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Commentary

Dystroglycan is a prominent cell surface protein that mediates attachment to the extracellular matrix. Although broadly expressed, glycosylated dystroglycan is critically important for muscle cell adherence to its surrounding matrix. A subgroup of muscular dystrophies, which often manifest in infancy, is associated with reduced glycosylation of dystroglycan. In this issue of the *JCI*, Beedle et al. used conditional gene targeting of *Fktn*, the gene responsible for Fukuyama congenital muscular dystrophy, to investigate a developmental requirement for glycosylation of dystroglycan.

The attachment disorders of muscle: failure to carb-load

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The dystrophin glycoprotein complex is the major transmembrane linkage in muscle cells that allows the plasma membrane to attach to the surrounding matrix. Disruption of dystrophin, which occurs in Duchenne muscular dystrophy, renders the sarcolemma fragile and susceptible to contraction-induced damage because the intracellular connection to the membrane is disrupted. In a related class of genetic diseases, mutations that disrupt the post-translational modifications on the transmembrane protein dystroglycan also lead to muscular dystrophy (Figure 1). Dystroglycan is a heavily glycosylated protein that mediates muscle attachment by linking directly to matrix components with laminin globular domains including laminin-α2, agrin, and perlecan. The extracellular α-dystroglycan subunit undergoes extensive post-translational modification, including the unusual deposition of carbohydrate sugars onto serine/threonine residues known as *O*-linked glycosylation. Mutations in seven different genes that encode known or suspected glycosyltransferases lead to congenital muscular dystrophy (1). The congenital muscular dystrophies may be evident at birth, often presenting as floppy infant syndrome with reduced muscle tone (2). Progressive muscle degeneration is variably present in the congenital muscular dystrophies and may be accompanied by extramuscular features including eye and brain defects. Disruption of dystroglycan's normal post-translational processing targets tissues and cells beyond muscle, and matrix binding to dystroglycan is an important feature in many different cell types, including the brain cells (3).

Early muscle-specific deletion of fukutin replicates the muscle phenotype in Fukuyama congenital muscular dystrophy

Fukuyama congenital muscular dystrophy (FCMD, OMIM 607440) is one of the most common autosomal recessive disorders in Japan (~1:10,000) and arises from a retrotransposon insertion in the 3′ UTR of the *FKTN* gene (4, 5) leading to missplicing of the mRNA and loss of function. *FKTN* encodes an enzyme resident in the secretory system that mediates *O*-linked glycosylation. FCMD is not only associated with congenital muscular dystrophy, but is also accompanied by micropolygyria, indicating the gene's importance in brain formation. In the mouse, complete deletion of *Fktn* is not compatible with survival (6). In this issue, Beedle et al. describe targeted disruption of *Fktn* using three different approaches yielding a range of phenotypes (7). Deletion of the *Fktn* gene early in muscle development, using the *Myf5* promoter to drive Cre-mediated recombination at murine E8, resulted in the most severe phenotype. This early muscle-specific deletion of *Fktn* resulted in reduced body mass, reduced grip strength, and histologic evidence of muscular dystrophy. Notably, the muscle fibers in the Myf5-Cre/Fktn mice were smaller, and the authors speculate that this reflects a delay in full maturation, reminiscent of what is thought to occur in human FCMD patients.

In contrast, deletion of *Fktn* later in muscle development using the muscle creatine kinase (MCK) promoter produced a less severe phenotype. The MCK promoter deleted the *Fktn* gene close to the time of birth, at E17. Although histological features of muscular dystrophy were present, there was no reduction in body mass or reduction in strength. The authors were careful to note technical caveats for each of the methods used for targeted deletion, namely that the degree of excision may differ. For example, mice carrying the Myf5-Cre used to delete early in development are also haploinsufficient for *Myf5*, an important muscle regulatory factor critical for muscle development and regeneration. Further, detecting *Fktn* deletion efficiency was technically challenging because of the lack of specific anti-*Fktn* antibodies. Even with these considerations, the data suggest a critical developmental window that is highly sensitive to *Fktn* level. As the major target of *Fktn* activity is the heavily glycosylated dystroglycan, these data reinforce the hypothesis that normal development requires interaction between dystroglycan and the matrix.

