MPS 2021

16th International Symposium on MPS and Related Diseases

Virtual Conference | Jul 23-25, 2021 | Barcelona

POSTER Nr: 40

Targeted RNA-based therapies for Mucopolysaccharidosis

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Over the last years, most of our work has been focused on the development of alternative, RNA-based therapies for a number of LSD, being MPSIII one of the most relevant. Two major possible drug types, depending on the genotype that underlies pathology, are being used: **U1snRNAs** and **siRNAs**. The first are specifically designed to overcome particular splicing mutations and the second to promote an overall decrease of the accumulating substrate. Here we present an overview on our results with both approaches on MPS III.

METHODS

U1snRNAs

Mutation-specific approach to correct abnormal splicing process in MPS IIIC caused by the c.234+1G>A mutation

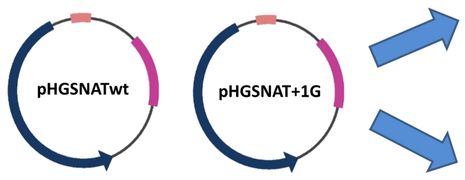
We demonstrated in patient fibroblast cells that a modified U1snRNA vector (comprising exon 1 to exon 3) designed to improve the definition of exon 2 5' ss of the *HGSNAT* can restore the splicing defect caused by the mutation c.234+1G>A (Matos *et* al., 2014).

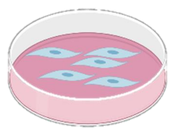
GOAL: Evaluate *in vivo* the therapeutic potential of the modified U1snRNA by testing it in mice expressing the human splicing defect

Generation of full-length (*HGSNAT* cDNA sequence plus part of introns 1 and 2) *HGSNAT* constructs of **wild-type (wt) (pHGSNATwt)** and **c.234+1G>A (pHGSNAT+1G)** by cloning the **wt** or the **mutated *HGSNAT* splicing-competent cassettes** into the **pcDNA 3.1 backbone**

In vitro

Transfection of 3µg of each construct using Lipofectamine® 2000





HEK 293T cells

In vivo

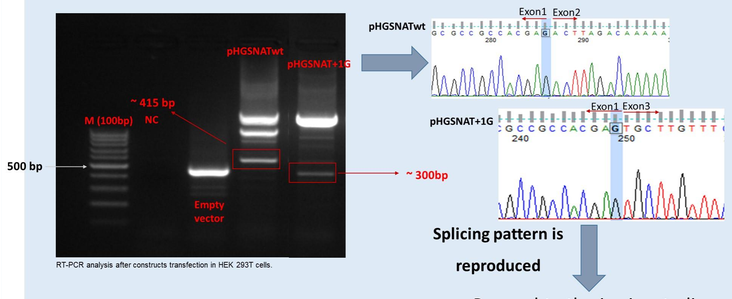
Administration in mice by hydrodynamic injection (using the tail vein)

C57BL/6 mouse strain

RESULTS

In vitro

Transfection of HEK 293T with **pHGSNATwt** and **pHGSNAT+1G** constructs:



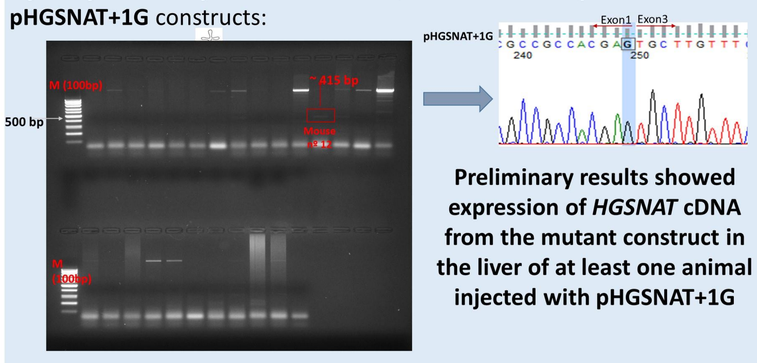
RT-PCR analysis after constructs transfection in HEK 293T cells.

Splicing pattern is reproduced

Proceed to the *in vivo* studies

In vivo

Hydrodynamic injection of C57BL/6 mice with **pHGSNATwt** and **pHGSNAT+1G** constructs:



Preliminary results showed expression of *HGSNAT* cDNA from the mutant construct in the liver of at least one animal injected with pHGSNAT+1G

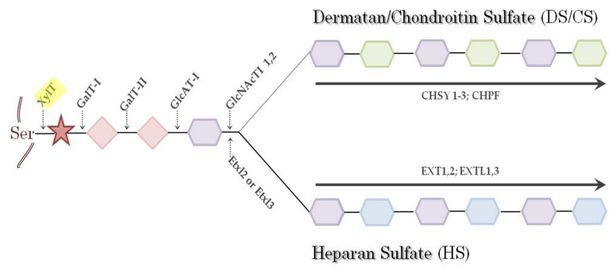
The **lack of pHGSNAT cDNA expression in the majority of animals** was probably due to **sub-optimal hydrodynamic injection conditions**. In fact, when we tried to administrate a volume of 10% of the mice body weight, animals died. So, we **lowered the volume to 7%**. Also noteworthy, the mouse strain we worked with (C57BL/6) is **aggressive**, has a **hyperactive behaviour** and has **black color**, which difficult the visualization of the tail vein.

