Telomerase Immortalized Human Amnion- and Adipose-Derived Mesenchymal Stem Cells: Maintenance of Differentiation and Immunomodulatory Characteristics

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Abstract

Cell banking of mesenchymal stem cells (SCs) from various human tissues has significantly increased the feasibility of SC-based therapies. Sources such as adipose tissue and amnion offer outstanding possibilities for allogeneic transplantation due to their high differentiation potential and their ability to modulate immune reaction. Limitations, however, concern the reduced replicative potential as a result of progressive telomere erosion, which hampers scaleable production and long-term analysis of these cells. Here we report the establishment and characterization of two human amnion-derived and two human adipose-derived SC lines immortalized by ectopic expression of the catalytic subunit of human telomerase (hTERT). hTERT overexpression resulted in continuously growing SC lines that were largely unaltered concerning surface marker profile, morphology, karyotype, and immunosuppressive capacity with similar or enhanced differentiation potential for up to 87 population doublings. While all generated lines showed equal immunomodulation compared to the parental cells, one of the amnion-derived immortalized lines resulted in significantly increased immunogenicity. Although telomerase proves as important tool for immortalizing cells, our data emphasize the need for careful and standardized characterization of each individual cell population for cell banks.

Introduction

During the recent years human adult stem cells (SCs) have evolved as important tools for several cell-based therapies—in particular, for tissue engineering approaches. Human mesenchymal SCs (hMSCs) are among the most promising candidates. Their differentiation potential toward various cell types by specific stimuli has been proven.1,2 In contrast to embryonic SCs, they raise no ethical issues, and they are regarded as safe concerning their tumorigenic potential.3 Although most initial studies have been conducted with hMSCs from bone marrow that are still considered as gold standard, hMSCs from alternative sources have gained growing attention because their procurement is easier and associated with less donor-site morbidity. Human adipose-derived SCs (ASCs) and human amniotic mesenchymal stromal cells (hAMSCs) show typical hMSC characteristics, including a common surface marker profile and differentiation potential *in vitro* and *in vivo*.4,5 Additionally, these cell sources arise as waste material after liposuction procedures and during cesarean sections. Further, there are several reports on hMSCs from both tissue sources demonstrating not only low allogeneic responses but also dosedependent suppression of activated lymphocyte proliferation *in vitro*.4,6,7 Especially, amniotic SCs have attracted interest because of a possible concurrent therapeutic application with umbilical cord blood-derived hematopoietic SCs from the same donor to enhance engraftment.8

However, SCs needed as therapeutic doses, especially in adults, may require extensive *in vitro* expansion. In this regard, one major drawback of these cells is their low proliferative capacity and limited *in vitro* life span before reaching an irreversible growth arrest, also termed "replicative senescence."2,4 Additionally, long-term cultures of hMSCs may show altered or reduced responsiveness to differentiation signals.9

One strategy to circumvent these limitations is the introduction of the catalytic subunit of human telomerase (hTERT), which has been reported to extend the cellular life span of numerous cell types, including normal fibroblasts, endothelial cells,10,11 and tumor cells, 12,13 but also of SCs.14,15 It has been shown that hTERT-immortalized hMSCs originating from sources such as bone marrow and adipose tissue maintain their differentiation potency. 14,16,17

However, whether hTERT introduction interferes with immunomodulatory functions has not been addressed so far, although this is a crucial factor for using these cell lines in allogeneic cell therapies. Therefore, if cell banking is intended, it is important to monitor the cells' ability to alloactivate peripheral blood mononuclear cells (PBMCs) as well as to modulate the proliferation of activated PBMCs.

Here we report the establishment of the first immortalized SC lines derived from amniotic membrane by transduction with hTERT. In addition, hTERT-immortalized cell lines from adipose tissue were generated and compared to the amniotic cell lines. This strategy was successful in creating immortalized cell lines with largely retained characteristics of the parental cells with regard to morphology, surface marker profile, and immunosuppressive capacity, and showed similar or improved differentiation potential. However, one of two hAMSCtelo lines resulted in a significantly higher immunogenicity compared to the nontransduced controls, although the surface marker profile currently regarded as most important for hMSC characterization did not differ from the parental cells. This suggests that yet unknown markers will have to be identified to predict immunogenicity of the cells. In summary, the novel cell lines give proof of principle that hTERT is a promising tool to generate sufficient material for SC banking and tissue engineering, but concomitantly emphasize the need for careful and standardized characterization.

Materials and Methods

The collection of placentae, adipose tissue, and peripheral blood was approved by the local ethics board.

Human amniotic mesenchymal stromal cells

Human placentae were obtained during caesarean sections. Amniotic membrane was peeled off the placenta by blunt dissection and washed several times in phosphate-buffered saline (PBS). hAMSCs were isolated as described previously[7] and cultured in EGM-2 (Lonza, Verrier, Belgium) at 37°C, 5% CO_2, and 95% air humidity to a subconfluent state. Population doublings (PDs) were calculated using the following formula: PD = [ln (final cell density/initial cell density)]/ln 2. The PD level results from cumulative PDs.

