

# Proliferation and differentiation potential of human adipose-derived mesenchymal stem cells isolated from elderly patients with osteoporotic fractures

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## Abstract

Aging has less effect on adipose-derived mesenchymal stem cells (ADSCs) than on bone marrow-derived mesenchymal stem cells (BMSCs), but whether the fact holds true in stem cells from elderly patients with osteoporotic fractures is unknown. In this study, ADSCs and BMSCs of the same donor were harvested and divided into two age groups. Group A consisted of 14 young patients ( $36.4 \pm 11.8$  years old), and group B consisted of eight elderly patients ( $71.4 \pm 3.6$  years old) with osteoporotic fractures. We found that the doubling time of ADSCs from both age groups was maintained below 70 hrs, while that of BMSCs increased significantly with the number of passage. When ADSCs and BMSCs from the same patient were compared, there was a significant increase in the doubling time of BMSCs in each individual from passages 3 to 6. On osteogenic induction, the level of matrix mineralization of ADSCs from group B was comparable to that of ADSCs from group A, whereas BMSCs from group B produced least amount of mineral deposits and had a lower expression level of osteogenic genes. The p21 gene expression and senescence-associated  $\beta$ -galactosidase activity were lower in ADSCs compared to BMSCs, which may be partly responsible for the greater proliferation and differentiation potential of ADSCs. It is concluded that the proliferation and osteogenic differentiation of ADSCs were less affected by age and multiple passage than BMSCs, suggesting that ADSCs may become a potentially effective therapeutic option for cell-based therapy, especially in elderly patients with osteoporosis.

**Keywords:** adipose tissue-derived stem cell (ADSC) • aging • bone marrow-derived mesenchymal stem cell (BMSC) • osteogenic differentiation • osteoporosis • p21 • proliferation • senescence-associated  $\beta$ -galactosidase

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## Introduction

Studies on pluripotent stem cells have created many new possibilities for cell-based therapies. Because the use of human embryonic stem cells is ethically controversial, focus has shifted to the use of adult stem cells, especially bone marrow-derived mesenchymal stem cells (BMSCs) [1–11]. However, the proliferation and osteogenic differentiation potential of BMSCs were associated with aging, which render their use in autologous cell therapy unsuitable in elderly patients [12–16]. Human adipose-derived mesenchymal stem cells (ADSCs) have been identified as an alternative source of post-natal progenitor cells and are thought to have several advantages over BMSCs, including ease of isolation, relative abundance, rapidity of expansion in culture and ability to be cryo-preserved [17–20]. Unlike BMSCs, ADSCs maintain their osteogenic capability in the elderly [18, 21, 22]. Similar osteogenic potential has been observed in ADSCs isolated from juvenile and adult mice, which researchers have used to treat critical-sized calvarial defects [21, 23]. Nevertheless, studies on human ADSCs were scarce and were based only on a limited number of cases [18].

Because of the growing attention on ADSCs and its advantages compared to BMSCs, a thorough understanding of the biological properties of human ADSCs in relation to age is crucial, especially when ADSCs are to be used in the treatment of elderly patients. In this study, human ADSCs and BMSCs were derived from both young patients and elderly patients with osteoporotic fractures. The proliferation and osteogenic differentiation potentials of ADSCs and BMSCs were compared to determine the effect of aging and osteoporosis on human ADSCs and BMSCs. To the best of our knowledge, this is the first study to characterize human ADSCs and BMSCs isolated from elderly patients with osteoporosis, and to compare the proliferation potential of ADSCs and BMSCs from the same patient. Results from this study should provide implications for the clinical application of ADSCs to skeletal regeneration in elderly patients.

## Materials and methods

### Procurement of samples

The protocol for this study was approved by the institutional review board of Kaohsiung Medical University Hospital. Mesenchymal stem cells were isolated from adipose or bone marrow tissues obtained with informed consent from patients undergoing hip surgeries. We excluded patients with liver or renal function insufficiency, hormone disorders, diabetes mellitus, pregnancy and past-history of neurological impairment or those who had taken glucocorticoid medications. The donors were divided into two age groups. Group A consisted of 14 patients (eight males and six females,  $36.4 \pm 11.8$  years old) suffering from dysplastic hip osteoarthritis or hip fracture due to major trauma unrelated to osteoporosis. Group B consisted of eight elderly patients (three males and five females, aged  $71.4 \pm 3.6$  years old) with osteoporotic fractures at the

intertrochanter or neck of femur due to low-energy trauma such as falls at home. Obesity was not observed in all patients, whose average body mass index (BMI) was 23.5 (ranging from 20.8 to 25.7). ADSCs were isolated from adipose tissue samples provided by 10 patients in group A (ADSC-A) and seven in group B (ADSC-B). BMSCs were isolated from bone marrow tissues provided by eight patients in group A (BMSC-A) and four in group B (BMSC-B).

### Isolation of ADSCs from adipose tissues

We have previously reported the detailed procedures of the isolation and characterization of ADSCs [24, 25]. Briefly, subcutaneous adipose tissue (5 g) was excised from the gluteal area during surgery, minced with scissors, and digested with type IA collagenase. The digested tissue was centrifuged at 1000 rpm for 5 min., and the pellet was resuspended in K-NAC medium [24, 26] (Gibco-BRL, Grand Island, NY, USA) and plated in a 100-mm culture dish. The medium was refreshed 24 hrs later and changed every 2 days thereafter.

