Glycometabolic Biochemistry Laboratory (2024)

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(0) Research field
CPR Subcommittee**:** Biology
Keywords: glycoproteins, asparagine-linked glycans, metabolism, peptide:*N*-glycanase, Ngly1

(1) Long-term goal of laboratory and research background

Peptide:*N*-glycanase (PNGase) releases asparagine-linked (N-linked) glycans from glycoproteins/ glycopeptides. The cytoplasmic PNGases (NGLY1/Ngly1 in human/mouse or rat), ubiquitously found throughout eukaryotes, are now widely recognized as a component implicated in the ERAD (ER-associated degradation) process, which constitute one of the quality control machineries for newly synthesized misfolded glycoproteins exported out of the ER lumen. While the biosynthetic pathway for N-glycans has been clarified in detail, the catabolic pathway for the "free" N-glycans released by the cytoplasmic PNGase remains largely unknown. Although this "non-lysosomal" metabolic path for N-glycan may represent one of the very basic biological phenomena in eukaryotes, there are still many more enzymes/transporters that remains to be identified. We are currently trying to identify other players involved in this process and also taking a number of approaches to analyze the physiological importance of this non-lysosomal metabolic pathway.

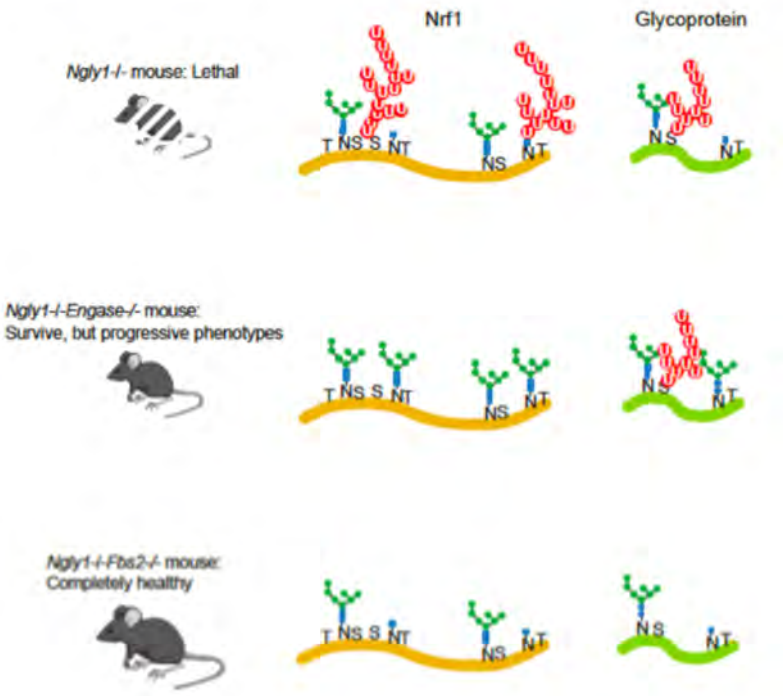
(2) Current research activities (FY2024) and plan

Previously we demonstrated that motor functions of *Ngly1*$^{-/-}$ rats was greatly improved by intracerebroventricular administration of an adeno-associated virus vector expressing human NGLY1 (Asahina, et al. ***Mol Brain*** 2021). This result suggests that early diagnosis of NGLY1 deficiency and early therapeutic intervention may improve symptoms resulting from NGLY1 defects. Therefore, establishing a novel NGLY1 assay in biological samples is in urgent need. We developed novel assays that can contribute to early diagnosis.

First, we developed a novel FRET-based assay system using a probe that "**emits fluorescence when reacting with NGLY1**" [1]. Moreover, we developed a simple and highly sensitive NGLY1 assay system using an anti-tag antibody [2]. Both methods are capable of detecting NGLY1 activity from crude biological samples with the methods compatible with most clinical laboratories.

Epileptic seizures are symptoms often found in NGLY1 deficiency patients. It was found that *Ngly1*$^{-/-}$ mouse also exhibit spontaneous seizure-like behaviors. Gene expression analysis revealed significant decrease in expression of oxytocin in the hypothalamus of *Ngly1-/-* mice. Curiously, the seizure-like behaviors in *Ngly1*$^{-/-}$ mice were transiently suppressed by an intranasal administration of oxytocin. These findings suggest the therapeutic potential of oxytocin for epileptic seizure in patients with NGLY1 deficiency [3].