Whole-body deletion of *Fktn* was also induced in the mouse using tamoxifen-sensitive Cre expression beginning at 6 weeks of age. This resulted in typical muscular dystrophy features appearing approximately 6 weeks after gene deletion was induced, including an increase in centrally nucleated fibers. In the normal myofiber, nuclei are found at the periphery, and a centrally nucleated fiber is often an indication of recent regeneration (8). Mice with globally deleted *Fktn* had elevated serum creatine kinase (CK), consistent with muscle membrane disruption. Whole-body *Fktn* disruption produced histological features of muscular dystrophy including variably sized fibers and pockets of necrosis within the muscle. The authors speculated that aspects of muscle dysfunction in the whole-body-deleted *Fktn* mice may derive from loss of *Fktn* in peripheral nerve. Because these phenotypes mirror the clinical findings in FCMD patients, these mice provide a sound model for studying

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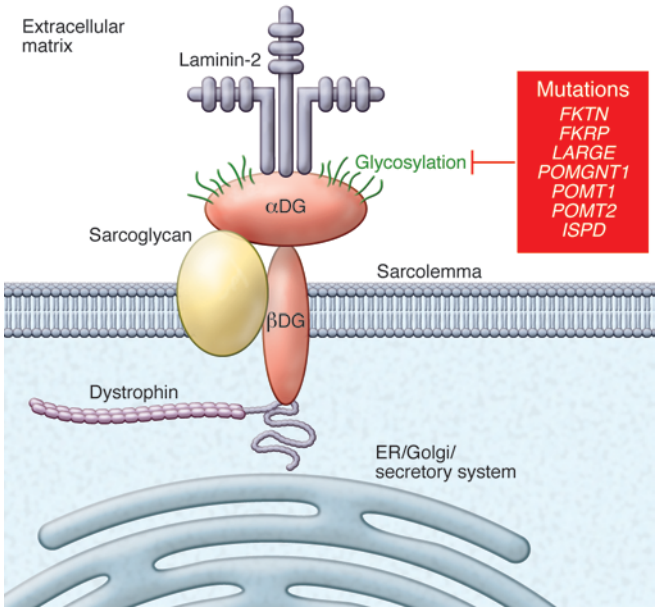

Figure 1
Shown is a schematic of dystroglycan and its position within the muscle cell membrane. Dystroglycan is composed of α and β subunits (αDG and βDG). βDG interacts directly with dystrophin, the protein product of the Duchenne muscular dystrophy locus. The sarcoglycan transmembrane complex stabilizes the interaction between αDG and βDG. Glycosylation of αDG is critical for mediating attachment to matrix components including laminin. Mutations in at least seven different genes are associated with reduced αDG glycosylation (listed in the red box), and protein products of these genes encode glycosylases resident to the secretory system. Decreased αDG glycosylation reduces attachment between the membrane and surrounding matrix. Beedle et al. (7) now report three different deletion strategies for *FKTN*, and show that *FKTN* loss is associated with dysregulated glycosylation of αDG and muscular dystrophy.

replacement and other therapies in muscle (Table 1).