### Isolation of BMSCs from bone marrow

We have previously reported the detailed procedures of the isolation and characterization of BMSCs [27–29]. Generally, bone marrow sample (5 ml) obtained by tapping from the iliac crest was mixed with 25 ml DMEM and centrifuged at 12,000 rpm for 5 min. After removal of the supernatant, cell pellet was mixed with DMEM and Percoll (70% in PBS), followed by centrifugation at 1560 rpm for 15 min. The pellet was then resuspended in K-NAC medium and plated in a 150-mm culture dish. The medium was changed every 2–3 days.

### Determination of cumulative population doubling level

The proliferation potential of ADSCs and BMSCs was determined by cumulative population doubling level (CPDL), which was calculated as:  $\ln(N_f/N_i)/\ln 2$ , where  $N_i$  and  $N_f$  are initial and final cell numbers (the cell number on the day of subculture), respectively, and  $\ln$  is the natural log [26]. Starting from the 3rd to the 9th passage, the standard protocol for each subculture was described as follows. ADSCs or BMSCs were seeded in a 150-mm dish at an initial cell number of  $10^5$  ( $10^5$  cells/150-mm dish), and the culture medium was changed every 2 days. The doubling time and cell number was determined at each passage on the 7th day. However, once the cells were unable to reach confluence or once a doubling time of over 100 hrs was obtained in two consecutive passages before achieving the 9th passage, CPDL calculation was discontinued, and the culture was considered to have failed at that passage [26].

### Osteogenic differentiation

ADSCs and BMSCs were thawed at the 3rd passage and expanded to the 5th passage for osteogenic differentiation analysis. The cells were seeded at  $2 \times 10^4$  cells/well in a 12-well plate and maintained in K-NAC medium

until reaching 80% confluence. To induce osteogenic differentiation, culture medium was switched to osteogenic induction medium (OIM) consisting of 0.1  $\mu$ M dexamethasone, 50  $\mu$ M l-ascorbate-2-phosphate and 10 mM  $\beta$ -glycerophosphate in DMEM. The level of extracellular matrix calcification at different time points (0, 7 and 14 days) was determined by alizarin red S staining, and the expression of osteogenic genes at different time points (1, 2, 4, 7 or 10 days) was quantitated by real-time polymerase chain reaction (PCR).

## Alizarin red S staining

Cells were fixed with 4% paraformaldehyde at room temperature for 10 min. After washing once with ddH<sub>2</sub>O, 1 ml alizarin red S solution (2%, pH 4.2) was added to each well in a 12-well plate. The staining solution was removed 10 min. later, and each well was washed with ddH<sub>2</sub>O for four to five times. The plate was then air-dried at room temperature [29, 30]. The amount of matrix mineralization was determined by dissolving the cell-bound alizarin red S in 10% acetic acid and quantifying spectrophotometrically at 415 nm.

## Real-time PCR

The mRNA level of genes related to osteogenesis, including BMP-2, Runx2, osteocalcin and alkaline phosphatase (ALP), and those related to aging, including p53, p21 and p27, were quantitated by real-time PCR using an iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). In each assay, 1  $\mu$ g total RNA was treated with 2U DNase I (Ambion, Carlsbad, CA, USA) and reverse-transcribed by Clontech RT-for-PCR kit (BD Biosciences, San Jose, CA, USA) using oligo dT as primers. Real-time PCR reaction mixtures were prepared with iQ SYBR Green Supermix (Bio-Rad Laboratories). PCR primer sequences were listed in Table 1, with primer specificity confirmed on the NCBI Primer-BLAST website. Real-time PCR was performed with cDNAs from at least three independent experiments. Melting curve analysis was performed for each reaction to ensure a single peak. Amplicons were visualized with electrophoresis on a 1.4% agarose gel to ensure the presence of a single amplicon. Fold changes (*x*-fold) in gene expression level were calculated by the  $2^{-\Delta\Delta Ct}$  method [31]. Analysis of variance was performed as in previous studies using Excel 2003 software (Microsoft Corp, Cupertino, CA, USA) [28].

## Senescence-associated $\beta$ -galactosidase staining

Cells were washed with PBS and fixed in 10% formalin for 3–5 min. at room temperature. After rinsing with PBS to remove residual formalin, the cells were incubated at 37°C in the absence of CO<sub>2</sub> for 12–16 hrs in freshly prepared senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) staining solution (1 mg/ml 5-bromo-4-chloro-3-indolyl  $\beta$ -D-galactoside (X-Gal), 40 mM sodium citrate, 40 mM citric acid, 150 mM NaCl, 2 mM MgCl<sub>2</sub>, 5 mM potassium hexacyanoferrate II and 5 mM potassium hexacyanoferrate III, pH 6.0). The ratio of SA- $\beta$ -gal-positive cells was calculated as  $(N_s/N_t) \times 100\%$ , where  $N_s$  is the number of SA- $\beta$ -Gal-positive cells and  $N_t$  is the total cell number in a microscopic field. At least five different microscopic fields were randomly taken to determine the ratio of SA- $\beta$ -gal-positive cells.

