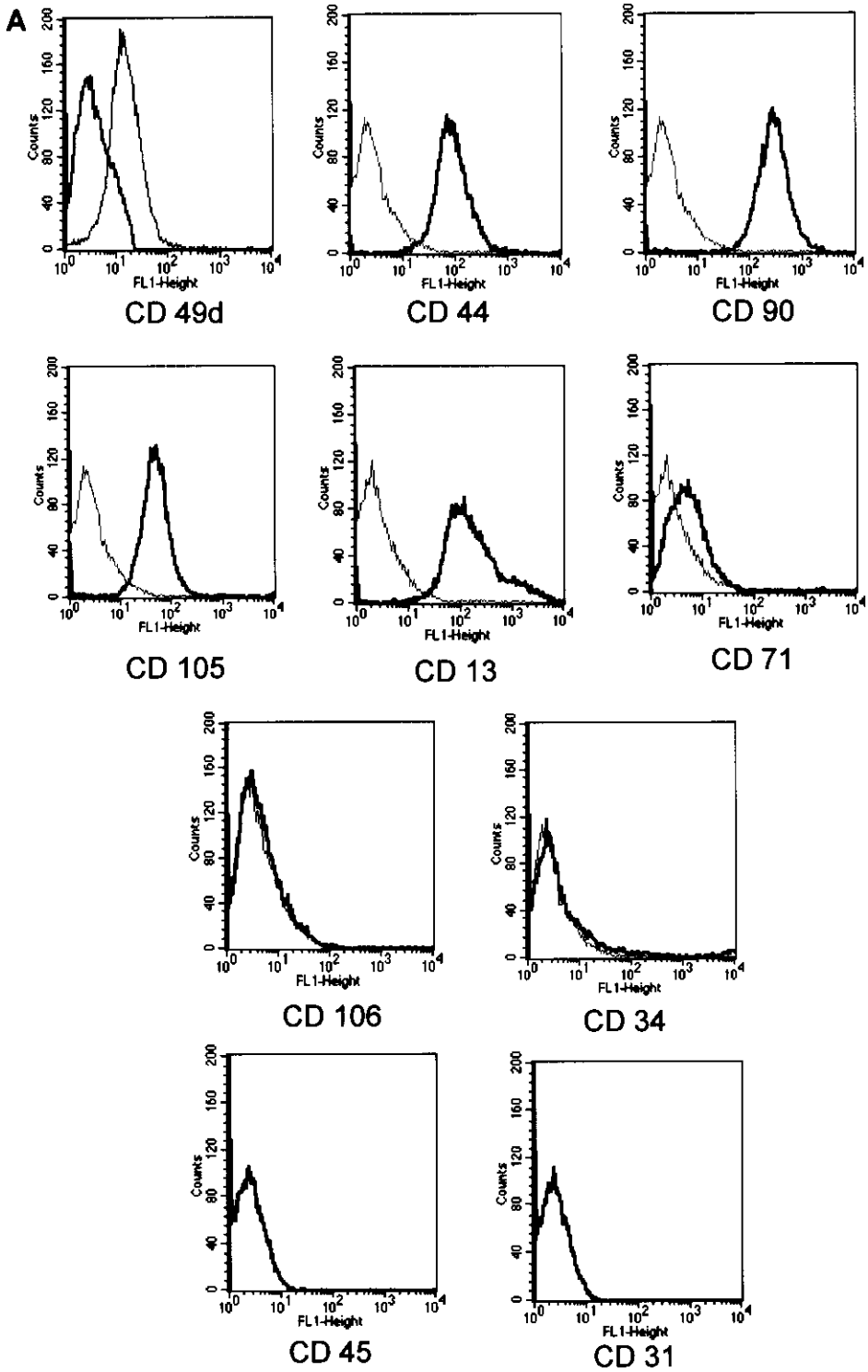

Figure 1. Cytolfuorimetric analysis of CD markers on adipose-derived stem cells. After purification, stem cells were analyzed by FACS to evaluate the level of endogenous stemness-related CD markers CD49f, CD44, CD90, CD105, CD13, and CD71 at passage 2 (A) and 7 (B) of in vitro culture. Cells were also analyzed for the expression of markers CD106, CD34, CD45, and CD31 at both passages of in vitro culture that, as expected, resulted negative (A, B). The result shown is representative of seven independent experiments. The mean of fluorescence and relative SD are reported in Table 3.