Human adipose-derived SCs

Subcutaneous adipose tissue was obtained during outpatient tumescence liposuction under local anesthesia. ASCs were isolated as described before[7] and cultured in Dulbecco's modified Eagle's medium (DMEM)–low glucose/Ham's F-12 supplemented with 2 mM L-glutamine, 10% fetal calf serum (FCS; PAA, Pasching, Austria, or PAN-Biotech, Aidenbach, Germany), 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 1 ng/mL recombinant human basic fibroblast growth factor (rhFGF; R&D Systems, Vienna, Austria) at 37°C, 5% CO_2, and 95% air humidity to a subconfluent state. PDs were calculated as stated above.

Differentiation into osteogenic and adipogenic lineage

For osteogenic differentiation, cells were seeded at 1×10^3 to 1×10^4 cells/cm^2 and cultivated in osteogenic differentiation medium (DMEM-low glucose, 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.1 μM dexamethasone, 50 μM ascorbat-2-phosphate, and 10 mM β-glycerophosphate) for human ASCs and MesenCult Human Osteogenic Stimulatory Kit (StemCell Technologies, Vancouver, Canada) for hAMSCs for up to 3 weeks.

Differentiation of ASCs toward adipogenesis was adapted from Pittenger *et al*.[1] as described previously.[7] Briefly, cells were cultured in three repetitive cycles of 48–72 h in adipogenic differentiation medium (DMEM-high glucose, 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 10 μg/mL insulin, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 100 μM indomethacin) followed by 24 h in adipogenic medium (DMEM-high glucose, 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 10 μg/mL insulin). Cells were kept in adipogenic medium for up to 3 weeks. Parallel cultures in control medium (DMEM-low glucose/HAM's F12, 1:1, supplemented with 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin) served as negative controls. Adipogenic differentiation of hAMSCs was performed according to Portmann-Lanz *et al*.[18] using following medium: DMEM-high glucose, 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 5.8 μg/mL insulin, 1 μM dexamethasone, 0.5 μM isobutyl-methylxanthine, and 200 μM indomethacin.

Adipogenic differentiation was detected by staining of lipid droplets with Oilred O (OO); osteogenic differentiation was demonstrated by Alizarin Red (AR) and von Kossa (vK) staining of mineral depositions, all described in detail previously.[7] For quantitative evaluation, OO stainings were extracted in 250 μL/cm^2 isopropanol for 15 min at room temperature and measured at 510 nm. AR was measured after extraction using 250 μL/cm^2 20% methanol/10% acetic acid for 15 min at 450 nm. For quantification of intracellular

alkaline phosphatase (AP) activity, washed cells were frozen at −20°C for 30 min and incubated in 0.5% Triton X-100 (in PBS) for 1 h at room temperature. After 1 h incubation with 4-nitrophenolphosphat, samples were measured at 405/620 nm. All described measurements were performed in quadruplicates and measured in triplicates.

Quantitative real-time PCR

Total RNA was isolated using TriReagent (Sigma, Vienna, Austria) according to the manufacturer's instructions. RNA content and integrity was assessed using RNA 6000 Nano Chips Kit (No. 5065-4476; Agilent Technologies, Boeblingen, Germany) on an Agilent 2100 Bioanalyzer. Only high-quality RNA was transcribed to cDNA according to the High Capacity cDNA Archive Kit protocol (Applied Biosystems, Brunn am Gebirge, Austria). Quantification of specific cDNAs was conducted using a LightCyclerTM 480 (Roche, Vienna, Austria) and TaqMan gene expression assays (Applied Biosystems) for following genes: alkaline phosphatase (ALPL), bone γ-carboxyglutamate protein, osteocalcin (BGLAP), peroxisome proliferative activated receptor γ (PPARγ), and leptin (Lep) using the following conditions: denaturation at 95°C/10 min; 50 cycles of 95°C/10 s and 60°C/45 s; cooling to 40°C/30 s. Slope speed was 20°C/s. Standard curves were prepared for quantification, and expression values were normalized to the housekeeping gene hypoxanthine–guanine phosphoribosyl-transferase.

Ectopic expression of hTERT

A retroviral transfection system was chosen for introduction of hTERT. Therefore, the cDNA of hTERT (kindly provided by Geron, Menlo Park, CA) was inserted into the retroviral vector pLXSN (Clontech Laboratories, Mountain View, CA), and retroviral particles were generated as described previously.[12] Gene transfer was performed at early PD (<PD8) according to the manufacturer's instructions (Clontech Laboratories). Twenty-four hours posttransduction, transfectants were selected using 200 μg/mL Geneticin Sulfate G418 and arising cell clones were grown as mass culture. PD of transduced cell lines were calculated starting with the first-passage posttransduction (PDpT) using the formula stated above.

Telomerase activity/telomeric repeat amplification protocol assay

Telomerase activity (TA) was determined using a modification of the real-time telomeric repeat amplification protocol (TRAP) assay as described in detail previously[12] and calculated relative to that of HEK293 cells (positive control).