**Table 1** Primer sequences for real-time PCR

Genes	Primers
Human p21	F: GACACCACTGGAGGGTGACT
	R: CAGGTCCACATGGTCTTCCT
Human p53	F: GTTCCGAGAGCTGAATGAGG
	R: TGAGTCAGGCCCTTCTGTCT
Human p27	F: ATGTCAAACGTGCCAGTGTCT
	R: TCTCTGCAGTGCTTCTCCAA
Human Runx2	F: AGATGGGACTGTGGTTACTG
	R: GTAGTCTACTGGGGAGGATT
Human BMP2	F: CGAATGACTGGATTGTGGCT
	R: TGAGTTCTGTCTGGGACACAG
Human osteocalcin	F: GTGCAGAGTCCAGCAAAGGT
	R: CGATAGGCCTCCTGAAAGC
Human alkaline phosphatase	F: CCTCCTCGGAAGACACTCTG
	R: GCAGTGAAGGGCTTCTTGTC
Human GAPDH	F: CAATGACCCCTTCATTGACC
	R: TTGATTTTGGAGGGATCTCG

The cycling conditions are as follows: 95°C for 5 min., followed by 35 cycle of 95°C for 10 sec., 61°C for 15 sec. and 72°C for 15 sec.

## Statistical analysis

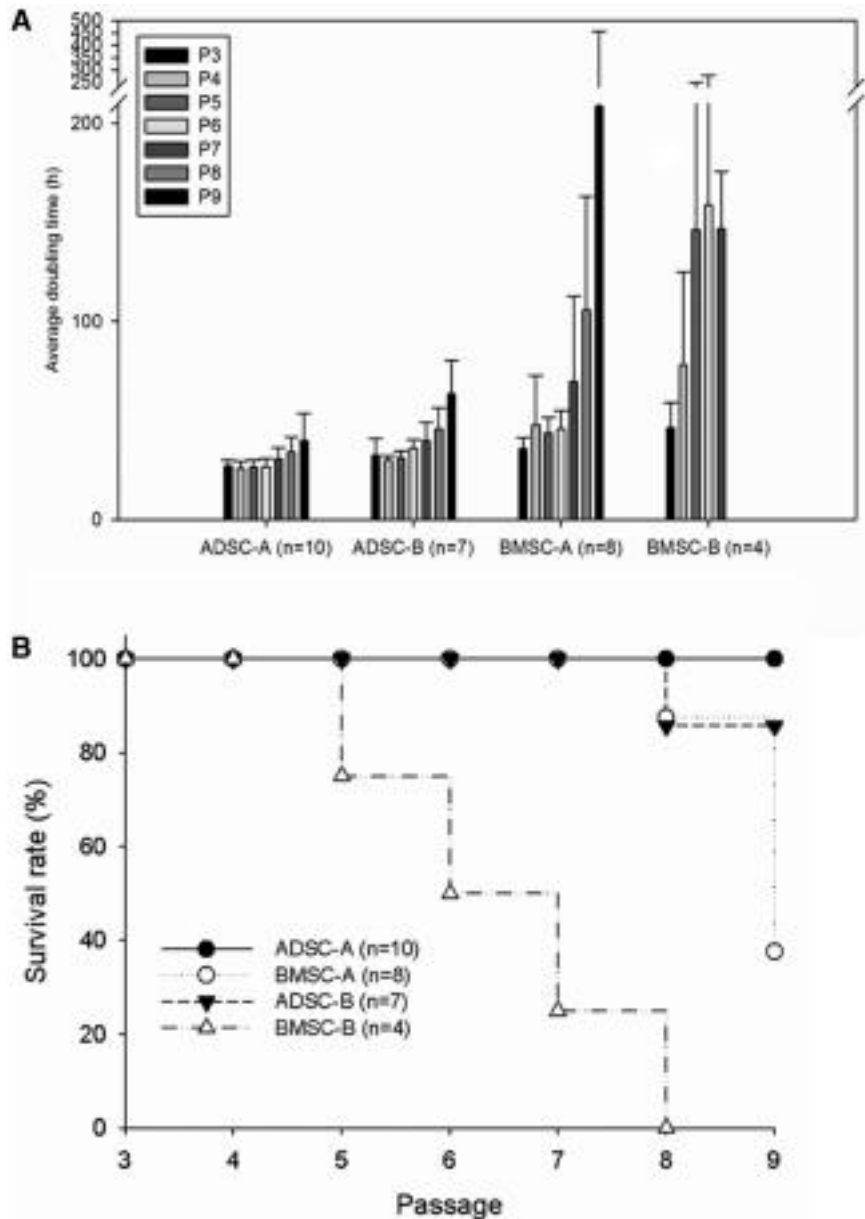
The doubling time of ADSCs and BMSCs was compared by two-way ANOVA with variables of age or cell types, followed by Tukey's test using the JMP 6.0 software (SAS Institute, Cary, NC, USA). Statistical significance in alizarin red S staining and gene expression was determined by ANOVA, and the differences between means were tested using Scheffe's method. A level of significance of  $P < 0.05$  was accepted as significant. The doubling time from passages 3 to 6 of ADSC and BMSC pairs from the same patient was compared by repeated measures analysis of variance. The log-rank test was used to compare the survival rate of ADSCs with those of BMSCs from passages 3 to 9. The difference between the slopes of the plots of accumulated cell numbers was determined by the *F*-tests of the SAS system.

