**Adipose Tissue-Derived Stromal Cell Function is Maintained in Senile Osteoporotic SAMP6 Mice**

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**INTRODUCTION**

Bone formation is reliant on the presence of functionally active mature osteoblasts derived from progenitor cells within the bone marrow termed bone marrow stromal cells (BMSCs), through a process of osteogenic differentiation. The observation that BMSCs isolated from aged osteoporotic patients have a higher propensity toward adipogenesis than osteogenesis implies that the structural abnormalities associated with osteoporotic bone maybe as a consequence of inadequacies in bone cell differentiation. The use of adult menenchymal stromal cells (MSCs) for the purpose of developing new bone reconstruction therapies is now prevalent in areas of both experimental and clinical research. Adult stem cells from adipose tissue, termed adipose tissue-derived stromal cells (ADSCs), have the capacity for osteogenic differentiation and may be easily accessed from subcutaneous fat stores through minimal invasive procedures. However, the application of ADSCs as a cell-based therapy for the purpose of enhancing orthopedic tissue repair and regeneration is at an early stage of research and as such, no studies have yet been carried out to evaluate the possible therapeutic benefits of treating osteoporotic bone with autologous ADSCs. In the present study, we have characterized ADSCs isolated from aged osteoporotic mice and evaluated their suitability as a source of osteogenic precursor cells.

**METHODS**

Experiments were performed using 20-week-old female and male senescence-accelerated mouse strain P6 (SAMP6) and control age-matched senescence-accelerated mouse resistant strain R1 (SAMR1). All animal research procedures were approved by the Animal Experimentation Committee of the Veterinary Office of the Canton of Zürich, Switzerland and followed the guidelines of the Swiss Federal Veterinary Office for the use and care of laboratory animals. The biological age and osteoporotic phenotype of mice were initially established using Q-FISH and microCT respectively. Stromal cells were isolated from subcutaneous inguinal fat pads by collagenase digestion and selected for by plastic adherence. Characterization of stem cell marker expression and multipotency was determined by FACS- determined using FISH and microCT respectively. ADSC proliferation (Figure 2a) and telomerase activity (Figure 2b) were monitored over 14 days through the quantitative measurement of alkaline phosphatase (ALP) activity normalized to cell protein levels. Adipogenesis was assessed at day 14 by Oil Red O staining. The level of staining was quantified by colorimetric analysis following isopropanol extraction and normalized to total cell DNA content.

**RESULTS**

SAMP6 mice showed osteoporotic-related traits as indicated by significant reductions in trabecular bone volume density (4.72±0.33 vs. 8.43±0.52, p<0.001), trabecular bone surface density (2.01±0.14 vs. 3.22±0.18, p<0.001) and trabecular number (1.38±0.05 vs. 1.9±0.06, p<0.001). In addition, Q-FISH analysis of interphase nuclei in live sections revealed significant reductions (p<0.01) in telomere length (Figure 1) indicative of premature aging in SAMP6 mice. ADSC populations from SAMP6 and SAMR1 were positive for several markers considered to be representative of mesenchymal stem cells including CD29, CD105 and Sca1, but were almost absent for the well-known hematopoietic markers CD34 and CD45 (data not shown). Cell morphology was comparable in undifferentiated ADSCs from SAMP6 and SAMR1 mice and multipotency was demonstrated by their ability to differentiate into adipocytes, osteoblasts and chondrocytes (data not shown). Furthermore, ADSC proliferation (Figure 2a) and telomerase activity at passage 1 (P1) and passage 4 (P4) (Figure 2b) were found to be similar between both strains. Quantitative analysis of ALP activity in cells undergoing osteogenic differentiation revealed no significant differences between mouse strains (Figure 3a). Similarly, adipogenesis as determined by Oil Red O staining was also comparable (Figure 3b).

**DISCUSSION**

The general fitness of BMSCs appears to decline with donor age and passage number as evidenced by increases in cellular aging markers and an inability to maintain osteogenic potential. Such observations have therefore led to speculation that deficiencies in resident BMSC differentiation may play a significant role in the development of age-related osteoporotic phenotypes.

Stromal cells isolated from adipose tissue represent a readily available source of MSCs with the ability to differentiate into numerous cell types including osteoblasts and fulfill all criteria deemed necessary for regenerative applications involving stem cells. In the present report, we isolated and characterized ADSCs from SAMP6 mice and assessed their differentiation capabilities in the context of bone and adipose cell formation. ADSCs isolated from both SAMP6 and SAMR1 mice were easily accessed from subcutaneous inguinal fat pads and could be expanded in culture over several passages. They consistently expressed the characteristic surface markers associated with non-differentiated MSCs and readily differentiated into adipocytes, osteoblasts and chondrocytes thus confirming their multipotency. Proliferation rates and telomerase activity levels were not compromised in ADSCs from aged SAMP6 mice. Similarly, unlike BMSCs, ADSCs isolated from aged osteoporotic SAMP6 mice had the capacity to retain their osteogenic differentiation potential in vitro. In conclusion, adipose tissue may represent a promising autologous cell source for the development of novel bone regenerative therapeutic strategies in the treatment of age-related osteoporosis.