

## Heparan sulfate promotes the differentiation of muscle cells and contributes to maintain motor function in mice

Mariko Yokoyama<sup>1</sup>, Takeo Yoshikawa<sup>1</sup>, Takuro Matsuzawa<sup>1</sup>, Yu Yamaguchi<sup>2</sup>, Kazuhiko Yanai<sup>1</sup>

<sup>1</sup>Dept. Pharmacol., Grad. Sch. Med., Tohoku Univ., <sup>2</sup>Sanford Burnham Prebys Medical Discovery Institute

Heparan sulfate (HS) is a sulfated linear polysaccharide at the cell surface and in the extracellular matrix. HS plays an important role in various physiological and pathophysiological processes. Although previous studies showed the existence of HS in skeletal muscles, the roles of HS in these tissues remain unclear.

First, we examined the role of HS in the differentiation of muscle cells using C2C12 cells, a mouse myoblast cell line. CRISPR/CAS9 technology was used to delete Ext1, which encodes a heparan sulfate synthase. HS deletion dramatically impaired myoblast differentiation, demonstrating the essential role of HS *in vitro*. In order to confirm the importance of HS *in vivo*, we created skeletal muscle specific Ext1 knockout mice by Cre-loxP system (cKO). Muscle weakness of cKO was observed in treadmill tests and wire hang tests. Contraction of isolated soleus muscles from cKO was also impaired. Histological observation revealed that the cross sectional areas of various muscles were smaller in cKO. Electromicroscopic observation showed that myofibrils were thinner in cKO. Finally, we examined muscle differentiation after muscle injury by BaCl<sub>2</sub> injection to tibialis anterior muscle (TA). We showed the reduced expression level of myosin heavy chain and the increased number of centronucleated cells in cKO TA, indicating that the muscle regeneration after injury was attenuated in cKO.

These results demonstrate that HS plays an important role in skeletal muscle, especially in differentiation.