Adipose Stem Cell Mediated Calvarial Defect Repair In An Osteoporotic Rat Model

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Disclosures:  M. Pei: None. J. Li: None. D. McConda: None. N. Clovis: None. S. Danley: None.

Introduction: Osteoporosis (OP) is a systemic skeletal disorder characterized by reduced bone mineral density (BMD), microarchitectural deterioration of the skeleton, and increased risk of fracture. Repairing large bone defects is a big challenge in orthopaedic surgery, especially in those with a limited regenerative capacity such as calvarial defects in OP patients. Recently adipose stem cells (ASCs) were shown to be less affected by the aging process and cell “stemness” could be maintained even in OP patients. The aim of this study was to compare ASCs from both normal (NORM) and ovariectomized (OVX) rats in osteogenic potential using both in vitro and in vivo models. We hypothesized that ASCs from osteoporotic rats could retain their in vitro proliferation and osteogenic potential and that premature tissue constructs from osteo-induced ASCs seeded into poly(lactic-co-glycolic acid) (PLGA) could repair critical-sized calvarial defects in osteoporotic rats.

Methods: Six-month-old female Sprague-Dawley rats underwent bilateral ovariectomy (OVX) (osteoporotic group) or sham operation (NORM) (normal group). Osteoporosis was confirmed by dual-energy X-ray absorptiometry measurement of entire spine BMD four months after surgery. ASCs were harvested from NORM and OVX rats and tested for their proliferation and osteogenic/adipogenic potential under monolayer culture conditions in vitro. Proliferation index and DNA content were measured to evaluate proliferative potentials. Alkaline phosphatase staining and activity, extracellular calcium accumulation and Alizarin Red S staining were used to determine osteogenesis. Both Oil Red O staining and quantification were used to measure adipogenesis. Osteo-induced or non-induced ASCs from both groups were seeded into PLGA scaffolds to form cell/matrix constructs for the repair of critical-sized calvarial defects (Φ 5 mm). Scanning electron microscopy (SEM) was used to observe the cell morphology and interaction with the scaffold. Six weeks and 32 weeks after implantation, defect healing was evaluated by radiodensitometric analysis, BMD measurement, μCT and Herovici’s staining.

Results: After successful establishment of a rat OP model (data not shown), we did not find a significant difference in the proliferative capacity of ASCs from NORM and OVX rats (Figure 1). Alkaline phosphatase (ALP) activity and bone gamma-carboxyglutamate protein (BGLAP) mRNA were significantly lower in osteogenically induced ASCs from OVX rats than those from NORM rats despite higher levels of both RUNX2 and SPP1 mRNA. In contrast, Oil Red O staining and quantitative data suggested that adipogenic capacity in ASCs from OVX rats were superior to those from NORM rats, which was also confirmed by adipogenic gene data including PPAR, CEBP, and LPL mRNAs (Figure 1). After 14-day osteogenic induction, the constructs using ASCs (from both NORM and OVX rats) and poly (lactic-co-glycolic acid) (PLGA) were implanted to repair critical size calvarial defects in NORM and OVX rats, respectively. Thirty-two weeks post implantation, the data from Herovici’s collagen staining and microcomputed tomography (μCT) (Figure 2) suggested that the OVX rats exhibited a higher ratio of bone volume to total volume (BV/TV) compared to NORM rats, especially for the implantation of the PLGA alone group; the implantation of ASC-PLGA constructs exhibited a higher BV/TV compared to the PLGA alone group, especially for the NORM group (Figure 3).
Legends:

Figure 1. Evaluation of proliferative and multi-lineage differentiation potentials of ASCs from NORM and OVX rats in vitro. (A) DNA content of both NORM and OVX rat groups were measured every two days. Proliferation index was analyzed for rat ASCs from both NORM (B) and OVX group (C) after 8 day expansion by flow cytometry. Osteogenesis and adipogenesis were analyzed after a 21 day induction. Adipogenic differentiation was evaluated with Oil Red O staining (D) and quantification (E). Osteogenic differentiation was evaluated by Alizarin Red S staining (F) and the expression of osteogenic marker SPP1 by real-time PCR (G). Data are shown as average ± standard deviation (SD) for n = 4. *p < 0.05 indicated a statistically significant difference.

Figure 2. Morphometric and histological evaluation of bone regeneration after transplantation of ASC PLGA constructs. MicroCT analysis and Herovici’s staining of newly regenerated bone in both normal (A, C) and OVX (B, D) rats 32 weeks post implantation.

Figure 3. Quantitation of bone regeneration after transplantation of ASC PLGA constructs. Ratio of bone volume to total volume in newly regenerated bone from both normal and OVX rats transplanted with PLGA only or with ASCs 32 weeks post implantation.

Discussion: ASC based tissue constructs from NORM rats are more beneficial for the repair of calvarial defects while implantation of PLGA scaffold contributed to defect regeneration from OVX rats.

Significance: Our study for the first time evaluated the osteogenic differentiation potential of ASCs derived from osteoporotic rats both in vivo and in vitro. The comparable proliferative and osteogenic potentials of OVX ASCs to NORM rats suggest that allogenic/autologous ASCs are a good candidate for osteoporotic bone defect repair.
ORS 2015 Annual Meeting
Poster No: 1455