Senescence-associated β-galactosidase assay

For determination of senescence-associated β-galactosidase assay (SA-β-gal) activity cells were fixed with 3% formaldehyde and stained as described in detail previously.[19]

Flow cytometric analysis

All nontransduced and hTERT-transduced SCs were characterized using antibodies against human CD14, CD34, CD45, CD73, CD90, HLA ABC, HLA DR (all from BD Pharmingen, Vienna, Austria), and CD105 (Abcam, Cambridge, United Kingdom). Briefly, 2.5×10^5 cells in 50 mL PBS/1% BSA (PAA) were incubated with antibody according to the manufacturer's instructions. Cells were washed with Cell Wash™ (BD Pharmingen) and resuspended in Cell Fix™ (BD Pharmingen). Samples were stored at 4°C in the dark until measured on a FACSCalibur (BD Pharmingen). Percentage of positive cells was determined, compared to a nonspecific isotype control.

Mixed lymphocyte reaction

PBMC isolation from whole blood and mixed lymphocyte reaction (MLR) were performed as described previously.[7] Briefly, 5×10^4 cells of two different allogeneic PBMC populations were cocultured in a final volume of 100 µL PBMC medium/well (RPMI1640, 9% FCS, 2 mM L-glutamine, 100 U/mL penicillin G, and 0.1 mg/mL streptomycin). About 10 µM BrdU was added on day 5, and BrdU ELISA (Roche) was performed on day 6 according to the manufacturer's instructions. To test immunomodulatory properties of allogeneic third-party hAMSCs or ASCs, these cells were seeded in the wells and allowed to adhere before adding PBMCs. SCs were added at SC/PBMC ratios of 1:1 (5×10^4 SC), 1:2, 1:4, 1:8, and 1:16.

Phytohemagglutinin activation assay

Phytohemagglutinin (PHA) activation assay was performed as already described.[7] Briefly, 5×10^4 PBMCs were seeded in a final volume of 100 µL medium/well and activated by 5 µg/mL PHA (Sigma) on day 3 of the culture. Coculture with SCs as well as quantification of PBMC proliferation were performed as described for MLR.

Alloreactive assay

To examine interaction between allogeneic SCs and unstimulated PBMCs, SCs were resuspended in PBMC medium and seeded in triplicates in 96-well flat-bottom plates at different cell ratios compared to PBMCs: 1:1 (5×10^4 SCs), 1:2, 1:4, 1:8, and 1:16 each in 50 µL. After adherence 5×10^4 PBMCs were applied in 50 µL. On day 4, 10 µM BrdU was added, and on the following day BrdU ELISA was accomplished.

Calculations

The inhibitory effect of SC was calculated as described.[7] For evaluation of the alloreactive assay, absorption of MLR stimulated PBMCs was set to a value of one. Relative to this, values of PBMCs in coculture with SC were expressed after subtraction of blank (correlating SC cultures without PBMCs).

Statistical analysis

Data were tested for Gaussian distribution and analyzed using one-way ANOVA and Tukey's multiple comparison test. To obtain additional information, the data sets of cells at low PD versus cultures at high PD were compared by two-tailed Student's *t*-test. A *p*-value less than 0.05 was considered as significant.

Results

Four immortalized SC lines (originating from two adipose tissue donors, ASC52 and ASC57, and two amniotic membrane donors, hAMSC76 and hAMSC83) were established by overexpression of hTERT.

Establishment of human amnion and adipose tissue–derived SC lines

Human SCs were isolated from amnion and adipose tissue and propagated *in vitro* until they reached replicative senescence. Representative growth curves of hAMSC76 and ASC57 are shown in Figure 1A. Senescence was evidenced by growth arrest, large and flat cell morphology (Fig. 1B), and SA-β-gal activity (Fig. 1C). Upon ectopic expression of hTERT, all SC populations were immortalized (so far expanded to at least PD60 with no signs of growth retardation; Fig. 2A). Further, hTERT overexpression maintained many characteristics of the original cellular phenotype. Figure 2B demonstrates fibroblastoid morphology of transduced cells (hAMSC76telo-PD78pT, hAMSC83telo-PD43pT,

ASC57telo-PD73pT, and ASC52telo-PD42pT) comparable to early passage parental counterparts. TA after transduction was verified by TRAP assay (Fig. 2C). In contrast to empty vector control cells (hAMSCneo and ASCneo), all hTERT-transduced cell lines expressed significant TA at early PDpT as well as higher PDpT (at least PD38pT) when compared to HEK293 control cells (in the range of 49–240% of HEK293).

Surface marker expression profile

Expression of selected hematopoietic (CD14, CD34, and CD45 negative) and mesenchymal (CD73, CD90, and CD105 positive) markers on hTERT-transduced cell lines was similar to their parental cultures, also after prolonged *in vitro* propagation (hAMSC76telo-PD84pT, hAMSC83telo-55pT, ASC52telo-PD70pT, ASC57-PD87pT; Table 1). Interestingly, the major population of hTERT-transduced hAMSCs lost expression of the mesenchymal marker CD90. Therefore, hAMSC83telo were characterized in more detail, that is, at several PD and for additional antigens (Supplemental Table S1, available online at www.liebertonline.com/ten). At early PD after transduction, hAMSC83telo were still homogenously positive for CD90; however, at PD55pT, only 8.7% of the cells expressed this marker. This subpopulation remained detectable after further 41 PDs (PD96pT). With the exception of SSEA-4, the expression of which increased after hTERT transduction, all other antigens tested showed no alteration.