## Results

### Comparison of the doubling time of human ADSCs and BMSCs from different age groups

Both ADSCs and BMSCs were derived from each donor to study the effects of age and osteoporosis on the proliferation of these stem cells. The doubling time of ADSCs was generally maintained below 70 hrs, and the aging effect in ADSCs was not significant until the 9th passage for both group A ( $P < 0.001$ ) and group B ( $P = 0.001$ ; Fig. 1A). On the other hand, the doubling time of BMSCs increased

**Fig. 1** Comparison of the proliferation potential of ADSCs and BMSCs from groups A and B. Donors of ADSCs and BMSCs were divided into two age groups: Group A, 14 patients (eight males and six females, aged  $36.4 \pm 11.8$  years old) with dysplastic hip osteoarthritis or hip fracture due to major trauma unrelated to osteoporosis; Group B, eight elderly patients (three males and five females, aged  $71.4 \pm 3.6$  years old) with osteoporotic fractures at the intertrochanter or neck of femur due to low energy trauma. **(A)** Change in average doubling time of human ADSCs and BMSCs with the number of passage. Error bars represent standard deviations. *n* in the *x*-axis represents the number of stem cell lines survived at each passage unless otherwise stated as follows: *n* = 6 at P8 and P9 of ADSC-B; *n* = 5 at P9 of BMSC-A; *n* = 3 at P6 of BMSC-B and *n* = 2 at P7 of BMSC-B. **(B)** Survival rate analysis of human ADSCs and BMSCs. Failure was defined as a doubling time of over 100 hrs for two consecutive passages or when cells ceased to grow and were unable to reach confluence.



with the number of passage, and was significantly longer than that of ADSCs ( $P = 0.015$  at the 6th passage). Several of the BMSC lines ceased to proliferate at high passage. In BMSCs of group A (BMSC-A), only five of the eight BMSC lines reached the 9th passage, whereas in BMSCs of group B (BMSC-B), only two of the four BMSC lines reached the 7th passage. No gender specificity was observed, as the change in doubling time with passage number of ADSCs and BMSCs were similar in male and female (data not shown). These results suggest that aging but not gender had a greater suppressive effect on the proliferation of BMSCs than on ADSCs.

The survival rate of ADSCs and BMSCs from the 3rd to the 9th passages was evaluated by the log-rank test (Fig. 1B and Table 2). Failure was defined as a doubling time of over 100 hrs in two consecutive passages or when cells ceased to grow and were unable to reach confluence. The survival rate of ADSCs from both age groups (ADSC-A and ADSC-B) was comparable at each passage, with ADSC-A maintaining 100% survival to the 9th passage. In contrast, the survival rate of BMSCs decreased significantly with the number of passage. The survival rate of BMSC-A decreased to 37.5% by the 9th passage, while that of BMSC-B declined to 25%

**Table 2** Statistical analysis of the survival rate of ADSCs and BMSCs from groups A and B (Fig. 1B) using either cell types or age as variables

Variables	Group	P
Cell types (ADSC versus BMSC)	A	0.004
	B	0.001
Age (group A versus group B)	ADSC	0.340
	BMSC	0.011

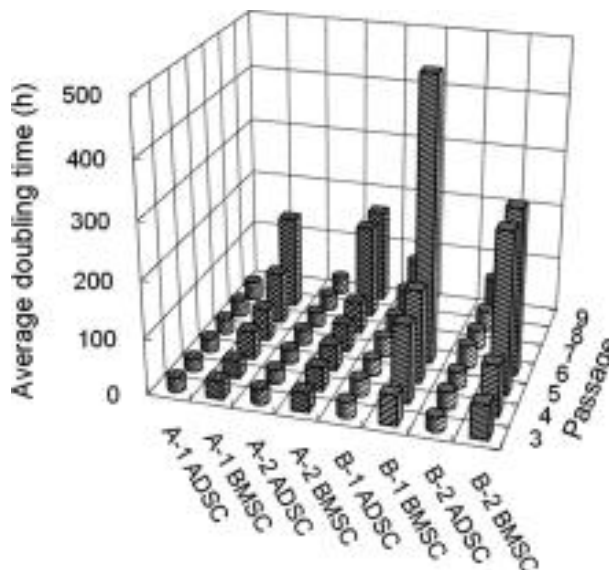
by the 7th passage, and proliferation ceased thereafter (Fig. 1B). Statistical analysis of the survival rate suggests that the effect of aging was more evident in BMSCs than in ADSCs (Table 2). In addition, there was no significant difference when the survival rate of stem cells from female patients was compared with that from male patients (data not shown).

### Paired doubling time comparison of ADSCs and BMSCs from the same patient

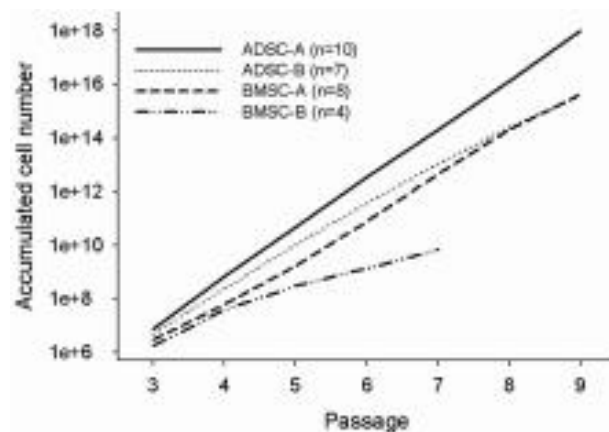
Paired doubling time comparison of ADSCs and BMSCs from the same patient was performed by repeated measures analysis of variance. Because some of the stem cell lines ceased to grow beyond passage 7, the doubling time of ADSC and BMSC pairs was compared from passages 3 to 6. We found that there was a significant increase in the doubling time of BMSCs compared to ADSCs in each individual from passages 3 to 6 ( $P = 0.019$ ; Fig. 2). No gender specificity was observed when the doubling time of ADSCs and BMSCs from the same donor was compared (data not shown).

