IS ANTISENSE OLIGONUCLEOTIDE-MEDIATED EXON SKIPPING A POTENTIAL THERAPEUTIC APPROACH FOR MUCOLIPIDOSIS II?

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INTRODUCTION: Mucolipidosis II (ML II) is a Lysosomal Storage Disorder caused by N-acetylglucosamine-1-phosphotransferase (GlcNAc-PT) deficiency, which impairs lysosomal hydrolases trafficking. Here, we explored an innovative therapeutic strategy based on the use of antisense oligonucleotides (ASOs) to promote targeted skipping of *GNPTAB* exon 19, which harbors c.3503_3504del, the most frequent disease-causing variant. Previously, in ML II patients' fibroblasts, we tested ASOs to induce exon 19 skipping, successfully generating an in-frame mRNA[1]. Now, our aim is to determine if this in-frame transcript leads to increased GlcNAc-PT levels.
Methodology: First, the GlcNAc-PT activity was measured in fibroblasts, but activity levels were similar in ML II and control fibroblasts (treated/non-treated) showing that the assay is not proper to measure endogenous levels. To overcome this, we designed 3 constructs: a WT (full *GNPTAB* cDNA), a del_ex19 (without exon 19) and a mutant (with the mutation c.3503_3504del) that were transfected in HEK293T cells. Then GlcNAc-PT expression was analyzed by Western Blot (WB).
Also, we measured the activity of several hydrolases and evaluated the expression of α-galactosidase A (α-Gal) by WB after ASO treatment.
To further validate this therapy we also generated a novel GlcNAc-PT antibody in rabbits.
Results: Our results showed that HEK293T cells were able to express all the constructs. The WB of both WT and del_ex19 constructs showed bands corresponding to the α/β precursor. However, only the WT construct expressed the β-subunit, suggesting that there is no GlcNAc-PT activity in the absence of exon 19. As expected, in the delTC construct WB no α/β precursor band was detected.
We also observed a slight increase in the activity of various lysosomal hydrolases in ML II fibroblasts after treatment. However, only the α-Gal values were statistically significant, but the WB analysis for this enzyme did not reveal any band in ASO-treated ML II cells.
We also developed a novel antibody for GlcNAc-PT. Preliminary results showed a β-subunit band both in control and patient fibroblasts (unexpected), but in overexpression both WT and del_ex19 constructs presented α/β precursor bands. So, further assays are needed to assess its specificity.
Conclusion: Our ASO-based approach effectively promotes exon 19 skipping. However, this strategy, as far as we have been able to prove, is not able to restore any GlcNAc-PT enzymatic activity. Further validation, including co-localization studies are planned to clarify these findings.

Keywords: Antisense Oligonucleotide Therapy; Exon-Skipping; Mucolipidosis II

1. Matos L et al. Hum Gene Ther, 2020, 31:775-83.