Poster #37
Thursday
4-5 pm

CLN2 Disease: S1S3 Phosphotransferase Mediated Phosphorylation, Uncovering, and Binding to CI-MPR of Tripeptidyl-peptidase 1 (TPP1)

Linda Lyons, Udayanga Wanninayake, Jonathan Roberts, Michael DiGruccio, Vaughn Weaver, Riley Marcinczyk, Russell Gotschall, Andrew Hedman, Lin Liu, Hung Do.

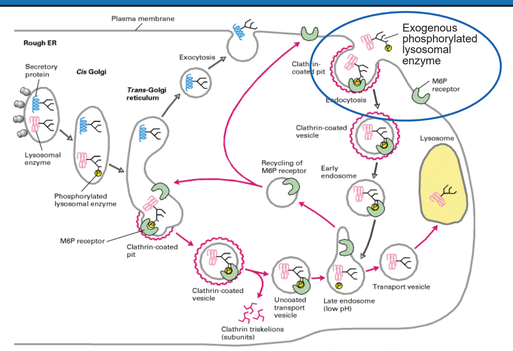
R&D, M6P Therapeutics, St. Louis, MO, 63108

M6P THERAPEUTICS

Abstract

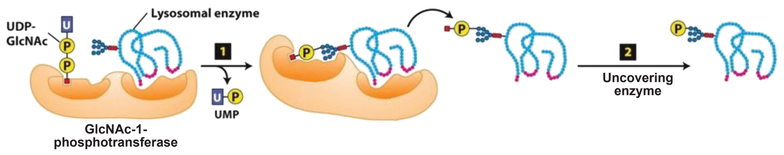
CLN2 disease, also known as late infantile neuronal ceroid lipofuscinosis type 2 (LINCL), is a rare, genetically inherited disorder that primarily affects the nervous system. It is caused by a mutation in the TPP1 gene and results in a deficiency of the enzyme TPP1, which leads to an accumulation of a fatty substance called ceroid in the brain and other tissues. Symptoms typically appear between the ages of 2 and 4, and may include seizures, progressive vision loss, motor function decline, language disturbances, and dementia. The disease is fatal, and there is currently no known cure other than treatment for managing symptoms and improving quality of life. Gene therapy (GT) and enzyme replacement therapy (ERT) are being studied as potential treatment options. ERT involves direct or systematic injection of a functional copy of an enzyme to the brain. However, inefficient uptake of the exogenous recombinant enzyme via the cation- independent mannose 6-phosphate receptor (CI-MPR), due to insufficient phosphorylation of N-Glycan terminal mannose of TPP1, limits the successful delivery of curative doses. M6P Therapeutics has developed a novel technology to co-express S1S3 phosphotransferase with TPP1 to enhance the level of phosphorylation. This approach has increased the affinity to CI-MPR receptor by 4-fold. Also, CI-MPR column chromatography profile revealed that S1S3 co-expression has increased the high binding TPP1 from 33% (wildtype) to 56%. Furthermore, removal of the masking N-acetylglucosamine (GlcNAc) portion from terminal mannose 6-phosphate (M6P) residues has increased this number to 73% compared to wildtype enzyme. Here we demonstrate how co-expression of rhTPP1 with S1S3 phosphotransferase increases the CI-MPR affinity and uncovering the M6P moieties to further increase the CI-MPR affinity. Our data suggests that compared to its wildtype counterpart, co-expression of the rhTPP1 in the presence of S1S3 phosphotransferase significantly increases the binding affinity to CI-MPR.

Natural M6P Receptor Pathway Enables Phosphorylated Exogenous Lysosomal Enzymes Cellular Uptake for Treatment of Lysosomal Storage Diseases



Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 17.7

Phosphorylation of Lysosomal Enzymes is Mediated by GlcNAc-1-Phosphotransferase That is Inherently Inefficient in Cells



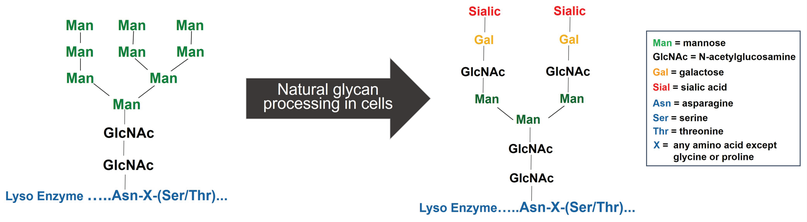
GlcNAc-1-phosphotransferase catalyzes the transfer of GlcNAc-1-phosphate from sugar donor UDP-GlcNAc onto certain terminal mannose residues of the N-linked oligosaccharides in cis-Golgi

NAGPA (N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase), also known as uncovering enzyme, removes covering GlcNAc group to reveal mannose 6-phosphate (M6P)

Section 17.7 Protein Glycosylation in the ER and Golgi Complex, Molecular Cell Biology, 8th edition, 2016