NRF1/NFE2L1 is a transcription factor activated in response to various stresses; for example, when proteasome activity is impaired, it is translocated to the nucleus and induces proteasome gene expression, leading to the formation of new proteasomes. It has been suggested that NGLY1-mediated deglycosylation is critical for full NRF1 activation. Furthermore, we showed previously that ubiquitinated NRF1 accumulates in *Ngly1*-KO cells. In this study we showed that non-canonical ubiquitination occurs on NRF1, mainly through binding to hydroxy group of Asn-linked GlcNAc, which was formed by the ENGase action. This reaction requires FBS2 for glycan recognition, while another ubiquitin ligase, ARIH1, form complex, branched ubiquitin chains. These results correlate well with the mice phenotypes so far observed (Figure). It was therefore strongly suggested that the pathogenic mechanism



of NGLY1 deficiency is mainly due to “abnormal ubiquitination” rather than an intrinsic defect of NGLY1 function [4].

Figure　Pathology of various *Ngly1*-KO mice [4]

(Top) Under NGLY1 deficiency, glycans are not removed from the glycoproteins in the cytosol, and accordingly, glycoproteins, including Nrf1, with atypical ubiquitination are accumulated. In the *Ngly1*-KO model mouse, this causes embryonic lethality. (Middle) When one of the therapeutic target genes, ENGase, is deficient, atypical ubiquitination is suppressed because the ubiquitin acceptor site (N-GlcNAc), produced by ENGase, is not formed, but ubiquitination via Ser/Thr near the glycan probably occurs in some glycoproteins, and therefore the suppression effect is only partial. In the model mouse, the embryonic lethality is partially rescued. (Bottom) When Fbs2 is deficient, atypical ubiquitination does not occur at all, and the phenotype is strongly suppressed. In the model mouse, embryonic lethality is almost completely suppressed, and *Ngly1 Fbs2* double KO mice grow normally [4].

Free N-glycans (FNGs) are known to occur widely in nature, and it has been well clarified regarding the biosynthesis/degradation of intracellular FNGs. On the other hand, it remains largely unknown for extracellular FNGs, such as FNGs in serum (Seino, *et al*. ***Glycobiology*** 2016). We carried out FNG analyses for salmon serum and demonstrated that there are similar repertoire of free oligosaccharides, including FNGs, with mammalian sera in fish serum, indicating that serum free glycans occur widely in vertebrates [5].

In the future, we will continue to aim at clarifying the molecular mechanism for the catabolism and N-glycans and their precursors (dolichol-linked oligosaccharides). We will also aim at unveiling the species-specific glycan biosynthetic and degradation pathway, such as the molecular mechanism for free O-mannose glycan formation in budding yeast, to provide novel insights into the functional importance of glycans from the standpoint of “comparative glycobiology”. We will also carry out in-depth analysis of phenotypic consequences of *Ngly1*-KO mice, to clarify the pathophysiology of NGLY1 deficiency.

(3) Members (FY2024)

(Chief Scientist)
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Kenichi Moto
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Yuko Suzuki
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Tsugiyo Matsuda

(4) Representative research achievements

(Lab members: double underline; T-CiRA/AMED-CREST members: single underline)

1. H. Hirayama, Y. Tachida, R. Fujinawa, Y. Matsuda, T. Murase, Y. Nishiuchi, and T. Suzuki* (2024) Development of a fluorescence and quencher-based FRET assay for detection of endogenous peptide:N-glycanase/NGLY1 activity. ***J. Biol. Chem.*** **300,** 107121. (doi: 10.1016/j.jbc.2024.107121)
2. H. Fujihira, K. Sato, Y. Nishiuchi, T. Murase, Y. Matsuda, Y. Yoshida, T. Kamei and T. Suzuki* (2024) ELISA-based highly sensitive assay system for the detection of endogenous NGLY1 activity. ***Biochem. Biophys. Res. Commun.*** **710,** 149826 (doi: 10.1016/j.bbrc.2024.149826)
3. Y. Makita, M. Asahina, R. Fujinawa, H. Yukitake, and T. Suzuki* (2024) Intranasal oxytocin suppresses seizure-like behaviors in a mouse model of NGLY1 deficiency. ***Commun. Biol.*** **7**, 460 (doi: 10.1038/s42003-024-06131-7)
4. Y. Yoshida*, T. Takahashi, N. Ishii, I. Matsuo, S. Takahashi, H. Inoue, A. Endo, H. Tsuchiya, M. Okada, C. Ando, T. Suzuki, N. Dohmae, Y. Saeki, K. Tanaka*, and T. Suzuki* (2024) Sugar-mediated non-canonical ubiquitination impairs Nrf1/NFE2L1 activation. ***Mol Cell*** **84,** 3115-3127 (doi: 10/1016/j.molcel.2024.07.013).
5. A. Honda, J. Seino, C. Huang, M. Nakano and T. Suzuki* (2025) Occurrence of free glycans in salmonid serum. ***Biochem. Biophys. Res. Commun.*** **742,** 151096 (doi:10.1016/j.bbrc.2024.151096)

Supplementary

Group photo of RIKEN Glycometabolic Biochemistry Laboratory

Laboratory Homepage
https://www.riken.jp/research/labs/chief/glycometab_biochem/index.html