### The accumulated cell number of human ADSCs and BMSCs from different age groups

ADSC-A and ADSC-B were capable of achieving a cell number of  $10^8$  within 4 passages and  $10^{13}$  within 7 passages (Fig. 3). BMSC-A and BMSC-B reached a cell number of  $10^8$  after 5 passages, but only BMSC-A attained to a population of  $10^{13}$  at the 8th passage, while the accumulated cell number of BMSC-B levelled off at  $10^9$ . Statistical analysis of the slope of the plots of accumulated cell numbers indicated that the growth rate of ADSC-A was comparable to that of ADSC-B, but the difference in cell accumulation between ADSC-B and BMSC-B was statistically significant ( $P = 0.038$ ). The growth of BMSC-B was apparently delayed, although significant difference between BMSC-A and BMSC-B was not detected, due possibly to a low case number. Significant difference was not found when we compared the accumulated cell number of ADSCs and BMSCs by gender (data not shown).



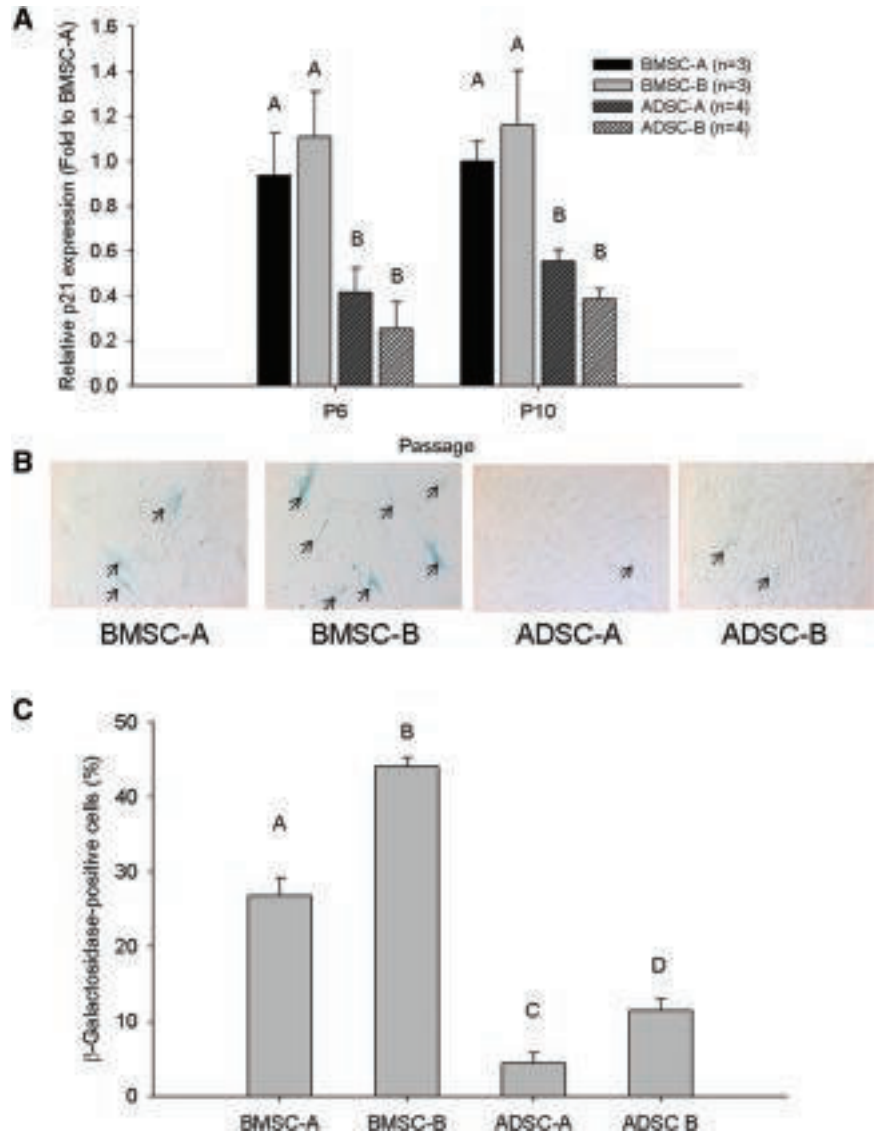
**Fig. 2** Paired doubling time comparison of ADSCs and BMSCs of the same patient. The doubling time of two representative ADSC and BMSC pairs from each age group was shown.



**Fig. 3** Comparison of the accumulated cell number of ADSCs and BMSCs from groups A and B. The average accumulated cell number of each ADSC and BMSC line was obtained at each passage and plotted against the number of passage (passages 3–9).  $n$  represents the number of stem cell lines survived at each passage unless otherwise stated in the legend of Figure 1.

### Expression of genes related to aging

The mRNA levels of p21, p27 and p53, which are associated with senescence, were evaluated by real-time PCR. Although no significant difference in the gene expression of p27 and p53 was observed in ADSCs and BMSCs ( $P > 0.05$ , data not shown), the mRNA level of p21 was significantly higher in BMSCs than in



**Fig. 4** The expression of biomarkers related to aging in ADSCs and BMSCs from groups A and B. **(A)** The mRNA level of p21 at passages 6 and 10 of ADSCs and BMSCs. **(B)** Representative senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) staining results of ADSCs and BMSCs. Arrows indicate SA- $\beta$ -gal-positive cells. **(C)** The ratio of SA- $\beta$ -gal-positive cells in ADSCs and BMSCs. Different uppercase letters designated on bars under comparison indicate significant difference between the stem cell lines or passages.