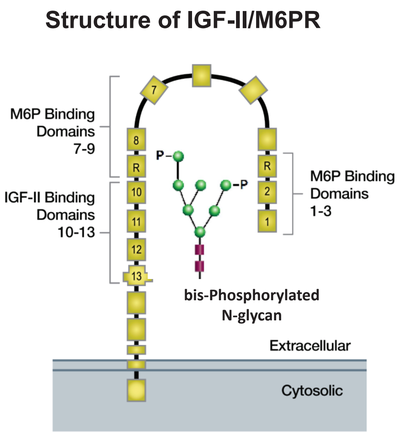
- GlcNAc-1-Phosphotransferase is a large and complex protein that is responsible for adding phosphate groups to mannose residues on N-linked glycans of newly synthesized lysosomal enzymes in Golgi to create mannose 6-phosphate (M6P)
- Phosphorylation of lysosomal enzymes is an inherently inefficient process in nature, it is further compromised when overexpressing lysosomal enzyme
- Increasing GlcNAc-1-Phosphotransferase activity within cells for producing therapeutic lysosomal enzymes with higher M6P content has not been achievable thus far with traditional ERT and gene therapy approaches

The Problem: Most N-glycans on Lysosomal Enzymes are Not Phosphorylated and Processed to Complex-Type Structures During Over-Expression

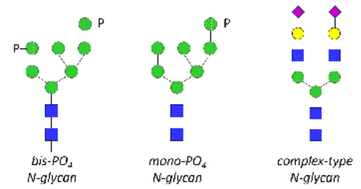


- Identical Man9 N-glycan structure is added to glycoproteins in endoplasmic reticulum during synthesis; N-glycan processing primarily occurs in Golgi
- Phosphorylation of recombinant lysosomal enzymes is highly inefficient in cells leading to mostly complex-type structures
- Complex-type N-glycans are typical of plasma proteins and do not enable cellular uptake or delivery of exogenous lysosomal enzymes to lysosomes
- No current technology can increase phosphorylation within cells

Structure of IGF-II/Cation-Independent M6P Receptor (IGF-II/CI-MPR) and Measured Binding Affinities of Carbohydrate Ligands



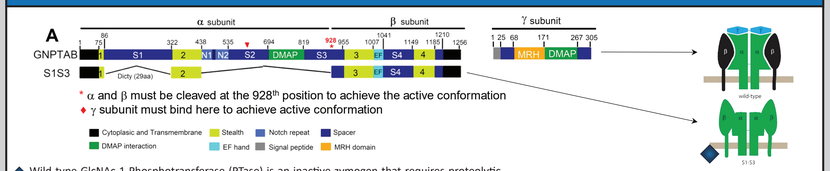
Not All Phosphorylated Glycans Are Equal for Binding M6PR



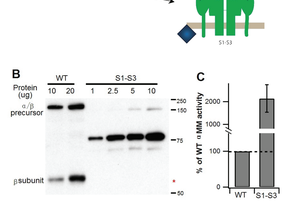
Ligand	Binding affinity (Apparent K_D, nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6000
complex type N-glycan	No binding

Adapted from Tong et al., 1989

S1S3 Variant Has Key Attributes That Enable Its Use for Development of Best-In-Class Recombinant Enzymes and Gene Therapies



- Wild-type GlcNAc-1 Phosphotransferase (PTase) is an inactive zymogen that requires proteolytic cleavage at a precise location (at residue 928) to achieve its active conformation
- No known way to increase proteolytic cleavage and enzyme activation within cells for increasing enzyme activity for improving phosphorylation
- S1S3 PTase is a truncated, pre-activated variant with significant advantages:
  - Has proper conformation without need for γ-subunit
  - Has 20-fold higher specific activity
  - Enables efficient phosphorylation of soluble lysosomal enzymes
  - Small gene size enables its incorporation into expression constructs for S1S3 PTase co-expression with therapeutic enzymes within cells for ERT and gene therapy applications



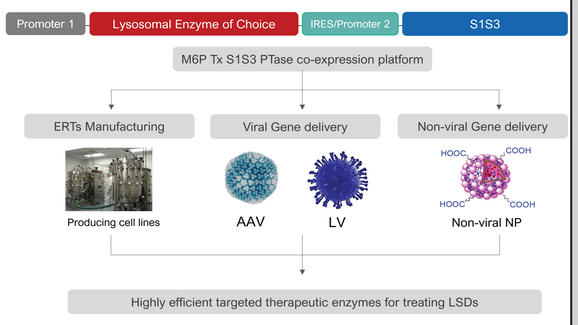
Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy, Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65.

