J Shoulder Elbow Surg (2013) 22, 197-205

ELSEVIER

Journal of Shoulder and Elbow Surgery

www.elsevier.com/locate/ymse

Bone marrow-derived cells from the footprint infiltrate into the repaired rotator cuff

Yoshikazu Kida, MD[a,b], Toru Morihara, MD[a], Ken-Ichi Matsuda, PhD[b], Yoshiteru Kajikawa, MD[a], Hisakazu Tachiiri, MD[a], Yoshio Iwata, MD[a], Kazuhide Sawamura, MD[a], Atsuhiko Yoshida, MD[a], Yasushi Oshima, MD[a], Takumi Ikeda, MD[a], Hiroyoshi Fujiwara, MD[a,*], Mitsuhiro Kawata, MD[b], Toshikazu Kubo, MD[a]

[a]*Department of Orthopaedics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*
[b]*Department of Anatomy and Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*

Background: Cells from the bone marrow are considered important during the rotator cuff repair process, but the kinetics of bone marrow-derived cells in this process is unknown.
Purpose: To analyze the kinetics of bone marrow cells during the rotator cuff repair process, to review whether or not they are histologically involved in rotator cuff healing, and to analyze the biomechanics of the repaired tissues.
Methods: Bone marrow chimeric rats that express green fluorescent protein (GFP) only in bone marrow- and circulation-derived cells were created. Bilateral supraspinatus tendons were separated from the greater tuberosity of the humeral head to produce a rotator cuff transection model. Drilling into the bone marrow was performed in the greater tuberosity of the right humerus and the supraspinatus tendon was repaired (drilling group), while the supraspinatus tendon was repaired on the left shoulder without drilling (control group). We examined the histology of the rotator cuff, the ultimate force-to-failure, and the proportion of GFP-positive cells in the repaired rotator cuff at 2, 4 and 8 weeks after surgery.
Results: Mesenchymal cells were observed in the repaired rotator cuff at 2 weeks in both groups. There were more GFP-positive cells in the drilling group than the control group at 2, 4 and 8 weeks. The ultimate force-to-failure was significantly higher in the drilling group than the control group at 4 and 8 weeks.
Conclusion: Bone marrow-derived cells passed through holes drilled in the humerus footprint, infiltrated the repaired rotator cuff and contributed to postsurgical rotator cuff healing.
Level of evidence: Basic Science Study, in-vivo Animal Model.

Keywords: Rotator cuff repair; supraspinatus; GFP; bone marrow-derived cells; biomechanical strength; footprint

Ethical committee of Kyoto Prefectural University of Medicine approved our study. The approval number is M22-12.

*Reprint requests: Hiroyoshi Fujiwara, MD, Department of Orthopaedics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan.
E-mail address: fjwr@koto.kpu-m.ac.jp (H. Fujiwara).

1058-2746/$ - see front matter © 2013 Journal of Shoulder and Elbow Surgery Board of Trustees.
doi:10.1016/j.jse.2012.02.007

Rotator cuff tears are one of the most common disorders of the shoulder,[4] and frequently result in substantial pain and disability.[14,29] Successful healing of a torn rotator cuff is often difficult or delayed even after surgical rotator cuff repair.[10] This is attributed to a poor blood supply in the rotator cuff itself, which leads to insufficient migration of cells involved in the healing process of the torn rotator cuff.[2,9] To develop appropriate strategies for enhanced remodeling of torn rotator cuffs, it is necessary to investigate the kinetics of cells associated with rotator cuff healing. Intrinsic cells such as tendon cells, extrinsic cells such as synovial cells, and bone marrow-derived pluripotent cells have been reported to play a role in the healing process of tendon tissues.[3,6,13,20,26,32] We previously clarified that extrinsic cells from surrounding tissues infiltrate the grafted tendon over time in a green fluorescent protein (GFP) rotator cuff transplantation model.[19,35] Cells supplied from the subacromial bursa and bone marrow are considered important during the healing process after repair of a rotator cuff.[38] Extrinsic synovial cells in the subacromial bursa have been reported to be involved in rotator cuff healing, but the precise role and kinetics of extrinsic cells from bone marrow is still unknown.[16,37]

Surgical repair is often indicated for a torn rotator cuff. In the standard surgical procedures for rotator cuff repair, the end of the torn rotator cuff is grasped with sutures; and then, bone tunnels are made on the greater tuberosity. The sutures are passed through the bone tunnels; the rotator cuff is then fixed to the footprint on the greater tuberosity by tying the suture.[27]

Recently, arthroscopic rotator cuff repair using suture anchors has been developed, where the suture anchors are inserted into holes drilled in the greater tuberosity and the torn rotator cuff is fixed by suture.[34] These procedures induce cells derived from the bone marrow of the humeral bone to migrate via the bone tunnel and drilled anchor holes. The presence of mesenchymal stem cells in the bone marrow, which have the potential to differentiate into tendon tissues, has been reported.[5] Therefore, bone marrow-derived cells released from the humeral bone after these surgical procedures might be involved in the healing process of rotator cuff tissues.

