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Reports

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Fast assay to predict multipotent mesenchymal stromal cell replicative senescence dynamics

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ABSTRACT

The major obstacle to the application of mesenchymal stromal cells (MSCs) in regenerative medicine is the expansion of the donor-derived cells *in vitro* to obtain high cell numbers in the shortest possible time. However, MSCs gradually undergo replicative senescence after a variable number of divisions that reduce their therapeutic efficacy, which needs to be determined before administration. The authors developed a fast and simple evaluation assay testing two senescence inducers, mitoxantrone (Mxt) and trichostatin A (TSA), to predict the onset of spontaneous replicative senescence of adipose-derived mesenchymal stromal cells (ASCs) and have confirmed the correlation between induced senescence and spontaneous replicative senescence in the assay using Mxt. This protocol facilitates the standardization of therapeutic ASCs and MSCs from other origins before application.

METHOD SUMMARY

Adipose mesenchymal stromal (stem) cells (ASCs) were isolated from the fat deposits of healthy donors at various anatomical locations, followed by long-term cultivation *in vitro* that differed in cumulative cell population doubling level and viability, as determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay and longevity (i.e., replicative senescence was determined after several passages using a β-galactosidase activity assay). This was compared in senescence induction assays, using mitoxantrone, a type II topoisomerase inhibitor, and trichostatin A, a histone deacetylase inhibitor. These fast and simple assays may replace the long-term replicative senescence potential assays that take several weeks for evaluation of results.

KEYWORDS:

adipose mesenchymal stromal (stem) cells (ASCs) ● cell longevity ● induced senescence ● mesenchymal stromal (stem) cells (MSCs) ● mitoxantrone ● replicative senescence ● trichostatin A

Mesenchymal stromal cells (MSCs), also called mesenchymal stem cells, are adult undifferentiated cells with self-renewal and clonogenic potential that are capable of differentiation into multiple cell lineages [1]. Due to these traits, MSCs are increasingly used in clinical practice for treatment in regenerative medicine, such as aesthetical surgery and in the treatment of many pathological conditions (e.g., tissue repair [2,3], autoimmune diseases [4], neurodegenerative diseases [5] and acute and chronic inflammation) [6]. Beneficial effects of MSCs on immune/inflammatory disorders of the lung were among the first to be reported [7], highlighting the potential of allogeneic use of MSCs in the treatment of COVID-19-related acute respiratory distress syndrome (ARDS) [8].

In addition to bone marrow, other sources of MSCs have been identified in a variety of fetal and adult tissues [1]. For any clinical application, the MSCs should fulfill the demand of the International Society for Cell Therapy for phenotypic and functional characterization [9]. MSCs have a limited lifespan due to their replicative senescence, which occurs after a cell type-specific number of population doublings in the *in vitro* culture conditions, thus representing a challenging task in the selection of the cell lines that will be most viable long-term. The onset of senescence in MSCs not only affects their longevity but also viability, self-renewal, differentiation capacity and secretome composition that, over time with cell aging, declines resulting in the impaired therapeutic potential of naive MSCs [10–15]. Unfortunately, the senescence status of cells cannot be predicted, unless the MSC's viability is followed *in vitro* through multiple passages and measurement of senescence cell fraction by the standard senescence marker, β-galactosidase (SA-bGal) activity [10,15].

Adipose-derived mesenchymal stromal cells (ASCs) are obtained from adipose tissue in rather high quantities and were therefore selected here for developing faster assays compared with routinely following *in vitro* spontaneous replication senescence to enable highly reliable standardization analyses, using senescence inducers. Here, the authors describe a method that would enable much more rapid characterization of MSC longevity and the immediate selection of good quality MSC cell lines after their isolation. The assays use mitoxantrone (Mxt), a type II topoisomerase inhibitor that disrupts DNA synthesis and repair via intercalation between DNA bases [16], and trichostatin A (TSA), a histone deacetylase inhibitor [17], both immediately inducing cell senescence. The data confirm a statistically

Table 1. Summary of donor characteristics and adipose-derived mesenchymal stromal cell yields.

Anatomical location	ASC code	Donor age (years)	Adipose tissue volume (ml)	Adipose tissue cells (n/ml)	Isolated cells (mean; n/ml)
Thighs	ASC-1	71	2.0	243,750	239,469†
	ASC-2	71	2.0	300,000	
	ASC-3	70	3.7	174,658	
Hips	ASC-4	35	1.5	91,666	74,305
	ASC-5	35	1.0	37,500	
	ASC-6	12	1.6	93,750	
Abdomen	ASC-7	35	1.0	75,000	41,667
	ASC-8	41	1.5	8333	

†The highest number of cells was isolated from the thighs compared with the hips and abdomen ($p < 0.05$).
ASC: Adipose-derived mesenchymal stromal cell.

significant correlation between chemically induced senescence in the Mxt assay and spontaneous replicative senescence of naive ASCs. This assay should be useful for the accelerated selection of high-quality ASCs for use in therapeutic applications. The assay is also applicable to other tissue sources of MSCs.

Materials & methods

Adipose tissue donors

Adipose tissue samples were collected from healthy donors who were undergoing esthetic surgery. The study was approved by the National Ethics Committee (Doc. no. 134/01/11). All the donors were female, with a mean age of 43 years, ranging from 12–71 years. Subcutaneous adipose tissue was taken from the thighs (n = 3), hips (n = 3) and abdomen (n = 2) from a total of seven donors (Table 1).

