2B13-1 Notch1 and Notch2 are essential for maintenance of the satellite cell pool in adult muscle

Tsubasa Hisamatsu, Shinya Masuda, Daiki Seko, Yuriko Kitajima and Yusuke Ono

Nagasaki University Graduate School of Biomedical Sciences

Purpose: Satellite cells are the resident stem cells in adult skeletal muscle. Notch signalling pathway is required for maintenance of satellite cell quiescence, as shown by recent studies from genetically knockout mice for the Notch target genes, Hey1 and HeyL, or the major transcriptional regulator of Notch signaling, RBPJ. Although satellite cells express three Notch receptors (Notch1-3) and Notch3 has recently been reported to act as a negative regulator for satellite cell proliferation, the function of Notch1 and Notch2 in satellite cells in vivo have not been evaluated yet.

Methods: Here, we examined the role of Notch1 and Notch2 on myogenic progression in adult muscle by using satellite cell-specific conditional knockout mice for Notch1 (N1-KO), Notch2 (N2-KO) or Notch1/Notch2 (DKO).

Results: We found that numbers of satellite cells are significantly decreased in both N1-KO and N2-KO mice, and almost completely depleted in DKO mice. Satellite cells isolated from N1-KO or N2-KO muscle lost the ability to self-renew and underwent premature myogenic differentiation in culture.

Discussion: Thus, these results indicate that Notch1 and Notch2 play a complementary and essential role in maintaining satellite cell pool in adult muscle.

Key words: Notch1; Notch2; Muscle; Satellite cells

2B13-2 Functional analysis of μ-Crystallin in skeletal muscle

Daiki Seko, Tsubasa Hisamatsu, Shinya Masuda, Yuriko Kitajima, Akihiro Taimura and Yusuke Ono

Nagasaki University

Purpose: Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder, resulting in muscle weakness and atrophy. Recent studies have shown that abnormal expression of μ-Crystallin (Crym) was observed in affected muscles in FSHD. However, physiological role of Crym in skeletal muscle has not been characterised yet. In this study, we investigated the role of Crym in muscle.

Methods: Satellite cells were isolated from extensor digitorum longus muscle in 8-12 weeks old C57BL/6 male mice and myogenic differentiation was induced in differentiation medium following satellite cell progeny expansion in growth medium. Crym was knock-downed by siRNA. Expressions of mRNA were quantified by Q-PCR. Crym distribution was visualised by immunostaining. Myotube contraction in culture was monitored by time-lapse analysis.

Results: Crym gene was highly expressed in brain and skeletal muscle tissues. In primary culture, Crym gene was up-regulated during myogenic differentiation. Consistent with this result, Crym protein was highly distributed in the cytoplasm of differentiating myotubes. Time-lapse images revealed that myotube contraction was disrupted by siRNA-mediated Crym knock-down. Reduced level of Crym protein by siRNA altered expressions of metabolic genes.

Discussion: Our results indicate that Crym may control muscle contraction and energy metabolism. Thus, abnormal expression of Crym might be associated with FSHD.

Key words: μ-Crystallin, FSHD, Skeletal muscle