Time Course of Expression of Insulin-like Growth Factor (IGF)-1 and Muscle-specific E3 Ubiquitin Ligases in Regenerating Skeletal Muscle after Eccentric Exercise

+1Okada A; 2Ono Y; 3Nagatomi Y; 3Kishimoto KN; 2Itoi E
2Tohoku University School of Medicine, Department of Orthopaedic Surgery
3Tohoku University School of Medicine, Department of Medicine & Science in Sports & Exercise
Senior author itoieiji@mail.tains.tohoku.ac.jp

Introduction:
The control of skeletal muscle mass is determined by a dynamic balance of anabolic and catabolic processes. In muscle regeneration, many muscle-specific proteins (M-cadherin, myogenin, MHC etc) are increased under the control of MyoD family of basic helix-loop-helix transcription factors. Ubiquitin-proteasome pathway plays a critical role in the adaptation of skeletal muscle to a persistent increase or decrease in muscle activity. Two muscle-specific ubiquitin ligases, atrogin-1 and Muscle Ring Finger-1 (MuRF 1), main factors in muscle catabolism, are known to be antagonized by insulin-like growth factor (IGF)-I in vitro. Previous report suggested that anti-IGF-I treatment reduced the number and size of regenerating myofibers after muscle injury. This means IGF-I play an important role in muscle regeneration. However the influence of IGF-I on two muscle specific E3s in regenerating skeletal muscle (in vivo model of muscle anabolism) has not been elucidated. To take the first step in clarifying the effect of IGF-I on muscle regeneration, we investigated the time course of expression of IGF-I and two muscle specific E3 ubiquitin ligases (atrogin-1 and MuRF1) in regenerating skeletal muscle after eccentric exercise.

Materials and Methods:
Animals and muscle damage protocols  Adult C57BL/6Cr male mice were housed in cages at 20-23 °C under a 12: 12-hour dark-light cycle. The soleus and gastrocnemius muscle of mice were subjected to forced eccentric contraction by electrical stimulation to induce muscle damage. Groups of 12 mice were sacrificed on Days 1, 3, 5, 7 and 14. Within a group, 6 mice were used for muscle protein and mRNA extraction, and 6 mice were used for histological examination.

Immunohistochemistry: Frozen gastrocnemius muscle cross-sections were cut into slices. Tissue sections were incubated with anti-gout IGF-I antibody (R&D). Regenerating myofibers were detected using developmental myosin heavy chain (MHCd) as a marker.

Real-Time PCR Total RNA was isolated and purified using RNeasy® Fibrous Tissue Midi Kit (QIAGEN). Real-Time PCR was conducted using the ABI Prism® 7700 Sequence Detection System. The change in expression of the target gene normalized to GAPDH was monitored. The data were analyzed using the ΔΔCt method (Livak JK 2001)

Western blot analysis Equal amounts of protein extracted from mice gastrocnemius muscle were electrophoresed on 15% SDS gel and sequentially blotted to a PVDF membrane (Bio-Rad). After blocking in 5 % non-fat milk for 1 h at room temperature, the membranes were incubated with goat anti-IGF-1 polyclonal antibody (1:2,000) or anti-beta-tubulin monoclonal antibody (1:600) at 4 °C overnight.

Results:
Immunohistochemistry showed IGF-I expressed in regenerating myofibers on Days 3 and 5. IGF-I protein expression was unchanged on Day1 and increased on Days 3, 5 after eccentric exercise. The expression of atrogin-1 mRNA was unchanged compared to the normal control on Day 1, but decreased after eccentric exercise. The expression of MuRF1 mRNA increased compared to the normal control on Day 1. Its transcript on Days 3, 5 and 14 decreased compared to that of control.

Discussion:
Previous report have shown IGF-I signaling downregulates the transcription of atrogin-1 and MuRF1 through the activation of PI3K / Akt / FOXO signaling pathways in vitro. Our hypothesis was that atrogin-1 and MuRF1 was down regulated under the control of IGF-1 in the regenerating skeletal muscle after eccentric exercise. Our results show the coincidence of two events, the decrease of two E3 ubiquitin ligases and the increase of IGF-I on Days 3 and 5 of muscle regeneration as evident by MHCd expression. Although further analysis is required, IGF-I may play an important role in muscle regeneration through the downregulation of the expression of atrogin-1 and MuRF1.

Acknowledgements We thank Mr. Katsuyoshi Shoji and Ms. Sachiko Satoh for their technical assistance.

References: