A novel S1S3 PTase co-expression gene therapy platform for lysosomal storage disorders

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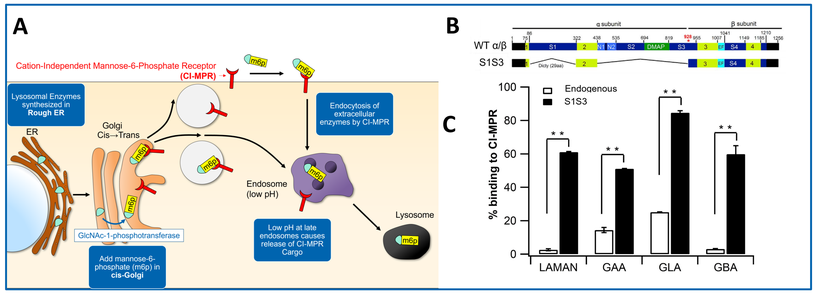
Abstract

Gene therapy for lysosomal storage disorders has a limitation in that overexpression of the missing protein by the modified cells results in the majority of the produced enzyme being poorly phosphorylated and secreted into the serum of patients. This low level of phosphorylation also prevents the circulating enzymes from being taken up by other cells to permit cross-correction. The development of novel methods to increase the M6P content of the produced enzyme by GlcNAc-1-phosphotransferase (PTase), and its targeting to the lysosome allows for improved gene therapies with greater efficacy. In this regard, the creation of S1S3, a hyperactive truncated form of PTase represented a significant advancement and innovation for treatment of lysosomal storage disorders, especially in the context of S1S3 co-expression gene therapies that permit production of lysosomal enzymes with high levels of M6P content. Using GLA, the lysosomal enzyme deficient in patients with Fabry disease, the utility of our S1S3 based platform for gene therapy is demonstrated using a Fabry mouse model. AAV based vectors that facilitate co-expression of S1S3 and GLA showed production of active α-Gal A enzyme in both wild-type and Fabry mice. However, in comparison to GLA expressed alone, the S1S3 platform produced an enzyme with increased M6P content, as evidenced by enhanced CI-MPR binding. In addition, the S1S3 co-expression gene therapy permitted lower doses of AAV to be administered to the Fabry mice to elicit substrate clearance compared to the viral vector harboring α-Gal A enzyme alone. These findings demonstrate the potential of the S1S3 based gene therapy platform and may permit the development of improved gene therapies for lysosomal storage disorders.

Background – S1S3 and phosphotransferase

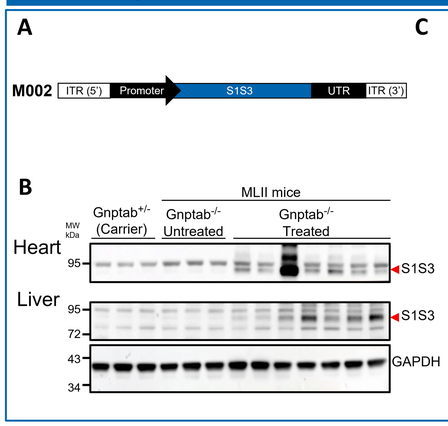
Soluble lysosomal hydrolases synthesized at the endoplasmic reticulum (ER) acquire high mannose glycans in the ER, which are further processed as they transit the Golgi. The enzyme N-acetylglucosamine-1-phosphate transferase (PTase) catalyzes the addition of mannose-6-phosphate (M6P) residues to these lysosomal hydrolases, which in turn are recognized by the two mannose-6-phosphate receptors (CI- and CD-MPR) at the trans Golgi network (TGN) for forward delivery to endosomes. The decreasing pH at endosomes causes the release of the hydrolases from the MPRs, and they subsequently enter the lysosome where they perform their function[1].