In order to overcome these issues we will now repeat the protocol:

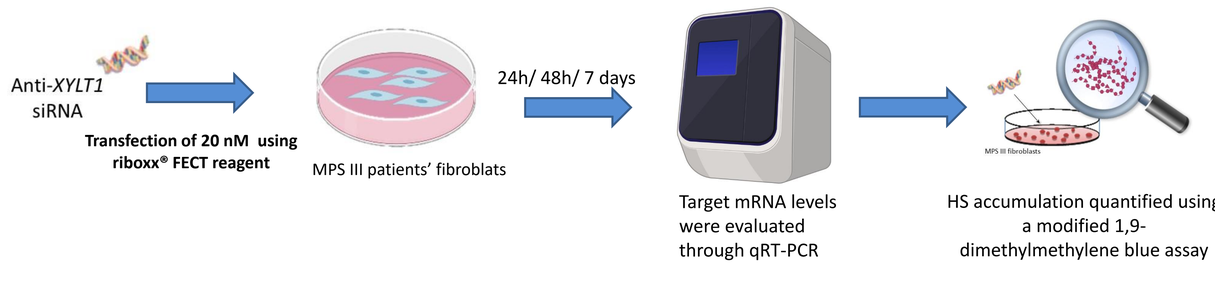
- **Using ICR mice (**albine strain, more easier to handle and known for its docile behaviour)
- **Increase** the volume of our injection for **8-9%** of the mice body weight

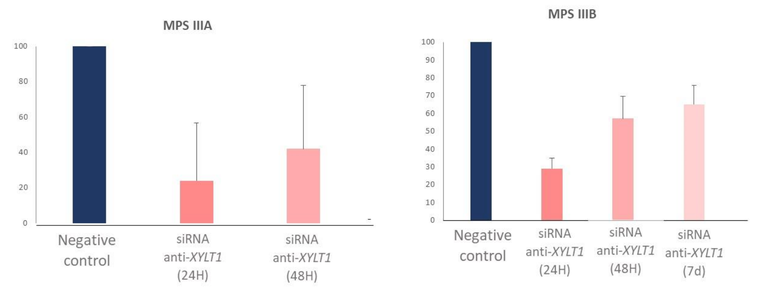
siRNAs

Selective downregulation of one gene involved in the very early stages of the glycosaminoglycans' (GAG) biosynthethic cascade to promote substrate reduction in Sanfilippo disease



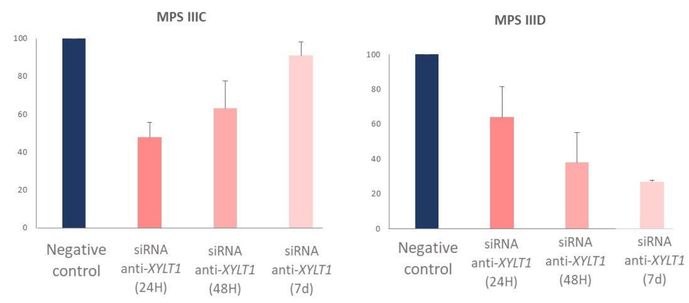
We designed and assayed in MPS III patients' fibroblasts a specific siRNA pool targeting *XYLT1,* a gene that encodes an enzyme involved in an early stage of the HS biosynthetic cascade: xylosyl-transferase 1.



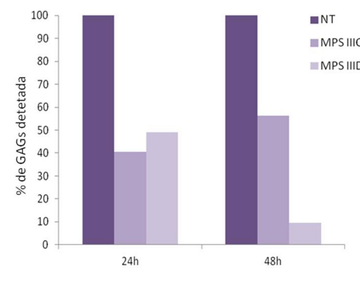


Proof of principle on the *in vitro* effect of anti-*XYLT1* siRNAs was obtained for Sanfillippo syndrome, with significant decreases of the target gene expression at different time points (24h-7 days).

In general, our results also reinforce the idea that there is variability amongst different cell lines in terms of response to treatment with siRNAs. In fact, we observe substantial differences between different cell lines treated with the same anti-*XYLT1* siRNA pool, at the same concentration. In studies by other authors, this sort of variability was observed even among cell lines from patients suffering from the same disease and with the same underlying genotype.



MPS IIIA, IIB, IIIC and IIID fibroblasts were transfected with 20nM of the anti-*XYLT1* siRNA pool and after 24, 48 hours (H) (for IIIA subtype) and 7 days (d) (for other subtypes), gene expression was analysed by real-time PCR. Results are expressed as the percentage of gene expression compared to non transfected cells (NT), using *GAPDH* as a reference.



After this first assessment on the effect of anti-*XYLT1* siRNA incubation at 24 and 48h over sulphated GAG levels, we are now evaluating its effect 7 days post-transfection and for other MPS subtypes, also with promising results.

Further validation

- qGAG by MS/MS;
- immunocytochemistry *(anti-HS antibody)*

Knowing that the **translation** of siRNAs from the bench to the clinic may be hindered by their limited cellular uptake, low biological stability and unfavorable pharmacokinetics, the development of an appropriate delivery method and a relevant disease model to test it is mandatory if we want to proceed with these preclinical studies. Therefore, **in the near future we will**:

- develop an appropriate **delivery method** for 'anti-GAG siRNAs' **targeted to neuronal receptors** (t-SNALPs)
- establish **patient-derived MPS III neuronal cell lines** through a non-invasive, cost effective approach using **dental pulp stem cells (DPSC).**

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