Isolation & culturing of adipose mesenchymal stromal cells

Isolation of ASCs from adipose tissue samples collected from healthy donors undergoing aesthetic surgery was performed according to the procedure described by Mahmoudifar *et al.* [18] with minor modifications. Briefly, the adipose tissue samples were washed with phosphate-buffered saline (PBS; PAA Lab, Pasching, Austria), cut into small pieces and dissociated with 0.1% collagenase I (Gibco, Life Tech. Corp., Paisley, UK) at 37°C with vigorous shaking. After 1 h, the collagenase was inactivated by adding heat-inactivated fetal bovine serum (FBS; PAA Lab), and the samples were centrifuged at 300 × g for 10 min. The pelleted cells were washed with erythrocyte lysis buffer (0.15 M NH4Cl, 0.1 mM EDTA, 0.01 M NaHCO3, pH 7.2–7.4; Sigma-Aldrich, Steinheim, Germany) and centrifuged at 300 × g for 5 min. The isolated cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) with 20% FBS, supplemented with 100 IU/ml penicillin, 0.1 mg/ml streptomycin and 2 mM L-glutamine (Gibco). The first ASC culture was denoted as passage 0. The medium was changed twice a week and the ASCs were replated at a density of 6000 cells/cm^2 after reaching 90% confluence.

Adipose-derived mesenchymal stromal cell characterization & differentiation

ASC identity was confirmed for all ASC lines at passage p7 by analysis of specific MSC cell surface marker expressions, such as the presence of CD13, CD29, CD44, CD73, CD90 and CD105 absence with less than 2% expression of the hematopoietic stem cell markers CD14, CD34, CD45 and HLA-DR. Briefly, one million ASCs were harvested, washed with PBS and labeled with antibodies or with appropriate isotype controls as instructed by the manufacturer (BD Biosciences, CA, USA). To exclude dead cells from the analysis, the cells were stained with propidium iodide (PI) and washed with PBS. The cells were analyzed by flow cytometry using a BD FACSCalibur™ and CellQuest Software (BD Biosciences). All ASC lines were tested for their selective differentiation into adipose, bone or cartilage-like cells as previously described [19].

Cumulative population doubling level

Growth profiling of the ASCs was performed by calculating their cumulative population doubling levels (CPDLs). The ASCs were counted at seeding and harvesting and their doubling levels were calculated according to the equation:

$$x = \{\log 10(NH) - \log 10(NI)\} \log 102$$

where NI is the initial ASC number at seeding and NH is the ASC number at harvesting [20]. The ASCs were passaged when they reached 90% confluence. The CPDL was obtained by adding the doubling level of the ASCs at each passage to that of the previous passage, with continuous cultivation from passage 1 until the cells ceased to proliferate (to at least passage 18).

Table 2. Adipose-derived mesenchymal stromal cells age-related cumulative population doubling levels at passages p6, p12 and p18.

Age group/(age average)	Cumulative population doubling level		
	p6	p12	p18
Older ASCs (70.7 years)	10.9 ± 5.8	26.7 ± 7.4	39.2 ± 8.8
Middle ASCs (36.5 years)	11.2 ± 2.2	26.0 ± 4.8	34.4 ± 4.0
Young ASCs (12 years)	9.5	19.1	24.7

Metabolic activity of adipose-derived mesenchymal stromal cells

The metabolic activity of the ASCs was determined using the MTT assay, as described by Mosmann *et al.* [21] with minor modifications. The concentration ranges for Mxt and TSA were selected based on data from Zhao *et al.* [22] and the preselection MTT test. Briefly, ASCs at passage p7 were seeded into 96-well plates (Corning, MA, USA) at a density of 5000 cells/well with 5 replicates for each condition and left to adhere for 24 h. Then 0.02–0.6 μM Mxt or 0.05–3.00 μM TSA was added. After 69 h, 0.5 mg/ml MTT (Sigma-Aldrich, Steinheim, Germany) was added to the ASCs, which were then incubated for 3 h at 37°C and 5% CO_2. The medium was removed and the formazan crystals were dissolved in dimethylsulphoxide (Sigma-Aldrich). The absorbance of the solution was measured at 570 nm (reference filter: 690 nm) using a Synergy™MX microplate reader (Bio-Tec Instruments Inc., VT, USA). The absorbance data are displayed as means ± standard deviation of 5 replicates from 3 independent experiments.

Replicative senescence & induced senescence of adipose-derived mesenchymal stromal cells

The spontaneous cell senescence that occurred under normal growth conditions *in vitro* was evaluated in ASCs at passages p6, p12 and p18. Briefly, the ASCs were plated on poly-lysine-coated coverslips at a density of 15,000 cells/coverslip in 24-well plates (Corning, MA, USA) and left to adhere overnight. The ASCs were fixed with 0.5% glutaraldehyde (Sigma-Aldrich, Steinheim, Germany) for 20 min at room temperature, and SA-β-gal activity at pH 6.0 was determined as previously described [20]. The fixed cells were washed twice with PBS and then incubated with 1 mg/ml 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside solution (X-gal substrate; Sigma-Aldrich). After 16 h, the ASCs were washed in distilled water and incubated with Hoechst 33342 (1:1000) to stain their nuclei. The percentage of senescent ASCs was determined by counting the senescent cells and nuclei per visual field under a light and fluorescent microscope, respectively. The results are presented as the ratio of the number of SA-β-gal-positive ASCs per the number of nuclei detected.

