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Adipose-derived stem cells alleviate osteoporosis by enchancing osteogenesis and inhibiting adipogenesis in a rabbit model

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Abstract

Background aims. Osteoporosis (OP) is characterized by a reduction in bone quality, which is associated with inadequacies in bone marrow mesenchymal stromal cells (BMSCs). As an alternative cell source to BMSCs, adipose-derived stem cells (ASCs) have been investigated for bone repair because of their osteogenic potential and self-renewal capability. Nevertheless, whether autologous ASCs can be used to promote bone regeneration under osteoporotic conditions has not been elucidated. *Methods.* The OP rabbit model was established by means of bilateral ovariectomy (OVX). Both BMSCs and ASCs were harvested from OVX rabbits and expanded *in vitro*. The effects of osteogenic-induced ASCs on the *in vitro* adipogenic and osteogenic capabilities of BMSCs were evaluated. Autologous ASCs were then encapsulated by calcium alginate gel and transplanted into the distal femurs of OVX rabbits ($n = 12$). Hydrogel without loading cells was injected into the contralateral femurs as a control. Animals were killed for investigation at 12 weeks after transplantation. *Results.* Osteogenic-induced ASCs were able to promote osteogenesis and inhibit adipogenesis of osteoporotic BMSCs through activation of the bone morphogenetic protein 2/bone morphogenetic protein receptor type IB signal pathway. Local bone mineral density began to increase at 8 weeks after ASC transplantation ($P < 0.05$). At 12 weeks, micro–computed tomography and histological evaluation revealed more new bone formation in the cell-treated femurs than in the control group ($P < 0.05$). *Conclusions.* This study demonstrated that ASCs could stimulate proliferation and osteogenic differentiation of BMSCs *in vitro* and enhance bone regeneration *in vivo*, which suggests that autologous osteogenic-induced ASCs might be useful to alleviate OP temporally.

Key Words: *adipose-derived stem cells, alginate, bone regeneration, osteoporosis, rabbit, tissue engineering*

Introduction

Osteoporosis (OP) is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, which leads to increased bone fragility and susceptibility to fracture [1,2]. With the increase of life expectancy, the prevalence of OP has exceeded that of osteoarthritis or of hard- and soft-tissue healing problems in the world's aging populations [3,4]. Current treatment of OP mainly includes pharmacological drugs, physical activity and adaption of nutrition, aiming at preventing the progression of OP by promoting bone forming and/or decreasing bone resorbing [2].

Although the detailed pathologic mechanism of OP remains unknown, some studies indicated that its occurrence is correlated to the osteogenic deficiency of bone marrow mesenchymal stromal cells (BMSCs) [5,6]. Bone regeneration is a complex process involving the intimate relationship between the activities of osteogenic and adipogenic progenitor cells, which are both derived from BMSCs [7]. The osteo-progenitor cell number, proliferating capability and differentiating potential of BMSCs decrease with aging or under osteoporotic conditions [8–12]. The balance between osteogenesis and adipogenesis of BMSCs is then disrupted, and the latter takes predominance. The increase of fat proportion in bone marrow subsequently

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induces apoptosis of osteoblasts and proliferation of osteoclasts, which results in further bone resorption and overall bone loss [13,14]. Therefore, it is speculated that OP might be ameliorated by supplementing osteo-progenitor cells to stimulate osteogenesis and inhibit adipogenesis, and the stem cell transplantation approach becomes attractive.

During the past decade, adipose-derived stem cells (ASCs) have been described as an alternative source to BMSCs because of their easy access, multilineage potential and self-renewal capability [15]. When induced *in vitro* into osteoblasts, ASCs could upregulate alkaline phosphatase (ALP) activity, produce osteogenic proteins and deposit mineralized extracellular matrix (ECM) [16–18]. In combination with proper scaffolds, osteo-differentiated ASCs were able to form osteoid and enhance bone repair *in vivo* [19–22]. The OP-ameliorating potential of ASCs has also been demonstrated in a syngenic murine model, and young ASCs were shown more effective in restoring bone mineral density (BMD) than were aged cells [23]. Nevertheless, the young and aged ASCs were not derived from OP mice. The mechanism of ASCs to enhance BMSC osteogenic potential and the bone microstructure changes after ASC transplantation remained to be elucidated. Moreover, mice cannot represent the gold standard in OP animal models because they lack the Haversian system and do not achieve true skeletal maturity [24,25].