ADSCs in both age groups ( $P < 0.0001$ , Fig. 4A). Besides, the level of p21 gene expression did not change with passage number (compare passage 6 with passage 10,  $P = 0.148$ ), suggesting that the difference in p21 expression between ADSCs and BMSCs already exists in early passage.

### Senescence-associated $\beta$ -galactosidase activity

The activity of SA- $\beta$ -gal serves as a specific biomarker for assessing replicative senescence in mammalian cells (Fig. 4B). Our results show that aging is correlated with an increase in SA- $\beta$ -gal activity in both ADSCs and BMSCs (Fig. 4B and 4C). Nevertheless,

in both age groups, the ratio of SA- $\beta$ -gal-positive cells in BMSCs was higher than that in ADSCs ( $P < 0.0001$ , Fig. 4B and 4C), suggesting that more senescent cells were present in BMSCs. These results indicate that higher p21 gene expression and SA- $\beta$ -gal activity may be partly responsible for the age-related decline in the proliferation capability of BMSCs.

### Osteogenic potential of ADSCs and BMSCs

The change in mRNA levels of osteogenic genes with time during osteogenic differentiation was determined by real-time PCR. The basal expression of Runx-2, osteocalcin and ALP at day 0 was nearly the same in both ADSCs and BMSCs (Fig. 5). BMP-2 gene expression

was increased at day 1 ( $P = 0.007$ ), but no significant difference was detected between ADSCs and BMSCs from both groups A and B (data not shown). Except for BMSC-B, the mRNA level of Runx-2 in BMSC-A, ADSC-A and ADSC-B increased at day 1 ( $P < 0.001$ ) and decreased at day 2 and 4 (Fig. 5A), whereas the expression of osteocalcin and ALP peaked at day 4 and 10, respectively (Fig. 5B and 5C). On the other hand, the expression of Runx-2, osteocalcin and ALP in BMSC-B remained at a similar level to day 0 (Fig. 5).

ADSCs and BMSCs derived from both groups A and B were cultured in osteogenic differentiation medium and stained with alizarin red S to determine the level of extracellular matrix calcification. Matrix mineralization in both BMSCs and ADSCs was observed at day 14 and 21, except for BMSC-B (Fig. 6A). The amount of alizarin red S stain was comparable in ADSC-A, ADSC-B and BMSC-A, and was significantly higher than that of BMSC-B ( $P < 0.001$  at day 21, Fig. 6B). The significantly delayed osteogenic differentiation of BMSC-B may be correlated with the absence of up-regulation of osteogenic genes (Fig. 5). These results indicate that the osteogenic potential of BMSCs is similar to ADSCs in young patients, but decreased significantly in the elderly, while that of ADSC-B remained comparable to ADSC-A.

## Discussion

Massive bone defects, non-united fractures or systemic bone disorders such as osteoporosis often pose a critical challenge to clinicians. New techniques involving gene therapy, cell therapy and tissue engineering are being developed to improve bone regeneration. Adult mesenchymal stem cells derived from various sources, including bone marrow, subcutaneous fat, infrapatellar fat, cruciate ligament, muscle and synovium [32, 33], are potential candidates for stem cell therapy. Although BMSCs are being widely used in cell-based therapy and tissue engineering [3, 34–38], there is increasing evidence suggesting that ADSCs possess biological properties similar to BMSCs, and have many advantages over BMSCs, including better proliferation and less aging effects. Recently, it was reported that the population doublings of BMSCs were less than ADSCs, and the level of apoptosis or aging increased with the number of passage of BMSCs [39–41]. Moreover, the osteogenic potential of BMSCs was correlated with age both in murine and in human [42, 43]. These drawbacks may restrict the application of BMSCs in bone tissue engineering, especially in elderly patients with bone loss and higher risk of fractures. In this study, we focused on the proliferation and differentiation potential of ADSCs from elderly patients suffering from osteoporotic fractures, which are of critical importance to the efficiency of stem cell therapy and tissue engineering in geriatrics.

Factors including BMI, source, gender, age and culture conditions affect the proliferation and differentiation of mesenchymal stem cells. Previous studies have shown that the proliferation rate of ADSCs from young donors was higher than those obtained from elder donors, and both adipogenic and osteogenic differentiation capabilities of ADSCs were correlated with age [44–46]. Van Hermelen *et al.* reported that age correlated negatively to the percentage of proliferation of subcutaneous ADSCs but not omental