CD34 and CD45 are cell surface markers that can identify bone marrow-derived pluripotent stem cells.[7] However, tracking the kinetics of these bone marrow-derived stem cells has been difficult, because CD34 and CD45 are not expressed after differentiation into mesenchymal cells.[12] A GFP bone marrow chimeric (GFP-BMC) model for identifying these bone marrow-derived cells was created recently using GFP transgenic rats.[18] Bone marrow-derived cells in GFP-BMC rats continue to express GFP signals even after differentiation into the final phenotype. This allows these bone marrow-derived cells to be recognized over a long period of time and thus facilitates analysis of their kinetics. Such chimeric models have been used to analyze the kinetics of bone marrow-derived cells involved in the healing process of various tissues.[1,8,15,18,21]

The objective of this study was to analyze the kinetics of GFP-positive bone marrow-derived cells following surgical rotator cuff repair, with and without holes drilled on the footprint at the greater tuberosity, using a GFP-BMC rat model to track the bone marrow-derived cells. In addition, the biomechanical strength of the repaired rotator cuff was investigated to evaluate the contribution of bone marrow-derived cells to rotator cuff healing. We hypothesized that bone marrow-derived cells passed through the holes drilled at the greater tuberosity could be involved in the healing process of repaired rotator cuff tissues.

Methods and materials

Generation of the transgenic bone marrow chimeric animal model

GFP transgenic rats (GFP rats) (Japan SLC, Hamamatsu, Japan)[30] (n = 15) and Sprague-Dawley wild-type rats of the same strain (SD rats) (n = 30) were used to create GFP-BMC rats as described in previous reports (Fig. 1).[18,21,22] Briefly, recipient SD rats were exposed to 10 Gy of gamma-ray irradiation (Gammacell 40 Exactor; Nordion International Inc., Kanata, ON, Canada) under deep anesthesia to achieve bone suppression. Bone marrow - derived cells were subsequently retrieved from the tibia, femur, and humerus of donor GFP rats under sterile technique. A 70 μm nylon mesh was used to remove impurities from the bone marrow - derived cells collected from the donor GFP rats, the cell count was adjusted to a concentration of 1.0×10^8/mL using a hemocytometer, and 1 mL was intravenously transplanted to the irradiated SD rats (GFP-BMC rats). The GFP chimeric ratio (GFP-positive cell ratio) in the peripheral circulation and bone marrow of the GFP-BMC rats was measured 4 weeks after bone marrow transplantation using the following method. After washing and hemolysis, samples from the peripheral circulation and bone marrow were suspended in PBS-containing propidium iodide (PI) (Sigma–Aldrich Corp., St. Louis, MO, USA) to identify and gate out dead cells. Cell suspensions were analyzed using a FACSCalibur (Becton Dickinson, Frankli Lakes, NJ, USA) with excitation at 488 nm and fluorescence detection at 530 nm. All flow cytometric data were analyzed using Cell Quest software (Becton Dickinson, Frankli Lakes, NJ, USA).[21]

The animals were housed in our institution's animal facility in accordance with the policies and procedures set out in the ''Guide for the Care and Use of Laboratory Animals'' by the National Institutes of Health.

Surgical procedure

Four weeks after bone marrow transplantation, the GFP-BMC rats were anesthetized by intraperitoneal injection of sodium pentobarbital. Bilateral supraspinatus tendons were sharply transected at the insertion on the greater tuberosity (Fig. 1).[11,36] Any remaining soft tissue at the insertion site was removed by scraping with a scalpel blade. On the right shoulder, an additional drilling procedure was performed at the insertion site of the supraspinatus tendon prior to supraspinatus tendon repair (drilling group). Six holes were drilled, using a 0.5-mm diameter drill bit attached to an electric drill. The holes were drilled in double rows (3 holes per row) on the greater tuberosity according to the double-row procedure,[34] for which favorable clinical results for rotator cuff