Dual promoter AAV gene therapy platform for producing soluble lysosomal enzymes with high M6P content in vivo to enable broad cellular uptake and cross-correction

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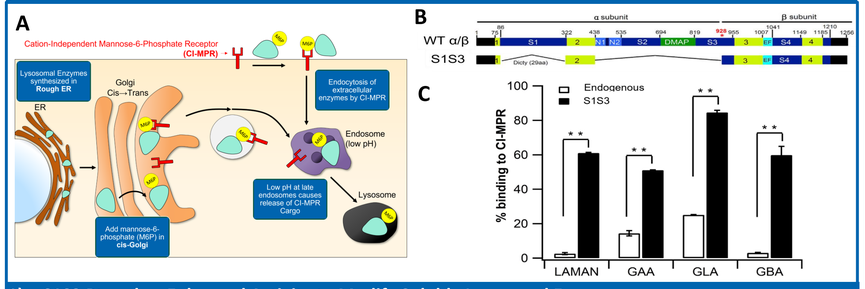
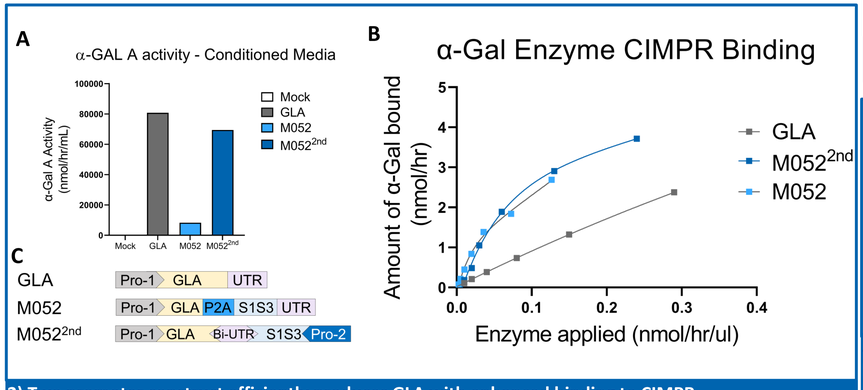
Introduction

GlcNAc-1-phosphotransferase (PTase) is a resident Golgi enzyme that adds phosphates to mannose residues on N-linked oligosaccharides to produce mannose 6-phosphate (M6P) on newly synthesized soluble lysosomal enzymes. M6P is a natural motif used by lysosomal enzymes to enable binding to M6P receptors and transport to lysosomes[1]. Modulation of Ptase activity in cells has proven difficult to produce lysosomal enzymes with high levels of M6P for therapeutic applications. Recently, a truncated PTase (designated as S1S3 PTase) was created that has enhanced phosphorylation activity (20X higher than wildtype PTase) (see reference 2 and panel 1). Importantly, the reduced size of the S1S3 PTase gene make it possible to be incorporated in vectors for co-expression of S1S3 PTase in AAV gene therapy applications to produce therapeutic lysosome enzymes with high levels of M6P *in vivo*. We have designed vectors using bicistronic methods (IRES or 2A sequences) or more recently using optimized promoter pairs for improved expression of both genes. This work has enabled us to develop new dual promoter AAV vectors with improved packaging and gene expression to produce highly phosphorylated lysosomal enzymes, including α-Gal A, β-glucocerebrosidase, modified β-hexosaminidase (HexM), α-N-acetylglucosaminidase among others. Thus, we have developed a novel dual promoter AAV gene therapy approach that reliably produces therapeutic lysosomal enzymes with high M6P levels for potential cross-correction *in vivo*.



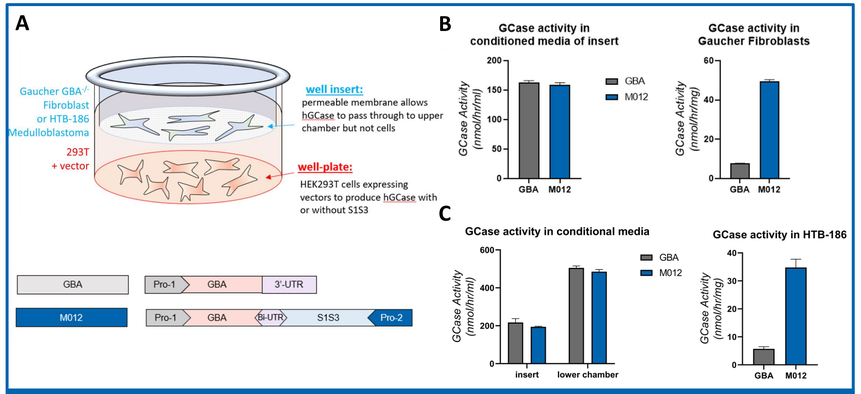
1) S1S3 Ptase has Enhanced Activity to Modify Soluble Lysosomal Enzymes

(A) M6P pathway (B) S1S3 truncated PTase compared to WT enzyme[2]
(C) Co-expression of S1S3 enhances binding of soluble lysosomal enzymes to CIMPR[2]

