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ORIGINAL ARTICLE

Role of transplanted bone marrow cells in development of rotator cuff muscle fatty degeneration in mice

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Background: Rotator cuff muscle fatty degeneration after a chronic tendon tear is an irreversible pathologic change associated with poor clinical outcomes of tendon repair, and its exact pathogenesis remains unknown. We sought to investigate the role of transplanted bone marrow cells in the development of fatty degeneration, specifically in adipocyte accumulation, using a mouse model.

Methods: Fourteen mice were divided into 2 bone marrow chimeric animal groups: bone marrow transplantation (BMT) group and reverse BMT group. For the BMT group, C57BL/6J wild-type mice underwent whole body irradiation followed by BMT into the retro-orbital sinus from green fluorescent protein (GFP)-transgenic donor mice. For the reverse BMT group, GFP-transgenic mice received BMT from C57BL/6J wild-type donor mice after irradiation. The supraspinatus tendon, infraspinatus tendon, and suprascapular nerve were surgically transected 3 weeks after transplantation. The rotator cuff muscles were harvested 13 weeks after transplantation for histologic analysis and GFP immunohistochemistry.

Results: On histologic examination, both groups showed substantial fatty degeneration, fibrosis, and atrophy of the cuff muscles. The BMT group showed no noticeable GFP immunostaining, whereas the reverse BMT group showed significantly stronger GFP staining in most adipocytes ($P < .001$). However, both groups also showed that a small number of adipocytes originated from transplanted bone marrow cells. A small number of myocytes showed a large cytoplasmic lipid vacuole resembling adipocytes.

Conclusions: This study's findings suggest that most adipocytes in fatty degeneration of the rotator cuff muscles originate from sources other than bone marrow–derived stem cells, and there may be more than 1 source for the adipocytes.

Level of evidence: Basic Science Study; Histology; Animal Model

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Keywords: Rotator cuff tear; fatty degeneration; adipocytes; bone marrow transplantation; green fluorescent protein; bone marrow–derived stem cells

Abnormal fat accumulation, atrophy, and fibrosis are the hallmark changes that occur in the rotator cuff muscles after a chronic tendon tear.[13,21,34] In this condition, variable amounts of the rotator cuff muscles are irreversibly replaced by fat, depending on the severity of the condition, often resulting in permanent loss of functional muscle units.[21,34] The severity

The study was approved by Penn State Institutional Animal Care and Use Committee: Protocol No. 47136. All animal procedures were performed in accordance with the guidelines of the National Institutes of Health.

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of this so-called fatty degeneration has been shown to correlate with poor clinical outcomes of rotator cuff repair.[13,15,16] Recent animal studies have demonstrated that reduced fatty degeneration and atrophy could be achieved through inhibition of certain genes,[10,25] application of adipose-derived stem cells,[29] application of perivascular stem cells,[11] or improvement of hypercholesterolemia.[5] Despite the recent surge of research interest, the exact pathogenesis of fatty degeneration of the rotator cuff muscles remains unknown, and there are currently no known methods to effectively prevent or to reverse this detrimental change in human patients.

Fat accumulation in skeletal muscle can be in the form of either intramyocellular lipid droplets (within muscle fibers) or adipocytes located in the perimysium or within fascicles (replacing muscle fibers).[2,47] Studies have shown that adipocytes can arise from various local muscle mesenchymal cells, including satellite cells,[4,19,38] fibro/adipogenic precursors[44-46] (eg, platelet-derived growth factor receptor α–positive nonmyogenic mesenchymal progenitor cells), certain muscle interstitial cells (eg, pericytes,[12] mesoangioblasts,[7,33] myoendothelial cells,[41] and PW1-expressing cells[28,30]), and resident fibroblasts.[2] Bone marrow–derived stem cells have also been known to participate in the healing process of skeletal muscle injuries.[1,6] In addition, it has been shown that bone marrow–derived stem cells can readily differentiate into adipocytes and contribute to adipogenesis in humans.[35,36,42]

Fatty degeneration in the human rotator cuff muscles demonstrates abnormal accumulation of adipocytes in the perimysium and within fascicles, and the precise origin of these adipocytes has not been fully elucidated. Given the substantial negative impact of fatty degeneration on clinical outcomes of rotator cuff tear patients, it is imperative to understand the pathomechanism of fat accumulation. The purpose of this study was to investigate the role of transplanted bone marrow cells in the development of fatty degeneration, specifically in adipocyte accumulation. To this end, we used an established animal model for rotator cuff fatty degeneration on bone marrow chimeric mice developed using green fluorescent protein (GFP) transgenic mice. We hypothesized that bone marrow–derived stem cells would not be a dominant source of newly formed adipocytes in fatty degenerated rotator cuff muscles.

