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Finding of *O*-Mannosylglycan in Mammals and Its Glycopathology

profile

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Tamao Endo graduated from the Faculty of Pharmaceutical Sciences, the University of Tokyo in 1977 and obtained his Ph.D. at the same institution in 1982 (Prof. Shoshichi Nojima). He was a postdoctoral fellow at the Baylor College of Medicine (Prof. Donald M. Marcus), and a research associate in the Institute of Medical Science, the University of Tokyo (Prof. Akira Kobata). Since 1994, he has been the head of the Department of Glycobiology, Tokyo Metropolitan Institute of Gerontology (TMIG). Last April, TMIG was reestablished as a new foundation and he is the head of the Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Foundation for Research on Aging and Promotion of Human Welfare. His current research interests are glycobiology on aging, dementia, and development.

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α -Dystroglycan is an extracellular peripheral membrane glycoprotein anchored to the cell membrane by binding to a transmembrane glycoprotein, β -dystroglycan. These two dystroglycan subunits were originally identified as members of the sarcolemmal dystrophin-glycoprotein-complex (DGC). α - and β -dystroglycans are encoded by a single gene, and cleaved into separate proteins by posttranslational processing. The α -dystroglycan- β -dystroglycan complex is widely expressed in a broad array of tissues and thought to act as a transmembrane linker between the extracellular matrix and intracellular cytoskeleton, because α -dystroglycan binds to laminin, and the intracellular domain of β -dystroglycan interacts with dystrophin in skeletal muscle (1).

α -Dystroglycan is heavily glycosylated, and chemical modification by treatment with periodic acid or trifluoromethanesulfonic acid resulted in the loss of laminin-binding, suggesting that the sugar moiety is essential for its binding. Recently, we demonstrated that the sialic acid residues, which are probably attached to *O*-linked oligosaccharides, were essential for this binding.

Further, we have elucidated the structure of *O*-linked oligosaccharides of bovine peripheral nerve α -dystroglycan as a novel *O*-mannosyl glycan, $\text{Sia}\alpha 2\text{-}\beta\text{Gal}\beta 1\text{-}4\text{GlcNAc}\beta 1\text{-}2\text{Man}$ (2). In another study, we found the same *O*-mannosyl glycan in rabbit skeletal muscle α -dystroglycan (3). After our report on the sialyl *O*-mannosyl glycan, a series of mammalian type *O*-mannosyl glycans, with heterogeneous Man-branching (2-substituted and 2,6-disubstituted mannoses) and peripheral structures [$\text{Sia}\alpha 2\text{-}\beta\text{Gal}\beta 1\text{-}4\text{GlcNAc}\beta 1\text{-}2\text{Man}$, $\text{Gal}\beta 1\text{-}4\text{GlcNAc}\beta 1\text{-}2\text{Man}$, $\text{Gal}\beta 1\text{-}4(\text{Fuc}\alpha 1\text{-}3)\text{GlcNAc}\beta 1\text{-}2\text{Man}$, $\text{HSO}_3\text{-}\beta\text{GlcA}\beta 1\text{-}3\text{Gal}\beta 1\text{-}4\text{GlcNAc}\beta 1\text{-}2\text{Man}$, $\text{Sia}\alpha 2\text{-}\beta\text{Gal}\beta 1\text{-}4\text{GlcNAc}\beta 1\text{-}2(\text{Sia}\alpha 2\text{-}\beta\text{Gal}\beta 1\text{-}4\text{GlcNAc}\beta 1\text{-}6)\text{Man}$] have been found. In summary, *O*-mannosylation is known as a yeast-type modification, and oligomannose-type *O*-mannosylated glycoproteins are abundant in the yeast cell wall. On the other hand, mammalian *O*-mannosylation is a rare type of protein modification that is observed in a limited number of glycoproteins of brain, nerve, and skeletal muscle. Future studies are needed to clarify the distribution of such *O*-mannosyl glycans in various tissues.