Karyotype stability and tumorigenicity

Analysis of the cellular karyotype revealed that hTERT transduction did not induce abnormalities in chromosomal number or structure because both untransduced SCs and the hTERT cell lines showed a normal karyotype. Additionally, soft agar assays showed no indication for a tumorigenic conversion upon hTERT transduction (data not shown).

Differentiation characteristics

After introduction of hTERT, amnion- and adipose-derived SC lines showed a similar differentiation potential toward the adipogenic and osteogenic lineage when compared to the nontransduced counterparts. hAMSCs generally showed a low differentiation potential toward the adipogenic lineage as demonstrated by OO staining. Although singular hAMSC76telo cells gained the capacity for lipid accumulation (Fig. 3A, left picture), these rare events were not quantifiable (data not shown). In contrast, ASCs, whether hTERT transduced or not, were characterized by adipogenic differentiation as observed by OO staining (Fig. 3A, right pictures). These data are further strengthened by a significant lipid accumulation as detected by spectrophotometric quantification after isopropanol extraction (Fig. 3B). On the level of differentiation marker genes, quantitative real-time PCR revealed low levels of PPARγ expression in hAMSCs (Fig. 4A, left picture), while in ASCs expression was higher and clearly responsive to differentiation media (Fig. 4A, right pictures). In regard to leptin transcription, both hAMSC and ASC lines show induction during adipogenic differentiation (Fig. 4B).

When tested for osteogenic differentiation, low, albeit significant, mineral deposition of all hAMSC lines was observed, while all ASC lines showed high levels of mineralization as analyzed by vK staining (Fig. 5A) as well as quantification of AR staining (Fig. 5B). In immortalized hAMSC lines, low but significantly increased AP activity was observed (Fig. 5C, left picture), while AP activity was significantly induced in normal and immortalized ASC lines (Fig. 5C, right picture). At mRNA level, AP was detected only at very low levels in all hAMSC lines, whereas ASCs showed higher and induced AP expression (Fig. 6A). Osteocalcin mRNA was induced in all hASMCs during differentiation, while in ASC lines, only ASC52telo and ASC57 reacted with osteocalcin upregulation (Fig. 6B).

Noteworthy, for optimizing ASC57telo differentiation toward both lineages, standard FCS (PAA) was replaced by an alternative FCS charge (PAN) during expansion of the telomerized cell line. While this media modification reduced the proliferative rate of the cell line, it allowed for mineralization under osteoinductive conditions.

Immunomodulatory characteristics

To test immunomodulation of the hTERT-immortalized SC lines, their suppressive effect on MLR- or PHA-activated lymphocyte proliferation was analyzed (Fig. 7). All tested immortalized lines inhibited MLR-activated PBMC proliferation in a cell dose–dependent manner. hTERT-transduced hAMSCs inhibited significantly at a 1:8 SC/PBMC ratio (Fig. 7A), and parental hAMSCs even at 1:16. Both ASCs and ASCtelo acted less immunosuppressive, inhibiting activated PBMC proliferation significantly at a ratio of 1:4 (Fig. 7B).

Similarly, when SCs were cocultured with PHA-activated PBMCs, the inhibitory potency of hAMSCs and ASCs was unaltered after hTERT overexpression. hAMSCs inhibited PBMC proliferation significantly at a ratio of 1:8 (Fig. 7C); for significant ASC-mediated inhibition, a 1:2 cell dose was necessary (Fig. 7D). Analysis of hTERT-immortalized ASC52telo at different time points during culture (PD25pT, PD37pT, PD43pT, and PD75pT) further showed that the immunosuppressive activity did not significantly alter during prolonged *in vitro* cultivation (Supplemental Fig. S1, available online at www.liebertonline.com/ten).

Immunogenic characteristics

To investigate the immunogenicity of the hTERT-transduced lines, alloreactive assays were performed. Alloreactive responses to hMSCs are usually mild, taking place only in singular cases.[7,20,21] Indeed, we found that ASCs provoked typically a mild, but not significant, activation of PBMCs independent of telomerase transduction (Fig. 8B). However, one of the immortalized hAMSCs, hAMSC76telo resulted in a significant lymphocyte response (Fig. 8A) in contrast to the parental lines. Interestingly, the surface marker profile was identical for both hAMSCtelo lines, indicating that additional markers with predictive character for immunogenicity of hAMSCs have to be identified.

Discussion

During recent years, human adult MSCs have been established as one of the most promising tools in regenerative medicine. First successful cell-based therapies for diseases, including myocardial infarction, multiple sclerosis, amyotrophic lateral sclerosis, graft versus host disease, osteogenesis imperfecta, and Crohn's fistula, have been conducted.[22-27] Applicability of these cells for allogeneic transplantation and SC-based therapies could further be boosted by standardized collection, quality control, and careful selection of functional and safe cell banking products. However, to provide sufficient SC numbers for cell banking and cell-based therapies, their limited replicative potential has to be overcome. In this regard, ectopic expression of hTERT has proven valuable. Besides elongation of cellular life span, improvement of growth characteristics, stabilization of the karyotype, and maintenance of the original cellular phenotype,[10,11,28-30] hTERT has also been demonstrated to retain or even improve differentiation potential.[14,16,17,31] However, one key issue has not been addressed so far. It is not clear, whether immunomodulation mediated by MSCs is still active after hTERT introduction, a crucial point before allogeneic transplantation therapies should be considered.

