Fatty Degeneration and wnt10b Expression in Supraspinatus Muscle after Surgical Repair of Torn Rotator Cuff Tendon.

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Introduction: Several lines of evidence indicated that fatty degeneration of rotator cuff muscles is one of prognostic factors for poor recovery after rotator cuff repair. Previously, elevated expression levels of PPARγ and C/EBPα were confirmed in the supraspinatus (SSP) muscles after rotator cuff resection. Prior to the elevation of these adipogenic markers, the expression of wnt10b, which was related to regulation of adipogenesis, was decreased in the SSP muscles. Also in vitro experiments using cultured myoblasts suggested that wnt10b plays a key role in the fatty degeneration of skeletal muscles. However, the expression of wnt10b in the SSP muscle after surgical repair of torn tendons has not been elucidated. In the current study, we first investigated the gene expression profiles of PPARγ, C/EBPα, and wnt10b in different parts of rabbit SSP muscles after tendon detachment. Next, we assessed expression of same genes after rotator cuff repair with different intervals from initial detachment. Fatty degeneration of SSP muscle were examined histologically by OilRed-O staining.

Methods: Surgical procedures: Experimental procedures were approved by local ethics committee. In the rotator cuff tear model, the rabbit SSP tendon was transected close to its insertion (Fig1A). The proximal stump of the SSP tendon was wrapped with a polyvinylidene fluoride (PVDF) membrane to prevent spontaneous reattachment (Fig1B). In the rotator cuff repair model, the SSP tendon was reattached to the greater tuberosity at different time points from initial surgery. Bony trough was made between articular cartilage of the humeral head and the tuberosity. The tendon stump was pulled into the trough through 1mm diameter three drill holes with 2-0 nylon (Fig1C). Experiments for rotator cuff tear and repair were performed in the right shoulder. The left shoulder underwent a sham procedure (Control side).

Sample harvesting: The SSP muscles were excised from scapula encapsulated by muscle fascia (Fig1D). The most distal 5mm tendinous part was discarded. Rest of harvested SSP muscles were divided into three portions; distal, middle, proximal. Five-millimeter cubic muscle tissues sampled from central and peripheral part of distal and middle portions were subjected to molecular analysis (Fig1E). Adjacent muscle tissue of distal portion was used for histological analysis.

Experimental periods: In the rotator cuff tear model, SSP muscles were harvested at 1, 2, and 3 weeks after detachment surgery. In the rotator cuff repair model, repair surgery was performed at 1, 2, and 3 weeks after initial surgery. SSP muscles were harvested 5 weeks after reattachment surgery. Oil Red-O staining and quantification: Frozen sections were made and stained by the Oil red-O solution. The extent of Oil red-O staining were quantified by Oil red-O elution. A hand-made polypropylene cylinder was fixed on the slides surrounding a stained muscle section. Oil red-O stain was extracted with 100 µl of
100% isopropyl alcohol placed in the cylinder (Fig 1F). Its absorbance was measured by a spectrophotometer at a wavelength of 510 nm.

Quantitative RT-PCR: Total RNA was extracted from harvested SSP muscles. Quantitative real-time PCR were performed using ABI StepOnePlus and Power SYBR Green PCR MasterMix (Applied Biosystems) routinely in duplicate. The primers were designed based on the sequences in the GenBank database. Statistics: When p-value of t-test was less than 0.05, the result was considered as significant.

Results: Fatty degeneration of SSP muscle after cuff tear: Oil red-O stained oil deposits remarkably increased after 3 weeks after resection (Fig 2A). The quantification of Oil red-O exhibited significant increase at three weeks after detachment (Fig 2B). Gene expression profiles in SSP muscles examined by RT-PCR revealed a decreased expression of wnt10b in the cuff-tear side from 1 to 3 weeks in both central and peripheral areas of distal portions. PPARγ mRNA expression exhibited significant increase at 2 and 3 weeks in peripheral and central area of distal portion and at 2 weeks in central area of middle portion. C/EBPα mRNA expression showed a significant increase at 2 weeks in the central and peripheral area and 3 weeks in central area of distal portion. No significant difference in wnt10b and C/EBPα mRNA expression was seen in middle portion (Fig 2C).

Fatty degeneration of SSP muscle after tendon repair: In rotator cuff repair model, adipose cells showing remarkable number of Oil red-O positive cells existed on samples of 2- and 3-week detachment followed by 5-week reattachment (Fig 3A). Quantification of Oil red-O in these samples also exhibited significant increase (Fig 3B). Depletion of wnt10b was seen in central area at 1-, 2- and 3-week detachment followed by 5-week reattachment. Elevation of PPARγ and C/EBPα mRNA expression in the detached muscles were observed in both central and peripheral areas at 2- and 3-week detachment followed by 5-week reattachment (Fig 3C).

Discussion: Our investigation on rabbit SSP muscles was consistent with the results from previously reported rat model. In our findings of tendon repair model, oil deposits existed remarkably in the SSP muscles at 2- and 3-week detachment followed by 5-week reattachment. The expression of PPARγ and C/EBPα started to show significant increase from 2 weeks after detachment. The expression of wnt10b remained decreased after 5 weeks after repair only in central area of distal portion of SSP muscles. These results may suggest that elevation of adipogenic markers determines the irreversible fate of fatty degeneration in SSP muscles.

Significance: Gene expression of PPARγ and C/EBPα in rabbit SSP muscle showed significant increase from 2 weeks after detachment of tendon and remained increased after tendon reattachment. Elevation of these adipogenic markers may indicate irreversible process of fatty degeneration of rotator cuff muscles after tendon tear.
Figure 3