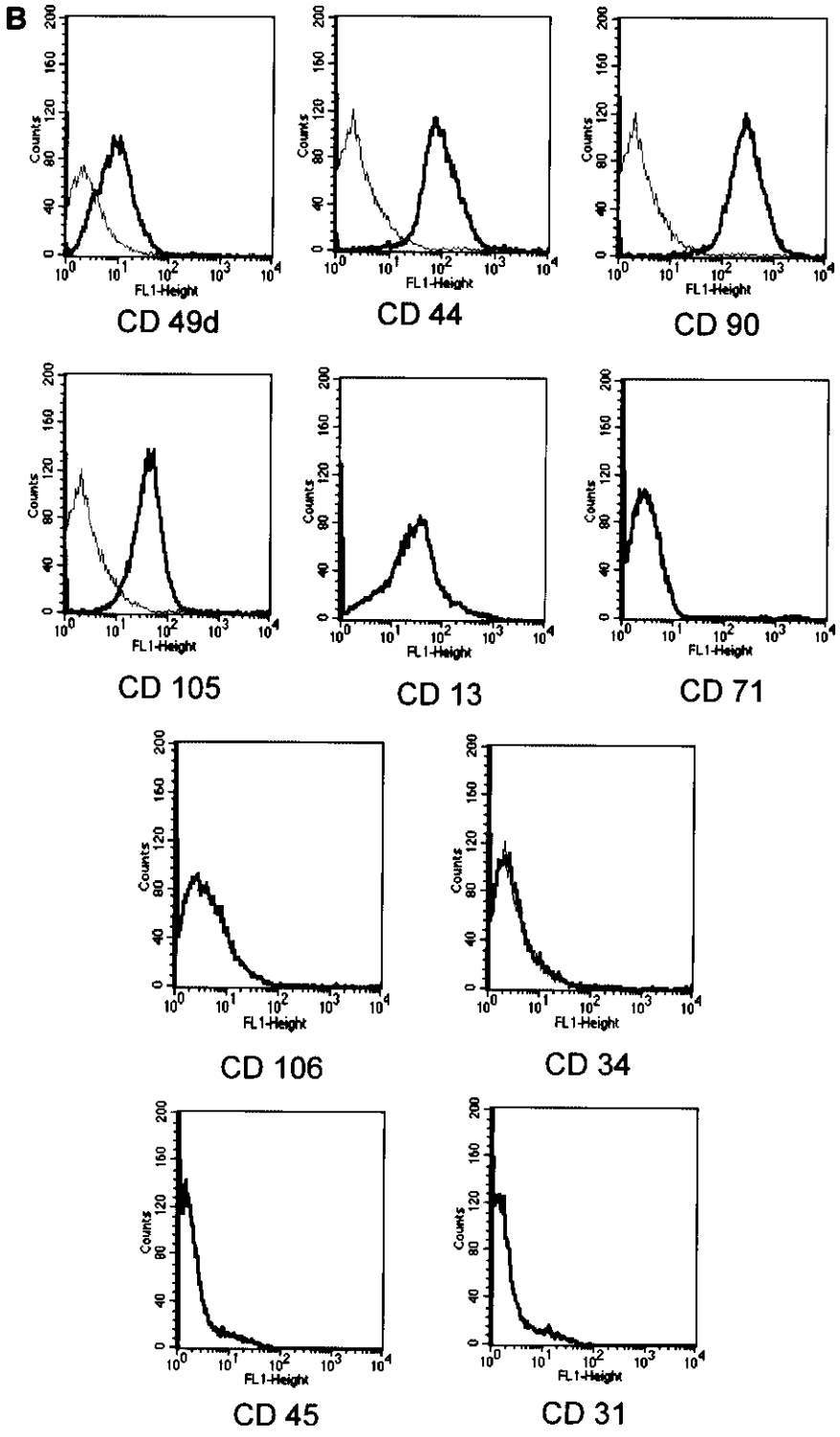

Figure 1. Continued.