Analysis of the biosynthetic pathway of the *O*-mannosyl glycans in mammals is important for elucidating not only the regulation of expression but also the biological functions of these glycans. The identification and characterization of the enzymes involved in the biosynthesis of mammalian type *O*-mannosyl glycans will be an important step forward elucidating these glycans. A key difference between mammalian and yeast-type *O*-mannosyl glycans is that those in mammals have the $\text{GlcNAc}\beta 1\text{-}2\text{Man}$ linkages. This linkage is assumed to be catalyzed by a glycosyltransferase, UDP-*N*-acetylglucosamine: protein *O*-mannose $\beta 1,2$ -*N*-acetylglucosaminyltransferase (POMGnT1). POMGnT1 catalyzes the transfer of *N*-acetylglucosamine from UDP-GlcNAc to *O*-mannosyl glycoproteins, according to the reaction: $\text{UDP-GlcNAc} + \text{Man-R} \rightarrow \text{GlcNAc}\beta 1\text{-}2\text{Man-R} + \text{UDP}$ in which R is protein. After we succeeded in developing an enzyme assay for POMGnT1, its activity was found in brain homogenates of several mammals (4). It should be noted that $\text{GlcNAc}\beta 1\text{-}2\text{Man}$ linkages are also found in N-glycans, where they are catalyzed by two enzymes, UDP-*N*-acetylglucosamine: α -3-D-mannoside β -1,2-*N*-acetylglucosaminyltransferase I (GnT-I) and UDP-*N*-acetylglucosamine: α -6-D-mannoside β -1,2-*N*-acetylglucosaminyltransferase II (GnT-II). However, we found that recombinant GnT-I and GnT-II had no ability to catalyze the $\text{GlcNAc}\beta 1\text{-}2\text{Man}$ linkage in *O*-mannosyl glycans, suggesting that a new enzyme must be responsible for the formation of this linkage. Thus, we cloned the human *POMGnT1* gene on the basis of human cDNA sequences homologous to human *GnT-I* (5). The nucleotide sequence indicated

that POMGnT1 is a 660 amino acid protein with a calculated molecular mass of 71.5 kDa. A hydrophobicity analysis and secondary structure prediction of the amino acid sequence suggested that human POMGnT1 is a type II membrane protein. This topology was similar to the topologies of other Golgi glycosyltransferases cloned to date.

The human *POMGnT1* gene exists at 1p33, and we find that the *POMGnT1* gene is located within the small candidate interval for muscle-eye-brain disease (MEB: OMIM 253280). MEB is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities and brain malformation (type II lissencephaly) (6). Patients with MEB show congenital muscular dystrophy, severe congenital myopia, congenital glaucoma, pallor of the optic discs, retinal hypoplasia, mental retardation, hydrocephalus, abnormal electroencephalograms and myoclonic jerks. All infants with MEB are floppy with generalized muscle weakness, including facial and neck muscles, from birth. Muscle biopsies show dystrophic changes, and brain MRIs reveal pachygyria-type cortical neuronal migration disorder, flat brainstem and cerebellar hypoplasia. Since defects of DGC cause muscular dystrophies (1) and *O*-mannosyl type glycan is required for the laminin binding of α -dystroglycan in DGC (2), it is possible that mutations in the *POMGnT1* gene are related to MEB.

To test this hypothesis, we screened the whole coding region and the exon/intron flanking sequences of the *POMGnT1* gene for mutations in six patients with MEB. We identified six independent disease-causing mutations in these patients and we have not detected these six substitutions in any of 246 normal chromosomes, indicating that these mutations are pathogenic and that the *POMGnT1* gene is responsible for MEB (5). To confirm that the mutations observed in patients with MEB are responsible for the defects in the synthesis of *O*-mannosyl glycan, we expressed the most frequent mutation and found a loss of enzymatic activity (5). Additionally, we found a selective deficiency of α -dystroglycan in MEB patients (7). This finding suggests that α -dystroglycan is a potential target of POMGnT1 and that altered glycosylation of α -dystroglycan may play a critical role in the pathomechanism of MEB.