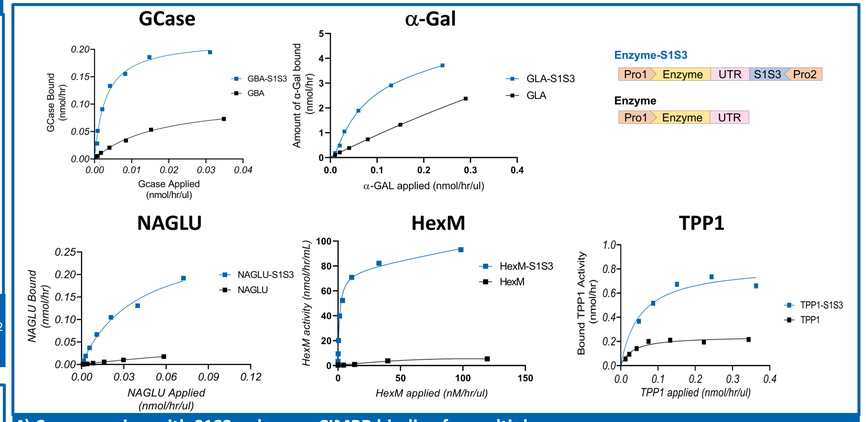


2) Two promoter construct efficiently produces GLA with enhanced binding to CIMPR

(A) Enzyme activity in conditioned media. (B) CIMPR binding for conditioned media (C) Vector designs

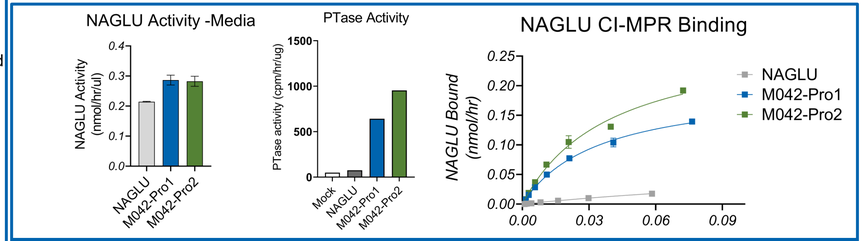


3) Higher phosphorylated GCase from M012 vector has enhanced uptake in cells
(A) Schematic for co-culture of vector expressing 293 cells co-cultured with
(B) Gaucher Fibroblasts or (C) HTB-186 medulloblastoma cells.
GCase activity was examined in media and cells – (B) Gaucher Fibroblasts, (C) HTB-186 cells.



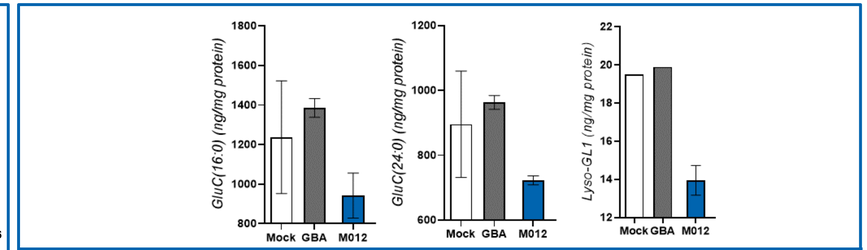
4) Co-expression with S1S3 enhances CIMPR binding for multiple enzymes

CIMPR binding for multiple enzymes from media. (Top right) Vectors for expression

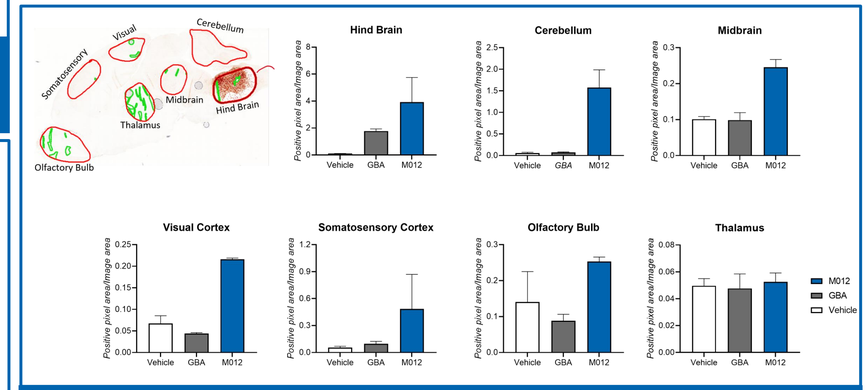


5) S1S3 expression correlates with enzyme binding to CIMPR

NAGLU was expressed alone or co-expressed with S1S3. S1S3 was expressed by two different promoters (Pro1 or Pro2). Cell lysates or conditioned media were examined for NAGLU activity, PTase activity, or binding to CIMPR. NAGLU CIMPR binding is correlated with S1S3 activity.



6) Higher phosphorylated GCase from M012 vector enhances substrate clearance in Gaucher Fibroblasts
After co-culture as in (panel 3B), Gaucher fibroblasts were processed for substrate analysis using LC/MS. M012 shows enhanced substrate reduction of Glucosylceramide (GlcC) and Glucosylsphingosine (lyso-GL1) compared to Mock or GBA expressed alone.



7) GCase produced by M012 shows broad distribution in brain tissue after ICM administration
Analysis of wild-type mouse brain tissue after AAV9 treatment. Images were analyzed in seven brain regions (red outlines) for IHC staining of GCase (brown).

Conclusion

We present the development of S1S3 PTase co-expression gene therapy as a novel strategy to allow for the expression of soluble lysosomal enzymes with increased M6P content in the treatment of lysosomal storage disorders. Co-expression with two promoters produces enzymes with enhanced M6P content, enhanced binding to CIMPR, and enhanced uptake in cells. In a co-culture system, expression of S1S3 correlates with enhanced uptake and enhanced substrate reduction in patient fibroblasts. In vivo we can also see broad detection of GCase in brain from AAV with co-expression of GCase with S1S3. Altogether these results suggest that co-expression of S1S3 has the potential to facilitate cellular uptake of exogenous therapeutic lysosomal enzymes for broad cross-correction in nearly all cells, including those in brain to provide a novel approach for addressing CNS manifestations.

Acknowledgements

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References

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2. Liu L et al. 2017 *Mol Ther Methods Clin Dev*. 5:59-65.