Table 3. CD Markers Analysis in Seven Stem Cells Lines

	Passage 2 of In Vitro Culture		Passage 7 of In Vitro Culture	
CD Marker	Mean of Fluorescence	SD	Mean of Fluorescence	SD
49d	18.86	1.11	13.24	1.53
44	104.77	1.74	110.72	3.93
90	304.39	1.93	343.19	7.11
105	69.36	1.11	66.14	2.87
13	333.75	3.63	44.10	2.26
71	22.76	1.24	9.95	2.45
106	8.55	0.62	9.22	0.78
34	4.40	0.36	4.22	0.37
45	2.74	0.54	3.36	0.42
31	2.93	0.62	3.98	0.75

Cells were washed three times with ice-cold PBS and lysed with NP40 buffer [1% Nonidet P40, 10% glycerol, 137 mM NaCl, 20 mM Tris HCl (pH 7,4), 50 mM NaF, 1 mM PMSF, 5 mM Na_3VO_4, 50 mM protease inhibitors (Sigma-Aldrich)] for 30 min at 4°C. Total cell lysates were clarified by centrifugation at 14,000 rpm for 30 min. Aliquots of cell extracts containing equivalent amount of proteins were resolved by SDS-PAGE and transferred to nitrocellulose. Total proteins were then probed with Abs direct to p53 family members, and with the rabbit polyclonal Ab directs to P-Akt and P-MAPK, respectively. As secondary Abs, the horseradish peroxidase-coniugated goat anti-mouse or rabbit were used; the chemiluminescence was resolved by enhanced chemiluminescence ECL kit (Amersham, Milan, IT). Total proteins were normalized by anti-actin, total-Akt or MAPK Abs.

Senescence-Associated β-Galactosidase Assay

Adipose-derived stem cells at passages 2 and 7 or 11 in vitro underwent β-galactosidase (β-gal) staining. In this assay, β-gal stains senescent cells at pH 6.0. When cells were subconfluent (1×10^6 cells per flask), monolayers were repeatedly washed with PBS, fixed in 2% formaldehyde/0.2% glutaraldehyde for 3–5 min at room temperature, and washed twice in PBS. Cells were then incubated at 37°C (without CO_2) with the senescence-associated β-gal staining solution: 1 mg of 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) per milliliter in dimethylformamide; 40 mm citric acid/sodium phosphate (pH 6.0); 5 mm potassium ferrocyanide; 5 mm potassium ferricyanide; 150 mm sodium chloride; 2 mm magnesium chloride. The X-Gal solution had to be added just before use. The cells were observed for the blue-green staining after 12–16 h.

Statistical Analysis

All experiments were performed as single experiment and the results shown in the figures are representative of several experiments we performed in different adipose-derived stem cell preparation. However, numerical data were reported as means of seven or five experiments, as reported above, ±SD.

RESULTS

Cytofluorimetric Analysis of Purified Adipose Stem Cells

After purification by subtraction using magnetic bead-conjugated antibodies recognizing CD45 and CD31, purified adipose-derived stem cells were counted, as reported in Table 2, and plated at the concentration of 10^5 cell/ml. Purified stem cells were harvested at passage 2 and 7 of in vitro culture and analyzed by FACS to evaluate the stemness-related CD markers to verify whether these cells were able to maintain the characteristics of staminality. As shown in Figure 1A, purified adipose-derived stem cells express, at passage 2, high level of CD49f, CD44, CD90, CD105, CD13, and CD71. As shown in Figure 1B, these levels of expression were maintained until passage 7 corresponding to at least 3 months of in vitro culture. As expected, the purified adipose-derived stem cells were negative for the expression of endothelial and hematopoietic stemness-related markers, such as CD106, CD34, CD45, and CD31, independently from the passage of in vitro culture (Fig. 1A, B). The results shown in Figure 1 are representative of seven independent experiments. Table 3 reports the mean fluorescence value corresponding to each CD marker with the relative SD.

Adipogenic Differentiation

Because one of the aims of this work was to evaluate the capability of purified adipose-derived stem cells to differentiate towards the adipogenic lineage in vitro, the cultured cells at passage 2 and 7 were incubated with 1-methyl-3 isobutylxanthine, 1 μM dexamethasone, 10 μg/ml insulin, and 100 μM indomethacin for 2 weeks. The adipogenic differentation was visualized by lipid droplets stained with Oil-Red O. As reported in Figure 2A and B (right panels), purified adipose-stem cells were able to differentiate towards adipocitic lineage, as indicated by the lipid droplets, at both passages in vitro, compared to untreated proliferating cells (left panels). These results are representative of five independent experiments. The percentage of differentiated cells (90% compared to proliferating cells ± SD) (Fig. 2, lower panels) indicates that the purified adipose-derived stem cells are able to maintain a good level of staminality at least for 3 months of in vitro culture, as indicated by the high