Recent investigations have revealed that some muscular dystrophies may be caused by abnormal glycosylation of α -dystroglycan including Fukuyama-type congenital muscular dystrophy (8), MDC1C (9), and the myodystrophy (*myd*) mouse (10), although the detailed defect is still unclear. Identification of these defects provides new directions for unraveling a glycopathomechanism for muscular dystrophy.

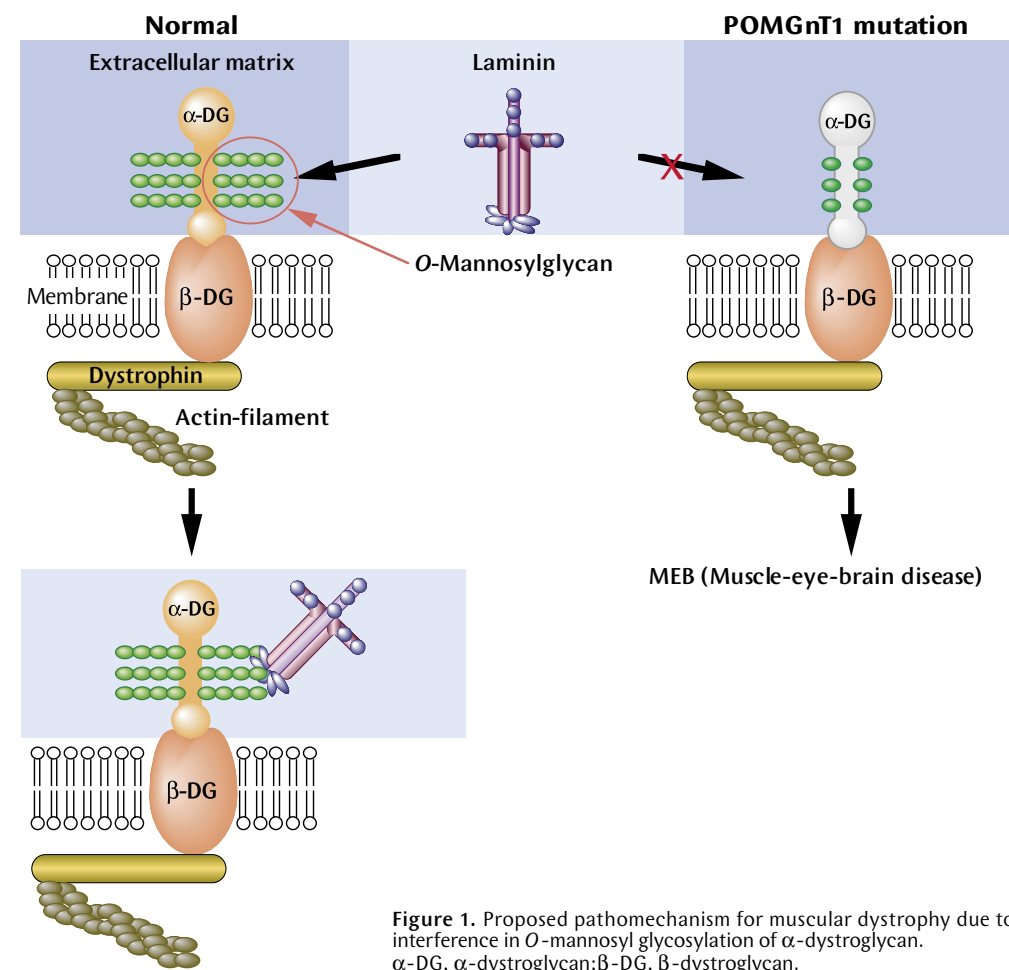


Figure 1. Proposed pathomechanism for muscular dystrophy due to interference in *O*-mannosyl glycosylation of α -dystroglycan. α -DG, α -dystroglycan; β -DG, β -dystroglycan.

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