M6P Therapeutics' Unique S1S3 PTase Co-Expression Platform for Producing Therapeutic Lysosomal Enzymes with High M6P Levels M6P for ERTs and from GTs

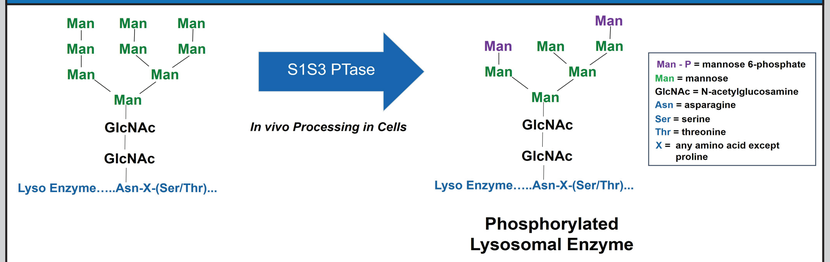
01 M6P platform avails co-expression of S1S3 PTase with majority of soluble lysosomal enzyme

02 S1S3 PTase co-expression platform approach can be utilized for ERTs and GTs

03 Platform enables high M6P content for ERTs and therapeutic enzymes from GTs



The Solution: S1S3 PTase Co-Expression Platform Ensures Production of Therapeutic Lysosomal Enzymes with High Levels of M6P



Co-expression of S1S3 PTase with therapeutic protein produces lysosomal enzyme with high levels of M6P to enable much improved drug targeting

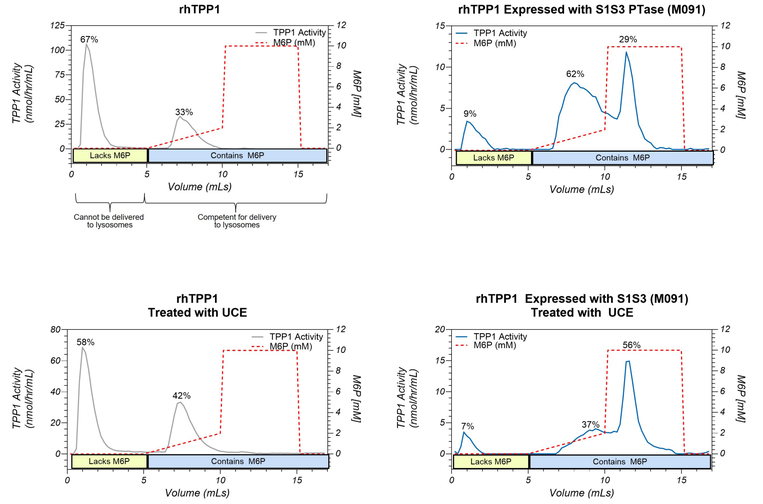
Cation-independent M6P receptor (CI-MPR) is present on nearly all cells for cellular uptake of exogenous M6P-bearing lysosomal enzymes

Project Goals

- *Evaluate TPP1 ephosphorylation*
- *Evaluate TPP1 co-expressed with S1S3 PTase phosphorylation*
- *Perform biochemical characterization of both TPP1 versions*

Cross-Correction via CI-MPR Uptake Should be More Efficient with Well Phosphorylated TPP1 Co-expressed with S1S3 PTase

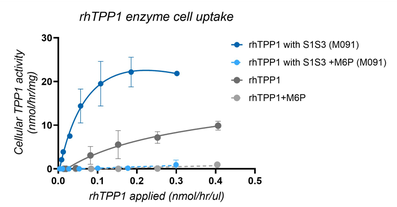
- CI-MPR binding was evalauted by CI-MPR affinity chromatography.
  - Briefly, purified bCI-MPR affinity resin was prepared by coupling CI-MPR to NHS activated Affi-Gel 15 (Bio-RAB) @ 1 mg/ml resin.
  - A 1 mL column was prepared and a two buffer system was use. Buffer A (NaPhosphate, pH 6.8) was used for binding and any bound TPP1 was eluted with Buffer B (NaOAc, pH 4.8 + 10 mM M6P) as indicated by the red dashed line.
  - 1 mg of TPP1 was loaded onto the column and the chromatograph developed as indicated by the dotted line on the graphs below. Fractions were collected and TPP1 activity measured.



- TPP1 co-expressed with S1S3 PTase increased the amount of phosphorylated TPP1 by >50% as indicated by increased CI-MPR binding
- 29% of the TPP1 co-expressed with S1S3 PTase required 10 mM M6P to compete it off. Indicating strong affinity for CI-MPR
- UCE treatment reveals that >25% of TPP1 co-expressed with S1S3 PTase has a terminal GlcNAc "covering" M6P compared to 9% of TPP1 expressed alone.
- This may improve therapeutic efficiency by protecting M6P until until TPP1 reaches the affected tissue and can be uncovered by UCE found on the surface of the cell

M6P S1S3 PTase Co-Expression Platform Produces Highly Phosphorylated TPP1 for Significantly Better Cellular Uptake

- Increasing amounts of TPP1 was added to CLN2 patient-derived fibroblasts for evaluating TPP1 cellular uptake
- Highly phosphorylated TPP1 produced by S1S3 co-expression platform has superior cellular uptake in patient fibroblast cells via M6P receptor pathway



Conclusions

- TPP1 co-expressed with S1S3 PTase increased CI-MPR receptor binding by >50%.
- Treatment of TPP1 with UCE suggests that >25% of the glycans on TPP1+S1S3 PTase is covered. Thereby protecting TPP1 from potential serum or interstial phosphatases.

RARE DISEASE DAY®