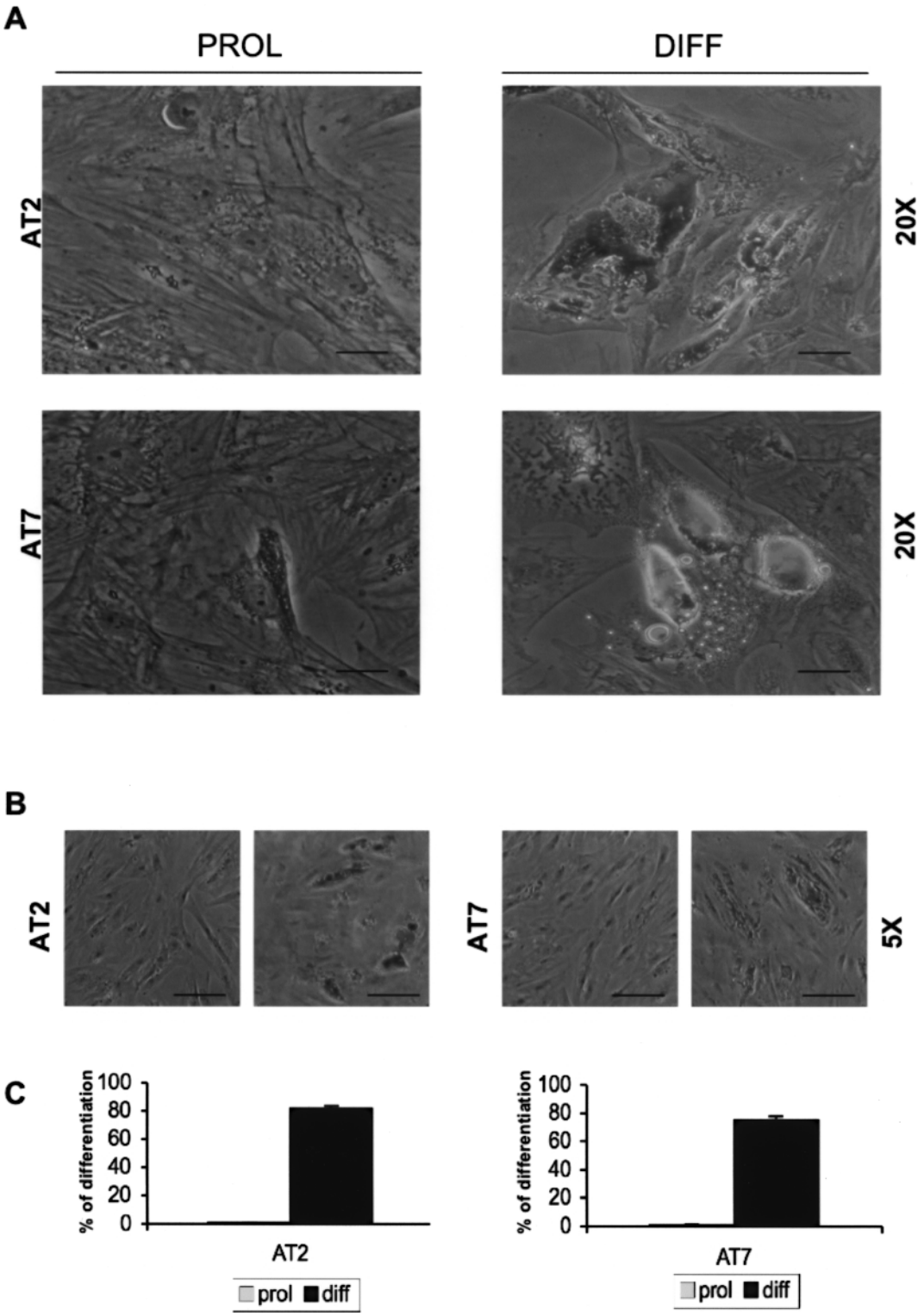

Figure 2. The adipose-derived stem cells were analyzed for the capability to differentiate toward the adipocytic lineage. (A) The figure shows control proliferating (left panel) and differentiated (right panel) cells, following 2 weeks in the presence of differentiation culture medium, at passage 2 and 7 of in vitro culture (20× of magnification). Scale bars: 100 μm. (B) Proliferating and differentiated cells at 5× of magnification. Scale bars: 25 μm. (C) The result showed in the figure is representative of five independent experiments. The percentage of differentiated cells was reported in the graphs at both passages of in vitro culture ± SD.