To induce senescence, the ASCs at passage 7 were plated onto poly-lysine-coated coverslips (15,000 cells/coverslip) and left to grow overnight. The next day, the ASCs were exposed to 0.02, 0.2, 0.4 or 0.6 μM Mxt (Sigma-Aldrich, Steinheim, Germany) or 0.05, 0.5, 1, 2 or 3 μM TSA (Sigma-Aldrich) for 72 h. The detection of SA-β-gal-positive ASCs was performed as previously described.

Statistical analysis

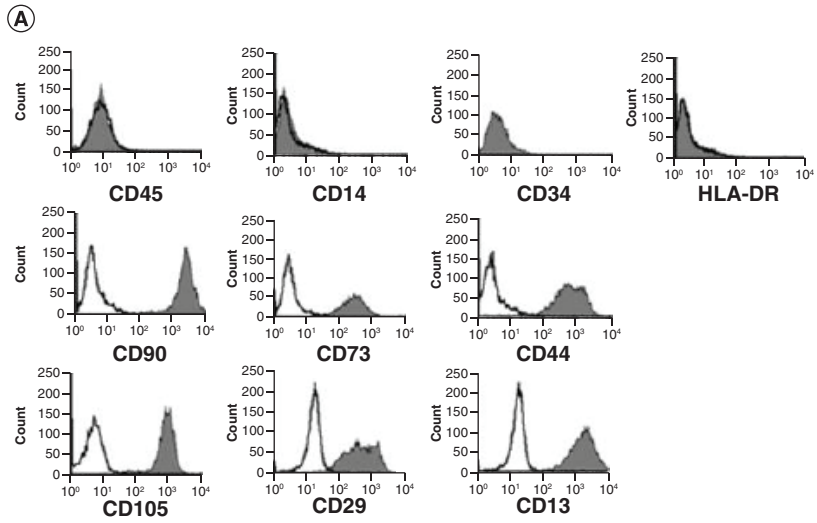
To test for spontaneous senescence progress in each cell line at passage p6, p12 and p18, determined by the number of SA-β-gal-positive cells and to test correlation of ASCs harvest site and CPDL, ANOVA with Tukey's posthoc tests was used. To test the effects of Mxt and TSA on ASC viability and senescence, treated ASCs were compared with untreated ASCs using ANOVA with Dunnett's posthoc tests. To test for correlations between ASCs replicative senescence at passage p12 and induced senescence in ASCs as a response to Mxt and TSA exposure, ANOVA with Fisher's LSD test was used. GraphPad Prism version 6 for Windows was used (GraphPad Software, CA, USA). Data are displayed as means ± standard deviation, with $p < 0.05$ considered statistically significant.

Results & discussion

Growth profiling & replicative senescence of adipose-derived mesenchymal stromal cells

After ASCs phenotype confirmation by selective MSC markers (Figure 1A & B) and specific differentiation profile into adipocytes, osteocytes and chondrocytes (Figure 1C) used to characterize MSCs as multipotent mesenchymal stromal cells, the proliferation capacity of each ASC line (ASC-1 to ASC-8) was determined. The cells were passaged until they ceased to proliferate. The growth curves determined in terms of CPDLs differed considerably among the ASC lines (Figure 2A). ASC-3 exhibited the highest proliferation rate, followed by ASC-5 and ASC-8. The remaining cell lines (ASC-1, ASC-2, ASC-4, ASC-6 and ASC-7) had similar proliferation profiles and formed a cluster of cells that showed nearly identical CPDLs after 100 days of cultivation. Comparison of the proliferation rates of ASC lines derived from three different anatomical sites showed that the proliferation of cells was not significantly different even after the highest number of passages, at p18 (Figure 2B). Similarly, when ASC lines were grouped according to the age of the donors (young, middle and old age), no significant differences were observed in terms of CPDL (Table 2). However, the number of ASC lines included in the study was too small to draw precise statistical conclusions.

Next, the presence of spontaneously senescent cells was evaluated by detection of SA-β-gal activity, the most frequently used standard for senescence detection. The proportions of SA-β-gal-positive cells varied among ASC lines at p6, p12 and p18, respectively (Figure 2C). However, the proliferation rates (CPDL) failed to reliably indicate the senescence status in ASCs at later passages (Figure 2). Thus, the CPDL assay should be replaced or only added to the more informative assay related inversely to the metabolic activity of cells.