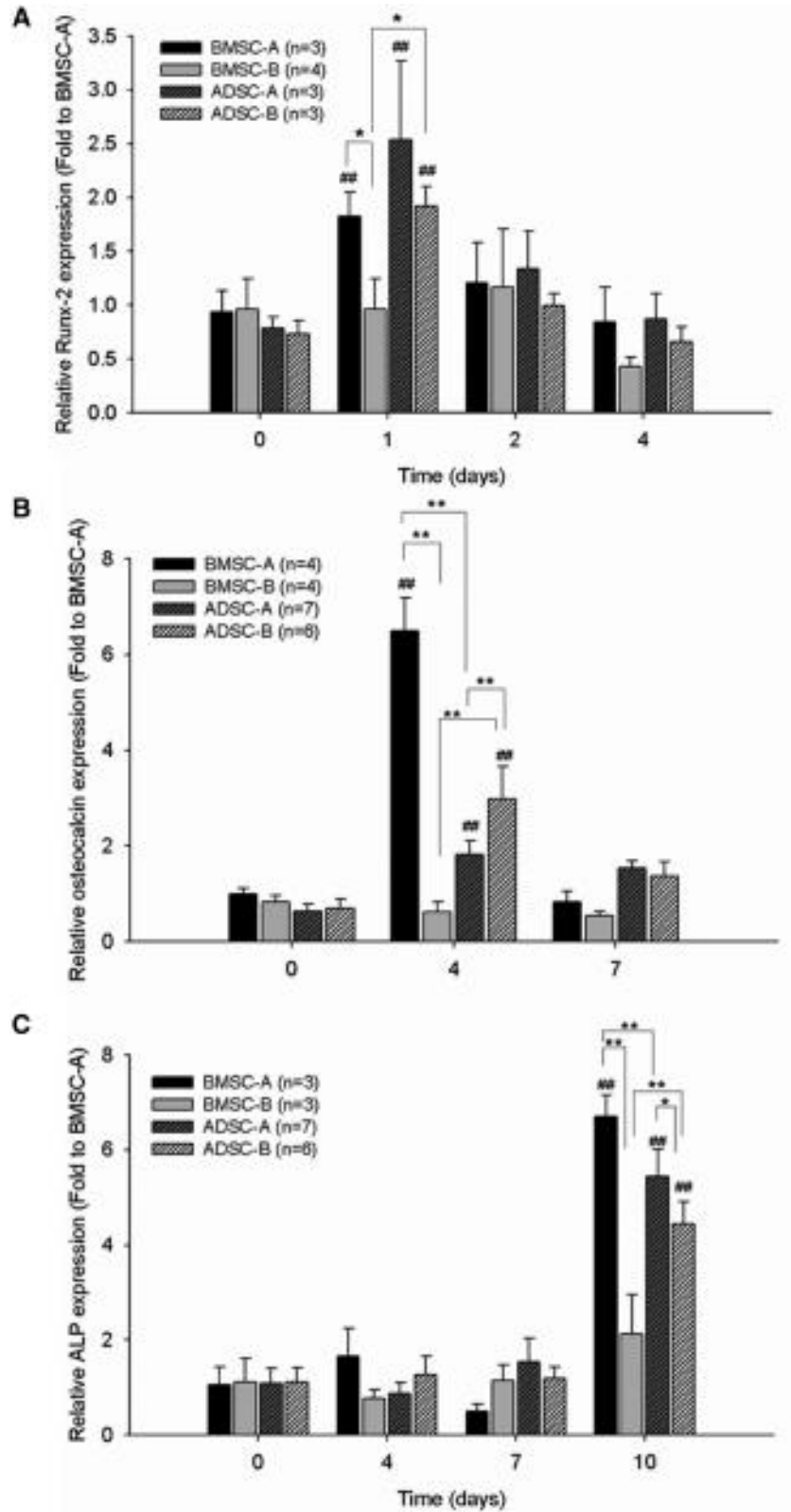
ADSCs. The adipogenic differentiation potential of omental ADSCs, but not subcutaneous ADSCs, decreased with age. Besides, they also found that ADSCs from obese patients were less capable of adipogenic differentiation than those from non-obese patients [44]. Huang *et al.* suggested that aging significantly suppressed proliferation and enhanced adipogenic differentiation of ADSCs, whereas the osteogenic potential was not significantly affected by aging [46]. Girolamo *et al.* reported no significant discrepancies in adipogenic differentiation between ADSCs isolated from young and elderly women, whereas the level of osteogenic differentiation was significantly reduced by aging.

Our results suggest that age was not an influential factor in the proliferation and osteogenic differentiation of ADSCs. Although significant difference between ADSC-A and ADSC-B was observed in the mRNA level of osteocalcin and ALP at day 4 and day 10, respectively (Fig. 5), the doubling time (Figs 1 and 2), accumulated cell number (Fig. 3) and level of matrix mineralization (Fig. 6) of ADSCs remained unaffected by age. The discrepancy between our findings and those in the literature may be explained by differences in the gender, age and health status of the donor, sources of the adipose tissue, and culture conditions of ADSCs. In addition, whether the adipogenic differentiation capability of ADSCs obtained in this study was correlated with age remains to be investigated.

There are few studies focusing on the comparison of human ADSCs and BMSCs derived from the same donor, especially elderly donors with bone-related diseases. This study is also the first to correlate the proliferation capability of ADSCs and BMSCs with passage number. It was found that the proliferation potential of ADSCs was higher than BMSCs, and that the growth of ADSCs was not affected by passage number, age and osteoporosis (Figs 1–3). When ADSCs and BMSCs from the same individuals were compared, the doubling time of BMSCs from passages 3 to 6 was significantly longer than that of ADSCs (Fig. 2). As reported in the literature, the mean population doubling time from days 3 to 9 during the logarithmic growth phase of PLA cells and MSCs cultured in DMEM were  $78 \pm 26$  hrs and  $86 \pm 23$  hrs, respectively [18]. In this study, the average doubling time of both ADSCs and BMSCs were generally shorter, supporting the proposal that the K-NAC medium accelerates the growth and prolongs the lifespan of stem cells [26].

