Special Lectures ❶

NGLY1 and non-lysosomal degradation of *N*-glycans, free *N*-glycans and dolichol-linked oligosaccharides

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The biosynthetic/processing pathways for *N*-glycans have been well characterized in mammalian cells. There are, however, issues that remain to be clarified concerning aspects of their degradation. While the molecular mechanism of the lysosomal degradation for *N*-glycoproteins has been well studied in relation to genetic disorders, evidence exists to suggest that there are also "non-lysosomal" degradation processes, which are now known to occur widely in eukaryotic cells.

The cytoplasmic peptide:*N*-glycanase (PNGase) is the enzyme involved in the non-lysosomal degradation of misfolded *N*-glycoproteins, a process called ERAD (ER-associated degradation) [1),2)]. In 2012, a patient harboring mutations of PNGase gene (*NGLY1*) was first reported. Symptom of these patients includes developmental delay, multifocus epilepsy, involuntary movement and liver dysfunction. This report clearly demonstrated that the cytoplasmic PNGase plays a pivotal role in normal human development.

The past decade we intensively analyzed *Ngly1*-deficient mice and found that they are embryonic lethal in C57BL/6 (B6) background. Surprisingly, the additional deletion of *Engase*, encoding another cytosolic deglycosylating enzyme involved in the non-

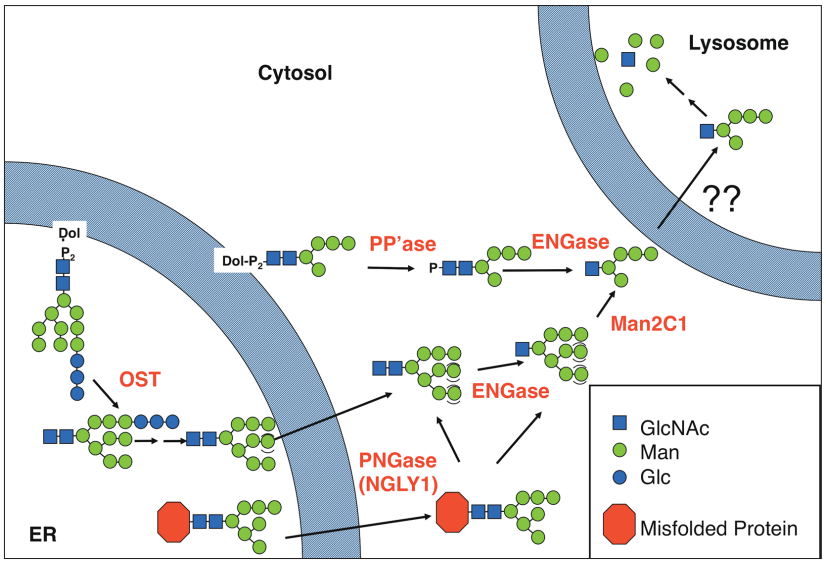

Figure 1
Non-lysosomal catabolism of *N*-glycoproteins, free *N*-glycans and dolichol-linked oligosaccharides in mammalian cells (Harada, et al., Cell Mol Life Sci 72, 2509-2533 (2015)). Dol: dolichol; ENGase: endo-β-*N*-acetylglucosaminidase; Man2C1: cytosolic α-mannosidase; OST: oligosaccharyltransferase; PNGase/NGLY1: peptide:*N*-glycanase

lysosomal degradation of *N*-glycoproteins called ENGase (endo-β-*N*-acetylglucosaminidase), resulted in the partial rescue of the lethality of the *Ngly1*-deficient mice [3]. Additionally, we also found that a change in the genetic background of B6 mice, produced by crossing the mice with an outbred mice strain (ICR) could rescue the embryonic lethality of *Ngly1*-deficient mice [3]. Viable *Ngly1*-deficient mice in a B6 and ICR mixed background, however, showed a very severe phenotype reminiscent of the symptoms of *NGLY1*-deficiency subjects. Again, many of those defects were strongly suppressed by the additional deletion of *Engase* in the B6 and ICR mixed background.

We also showed that in *Ngly1*-KO cells, ERAD process was compromised. Interestingly, not only delayed degradation but also the deglycosylation of a model substrate was observed in this cell. The unexpected deglycosylation was found to be mediated by ENGase. Surprisingly, the ERAD dysregulation in *Ngly1*-KO cells were restored by the additional KO of Engase gene. These observations collectively suggest that the ENGase represents one of the potential therapeutic targets for this genetic disorder [4].

Besides degradation of *N*-glycoproteins, there are also novel pathways exist in cells for the catabolism of free *N*-glycans (FNGs); free oligosaccharides related to *N*-glycans, as well as dolichol-linked oligosaccharides (DLOs). The recent years we have also analyzed the following novel catabolic pathways for FNGs and DLOs; (i) oligosaccharyltransferase-mediated release of FNGs in the ER [5),6)]; (ii) pyrophosphatase acting on dolichol-linked oligosaccharides [7),8)]; and (iii) formation and catabolism of sialyl FNGs inside and outside of cells [9),10)]. Occurrence as well as biological importance of those novel pathways have been largely overlooked, partly because there are several genes for enzymes/transporters that remained to be identified. I have a firm belief that clarification of those novel pathway, which should constitute the one of the most basic biological processes, will lead to not only providing better insight into the process, but also understand the human health and disease. In this symposium, I will overview our recent findings on NGLY1 and novel degradation pathways for FNGs/DLOs.

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