To get first insights whether telomerase activation interferes with immunomodulation *in vitro*, we focused on two promising adult SC types, human ASCs and hAMSCs. In a first step, both cell types were successfully immortalized by overexpression of hTERT alone. We obtained, for the first time, two different continuously growing SC lines derived from amnion. Similarly, adipose-derived SC lines were established by hTERT overexpression, demonstrating that this strategy results in scaleable generation of SC material. SC characteristics of the newly established cell lines, especially their differentiation and immunogenicity, were variable. hTERT-transduced ASC lines maintain their typical surface marker profile as reported previously,[5,16] whereas hAMSCs lost the mesenchymal marker CD90 in a subpopulation of telomerized cells during prolonged *in vitro* propagation. It has recently been published that upon cultivation in EGM-2 (also used in our study for hAMSC) a subpopulation of CD90 negative human bone marrow MSCs evolved after prolonged culture probably due to angiogenic growth factors in the medium.[32] Decrease in CD90 expression and concomitant increase of the embryonic SC marker SSEA-4 found in our immortalized hAMSC lines suggest an alteration of the phenotype during long-term culture in EGM-2.

ASC line ASC57telo responses to adipogenic and osteogenic stimuli were maintained, and adipogenic differentiation of ASC52telo was even enhanced compared to the parental line. This is in accordance to reports on enhanced mesenchymal differentiation capacity after hTERT immortalization of ASCs from different species.[16,33] Further, even among subclones derived from single ASCs, variations in the differentiation potential exist.[34] This may account for the increase in differentiation in some but not all hTERT- transduced lines. Both parental hAMSC isolates possessed low adipogenic differentiation potential, which has been described before (Stadler *et al.*, unpublished data). However, hTERT transduction led to increased lipid accumulation of hAMSC76 under adipogenic conditions. This is in contrast to the finding that mesenchymal stromal cells from chorion cotransduced with hTERT and Bmi-1 showed minimal adipogenic differentiation that even decreased with time in culture.[35]

The use of SCs in allogeneic settings immensely broadens the applicability of SC therapies. It has been reported that *in vitro* human ASCs and MSCs from amniotic or chorionic membrane are only mildly allogeneic and suppress PBMC proliferation in a cell dose–dependent manner.[6,7,20,36,37] With respect to these extraordinary properties, we observed only low allogeneic lymphocyte activation and further immuno-suppression of activated PBMCs by our immortalized cell lines. In three out of four cases these properties were similar to the parental cells, the exception being hAMSC76telo. Because the cell surface marker profiles of hAMSCtelo lines analyzed here were identical, we propose that a marker as predictor for immunogenicity remains to be identified and included in routine surface marker profiling.

In conclusion, the immortalized SC lines established in this study can be seen as a first step to a proof of principle for their applicability in cell-based therapy approaches. Because obvious donor and cell line–specific differences exist, SC material for cell banks will have to be routinely tested. Specifically, their differentiation potential and immunosuppressive effects are of major importance. However, additional caveats that limit the use are controversially discussed in literature. Especially, the tumorigenic potential of SCs in general and hTERT-transduced cells specifically is a matter of debate. Most reports find that SCs retain their differentiation potential, contact inhibition properties, and a stable karyotype and do not show tumorigenic potential even after extensive *in vitro* expansion. In other studies, spontaneous transformation of human ASCs after 4–5 months in culture and a high rate of tumorigenicity evolving as a consequence of hTERT introduction in human MSCs after approximately 3 years in culture were reported.[38,39] Hence, it can be expected that

given a high quality of the starting material concerning SC characteristics and genetic stability and by use of a reasonable culture time *in vitro* suitable SC material can be made available for cell banking by careful monitoring and characterization.