B

Marker	Marker expression according to ASC source (%)							
	ASC-1	ASC-2	ASC-3	ASC-4	ASC-5	ASC-6	ASC-7	ASC-8
CD45	1.8	1.9	0.4	1.2	1.0	1.3	1.4	0.8
CD14	0.9	0.7	0.4	1.6	1.8	1.8	1.9	1.9
CD34	0.2	1.4	0.6	1.8	1.4	1.9	1.2	1.6
HLA-DR	0.9	0.5	1.1	1.4	0.7	0.9	1.6	0.7
CD90	99.3	98.4	99.9	98.7	98.6	96.8	98.5	99.4
CD73	98.4	99.3	98.6	95.3	98.9	97.2	99.9	97.6
CD44	99.8	99.1	99.3	98.8	95.2	95.9	98.7	96.1
CD105	99.5	95.6	99.9	99.2	95.9	95.6	96.8	99.4
CD29	99.5	98.6	98.7	96.9	99.2	98.9	97.1	95.7
CD13	99.4	99.4	98.7	98.9	96.3	96.8	97.0	99.0

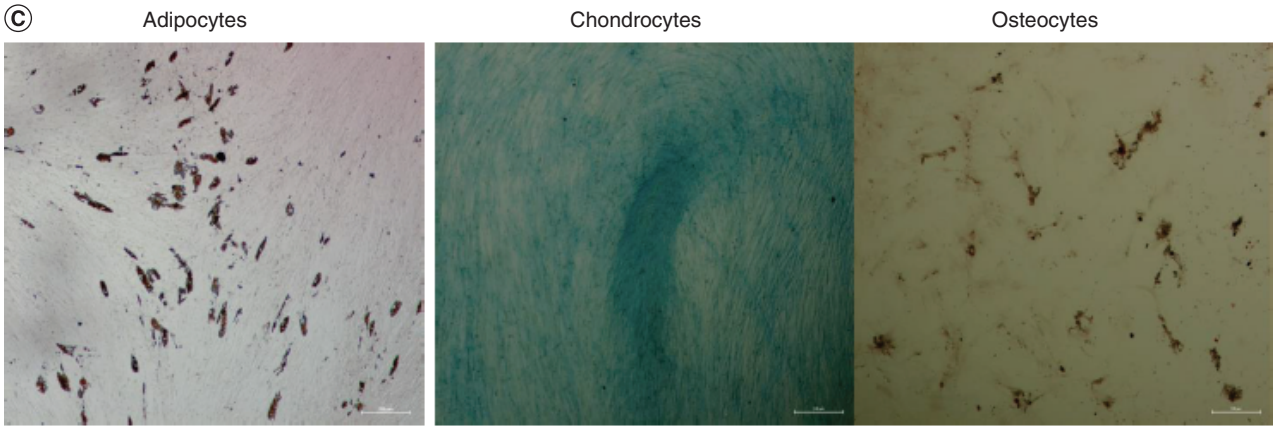


Figure 1. Characterization of adipose-derived mesenchymal stromal cells. (A) ASC surface marker expression, analyzed by flow cytometry for the characteristic MSC/ASC surface antigens CD45-FITC, CD14-FITC, CD34-FITC, HLA-DR-PE, CD90-PE, CD73-PE, CD44-PE, CD105-APC, CD29-APC, CD13-APC and the appropriate isotype (i.e., FITC-IgG1, FITC-IgG2a, PE-IgG1, PE-IgG2b and APC-IgG1), as described in the methods section. **(B)** Quantitative data for the MSC markers for all ASC lines used in the study, demonstrating their mesenchymal stromal cells origin. **(C)** Adipocytes, chondrocytes and osteocytes after differentiation of ASCs. Scale bars: 100 μm.
ASC: Adipose-derived mesenchymal stromal cell; MSC: Mesenchymal stromal cell.

Moreover, monitoring of senescence could be additionally assessed by new simple detection methods for phenotypic changes characteristic of cell senescence based on autofluorescence of cells using flow cytometry [23,24] or image-based assessment of change in morphology of senescent cells [25], which has also been shown to be a good indicator of cell senescence. To follow the mechanism of senescence in more detail, the above simple senescence detection techniques could be further supported by molecular, genomic, epigenomic and cytogenetic methods [15]. However, that was not the aim of this report.