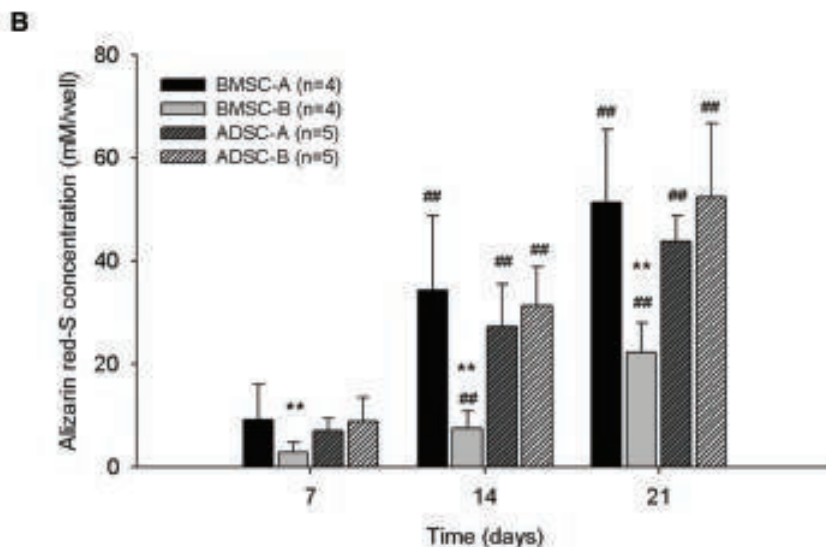
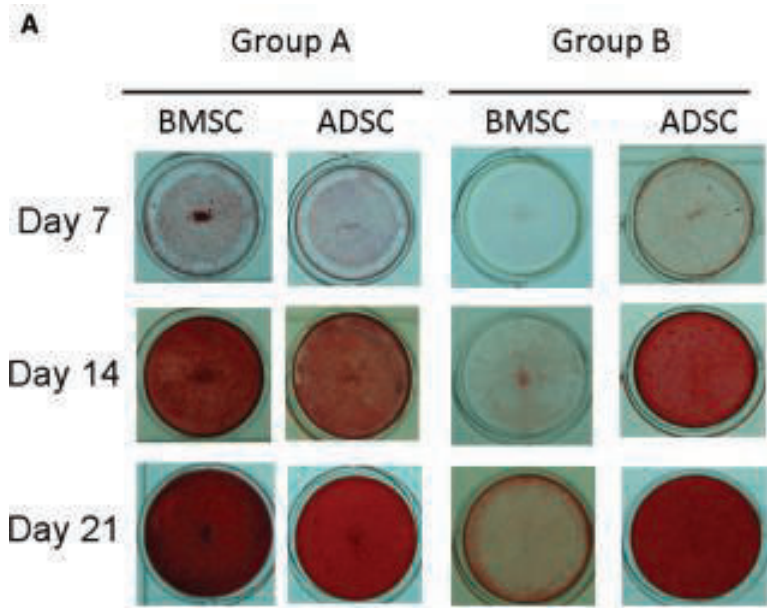
Studies have shown that there are intrinsic alterations in human MSCs with aging. The activity of SA- $\beta$ -gal, doubling time, number of apoptotic cells and expression of p53, p21 and BAX were elevated in BMSCs from elder patients [47]. Our findings support this assumption in that the SA- $\beta$ -gal activity of ADSCs and BMSCs from group B was significantly higher than those in group A. We further show that the activity of SA- $\beta$ -gal, as well as the expression level of p21, was higher in BMSCs compared to ADSCs, suggesting that pathways related to aging may be more activated in BMSCs than in ADSCs.

In summary, our study demonstrated that the proliferation and osteogenic differentiation of ADSCs were less affected by age than BMSCs. ADSCs from elderly patients with osteoporosis can be induced to differentiate into osteoblasts at a similar rate to ADSCs and BMSCs from younger donors. In addition, the levels of biomarkers related to senescence, such as SA- $\beta$ -gal



**Fig. 5** The mRNA level of (A) Runx-2, (B) osteocalcin and (C) alkaline phosphatase (ALP) in ADSCs and BMSCs from groups A and B. The double hash sign (##) indicates highly significant difference ( $P < 0.01$ ) compared with the same cell line at day 0. The double asterisk (\*\*) indicates highly significant difference ( $P < 0.01$ ) compared with other stem cell lines on the same day.





**Fig. 6** Extracellular matrix mineralization of ADSCs and BMSCs from groups A and B at the 5th passage. **(A)** Representative alizarin red S staining results of ADSCs and BMSCs at day 7, 14 and 21 of osteogenic induction. **(B)** Quantification of the alizarin red S staining results. The double hash sign (##) indicates highly significant difference ( $P < 0.01$ ) compared with the same cell line at day 7. The double asterisk (\*\*) indicates highly significant difference ( $P < 0.01$ ) compared with other stem cell lines on the same day.

activity and p21 gene expression, were lower in ADSCs compared to BMSCs, which may contribute in part to the superior proliferation and differentiation potential of ADSCs. These results suggest that ADSCs may serve as an effective alternative to BMSCs in stem cell therapy, and autologous ADSCs from the elderly may become a promising therapeutic agent in geriatrics to treat skeletal diseases.

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## Conflict of interest

The authors confirm that there are no conflicts of interest.

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