論文の内容の要旨

Role of the dystrophin-glycoprotein complex in skeletal muscle integrity

（筋肉の統合性維持における dystrophin-glycoprotein complex の働き）

辛　欣

[Introduction]

The integrity of skeletal muscle is believed to be regulated through the function of the dystrophin-glycoprotein complex (DGC), because genetic abnormality in the components of DGC is known to cause severe muscular disorders in many types of muscular dystrophies. However, whether DGC is involved in the maintenance of muscle integrity in other occasions is not known. Also, it is not well understood whether there is any biochemical mechanism involved in the regulation of DGC formation and DGC function. β-Dystroglycan (β-DG) and α-DG are the essential components of DGC. β-DG is a transmembrane protein that interacts with cytoskeleton, while α-DG is located outside the membrane. α-DG is modified with *O*-mannosyl glycans including the core structure Siaα2-3Galβ1-4GlcNAcβ1-2Manα-Ser/Thr and mannose-6-phosphate indirectly linked to repeating units of [3Xylα1-3GlcUAβ1], which is required for the binding of laminin (an extracellular matrix component) to α-DG and for proper DGC function (Figure 1). Mutations in the genes encoding the enzymes involved in the synthesis of *O*-mannosylglycan structures disrupt the function of the DGC and are

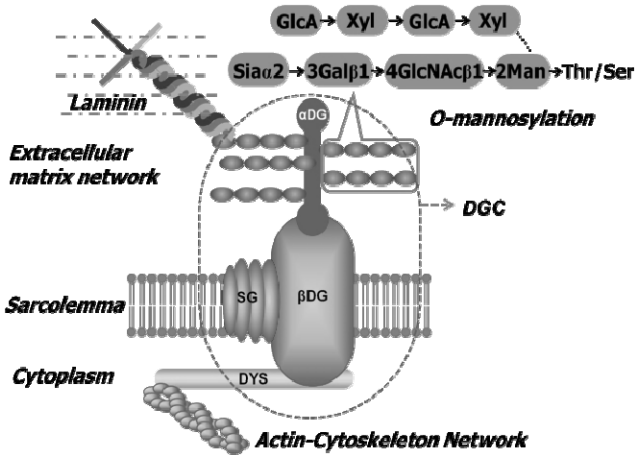

Figure 1. A representation of the DGC in skeletal muscle and some of the associated proteins. The DGC includes dystrophin (DYS), sarcoglycan complex (SG), β-DG and α-DG

causal for various forms of congenital muscular dystrophy.

Although muscular depression has been observed in aged muscle and when atrophy is artificially induced, mechanisms underlying these muscle dysfunctions were not previously elucidated. In the first part of the study, I aimed to clarify whether changes in the DGC occurred under these conditions. The relative contents of DGC components and glycosylation of α-DG were examined.

In the second part of the study, I evaluated whether protein *O*-linked mannose β1, 2-*N*-acetylglucosaminyltransferase (POMGnT1), which forms the GlcNAcβ1-2Man linkage of *O*-mannosyl glycans and is the key enzyme to form the *O*-mannosylglycans, was structurally and functionally modified by glycosylation.

[Methods and Results]

1. Biochemical changes in DGC during muscular depression

1-1 Effect of aging on the DGC contents in gastrocnemius muscle

To examine the effect of aging on DGC, I used 6-, 12-, 18-, 25- and 28-month old mice. Gastrocnemius muscle mass decreased in 25- and 28-month-old mice, but the difference was not significant. Membrane fractions from gastrocnemius muscles were subjected to immunoblot analyses for the relative expression levels of DGC components. Myosin is a predominant protein in muscle fiber and the amount of myosin in the total microsomal proteins did not change with age, whereas the contents of α-DG, β-DG and laminin decreased with age. Using IIH6 antibody, which specifically detects glycosylated α-DG, the amount of glycosylated α-DG was shown to decrease with age. Real-time PCR revealed that the expression level of *Dag1,* that encodes DG, did not change in muscles during aging, suggesting that the decreased expression of DGC components was not due to changes in the gene expression.