Acknowledgments

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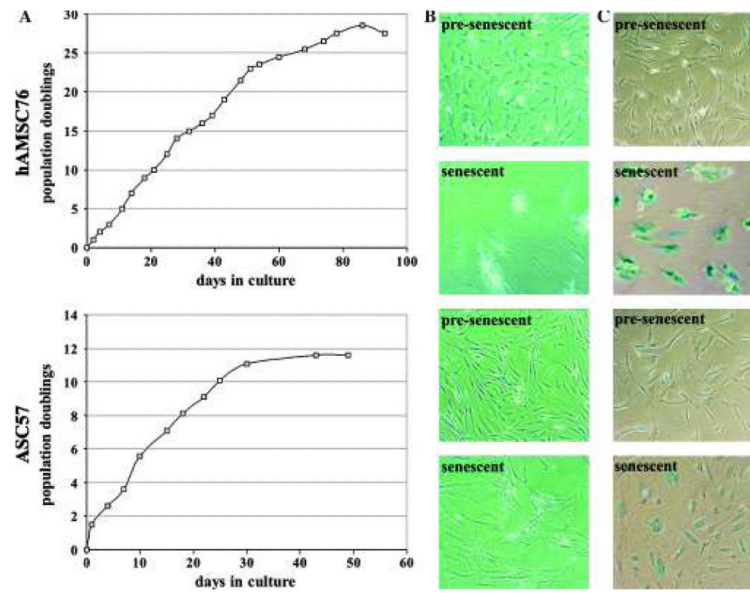
FIG. 1.
Growth characteristics and morphology of human amniotic mesenchymal stromal cells
(hAMSCs) and adipose-derived SCs (ASCs). (**A**) Cells were grown *in vitro* until replicative
senescence. Representative growth curves of hAMSC76 and ASC57 are shown. (**B**) Phase
contrast microscopy and (**C**) staining for SA-β-gal activity of early and late passage cells.
Magnification in (**B, C**), 100×. Color images available online at www.liebertonline.com/ten.
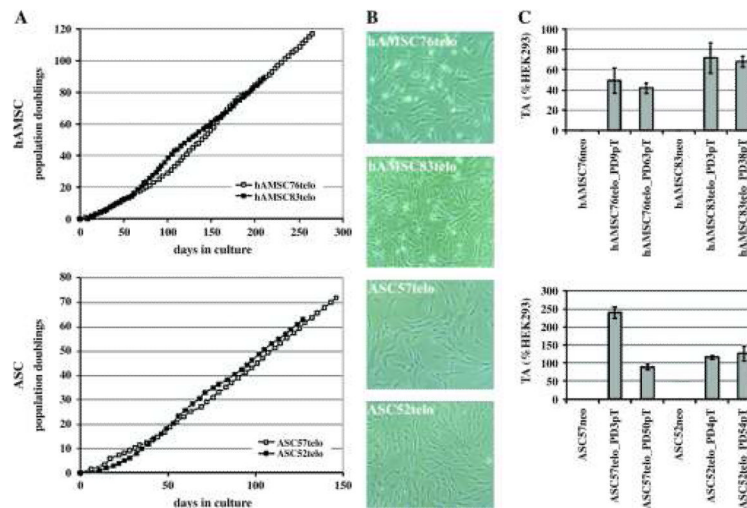
FIG. 2.
Growth potential and morphological characteristics of hTERT-transduced SC lines. (**A**) Growth curves of hTERT-transduced cell lines. (**B**) Phase contrast microscopy of hTERT-transduced immortalized cell lines. (**C**) TRAP assays at two different PDs posttransduction (PDpT) demonstrate TA in all cell lines. neo, vector control; telo, hTERT transduced; hAMSC, human amniotic mesenchymal stromal cells; ASC, adipose-derived SCs. Magnification in (**B**), 100×. Color images available online at www.liebertonline.com/ten.
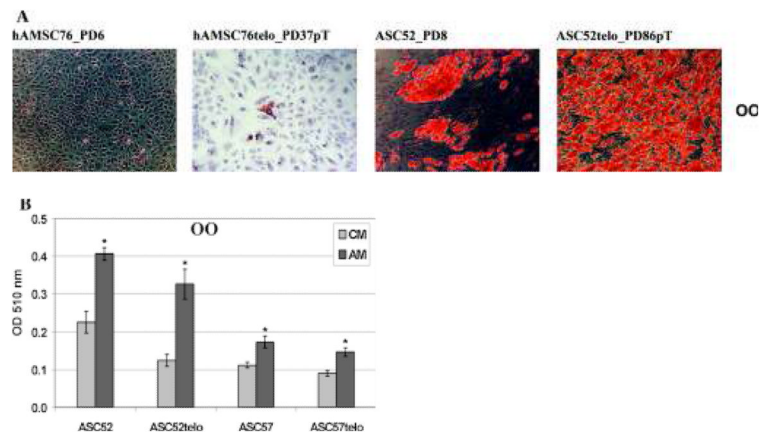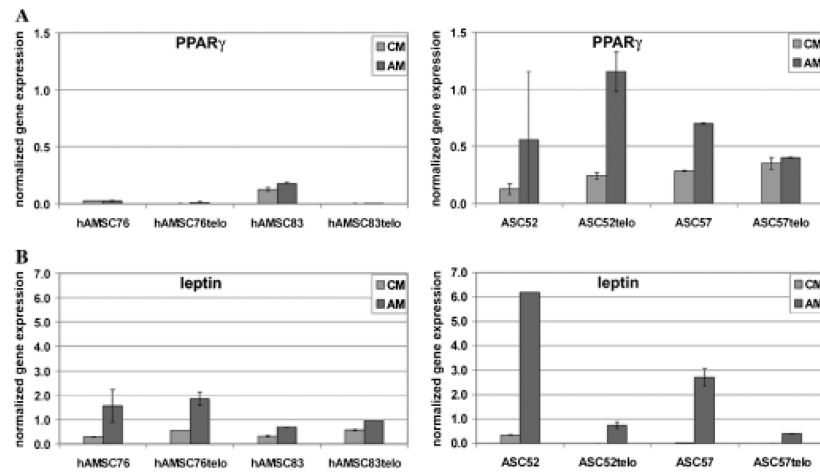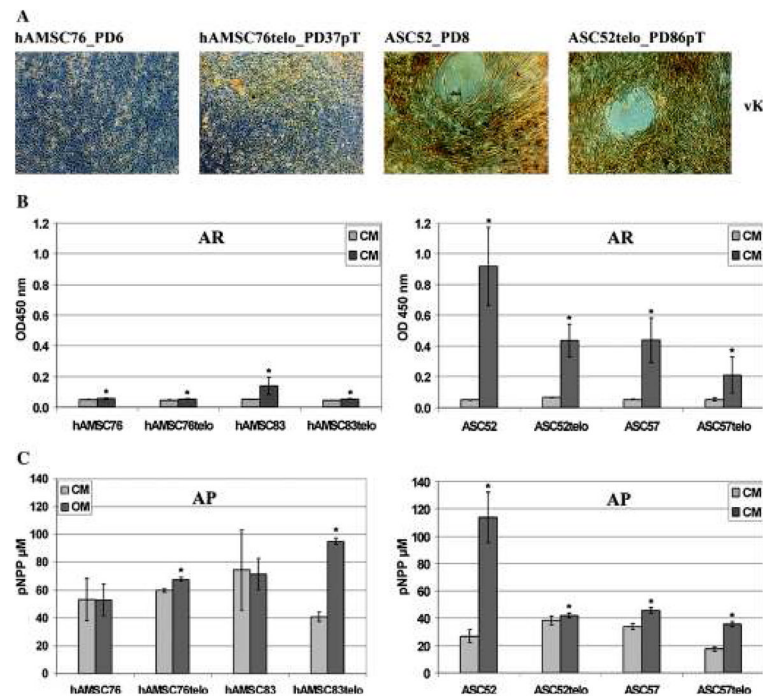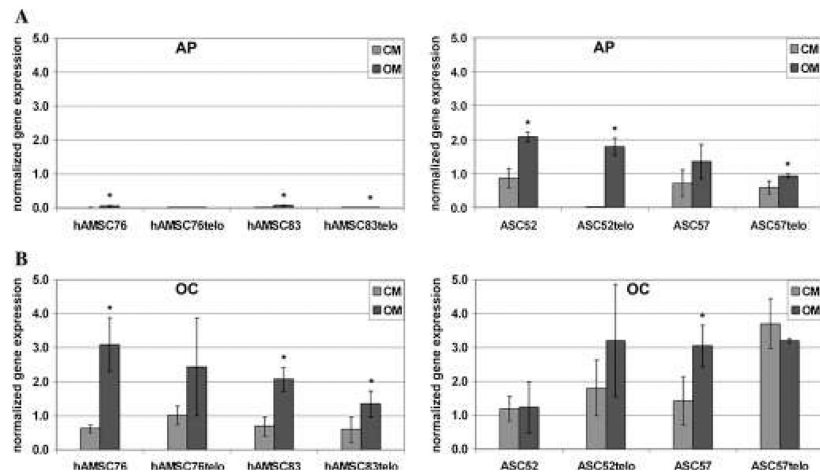
FIG. 3.
Adipogenic differentiation potential of non-transduced and hTERT-transduced human amniotic mesenchymal stromal cells (hAMSCs) and adipose-derived SCs (ASCs) 3 weeks after induction. (**A**) Representative pictures of Oilred O (OO) staining of hAMSC76-PD6, hAMSC76telo-PD37pT, ASC52-PD8, and ASC52telo-PD86pT. Magnification, 100×. (**B**) Adipogenic conversion demonstrated by Oilred O (OO) quantification of ASCs. Differences between control cultures (CM) and adipogenic differentiation cultures (AM) with $p < 0.05$ were regarded as significant. Color images available online at www.liebertonline.com/ten.