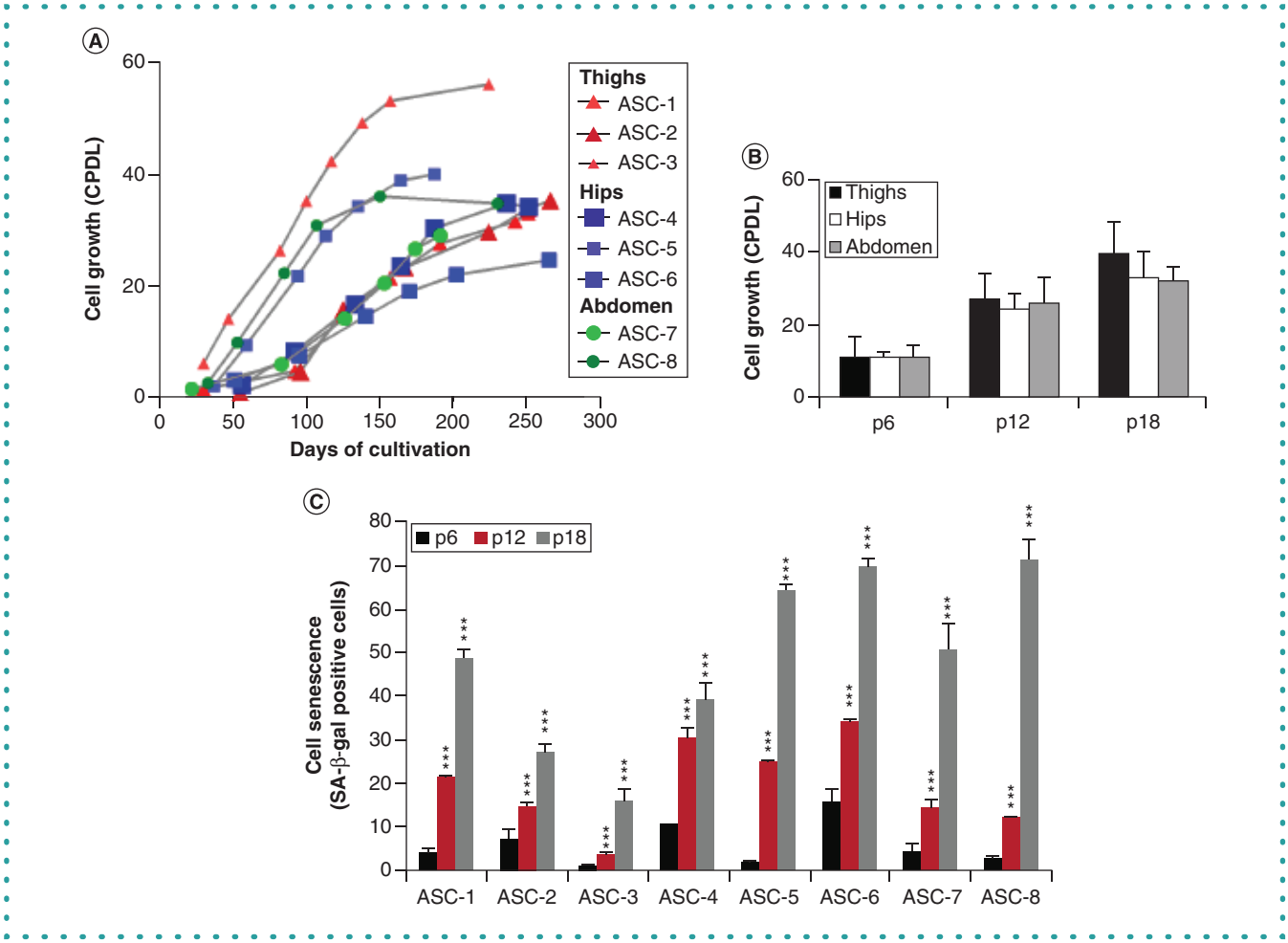


Figure 2. Growth curves and replicative senescence of adipose-derived mesenchymal stromal cells. (A) Long-term (250-day) growth curves of obtained ASC lines, quantified as described in the methods section. **(B)** CPDL of ASCs derived from different anatomical locations at the different passages, p6, p12 and p18. **(C)** SA-β-gal staining of the ASCs as a measure of spontaneous cell senescence during cell cultivation at early (p6) and late (p12 and p18) passage numbers. Quantification of senescent cells was performed by counting senescent cells and nuclei per visual field under a light and fluorescent microscope, respectively. Data represent the mean values ± SD of absorbance from three independent experiments.
***$p < 0.001$.
ASC: Adipose-derived mesenchymal stromal cell; CDPL: Cumulative population doubling levels.

Induced senescence of adipose-derived mesenchymal stromal cells

Since *in vitro* expansion is usually required to obtain sufficient numbers of cells for any particular application, the essential requirement for an ASC line at the time of application is that the cells retain genetic stability, viability and proliferation [26]. These properties strongly depend on cell-specific longevity (i.e., low or no senescence over several passages during ASC/MSC propagation *in vitro*) [14,23]. However, the changes are hardly noticeable at the initial stages of senescence before significant decreases in proliferation rate and altered morphology are observed [10,14,27]. At the molecular level, this is associated with gradual changes in the genetic and transcriptomic profile fingerprints of senescent cells. Cellular senescence-induced transcriptional profile changes in bone marrow–derived MSCs involve more than 5000 genes, including 31 miRNAs [12]. Differential miRNA expression in the aging versus young MSC-derived extracellular vesicles was observed, and these showed that aging MSC-derived extracellular vesicles were not suitable for the treatment of acute lung injury [28].

To avoid the prolonged expansion regimens needed to identify the most long-term, viable ASC/MSC lines, the current work established an evaluation assay that can be used to predict the onset of senescence. A rapid assay was developed using two senescence inducers, Mxt [16] and TSA [17,22], both of which affect cell cycle progression. The Mxt agent is an inhibitor of DNA topoisomerase II, which binds to DNA by intercalation, and the agent TSA is an inhibitor of histone deacetylase. As a consequence of the onset of senescence in ASCs after treatment with Mxt and TSA, their lower metabolic rate was detected.