1-2 Alteration of DGC during muscle atrophy

Muscle atrophy was induced by fixing the mouse legs with bandages or cutting the sciatic nerve in the limbs of mice. After two weeks treatment, the gastrocnemius muscle mass was lower in the atrophy models than in the control mice. The amount of myosin decreased in fixed muscle and denervated muscle. Immunoblot analyses of microsomes obtained from the gastrocnemius muscle revealed that the quantity of core α-DG did not change but the amount of glycosylated α-DG decreased and the electrophoretic mobility of core α-DG and glycosylated α-DG increased in the muscles of fixed legs. These results suggest that α-DG is less glycosylated in fixed muscle than in the control muscle. The amount of β-DG decreased in fixed muscle, but the amount of laminin did not change. The results also indicated that the relative quantities of core α-DG, glycosylated α-DG, and β-DG increased in denervated muscle. The electrophoretic mobility of glycosylated α-DG decreased in denervated muscle, indicating that α-DG is more glycosylated in denervated muscles than in controls. The amount of laminin did not change. The expression levels of *Dag1* decreased in fixed muscle and increased in denervated muscle, which is consistent with DGC protein expression. These results indicate that the amount of DGC proteins and glycosylation change during muscle depression, but the mechanisms are not consistent in different experimental systems.

2. **Identification of glycosylation of POMGnT1**

2-1 Identification of the glycosylation of POMGnT1 expressed in insect cells

It is known that POMGnT1 is not modified by *N*-glycan because it does not have a consensus sequence for *N*-glycosylation, but it is not clear whether *O*-glycans are attached. In order to assess *O*-glycosylation of POMGnT1, recombinant POMGnT1 was produced using a baculovirus/insect cell expression system and assessed for it binding to mannose-specific lectin (Con A), GalNAc-specific lectin (VVA), and Galβ1-3GalNAc-specific lectin (PNA). All of these lectins bound to POMGnT1, suggesting the presence of *O*-glycans. POMGnT1 was treated with sialidase and then subjected to lectin blot analyses with PNA and SSA, a sialic acid-specific lectin. SSA bound to POMGnT1 only before sialidase treatment and PNA bound to POMGnT1 regardless of sialidase treatment. These data suggest that POMGnT1 has sialylated and nonsialylated *O*-GalNAc-type glycans. Electrophoretic motility increased when POMGnT1 was treated with α-mannosidase, suggesting that POMGnT1 is also modified by *O*-mannosylation. Furthermore, when POMGnT1 was co-incubated with donor substrate UDP-[^{3}H]-GlcNAc, the transfer of [^{3}H]-GlcNAc on POMGnT1 was detected. Because only Ser/Thr-mannose on a protein can accept the GlcNAc and form the GlcNAcβ1-2Man linkage, POMGnT1 is an *O*-mannosylated protein. These results suggest that POMGnT1 expressed in insect cells has both *O*-GalNAc-type glycans and *O*-mannosyl glycans.

2-2 Identification of the glycosylation of POMGnT1 expressed in mammalian cells

Mammalian cells expressing POMGnT1 were treated with sialidase and then subjected to lectin blot analysis with SSA and PNA. The binding of SSA to POMGnT1 was weaker and the binding of PNA to POMGnT1 was stronger, indicating that mammalian POMGnT1 has sialylated *O*-GalNAc-type glycans. Mammalian cells expressing POMGnT1 underwent β-elimination in the presence of pyrazolone analogs and were applied to mass analysis. The results indicated that POMGnT1 is modified by sialylated *O*-GalNAc-type glycans, suggesting that mammalian cell-expressed POMGnT1 with *O*-GalNAc-type glycans.

[Discussion and future directions]

It is clear from the results that DGC contents in muscles and their glycosylation changed upon depression of skeletal muscles. However, the mechanism does not seem to be consistent in different experimental systems. DGC components decreased in the gastrocnemius muscle of aged mice, but increased in denervated muscle. In aging muscles, the myosin contents did not change, whereas DGC components decreased, indicating that DGC started to decrease before the loss of muscle fibers. Since muscle aging is characterized by diminished membrane integrity, decreased DGC is a possible cause to initiate fiber damage in aged muscles. A low level of glycosylation was observed with α-DG in bandage-induced atrophic muscles. Causal relationship in this phenomenon will be assessed by using *O*-mannosylation defective mice (POMGnT1 KO mice).

The results show that POMGnT1 contains *O*-GalNAc- and *O*-mannosyl-type glycans. Further studies focusing on the activity, subcellular distributions, and stability of the glycoforms of POMGnT1 are necessary after determining the glycan structures and glycosylation sites. POMGnT1 produced by *E. coli* was shown to have very low enzymatic activity, suggesting that *O*-glycosylation of POMGnT1 is necessary for its function.

In conclusion, relative contents and glycoforms of DGC are under strict control through a variety of mechanisms. The results will pave the foundation to understand the mechanism of muscle atrophy and to develop method to prevent muscular depression due to aging and other causes. One of important elements is POMGnT1, which is possibly regulated by the glycosylation. It will also reveal a novel mechanism leading to muscular dystrophies.