From bench to bioterium and back again: DEVELOPMENT OF A U1 snRNA-BASED THERAPEUTIC STRATEGY FOR MUCOPOLYSACCHARIDOSIS IIIC

Instituto Nacional de Saúde Doutor Ricardo Jorge

FACULDADE DE CIÊNCIAS UNIVERSIDADE DO PORTO

Juliana I. Santos[1,2]*, Liliana Matos[1,3]*, Paula A. Oliveira[4], Mariana Gonçalves[4], Mª João Pires[4], Mª Francisca Coutinho[1,3], Mª João Prata[2,5], Sandra Alves[1,3]

[1] Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal; [2] Biology department, Faculty of Sciences, University of Porto, Portugal; [3] CECA-ICETA - Center for the Study Animal Science, University of Porto, Portugal; [4] CITAB – Center for the Research and Technology of Agro-Environmental and Biological Sciences, Vila Real, Portugal; [5] i3S – Health research and innovation institute, University of Porto, Portugal

*These authors contribute equally to this work

Background

Poster

Splicing is an essential cellular process to generate mature transcripts from pre-mRNA. One of the most important factors for mRNA transcription is the U1snRNA, a spliceosomal component that recognizes 5' splicing donor sites (SDS) at specific regions in pre-mRNA. Splicing mutations represent one of the most frequent (~20%) genetic defects in Mucopolysaccharidosis IIIC (MPS IIIC), a Lysosomal Storage Disorder (LSD) caused by mutations in the *HGSNAT* gene, encoding an enzyme involved in heparan sulphate degradation.

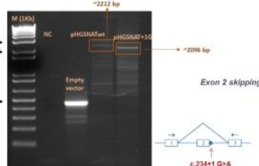
Exon-skipping has been demonstrated as, probably, the most frequent aberrant splicing defect, and occurs due to mutations in the 5' SDS. Application of modified U1snRNAs to improve recognition of mutated 5' SDS represent a potential therapeutic strategy to recover the normal splicing process.

Background

Poster

The c.234+1G>A is a frequent mutation among patients of countries around the Mediterranean basin (Portugal, Spain, Morocco and Tunisia). It's located in the + 1 position of intron 2 of *HGSNAT* gene and leads to the skipping of exon 2.

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



A partial recovery was achieved with a modified U1snRNA that completely matches the SDS.

Currently our goal is to evaluate *in vivo* the therapeutic potential of the modified U1 snRNA by testing it in mice expressing the human splicing defect.

Methods

Poster

In vitro

- Generation of full-length (containing the full *HGSNAT* cDNA sequence plus part of introns 1 and 2) splicing competent constructs of
  - wild-type (wt)
  - and c.234+1G>A *HGSNAT*

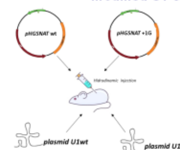
  How?

  By cloning the wt or the mutated *HGSNAT* splicing-competent cassettes into the pcDNA3.1 backbone

- Transfection of those constructs into COS-7 cells to check if the splicing pattern is reproduced

In vivo

- Use the constructed plasmid vectors to promote transient expression of the human *HGSNAT* wt or mutant alleles in c57bl/6 mice
- Co-injection of the wt or mutant vectors + modified U1 snRNA



Results

Poster

In vitro

Transfection of COS-7 with pHGSNATwt and pHGSNAT+1G constructs: the splicing pattern is reproduced ✓

~2212 bp

M (1Kb)

NC

pHGSNATwt

pHGSNAT+1G

3000 bp

2000 bp

500 bp

Empty vector

~2096 bp

Exon 2 skipping

c.234+1G>A

RT-PCR analysis after constructs transfection

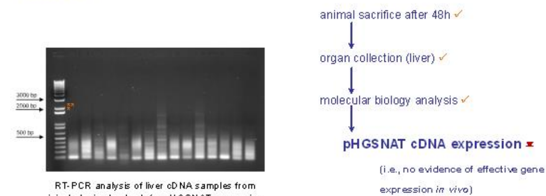
proceed to the *in vivo* studies.

Results

Poster

In vivo

Hydrodynamic injection of c57bl/6 mice with pHGSNATwt and pHGSNAT+1G constructs:



RT-PCR analysis of liver cDNA samples from injected mice to check for pHGSNAT expression

** expected amplicon length for the pHGSNATwt
* expected amplicon length for the pHGSNAT+1G

Conclusion/ Future perspectives

Poster

We have double checked every step of the overall protocol to assess pHGSNAT expression through RT-PCR and everything was OK!

So, the lack of pHGSNAT cDNA expression 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%.