Disrupted glycosylation of αDG is seen in all three models

The major target of *Fktn* activity is dystroglycan. Two subunits of dystroglycan, α and β (αDG and βDG), are produced from the same gene. βDG traverses the membrane and anchors the wholly extracellular αDG. Although the three models produced by Beedle and colleagues displayed differing phenotypic severity of muscle disease, all had similar biochemical profile in muscle in that glycosylation of αDG was markedly reduced. In each, βDG was readily detected and essentially unchanged by *Fktn* deletion. However, although the core unit of αDG was retained, its post-translational modifications were significantly depleted. There are two different *O*-mannosyl modifications on αDG, an *O*-mannosyl tetrasaccharide and the more recently described phosphodiester-linked branched structure (9, 10). Laminin binding by dystroglycan requires a phosphorylated *O*-mannosyl modification (9, 10), a type of modification that was previously described only in yeast. The αDG glycosylation defects that Beedle et al. describe in *Fktn*-deleted muscle were similar to those in muscle lacking *Large* (like-acetylglucosaminyltransferase), a bifunctional glycosyltransferase with both xylosyltransferase and glucuronyltransferase activities (11). The similar profile of αDG glycosylation in muscle deleted for either *Fktn* or *Large* is consistent with these two enzymes acting at the same step in αDG modification, and notably, mutations in *Large* are known to produce a similar spectrum of disease, as reflected by the phenotype of the *myd/myd* mouse (12, 13).

Genetic regulation of dystroglycan glycosylation: gene redundancy?

Although post-translational modification of αDG is known to be required for laminin binding, the precise residues and modifications sufficient to mediate the linkage have remained obscure. There are at least six genes that fall into this clinical and pathogenetic pathway. However, in many patients, the genetic defect that might explain muscle weakness and the hallmark finding of reduced αDG glycosylation is unknown. Most recently, the *ISPD* gene, encoding an isoprenoid synthetase domain, was the seventh gene implicated in the congenital muscular dystrophies (14). Based on homology to bacterially related genes, human *ISPD* may encode a new nucleotide sugar.

In addition, there may be redundancy among these enzymes, since overexpression or upregulation of compensatory enzymes may be corrective. Overexpression of *Galgt2*, the cytotoxic T cell (CT) GalNAc transferase that is normally enriched at the neuromuscular junction, protects dystrophin-deficient muscle against contraction-induced damage, and dystroglycan is thought to be the major target of this strategy (15). Mutations in fukutin-related protein (*FKRP*) also

Table 1
Comparison of *Fktn* deletion strategies in mice as reported by Beedle et al.

	Myf5 deletion	MCK deletion	Whole body deletion
Timing/location of deletion	Muscle only, E8	Muscle only, E17	Postnatal life (inducible)
Survival	Death by ~25 weeks	Normal	Normal
Body mass	Decreased	Trend toward increase	No change
Grip strength	Decreased	No change	No change
Myofiber central central nuclei	+++	++	++++
Serum CK	++	++	++++
Dystrophic histopathology in iliopsoas muscle	++	++	++

lead to a spectrum of muscle diseases that range from congenital to adult-onset (1).

Therapeutic implications

Enzyme delivery is an effective means of treating some muscle diseases, but whether αDG can be adequately targeted by enzyme replacement has not yet been addressed. The retroposon insertion responsible for FCMD induces missplicing of *FKTN*, and this could be corrected by antisense oligonucleotide treatment, which restored fukutin activity and normal αDG glycosylation (5). Therefore, combinatorial approaches to partially restore *FKTN* expression and augment activity through other enzymes may prove feasible for enhancing muscle function in the congenital muscular dystrophies. Furthermore, the work of Beedle suggests that the most effective targeting strategy might require treatment early in muscle development.