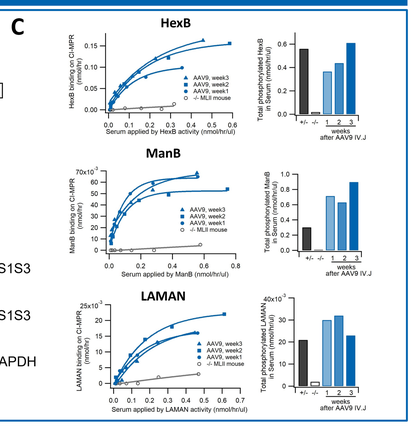
PTase functions as a dimeric-heterotrimer. S1S3 is a truncated hyperactive variant of PTase that efficiently catalyzes addition of M6P residues to lysosomal hydrolases.



S1S3 enhances M6P content of lysosomal hydrolases in cells.

(A) Cartoon depicting the trafficking itinerary of soluble lysosomal hydrolases. Lysosomal hydrolases synthesized at the ER acquire high mannose glycans which are further processed in the Golgi. The addition of the M6P modification at the Golgi permit recognition of the hydrolases by the CI- and CD-MPRs at the TGN, which deliver the enzymes to the endosomes. The decrease in pH at the endosomes causes release of the hydrolases, which subsequently enter the lysosome to perform their function. In addition, the CI-MPR on the cell surface can mediate the endocytosis of serum lysosomal hydrolases for their subsequent delivery to the lysosome.
(B) Schematic of WT and S1S3 PTase.
(C) Expi293 cells co-transfected with expression plasmids for the indicated lysosomal hydrolase along with either empty vector (endogenous) or the S1-S3 cDNA (S1S3) were assayed for efficiency of phosphorylation of the secreted hydrolases by measuring the extent of binding to CI-MPR beads[2].





Treatment of MLII mice lacking PTase activity with AAV-S1S3 restores phosphorylation of acid hydrolases *in vivo*

(A) Schematic of the AAV9 vector used for expression of S1S3 in MLII mice[3].
(B) Mice were injected by tail vein at 4 weeks of age and Western blot analysis performed 14 weeks post-injection to detect expression of S1S3 in the heart and liver of MLII treated animals.
(C) Lysosomal hydrolases (HexB, ManB, and LAMAN) were examined in the serum of MLII animals. Left – S1S3 expression enhanced CI-MPR binding of HexB, ManB and LAMAN. Right – S1S3 expression restores the total phosphorylated HexB, ManB and LAMAN enzymes in serum.

References

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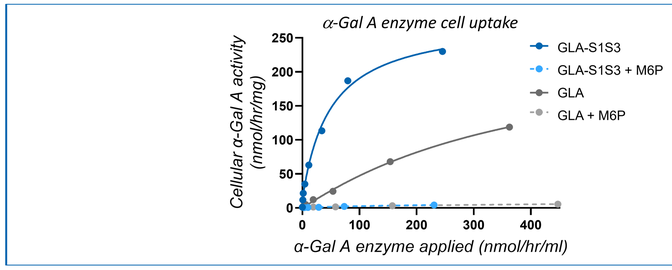
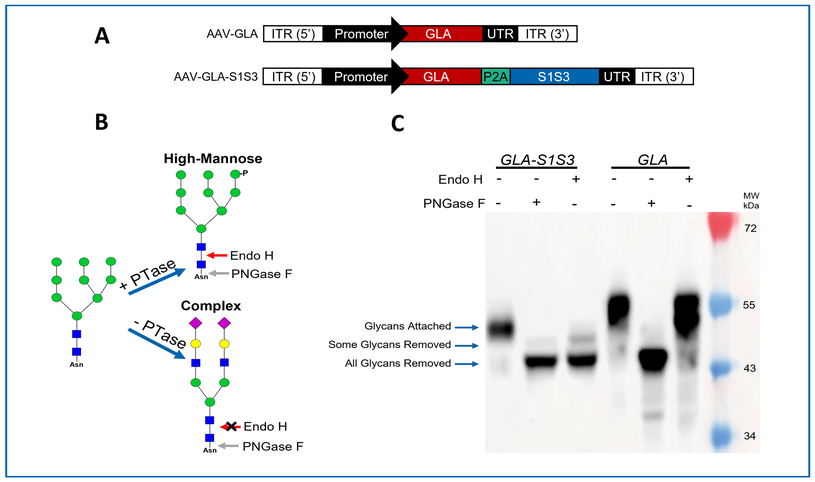
2. Liu L et al. 2017 *Mol Ther Methods Clin Dev*. 5:59-65.
3. Gelfman C et al. 2007 *IOVS*. 48: 5221-5228.
4. Ohshima T et al. 1997 *PNAS*. 94 (6): 2540-2544.