FIG. 4.
Expression levels of peroxisome proliferative activated receptor γ (PPARγ) (**A**) and leptin (**B**) 2 weeks after adipogenic induction of nontransduced and hTERT-transduced human amniotic mesenchymal stromal cells (hAMSCs) and adipose-derived SCs (ASCs). Expression levels in adipogenic medium (AM) or control medium (CM) are presented normalized to hypoxanthine–guanine phosphoribosyltransferase (HPRT). Means and SDs of two individual experiments are displayed.
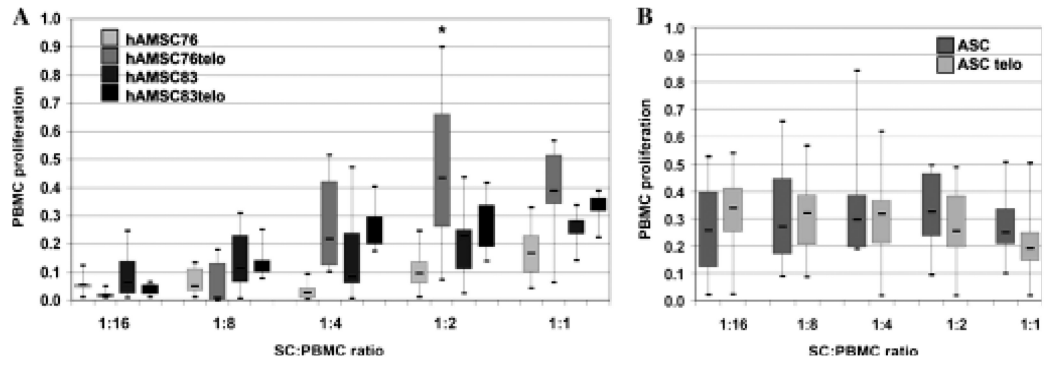
FIG. 5.
Osteogenic differentiation potential of nontransduced and hTERT-transduced human amniotic mesenchymal stromal cells (hAMSCs) and adipose-derived SCs (ASCs) 3 weeks after induction. (**A**) Representative pictures of von Kossa (vK) staining of hAMSC76-PD6, hAMSC76telo-PD37pT, ASC52-PD8, and ASC52telo-PD86pT. Magnification, 100×. Osteogenic differentiation demonstrated by Alizarin Red (AR) quantification (**B**) and alkaline phosphatase (AP) (**C**) of hAMSCs and ASCs. Differences between control cultures (CM) and osteogenic differentiation cultures (OM) with $p < 0.05$ were regarded as significant. Color images available online at www.liebertonline.com/ten.

