Decreased expression of Muscle Atrophy F-box (MAFbx) in regenerating skeletal muscle induced by eccentric exercise

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Introduction: The muscle specific ubiquitin ligase, Muscle Atrophy F-box (MAFbx), is upregulated in many catabolic states of skeletal muscle. The role of MAFbx during muscle regeneration after exercise-induced muscle damage, however, has not been elucidated. Exercise-induced muscle damage commonly occurs after activities with intensive eccentric muscle contractions. Skeletal muscle has a remarkable ability of regeneration following such damage. We investigated the time course of expression of MAFbx together with MyoD protein, a target of MAFbx, in the regenerating skeletal muscle after eccentric exercise.

Materials and Methods: Animals and muscle damage protocols. Right calf muscles of male mice (C57BL/6, 10-week-old) underwent forced eccentric contraction by electrical stimulation as a model of eccentric exercise. Mice were euthanized at 5 different time points 1, 3, 5, 7 and 14 days after the eccentric exercise. The right gastrocnemius muscles were resected. Those of untreated mice served as controls. RNA isolation and real-time PCR. Total RNA was isolated and purified using RNeasy® Fibrous Tissue Midi Kit (QIAGEN), and 1μl was then reverse-transcribed into first-strand cDNA using Quantitect® Reverse Transcription Kit (QIAGEN). The mRNA levels of MAFbx in the gastrocnemius muscle were assessed by real-time PCR. The change in expression of the target gene normalized to GAPDH was monitored. Real-time PCR using Taqman® gene expression assay was performed on the corresponding cDNA synthesized from each sample.

Immunohistological procedures. For the staining of developmental myosin heavy chain (MHCd), a marker of muscle regeneration, frozen sections were fixed in acetone for 5 minutes at –20°C. Sections were incubated with anti-MHCd antibody (Novocastra, 1:100) at 4°C overnight followed by a secondary antibody for 1 hour at room temperature. For the detection of MyoD, sections were fixed in 4% PFA at room temperature for 20 min. Sections were then incubated in anti-MyoD antibody (Santa Cruz, 1:400) followed by HRP conjugated goat-anti rabbit IgG(1:1000). Protein Sample Preparation and Western Blot Analysis. Gastrocnemius muscles were homogenized in a lysis buffer and protease inhibitor. The extracts were cleared by centrifugation. Equal amounts of protein in the supernatant (20 μg protein/ lane) were electrophoresed on a 10 or 12% SDS gel and blotted to a PVDF membrane (R&D). After blocking with 5% non-fat milk for 1 h at room temperature, the membranes were incubated with either goat anti-MAFbx antibody (Santa Cruz, 1:1,000), rabbit anti-MyoD antibody (1:500) or anti-beta-tubulin antibody (DSHB, 1:600) at 4°C overnight. Then the membranes were incubated with HRP-conjugated secondary antibody (1:2,000) for 1 h at room temperature and visualized by using an ECL immunoblotting kit (Amersham). The band intensity was assessed by using ImageJ. Statistical analysis. Data are expressed as means±SD (n=6). One-way ANOVA (Stat View ver5.0) was used to determine the significant effects of exercise on the measured variables. Tukey-Kramer test was used to assess individual differences between groups. Differences were considered significant at P<0.05.

Results: Immunohistochemistry showed MHCd was expressed on days 3 and 5. MAFbx mRNA remained at the control level on day 1 and then decreased after day 3. Western blot analysis of MAFbx demonstrated its protein content was decreased after day 3. MAFbx protein was decreased on Days 3 and 5.

Discussion: This is the first report of the temporal expression profile of MAFbx mRNA and protein in the degenerating-regenerating skeletal muscle after eccentric exercise. Immunohistochemistry for MHCd showed regenerating myofibers appeared on day 3. The mRNA and protein expression of MAFbx was decreased on days 3 and 5. The MyoD protein level was increased on days 3 and 5. These result revealed the coincidence of two events, a decrease of MAFbx and an increase of MyoD during muscle regeneration on day 3. Diminished MAFbx may work as a trigger in muscle regeneration by increasing transcripts of MyoD. Exercise-induced muscle damage commonly occurs after activities with intensive eccentric muscle contractions. To clarify the precise mechanism of muscle regeneration after eccentric exercise should be very useful for sports training. These findings provide critical insight into the molecular basis of muscle regeneration.

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Figure 1. Time dependent change in MAFbx (A) mRNA and (B) protein. MAFbx mRNA was decreased after Day 3. MAFbx protein was decreased on Days 3 and 5.

Figure 2. Time course of expression of MyoD. (A) Immunohistochemistry for MyoD showed nuclei of small cells around damaged myofibers and myotubes were positively stained. (B) Western blotting showed MyoD expression was increased from Days 3 and 5 compared to normal tissue.