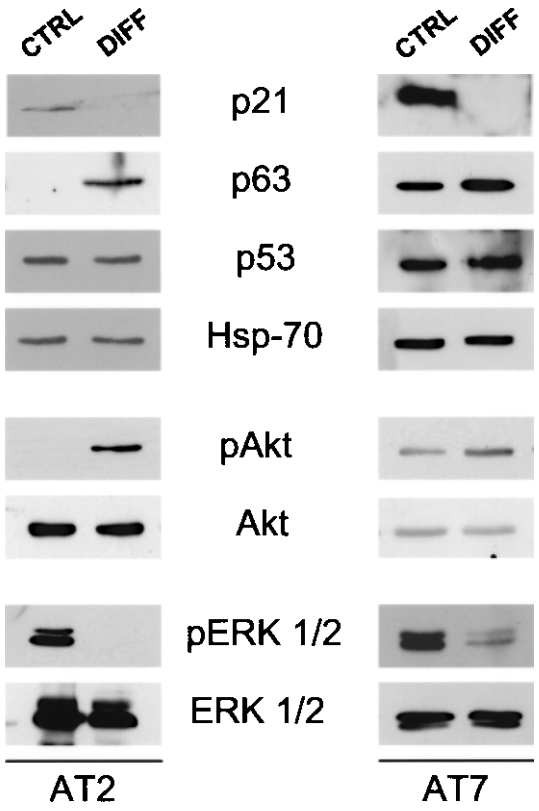

Figure 3. The p63 transcription factor is upregulated during adipocytic differentiation. The expression levels of p63, p53, p-Akt, p-ERK1-2, and $p21^{WAF1}$ were analyzed in proliferating and adipocytioc differentiated stem cells at passage 2 and 7 of in vitro culture, respectively. Hsp-70, total Akt, total ERK1/2, and actin Abs were use to normalize the loaded proteins. Overlapping results were obtained in five different adipose-derived stem cell lines.

capability to differentiate toward adipogenic lineage. In addition, we found that after 3 months of in vitro culture the cells slowed their proliferation and, in some cases, they stopped growth, suggesting that they might undergo senescence. However, the reactivity to β-galactosidase, a senescence marker, did not show any positivity until passage 11 of in vitro culture (data not shown). These data suggest that the slow down of cell proliferation observed at passage 7 is due cell cycle arrest, whose nature has to be defined.

The Transcription Factor p63 Plays a Role in Adipogenic Cell Differentiation

It was previously demonstrated that during adipocyte differentiation, when cells exit from the cell cycle and enter into a predifferentiation state of postmitotic growth arrest, there is a significant increase of the cyclin-dependent kinase inhibitor $p21^{WAF1}$ followed by a robust downregulation of $p21^{WAF1}$ when the cells are terminally differentiated (22). The complete inhibition of $p21^{WAF1}$ expression we found upon 2 weeks of differentiation at different passages of in vitro culture (2,7) was in agreement with previous results and confirmed the terminal differentiation status of our stem cells toward the adipocytic lineage (22). Even though the stem molecular signature of adipose-derived stroma cell was previously reported (26), and in the last year much information has been acquired concerning the involvement of transcription factors in the processes that regulate proliferation and differentiation of stem cells (23), it is not yet clear the role of p53 family members in this process.

Recently, a role for p53 in the mesenchimal differentiation programs has been reported (21). Thus, we evaluated the expression of p53 transcription factor family in the differentiation of adipose-derived stem cells towards adipogetic lineage. Interestingly, we found that p63 is expressed in proliferating stem cells and that it is upregulated in terminally differentiated adipocytes (Fig. 3), suggesting a role for this molecule in adipogenesis. We were unable to detect differences in p53 expression levels between proliferating and adipocytic cells (Fig. 3), and unable to detect p73 in both conditions (data not shown). The terminal differentiation towards adipogenic lineage we induced in adipose-derived stem cells was confirmed by the upregulation of phospho-Akt (36) and the downregulation of phospho-MAPK, as previously reported for insulin treatment (Fig. 3) (9). The low-level expression of p63 and the low activation of P-Akt at passage 7 in proliferating cells suggest that, at high passage of in vitro culture, the stem cells might be already committed to differentiate. We confirmed these results using five different adipose-derived stem cell lines. The loading proteins were normalized by the use of Abs direct to Hsp-70, total Akt and MAPK, respectively.

DISCUSSION

The administration of therapeutic cell types, such as stem and progenitor cells, has gained much interest for the repair of tissue damages caused by a variety of insults. In the last years, several treatments were developed to reduce the risk or the severity of tissue damage or that facilitates the healing of radiation injuries. These treatments have improved the quality of life of patients treated either for cancer or other pathologies. Plastic and reconstructive surgical procedures are thus performed to repair tissue defects or involution disorders resulting from resection as consequence of different pathologies. Among the strategies that have been used, autologous lipotransfer is actually largely used (15).

