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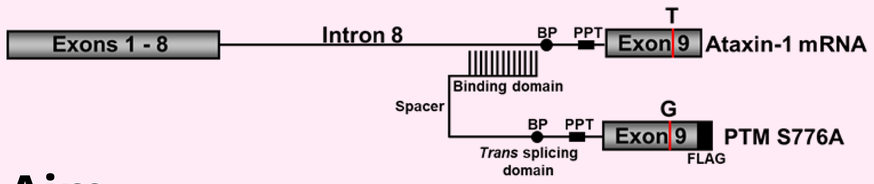
Evaluation of RNA *trans*-splicing as a therapeutic strategy for spinocerebellar ataxia type 1

Introduction

- Spinocerebellar ataxia type 1 (SCA1) is a caused by an expanded polyglutamine (polyQ) tract in the protein ataxin-1 encoded by the *ATXN1* gene
- Phosphorylation at S776 is critical for its neurotoxicity and acts synergistically with the polyQ tract in the pathogenic mechanism[1]
- We are evaluating the potential therapeutic value of substituting S776 for alanine using spliceosome-mediated RNA *trans*-splicing (SMaRT)
- SMaRT creates a hybrid mRNA through a *trans*-splicing reaction between an endogenous target pre-mRNA and an exogenously delivered pre-*trans*-splicing molecule (PTM)[2]



Aim

- **To reprogramme *ATXN1* RNA using SMaRT**

Results

PTMs successfully induced *trans*-splicing of:

- A human *ATXN1* minigene in SH-SY5Y cells **(Figure 1)**
- Endogenous human *ATXN1* in SH-SY5Y cells **(Figure 2)**
- Endogenous mouse *Atxn1* in wild type embryonic mouse cortical neurones transduced with a lentiviral vector co-expressing GFP **(Figure 3)**

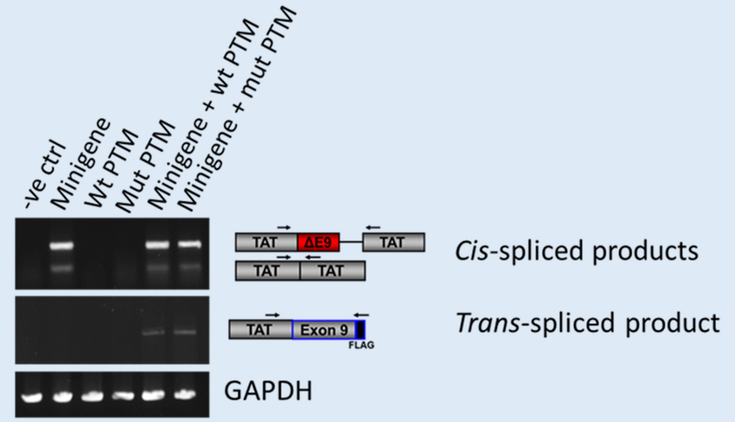


Figure 1.

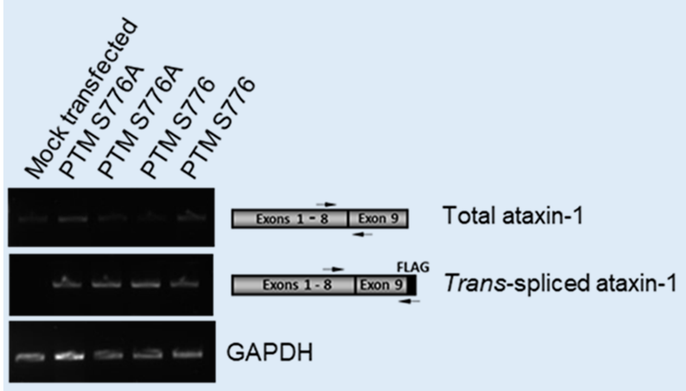


Figure 2.

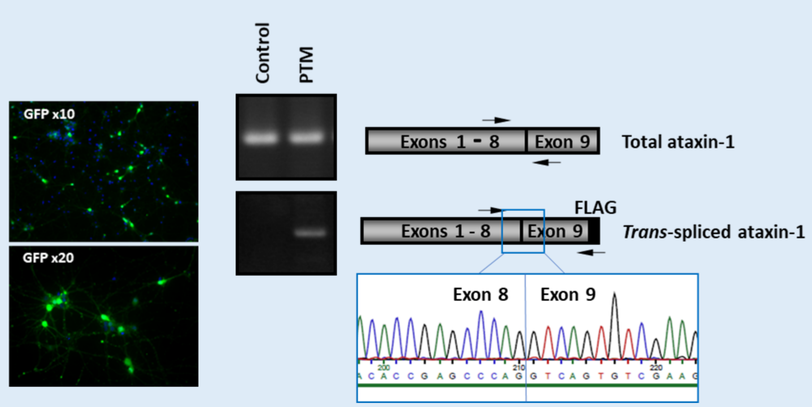


Figure 3.

All results in Figures 1 – 3 were verified by Sanger sequencing

Methods

- Wild-type and mutant PTMs (for mouse [S752] and human [S776]) were designed and constructed using traditional cloning into pcDNA3.1 or a lentiviral vector co-expressing GFP
- SH-SY5Y cells were transfected with a human *ATXN1* minigene and/or PTM
- Embryonic mouse cortical neurones were transduced with lentiviral vector co-expressing GFP and PTM
- *Trans*-splicing was assessed by RT-PCR using a target specific forward primer and a PTM-specific reverse primer

Discussion

- ❖ Ongoing work aims to confirm a change in phosphorylation status and test the effect of SMaRT on validated phenotypes of SCA1 patient-derived iPSC cell models[3]
- ❖ SMaRT can theoretically repair any mutation downstream of the PTM binding site and is suited for the dominant gain-of-function mutations of SCAs

References: [1]Emamian et al (2003) Neuron, Vol. 38, 375; [2]Espindola et al (2018) Cell Rep., Vol. 23, 709; [3]Buijsen et al (2018) Stem Cell Res., Vol. 29, 125