Materials and methods

Animals

C57BL/6-Tg(CAG-EGFP)1Osb/J mice (stock No. 003291; The Jackson Laboratory, Bar Harbor, ME, USA) hemizygous for the GFP transgene were bred with C57BL/6J wild-type mice (The Jackson Laboratory) to generate hemizygous GFP offspring. All tissues from these GFP transgenic mice, with the exception of erythrocytes and hair cells, appear green under excitation light. GFP-positive offspring were identified using an ultraviolet fluorescent lantern (UVL-4 UV lamp).

Generation of bone marrow chimeric mice using irradiation and bone marrow transplantation (BMT)

There were 2 bone marrow chimeric animal groups: BMT group and reverse BMT group (Fig. 1). For the BMT group, nine 10-week old C57BL/6 wild-type mice were irradiated with 2 split doses of whole body irradiation (600 cGy + 200 cGy with a 2.5-hour interval, 68.63 cGy/min) in an X-RAD biologic irradiator (Precision X-Ray, North Branford, CT, USA) using an F2 beam conditioning filter and a table height of 60 cm to deplete host bone marrow cells and to prime the marrow for engraftment of donor cells. In a pilot experiment using C57BL/6 wild-type mice, it was confirmed that this irradiation dose was myeloablative and lethal, leading to death of mice without BMT. The irradiation dose was chosen on the basis of previous studies.[18,20,22] Bone marrow of five 6-week-old syngeneic donor GFP transgenic mice was harvested from tibias and femurs. Transplantation of GFP-expressing bone marrow from the transgenic mice was used to enable tracking of cells within the recipient wild-type mice. To harvest bone marrow, soft tissues were first removed from bones using sterile scalpels and forceps. Bones were then cut at their shaft ends and flushed with phosphate-buffered saline (PBS) plus 5% embryonic stem cell–grade fetal bovine serum using a 27-gauge 1.5-inch needle. PBS was drawn into the syringe, and the marrow cavity was flushed repeatedly 3-6 times. Cell suspensions were created by repeatedly pipetting to break up cohesive cells and by passing the unfiltered suspension through a 70-μm nylon mesh to remove particulate matter. Cells were counted using a hemocytometer to estimate the total number of cells in the given volume. Cells were centrifuged at 900*g* for 10 minutes and resuspended in 1.0×10^7 cells/mL in PBS plus 5% embryonic stem cell–grade fetal bovine serum. Within 6 hours of irradiation, a 100- to 150-μL suspension of donor marrow was injected intravenously using a 27-gauge, 0.5-inch needle through the retro-orbital sinus of recipient mice under isoflurane anesthesia after pretreating the mice with 0.5% proparacaine hydrochloride ophthalmic analgesic solution (Alcon Laboratories, Fort Worth, TX, USA). Injections were performed above the medial canthus at approximately 30° from the horizontal, with the bevel away from the eye. After gradual syringe depression, the needle was removed, and the thumb and index finger were used to apply slight pressure to the closed eye for at least 10 seconds to prevent any backflow of blood or injectate from the sinus immediately after injection. All injections were performed under sterile conditions, and all surfaces in contact with mice were sprayed with MB-10 disinfectant solution. Cages were opened within Biosafety Level 2 safety cabinets, and mice were transferred to an anesthesia chamber cleaned with MB-10. After BMT, mice were maintained in sterile housing with acidified water treated with antibiotics (sulfamethoxazole-trimethoprim oral suspension, 200 mg/40 mg per 5 mL). For the reverse BMT group, the same irradiation procedure was performed on 6 GFP transgenic mice, which subsequently received BMT from 3 C57BL/6 wild-type mice in the same fashion as for the BMT group. All mice were weighed every other day for several weeks to track the health status. One mouse from the BMT group had to be euthanized because of its severe weight loss after the procedure.

Surgical creation of rotator cuff tendon tear and muscle denervation

At 3 weeks after BMT, the 14 recipient mice (8 BMT group mice and 6 reverse BMT group mice) underwent a surgical procedure on

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