FIG. 6.

Expression levels of alkaline phosphatase (AP) (**A**) and osteocalcin (OC) (**B**) 2 weeks after osteogenic induction of nontransduced and hTERT-transduced human amniotic mesenchymal stromal cells (hAMSCs) and adipose-derived SCs (ASCs). Expression levels in osteogenic medium (OM) and control medium (CM) are presented normalized to hypoxanthine–guanine phosphoribosyltransferase (HPRT). Means and SDs of three individual experiments are displayed. Differences between control cultures (CM) and osteogenic differentiation cultures (OM) with $p < 0.05$ were regarded as significant.
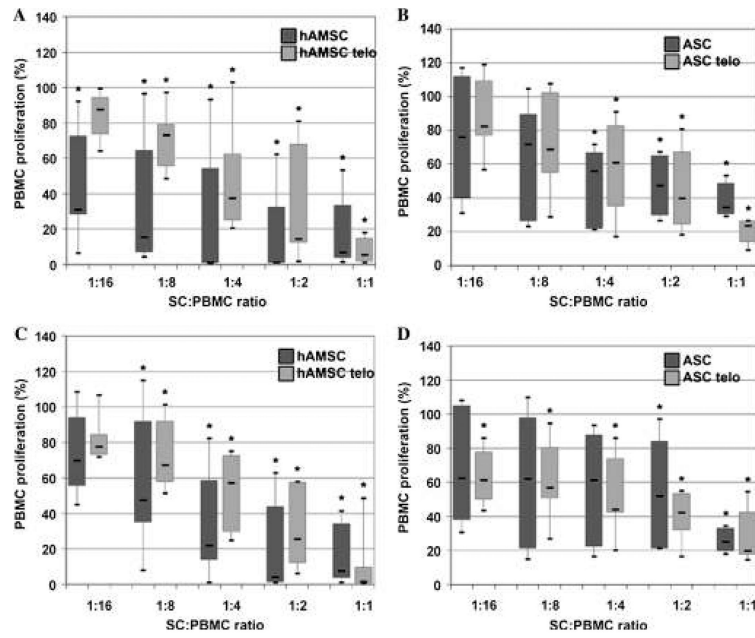
FIG. 7.
Immunomodulation of nontransduced and hTERT-transduced human amniotic mesenchymal stromal cells (hAMSCs) and adipose-derived SCs (ASCs). (**A**) hAMSCs (76, $n = 4$; 83, $n = 3$) and (**B**) ASCs (52, $n = 4$; 57, $n = 3$) inhibit mixed lymphocyte reaction (MLR) in a cell dose-dependent manner. (**C**) hAMSCs (76, $n = 4$; 83, $n = 4$) and (**D**) ASCs (52, $n = 4$; 57, $n = 3$) inhibit phytohemagglutinin-activated lymphocyte proliferation in a cell dose-dependent manner. PBMC proliferation is calculated as percentage of uninhibited proliferation. $p < 0.05$ was regarded as significant inhibition.

FIG. 8.
Allogeneic reaction of naive PBMC to different numbers of nontransduced and hTERT-transduced human amniotic mesenchymal stromal cells (hAMSCs) and adipose-derived SCs (ASCs). (**A**) hAMSC lines (76, $n = 6$; 83, $n = 6$) and (**B**) ASCs (52, $n = 10$; 57, $n = 6$); PBMC proliferation relative to the correlating MLR-stimulated PBMC activation (positive control set to one) is depicted. $p < 0.05$ was regarded as significant activation.

Table 1

Surface Antigen Expression of Nontransduced (Normal) and hTERT-Transduced (Telo) hAMSCs

Antigen	hAMSC76		hAMSC83		ASC52		ASC57	
	Normal	Telo	Normal	Telo	Normal	Telo	Normal	Telo
CD14	0.5	7.0	2.6	10.5	0.3	3.3	1.4	3.2
CD34	0.0	0.1	0.1	0.1	0.0	0.5	1.6	0.5
CD45	0.0	11.6	0.0	2.0	0.0	3.4	0.0	0.4
CD73	96.0	100.0	99.9	100.0	98.1	99.9	99.6	99.6
CD90	100.0	53.3	100.0	8.7	100.0	100.0	100.0	100.0
CD105	95.7	99.8	92.5	99.9	71.9	99.7	99.2	99.8
HLA ABC	98.9	99.8	99.4	99.7	95.6	99.6	93.0	98.6
HLA DR	0.5	2.6	0.2	2.3	0.3	5.2	2.2	10.0

hAMSC76, hAMSC83, ASC52, and ASC57 were analyzed at PD 6, 5, 3, and 8, and their hTERT-transduced counterparts at PD 84, 55, 70, and 87 posttransduction, respectively.