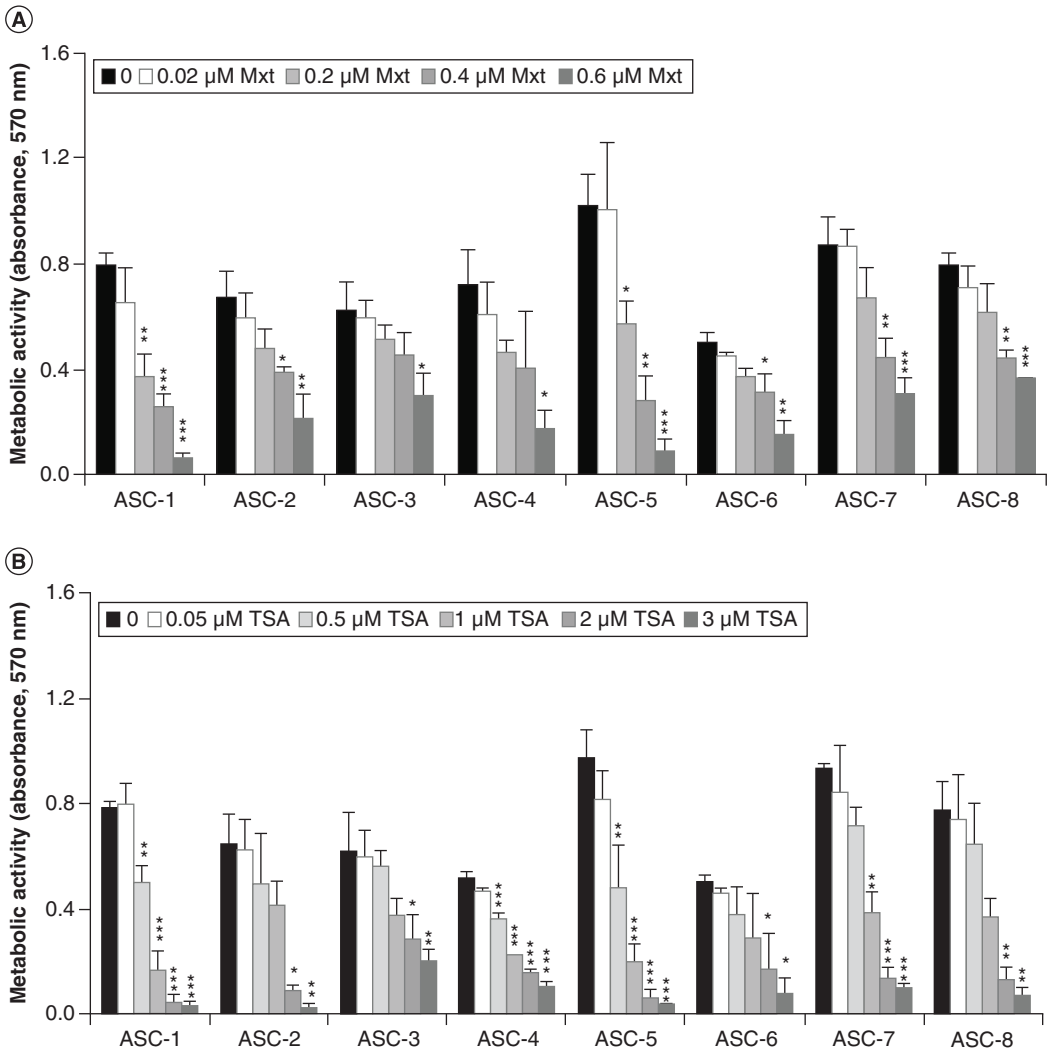


Figure 3. Metabolic activity of the adipose-derived mesenchymal stromal cells after exposure to the senescence inducers mitoxantrone and trichostatin A. Quantification of ASC metabolic activity after exposure to **(A)** mitoxantrone (Mxt) and **(B)** trichostatin A (TSA) using the MTT assay. Data represent the mean values ± SD of absorbance from three independent experiments.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
ASC: Adipose-derived mesenchymal stromal cell.

After 72 h of treatment with Mxt (concentrations ranging 0.02–0.6 μM) and TSA (concentrations ranging 0.05 to 3.00 μM), the viability of ASCs in all ASC lines decreased drastically in a dose-dependent manner, although to different extents (Figure 3A & B). Notably, cell viability and senescence were used, two distinct inversely correlated processes, to demonstrate the effects of treatment with each of the senescence inducers.

Correlation of replicative & induced senescence

We anticipated that the response of ASCs to senescence inducers exposure would reflect the dynamics of their replicative senescence status. Variability in the onset of senescence was observed among ASC lines after Mxt- and TSA-induced senescence (Figure 4A & B). The results in Figure 4A compare the number of spontaneously senescent cells at passage 7 and passage 12 (last red-colored column in each graph). The numbers of senescent cells induced by exposure to 0.02–0.6 μM Mxt showed that the proportion of senescent cells gradually and significantly increased in all ASC lines, although to varying degrees.

However, when comparing the number of spontaneously senescent cells at high passage 12 and the number of senescent cells upon exposure to Mxt in individual ASC lines (Figure 4A), comparable numbers of SA-β-gal positive cells were found in lines ASC-1, ASC-2, ASC-3, ASC-6 and ASC-7 already upon exposure at 0.02 μM Mxt and at 0.2 μM Mxt in lines ASC-3, ASC-4, ASC-5 and ASC-6. Only in