Adipose tissue is a highly specialized connective tissue whose primary function is to provide the body with energy source. The primary cellular component for adipose tissue is a large collection of lipid-filled cells

known as adipocytes. Other cellular components contained in adipose tissue are stroma-vascular cells, including endothelial and hematopoietic cells, and preadipocytes (5,19). Either preadipocytes or whole subcutaneous pads have been transplanted in patients to restore the volume of tissue lost at defect sites or for the treatment of degenerative chronic lesions induced, for example, by radiation (24,29,32). Even though autologous lipotransfer transplant is largely employed in the clinic, little is known regarding the possibility to transplant adipose-derived stem cells. Medical tissue regeneration requires an abundant source of human adult stem cells. There are several evidences that adipose stem cells contribute to the restoration of tissue vascularization and organ function. In addition, adipose tissue represents an abundant and accessible source of adult stem cells that are characterized by their ability to self-renew and to differentiate along multiple lineage pathways.

The CD marker analyses are essential to validate the quality of adipose stem cells purity. At the same time, little is known concerning the capability of these cells to maintain the characteristics of stemness in culture for clinical application. These mesenchymal stem cells are also multipotent and able to secrete proangiogenic growth factors and cytokines (4,28) necessary to reconstitute the tissue damage in all their components.

We purified mesenchimal stem cells from lipoaspirate by subtraction of CD45- and CD31-positive cells following Mylteny Biotech method with the aim of verifying how far they are able to maintain in culture the characteristics of stemness. The analysis of the CD markers we obtained from five different donors of adipose-derived stem cells cultures clearly indicate that the purified adipose-derived stem cells are able to maintain their characteristics of staminality for at least 3 months of in vitro culture, suggesting the possibility to use them for clinical application. Their stemness was confirmed by our finding demonstrating that these cells maintain a high capability to differentiate toward the adipogenic lineage also after long-term in vitro culture (90%). After 3.5 months of in vitro culture, the cells appeared suffering and started to grow slowly, indicating that after this period it is not advisable to further culture these cells for cell therapy. However, starting from the numbers of stem cells we obtained after purification (Table 2), 3 months of in vitro culture can be considered a long period to expand a number of cells sufficient for in vivo application.

The differentiation program toward the adipogenic lineage was also studied, in the same cell lines, through the analysis of the p53 family members because these transcription factors were previously involved in several differentiation programs (33). We showed for the first time that p63, one of the members of the p53 family, is strongly upregulated in terminally differentiated adipocytes, suggesting a role for this molecule in the mechanisms that regulate adipogenesis. The high level of p63 we found in terminal adipocytic differentiation also suggests a possible role for this molecule in inhibiting apoptosis and maintaining the differentiation status. This result is in agreement with previous results indicating a role for p63 in the mechanisms that regulate morphogenesis and differentiation (17,18,35).

Among this family of transcription factors, the role of p53 in mesenchymal differentiation programs is well documented as well as its role in inhibiting the adipogenic differentiation program (21). In agreement with these results, we did not find differences in the level of p53 expression between proliferating and terminally differentiated adipocytes cells, suggesting that p53 does not participate to this process. In agreement, the cyclin-dependent kinase inhibitor $p21^{WAF1}$ is upregulated when the differentiation program starts (22), but in terminal differentiated adipocytes it is completely downregulated, as we found after 2 weeks of culture to induce adipocytic differentiation. In agreement with previous findings, we demonstrated that terminal adipocytic differentiation is characterized by a strong activity of PI3K, as documented by the high level of P-Akt, and a strong downregulation of MAPK phosphorylation, as expected in cells that do not proliferate (9,36). Finally, as demonstrated by our β-galactosidase data, after 3 months of in vitro culture, our cells did not undergo senescence. However, the appearance of p63 expression and a low level of Akt phosphorylation in proliferating cells strongly support the idea that these cells are already committed to a differentiation program and consequently arrested in the cell cycle.

In conclusion, our in vitro results indicate that adipose-derived stem cells from lipoaspirate can be cultured for at least 3 months, maintaining their characteristic of staminality and suggest that they could be applied for cell therapy. However, these cells are in need of a more extensive biological characterization because they are multipotent and hold promise for a range of therapeutic applications.

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