INTRODUCTION

Full-thickness rotator cuff tear is one of the most common conditions affecting the shoulder joint. The clinical outcomes of rotator cuff repair have been promising, although structural failure after repair is a well-known and frequent complication. With various advanced repair techniques, there are biologic trials to enhance tendon-to-bone healing, but the results have not been successful as expected. Fatty degeneration of rotator cuff muscle after tendon tear is a well-known phenomenon, and this is closely related with outcome after repair even in integrity. Considering that our principal goal of rotator cuff repair is restore the insertion point of muscle to work as a motor, even excellent integrity would be worthless when complete muscle degeneration has been taken place. In these respects, we intended to regenerate the muscle, by way of using adipose-derived stem cells (ADSCs). Therefore, the purpose of this study was to evaluate effect of ADSCs after repair of subacute rotator cuff tear model in the rabbit by way of comparing expression of insulin-like growth factor type 1 receptor (IGF-1R) and myosin heavy chain (MyHC) in ADSC injected side and contralateral control side.

METHODS

Isolation and culture of rabbit ADSCs

Subcutaneous adipose samples were acquired from New Zealand male rabbits weighing approximately 3.5 kg. All experimental procedures were approved by the Experimental Animal Committee of the Clinical Research Institute of our institute (IACUC No: 10-0219). Adipose tissue was treated to select the ADSCs according to the previously procedure. ADSCs from passage 3 were labeled with a fluorescent cell membrane marker (Vybrant DiI; Molecular Probes, Eugene, OR) with the manufacturer's protocol. To perform the transplantation, cells were suspended to a concentration of 1x10^7 labeled cells in 500 μl of Hank's balance salt solution (HBSS, Sigma-Aldrich, St. Louis, MO).

Surgical procedures of animal model and injection of ADSC

Eight rabbits were included (5 for immunohistochemistry (IHC) and 3 for Western blot analysis) in the study. Bilateral tear model of rotator cuff tendon (supraspinatus) was created by surgical manner. Three weeks later, rotator cuff repair was done. The torn supraspinatus tendon was sutured to the greater tuberosity using trans-osseous technique with two 2-0 ethibond sutures (Ethicon-Johnson and Johnson, Somerville, NJ). After completion of repair, either side of shoulder was randomly selected and injection of the MSC (1x10^7) was injected to the contralateral supraspinatus muscle.

Immunohistochemistry (IHC)

On postoperative 3rd weeks, 5 rabbits were assigned for IHC. For the detection of IGF-1R and MyHC immunoreactivity, anti-IGF-1R (1:50, Abcam, Cambridge, UK) and anti-MHC [1:20, Leica Microsystems, Milton Keynes, UK] antibodies were used. Immunoperoxidase labeling was performed using a DAB kit (Dako, Carpenteria, CA), and were evaluated using an Olympus BX51 microscope (Olympus, Tokyo, Japan).

Western blot analysis

Three rabbits were assigned for Western blot analysis. Proteins were probed with anti-IGF-1R (1:50, Abcam, Cambridge, UK) and anti-MHC [1:20, Leica Microsystems, Milton Keynes, UK]. Peroxidase anti-rat IgG (vector, PI-1000, 1:3000 dilution) was used as a secondary antibody. Actin (1:300, Santa Cruz Biotechnology, Santa Cruz, CA) was used for an internal control.

RESULTS

Expression profiles of IGF-1R

At 3 weeks following rotator cuff repair, ADSC injected and saline injected muscles were analyzed using Western blot for determination of IGF-1R levels. The size of IGF-1R exists as 95 kD. ADSC injection increased the IGF-1R protein level (Fig. 1A). In order to determine the distribution of increased IGF-1R levels, ADSC injected and saline injected muscles were analyzed with IHC. IHC showed that ADSCs injection increased IGF-1R staining, which is located overlapping with staining of ADSCs (Fig. 1B). In Western blot analyzing, IGF-1R levels (95 kD) were increased in ADSCs injection side than saline injected muscle (A). In IHC analysis, IGF-1R staining is located overlapping with staining of ADSCs but there are no staining in saline injected muscle (B).

Expression of MyHC

Western blot for determination of MyHC levels showed that MyHC (200 kD) were higher in the ADSC injected muscle than in saline injected one (Fig. 2A). IHC showed that ADSCs injection in the rotator cuff increased MyHC staining, which is located overlapping with staining of ADSCs (Fig. 2B).

DISCUSSION and CLINICAL SIGNIFICANCE

Local injection of ADSC increased well-known muscle growth factors, that is, IGF-1R and MyHC, in rabbit’s rotator cuff repair model after subacute tear. This was evident by IHC and Western blot analysis. However, did not reached statistical significance owing to small number of cases. From this study result, we could speculate muscle regeneration can be expected in rotator cuff tear model by local administration of ADSCs. Anabolic effect of stem cell in the muscle could help treating muscle disorders as well as muscle degeneration after rotator cuff tear. Future study should be needed to verify histologic and functional change of the muscle with stem cell.

REFERENCES