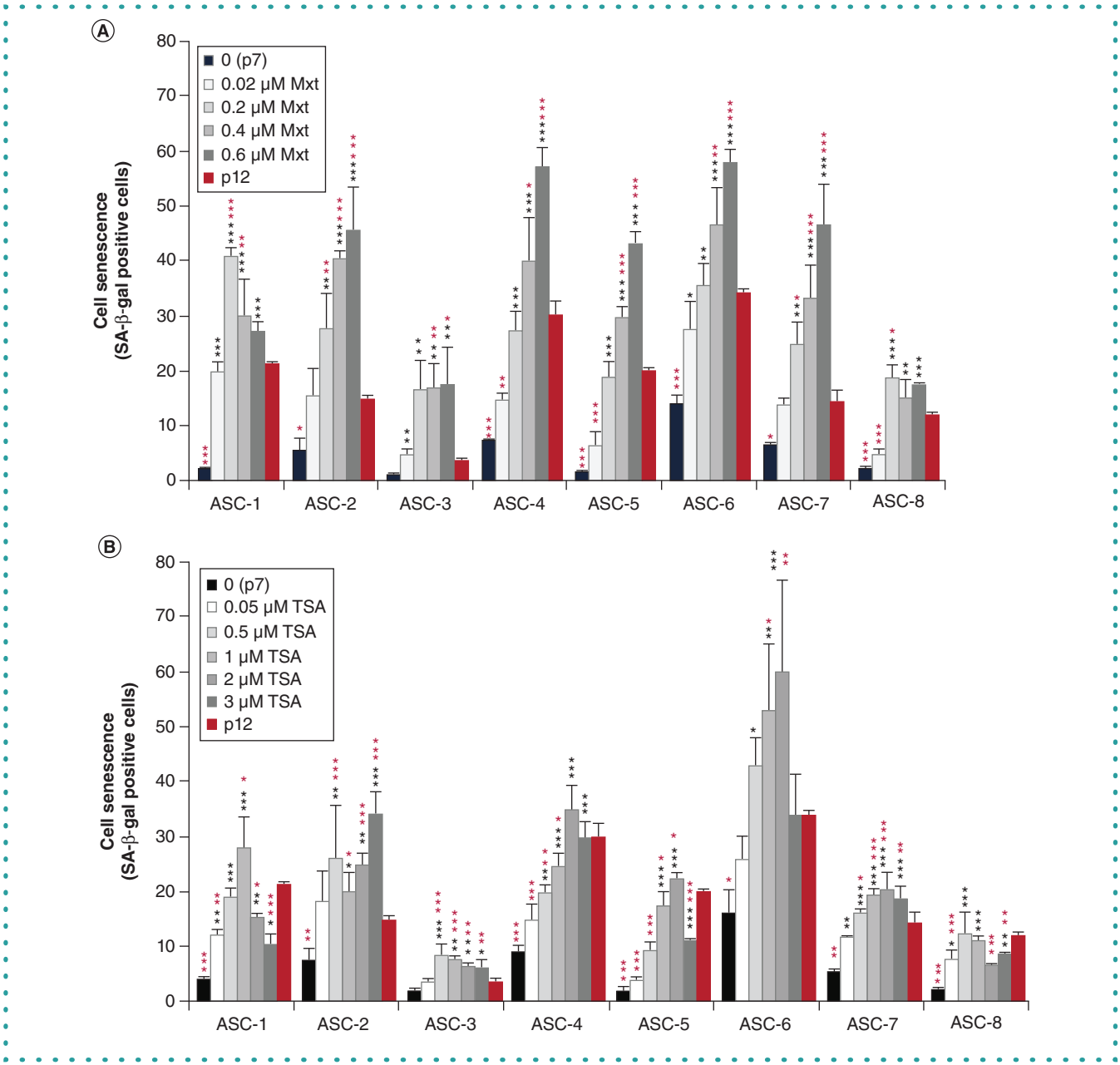


Figure 4. Senescence of the adipose-derived mesenchymal stromal cells during long-term cultivation and after exposure to senescence inducers.
SA-β-gal staining of the ASC's spontaneous cell senescence at late passage p12 and SA-β-gal staining of the ASCs after their exposure to **(A)** Mxt and **(B)** TSA at passage 7. Quantification of senescent cells was performed by counting senescent cells and nuclei per visual field under a light and fluorescent microscope, respectively. Comparison of senescent ASCs in control (black markings) and senescent ASCs at passage p12 (red markings) with respect to the ASCs exposed to Mxt and TSA, respectively. Data represent mean values ± SD from three independent experiments.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
ASC: Adipose-derived mesenchymal stromal cell.

ASC-8, was a comparable proportion of SA-β-gal-positive cells found at passage p12 cells at higher concentrations of Mxt (0.4–0.6 μM). Although the tested ASC lines varied in terms of the proportion of senescent cells at the early passage, exposure to Mxt between 0.02 and 0.4 μM already reflected the extent of spontaneous replicative senescence that would be achieved in the late passage p12 in all ASC lines.

Exposure of passage 7 ASCs to concentrations range 0.05–3.00 μM TSA also resulted in an increase of senescent cells in all ASC lines, again to various extents (Figure 4B). Again, faster-responding ASC lines such as ASC-2, ASC-3, ASC-6 and ASC-7 already correspond to the extent of SA -β-gal-positive cells at passage 12 upon exposure to 0.05 μM TSA and slower responding cells such as ASC-1, ASC-6,

ASC-8 at 0.5 μM TSA (Figure 4B). In ASC-4, the comparable numbers of SA-β-gal-positive cells at passage p12 cells and SA-β-gal-positive cells were observed after exposure to 1 and 2 μM TSA. In the ASC-5 line, no correlation was found between the SA-β-gal-positive cells at passage p12 and at all tested concentrations of TSA. These data showed poor consistency in the proportional increase of responsiveness of ASCs to TSA.