The aim of our present study was to investigate whether autologous ASCs could be applied to treat OP in larger animal models. We hypothesized that osteo-differentiated ASCs could reverse the imbalance between osteogenesis and adipogenesis in bone marrow and promote bone regeneration when transplanted into osteoporotic sites. An OP rabbit model was established by means of a bilateral ovariectomy (OVX) procedure in our study. Compared with rodents, rabbits have a moderate body size with sufficient adipose tissue for harvesting ASCs. In addition, they are the smallest animals with Haversian structures in cortical bone and can display seasonal estrogen-deficiency bone loss similar to that in larger mammals [26]. Three main questions were sought to be addressed in this study. First, could ASCs derived from OVX rabbits be served as osteo-progenitor cells? Second, what effects did ASCs have on the proliferation and differentiation capabilities of BMSCs under osteoporotic conditions? Finally, could autologous osteogenic-differentiated ASCs be used to alleviate OP in the rabbit model?

Methods

Ethical approval

All the animals enrolled in this study were cared for and processed in accordance with protocols approved by the Laboratory Animal Care and Use Committee and the Research Ethics Committee of Tongji University (2012–0083).

Rabbit OP model

A total of 20, 12-month-old, skeletally mature female New Zealand White rabbits were enrolled and randomized into 2 groups. Group 1 ($n = 13$) underwent bilateral OVX and group 2 ($n = 7$) was subjected to sham surgery. Each animal was housed in one cage and fed with standard chow (containing 0.8% calcium and 0.5% phosphate). Serum estrogen levels and BMD values were evaluated before and 8 months after surgery. The estrogen level was determined with the use of an electrochemical immunoassay kit (Roche, Mannheim, Germany). BMD of the rabbit distal femurs (10 mm from the distal end) was measured with the use of dual-energy X-ray absorptiometry (DXA) (Hologic Discovery A, Bedford, MA, USA). In addition, at 8 months after surgery, 1 animal in each group was randomly killed and the distal femurs were harvested for micro–computed tomography (micro-CT) measurement (uCT-80, Scanco Medical, Bassersdorf, Switzerland) and histological examination (hematoxylin and eosin staining) to confirm the osetopenic status.

Isolation, in vitro *expansion and characterization of rabbit ASCs*

At 8 months after OVX, rabbit ASCs were isolated and expanded *in vitro* as described previously [27]. Briefly, approximately 5 grams of subcutaneous adipose tissue in the groin area were harvested, washed 3 times with 0.1 M phosphate-buffered saline (PBS, pH 7.4) and treated with 0.075% type I collagenase (Washington Biochemical Corp, Lakewood, NJ, USA) at 37°C for 30 min. Enzymatic activity was neutralized with low-glucose Dulbecco's modified Eagle's medium (LG-DMEM, Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (HyClone, Logan, UT, USA). After being centrifuged at 1200*g* for 10 min, the yielding ASCs were resuspended in the basic culture medium (containing LG-DMEM, 10% fetal bovine serum, 100 mg/mL streptomycin and 100 U/mL penicillin; both from Sigma Aldrich, St Louis, MO, USA), and plated at 4×10^4 cells/cm^2 in Φ100-mm culture dishes (Falcon, B&D Bioscience, San Jose, CA, USA). The medium was changed twice per week, and cells were passaged on reaching 80–90% confluence by the use of 0.25% trypsin/ethylenediaminetetra-acetic acid solution (from Sigma) and seeded at a density of 1×10^4 cells per cm^2.

The phenotypic characterization of ASCs of passage 3 (P3) from both sham-surgery and OVX