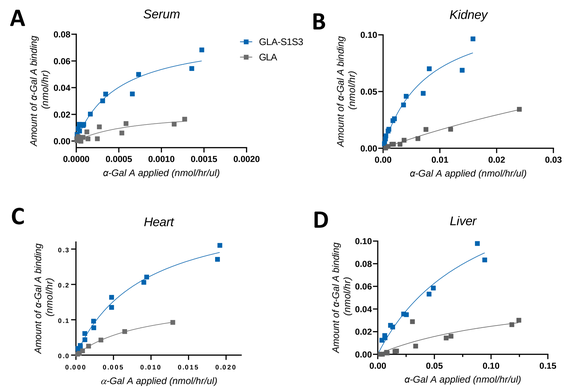
α-Gal A produced with S1S3 increases cell uptake via the CI-MPR pathway

Recombinant α-Gal A was expressed with or without S1S3 in 293T cells. The conditioned medium containing α-Gal A enzyme produced with S1S3 PTase (GLA-S1S3) or not (GLA) was incubated for 4 h in growth media to allow for uptake by human Fabry (*GLA*$^{-/-}$) patient fibroblasts, after which cells were lysed and α-Gal A activity was assayed. 5 mM Mannose 6-phosphate (M6P) was added to media for inhibiting CI-MPR mediated endocytosis. GLA-S1S3 showed better uptake compared to GLA alone.



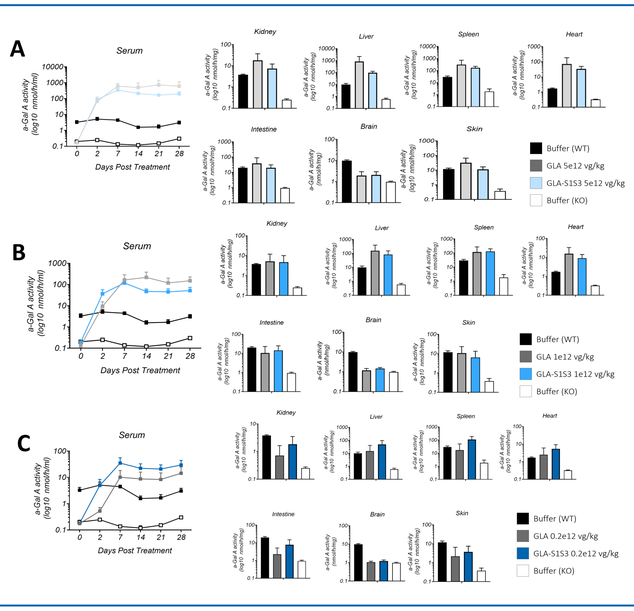
S1S3 alters N-Glycan profile on α-Gal A as determined by Endo H sensitivity

(A) Schematic of control AAV9 vector (GLA) and the AAV9 bicistronic vector (GLA-S1S3).
(B) Schematic of glycan processing that occurs in the presence of PTase activity (+PTase) wherein M6P is added to the high-mannose structure. Phosphorylation of certain terminal mannose residues prevents mannose trimming and conversion to complex-type glycans that are sensitive to Endo H. In the absence of PTase activity (-PTase), the glycans chains are further processed to complex forms that are resistant to Endo H treatment.
(C) Heart samples from WT animal treated with AAV-GLA or AAV-GLA-S1S3 were treated with PNGase F (which cleaves all glycans) or Endo H (which cleaves only high mannose glycans). α-Gal A molecular weight was examined by Western blot with α-Gal A specific antibody. α-Gal A co-expressed with S1S3 (GLA-S1S3) showed virtually all Endo H sensitive glycans, indicating mostly high mannose sugars as a result of PTase activity. α-Gal A expressed alone (GLA) is mostly Endo H resistant, indicating processing of glycans to complex forms.