Taken together, these results imply an association between the native, spontaneous replicative senescence of ASCs and induced senescence of ASCs triggered by the senescence inducer Mxt, but not by TSA. The limitation of the proposed protocol is that in this pilot study, its application of effects in a low number of ASCs and at passage 7 was demonstrated, whereas experiments at lower ASC passages would allow faster selection of quality cell lines, thus reducing workload and materials needed. To characterize each ASC line in terms of the senescence process in culture, we first examined the extent of replicative senescence in ASCs at passage 6 and at passage 7 and the extent of induced senescence in ASCs after exposure to various Mxt concentrations was determined, although the effects of senescence inducers should preferably be examined in identical and also lower ASC cell passages. In addition, the identified Mxt concentrations were not further validated on multiple ASC lines. Finally, the method has not yet been applied to bone marrow and other origins of tissues as the source of MSCs. The advantages of the method using this senescence inducer to determine the predisposition of a cell line toward early/late onset of senescence are that it is a technically simple and fast method in which ASCs and possibly MSCs of other tissue origins can be selected for clinical application in regenerative medicine and disease states.

Conclusion

Cell therapy represents an advanced medical tool that is increasingly used in experimental and clinical practice for the treatment of various diseases. MSCs are immunomodulatory cells used in the treatment of acute and chronic immune diseases, whereas autologous adipose tissue-derived MSCs (i.e., ASCs) are mainly used in regenerative medicine (e.g., aesthetic surgery).

Senescence is a progressive biological process that occurs both *in vivo* and *in vitro*. As the therapeutic potential of ASCs and MSCs diminishes with increased passages and progression to senescence, it is of utmost importance to use the primary cell cultures of the MSC lines that are the most senescence-resistant. However, to provide one to two million MSCs per kilogram of body weight for use in a single clinical setting [29] and to overcome the limitation of an extensive expansion of MSCs due to the onset of replicative senescence, cell lines should be subjected to a fast selection process that allows rapid identification of the most viable, long-lived cell lines. Therefore, senescence inducers could be used as a new analytical tool to preselect high-quality MSC lines for each application, avoiding lengthy and expensive *in vitro* expansion steps to validate replicative senescence.

This work is the first demonstration of an association between induced senescence and physiological longevity of ASCs isolated from adipose biopsies from different anatomical locations. The data showed that exposure of ASCs to the preferential senescence inducer Mxt, compared with TSA, can be used in a simple experimental method to identify the resistance of ASCs to the onset of replicative senescence. Although the tested ASC lines varied in their proliferation potential and in the proportion of senescent cells at early passage, exposure to Mxt reflected their tendency for faster progression of replicative senescence at late passage p12 in all ASC lines. Indeed, the results demonstrate a comparable number of SA-β-gal-positive ASCs due to replicative senescence at late passage 12 and SA-β-gal-positive ASCs after exposure to 0.02 μM, 0. 2 μM or 0.4 μM concentrations of Mxt. A limitation of the present study is that the status of replicative senescence of ASCs and the status of induced senescence of ASCs after Mxt exposure were not examined at the same passage and the identified Mxt concentrations were not validated in multiple ASC lines. Taken together, the advantages of this technically simple and fast method using senescence inducers lie in the prediction of the predisposition of a cell line to the onset of senescence of ASCs and possibly MSCs isolated from various tissue sources for allogenic or autologous application.

Future perspective

There is no doubt that advanced cell therapy is the most efficient approach to the treatment of tissue repair, certain autoimmune and neurodegenerative diseases and acute and chronic inflammation. First, Mxt should be tested in MSCs isolated from various tissue sources. Its broader application may also help tame ARDS in patients with COVID-19. Further, MSCs represent a pool of cells that can be used alone, attached to scaffolds or as delivery vectors that will be genetically engineered in the future to produce therapeutic proteins using CRISPR-Cas 9 technology. The longevity of sufficient amounts of these cells is still a bottleneck for wider application but can be overcome by Mxt assays to rapidly preselect the most viable MSC clones, ensuring their higher yield with superb clinical efficacy.

Author contributions

K Kološa conceptualized the study. A Leskovšek provided the tissue samples. T Rajar performed the experimental work. K Kološa analyzed the associated data and wrote the draft of this manuscript. T Lah provided grant support, input and contributed to the writing of the manuscript. All authors approved the final manuscript.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The collection and use of adipose samples were approved by the National Ethics Committee, Slovenia (doc. no. 134/01/11).

Data sharing statement

All related data are available under request.

Open access

Executive summary

- Adipose tissue is a valuable and easily accessible source of MSCs (i.e., ASCs) used for cell therapy and in regenerative medicine. However, the quality of ASCs is highly variable, depending on the age and anatomical location of the donors.
- ASCs, similar to other MSCs, have a limited lifespan due to replicative senescence, which affects their viability and longevity and should be predetermined to ensure their successful expansion in the shortest possible time, which is particularly relevant when a larger number of cells are required for autologous application.
- Early passage of ASCs/MSCs exposed to a chemical inducer of senescence, mitoxantrone, correlates with endogenous dynamics of replicative senescence of ASCs. This simple assay allows early and efficient selection of clones for further expansion of the most viable cells and replaces long-term culturing to evaluate their spontaneous onset of senescence. The value of this innovative approach in cell biotechnology is in particular important due to the increasing use of cell therapy using genetically engineered allogeneic MSCs, similar to Car-T cells, for the treatment of various immune diseases, such as COVID-19.

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