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1. Muntoni F, Torelli S, Brockington M. Muscular dystrophies due to glycosylation defects. *Neurotherapeutics*. 2008;5(4):627–632.
2. Collins J, Bonnemann CG. Congenital muscular dystrophies: toward molecular therapeutic interventions. *Curr Neurol Neurosci Rep*. 2010;10(2):83–91.
3. Smalheiser NR, Schwartz NB. Cranin: a laminin-binding protein of cell membranes. *Proc Natl Acad Sci U S A*. 1987;84(18):6457–6461.
4. Kobayashi K, et al. An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature*. 1998;394(6691):388–392.
5. Taniguchi-Ikeda M, et al. Pathogenic exon-trapping by SVA retrotransposon and rescue in Fukuyama muscular dystrophy. *Nature*. 2011; 478(7367):127–131.
6. Takeda S, et al. Fukutin is required for maintenance of muscle integrity, cortical histiogenesis and normal eye development. *Hum Mol Genet*. 2003; 12(12):1449–1459.
7. Beedle AM, et al. Mouse fukutin deletion impairs dystroglycan processing and recapitulates muscular dystrophy. *J Clin Invest*. 2012;122(9):3330–3342.
8. Holtzer H, Jones KW, Yaffe D. Research group on neuromuscular diseases. A report on various aspects of myogenic cell culture with particular reference to studies on the muscular dystrophies. *J Neurol Sci*. 1975;26(1):115–124.
9. Chiba A, et al. Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve alpha-dystroglycan. The role of a novel O-mannosyl-type oligosaccharide in the binding of alpha-dystroglycan with laminin. *J Biol Chem*. 1997;272(4):2156–2162.
10. Yoshida-Moriguchi T, et al. O-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. *Science*. 2010;327(5961):88–92.
11. Inamori K, Yoshida-Moriguchi T, Hara Y, Anderson ME, Yu L, Campbell KP. Dystroglycan function requires xylosyl- and glucuronyltransferase activities of LARGE. *Science*. 2012;335(6064):93–96.
12. Grewal PK, Hewitt JE. Mutation of Large, which encodes a putative glycosyltransferase, in an animal model of muscular dystrophy. *Biochim Biophys Acta*. 2002;1573(3):216–224.
13. Holzfeind PJ, et al. Skeletal, cardiac and tongue muscle pathology, defective retinal transmission, and neuronal migration defects in the Large(myd) mouse defines a natural model for glycosylation-deficient muscle – eye – brain disorders. *Hum Mol Genet*. 2002;11(21):2673–2687.
14. Willer T, et al. ISPD loss-of-function mutations disrupt dystroglycan O-mannosylation and cause Walker-Warburg syndrome. *Nat Genet*. 2012; 44(5):575–580.
15. Nguyen HH, Jayasinha V, Xia B, Hoyte K, Martin PT. Overexpression of the cytotoxic T cell GalNAc transferase in skeletal muscle inhibits muscular dystrophy in mdx mice. *Proc Natl Acad Sci U S A*. 2002; 99(8):5616–5621.

FAM83A and FAM83B: candidate oncogenes and TKI resistance mediators

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The growth and survival of tumor cells can depend upon the expression of a single oncogene, and therapeutically targeting this oncogene addiction has already proven to be an effective approach in fighting cancer. However, it is also clear that cancer cells can adapt and become resistant to therapy through compensatory activation of downstream pathways that relieve the cell of its addicted phenotype. In this issue of the *JCI*, two groups — Lee et al. and Cipriano et al. — identify two related candidate oncogenes that might both contribute to therapeutic resistance to tyrosine kinase inhibitors (TKIs). If validated, this information could help to identify new targets for therapeutic interventions in breast cancer and possibly other cancers and may also assist in the development of strategies designed to overcome resistance to currently available TKIs.

The notions of oncogene addiction and resistance to targeted agents are inextricably intertwined. The success of agents that target aberrantly expressed bone fide oncogenic receptor tyrosine kinases (RTKs), such as BCR/ABL in the case of CML or EGFR in the case of diverse epithelial malignancies, rests on the presumption that transformed cells have become, in contrast to their normal counterparts, addicted to a particular pathway for their survival (1). Two of the pathways most frequently involved in this phenomenon are the RAS/RAF/MEK/ERK and the PTEN/PI3K/AKT/mTOR cascades, which are among the most commonly dysregulated pathways in cancer. More recently, attention has begun to focus on so-called "orthogonal" pathways and their contribution to the transformed phenotype (2). These pathways protect neoplastic cells from multiple forms of oncogenic stress (e.g., proteotoxic, oxidative, DNA damage-related, etc.) which accompany expression of oncogenes (e.g., *c-Myc*) that confer survival or proliferation advantages on involved cells. However, an improved appreciation of the basis for the transformed phenotype also brings an understanding of the complex mechanisms capable of conferring resistance or sensitivity to targeted agents. For example,

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