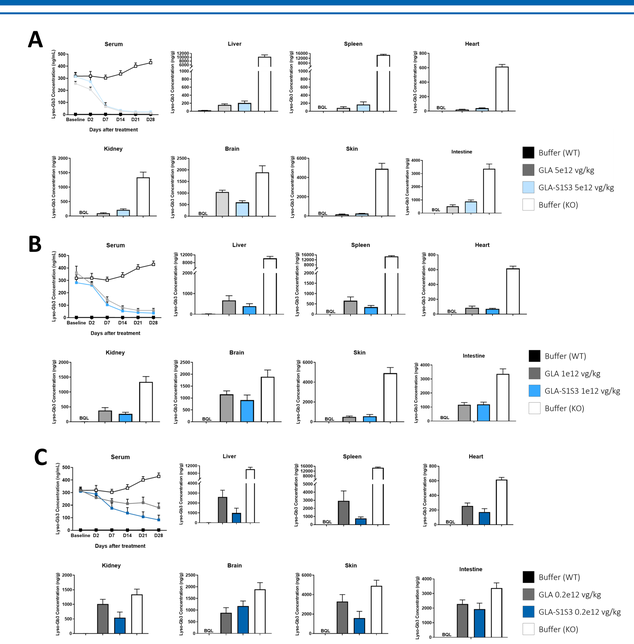
S1S3 co-expression increases α-Gal A CI-MPR binding in AAV9 treated animal tissues

(A) Binding of α-Gal A expressed alone (GLA) or α-Gal A co-expressed with S1S3 (GLA-S1S3) from serum of wild-type mice administered GLA or GLA-S1S3 AAV9, to CI-MPR immobilized on a 96 well plate. Serum from treated mice was incubated with CI-MPR, washed and α-Gal A binding examined by an activity assay. GLA-S1S3 showed enhanced binding to CI-MPR compared to GLA expressed alone in Fabry mice.
(B-D) Tissue homogenates from kidney, heart and liver were assessed for binding of GLA to CI-MPR as described in (A).



Dose response expression study of GLA-S1S3 AAV in Fabry mice

Wild-type mice were treated with only buffer while Fabry mice[4] were either treated with buffer or given AAV9 to express GLA or GLA-S1S3. Both buffer and AAV9 were administered via tail vein injection. Mice received three different doses of virus: (A) high (5e12 vg/kg), (B) medium (1e12 vg/kg) or (C) low (0.2e12 vg/kg). Serum was collected over a period of 28 days post-injection, and assayed for α-Gal A activity. At the end of the study, α-Gal A activity was assayed in tissue homogenates from kidney, liver, spleen, heart intestine, brain or skin. At both high and low doses, GLA and GLA-S1S3 had similar GLA activity in serum and tissues.



Higher phosphorylated α-Gal A translated to improved substrate clearance at low dose in Fabry mice

WT and Fabry mice were treated with buffer or AAV9 as described above. Serum and tissues were assayed for depletion of Lyso-Gb3 substrate, which accumulates in Fabry animals. At high (A) and medium (B) dose, GLA and GLA-S1S3 show similar depletion of Gb3 in serum and tissues. At the lowest dose, GLA-S1S3 appears to show stronger depletion of Lyso-Gb3 than GLA.

Conclusion

We present the development of S1S3 PTase co-expression gene therapy as a novel strategy to allow for the expression of lysosomal enzymes with increased M6P content in the treatment of lysosomal storage disorders. Treatment of Fabry mice with an AAV9 vector co-expressing GLA with S1S3 increased the M6P content of the α-Gal A, compared to treatment with an AAV9 expressing GLA alone. α-Gal A co-expressed with S1S3 (GLA-S1S3) from the serum and tissues of these mice displayed enhanced binding to CI-MPR indicating increased phosphorylation. The highly phosphorylated GLA translated to enhanced efficiency as shown by improved substrate clearance at low viral dose. These findings demonstrate that an S1S3 based gene therapy platform may permit the development of improved gene therapies for lysosomal storage disorders.