This mice strain 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 with ICR mice (albine strain, more easier to handle and known for its docile behaviour) with some alterations:

- Increase the volume of our injection for 8-9% of the mice body weight
- Dilute our construct in a commercial delivery solution to enhance the transfection efficiency

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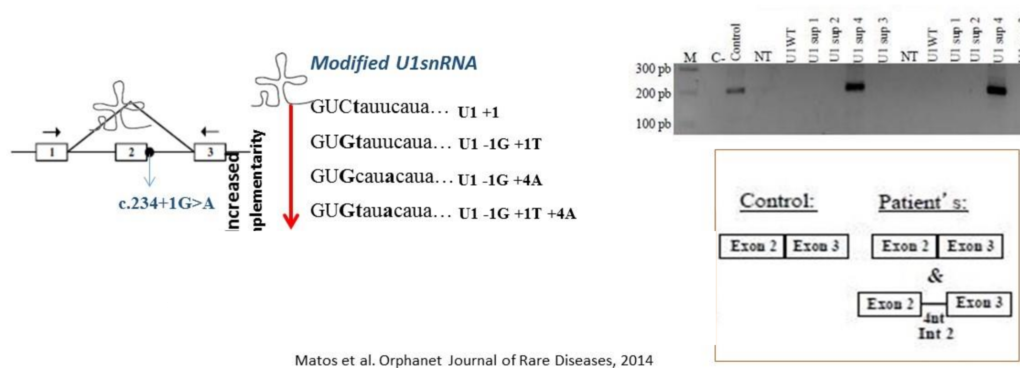

Matos et al. Orphanet Journal of Rare Diseases, 2014

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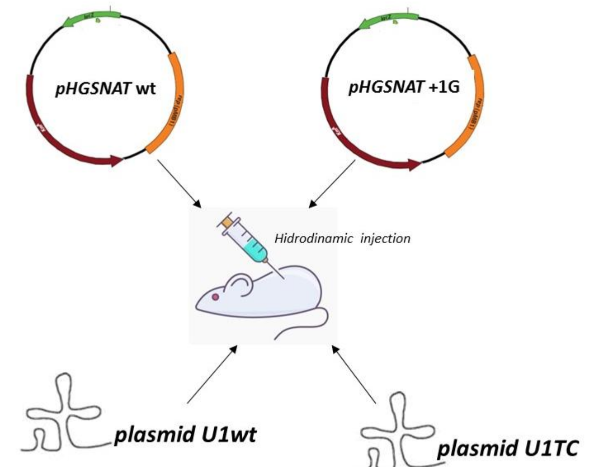
- **Co-injection of the wt or mutant vectors**

  +

  modified U1 snRNA



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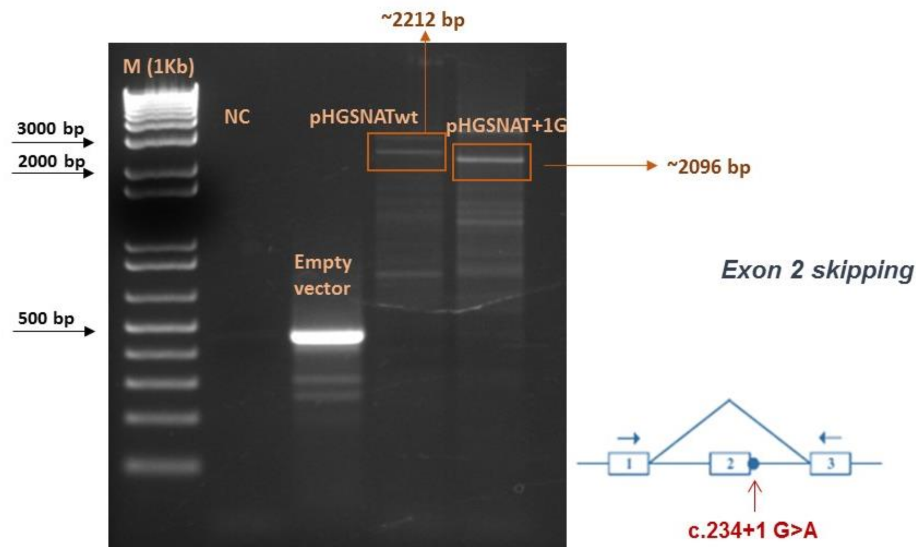

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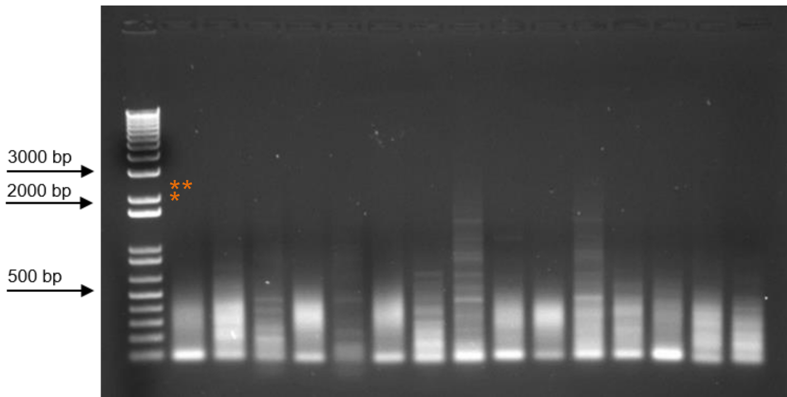

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** expected amplicon length for the pHGSNATwt

* expected amplicon length for the pHGSNAT+1G

animal sacrifice after 48h ✓

↓

organ collection (liver) ✓

↓

molecular biology analysis ✓

↓

pHGSNAT cDNA expression ✗

(i.e., no evidence of effective gene expression *in vivo*)

↓

REPEAT!

Poster

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