DELIVERY OF ANTISENSE OLIGONUCLEOTIDES USING CYCLODEXTRIN-BASED NANOPARTICLES: AN EFFECTIVE HUNTINGTIN LOWERING APPROACH

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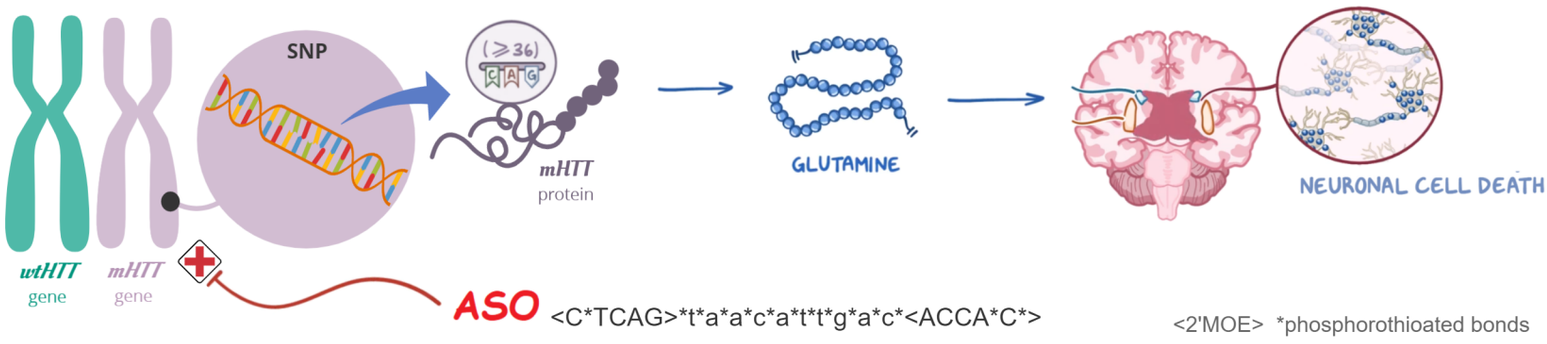
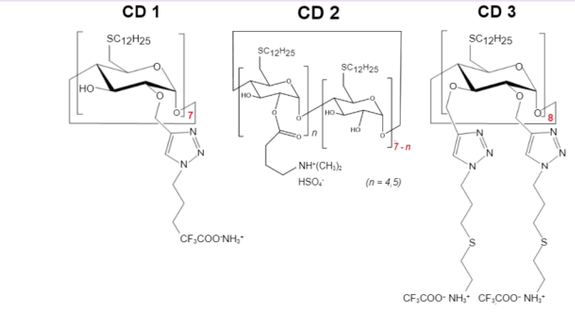
Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by a **CAG repeat expansion in the huntingtin gene (*HTT*)**, which codes for the **toxic mutant HTT (mHTT) protein**. Currently, there is no cure for patients with HD. Here, we designed and synthesized cyclodextrin (CD)-based nanoparticles loaded with antisense oligonucleotides (ASOs) targeting *HTT*. The CDs:ASO nanocomplexes were tested for allele-selective repression of *HTT* in GM04723 (CAG15/67) patient-derived fibroblasts using a single-nucleotide polymorphism (SNP)-specific RT-qPCR assay for rs362307 (SNP1), which is present in two-thirds of HD patients with European ancestry [1].



Results and Discussion



	CD 1	CD 2	CD 3
Size	175 ± 7.14 nm	174 ± 2.73 nm	148 ± 3.33 nm
PDI	0.31 ± 0.03	0.36 ± 0.03	0.26 ± 0.03
Charge	+11 ± 0.24 mV	+5 ± 0.30 mV	+15 ± 0.20 mV

Figure 1. The chemical structures of the amphiphilic CDs and the physicochemical properties of the CDs:ASO nanocomplexes.

The chemical structures of the CDs and physicochemical properties of the CDs:ASO nanocomplexes are shown in Figure 1. The gel retardation assay confirmed that all CDs bound the ASO at a mass ratio of 10:1.

The CD structure significantly affected ASO uptake, which was in the order **CD3:ASO (88.3%, blue) > CD1:ASO (75.3%, red) > CD2:ASO (33.7%, orange) > Free ASO (1.51%, grey)** (Figure 2A). We next investigated whether the differences in ASO cellular uptake directly correlated with mRNA silencing efficacy. As shown in Figure 2B, cells transfected with **CD1:ASO and CD3:ASO showed a decrease in SNP1 mRNA levels by 34 and 41% ($p < 0.05$), respectively.** The lower mRNA silencing efficacy of CD2:ASO nanocomplexes (27% reduction) was likely attributed to the lower ASO uptake, demonstrating a correlation between ASO cellular uptake and activity. Importantly, cellular viability was not affected by ASO treatment, with cell viability exceeding 92% (Figure 2C).

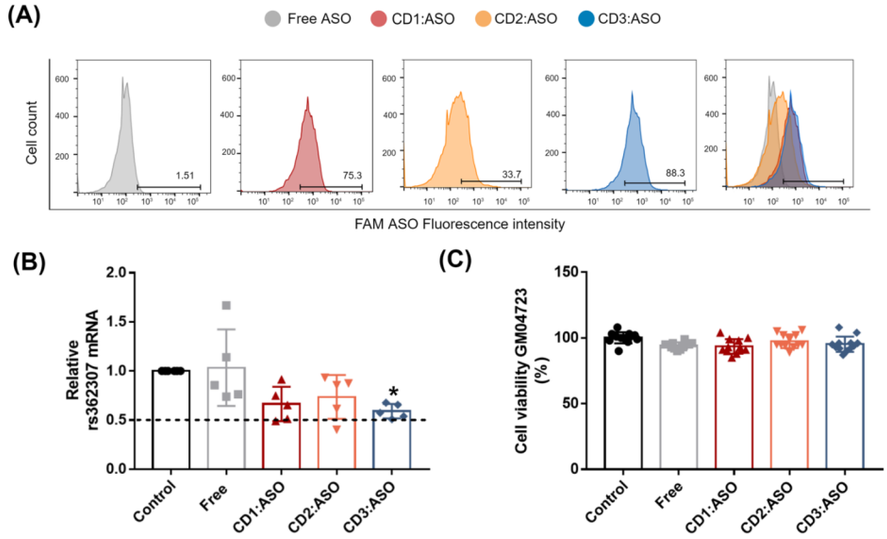


Figure 2. (A) Fibroblasts from an HD patient (GM04723) heterozygous at rs362307 were treated with ASO alone or complexed with CDs (100 nM final concentration) for 6 h and the cellular uptake was confirmed by flow cytometry. (B) mRNA levels of rs362307 were measured 72 h post-transfection by SYBR Green qPCR and normalized to GAPDH. Mean ± S.E.M of 5 technical replicates performed in duplicate. (C) Effect of the formulations in the viability of cells treated during 72 h Living cells were determined using a CellTiter-FLuor assay and expressed as a % of the control. Mean ± S.E.M of 4 technical replicates performed in triplicate. One-way ANOVA with Tukey's multiple comparisons test. * $p < 0.05$.

Conclusion

Overall, the results showed that the double-charged chain γ-CDs (CD3:ASO) exhibited higher cellular uptake and lower SNP1 mRNA levels in HD-patient derived fibroblast cell line in comparison to β-CDs (CD1:ASO and CD2:ASO). Future work will investigate the precise mechanism of action. The data demonstrate the potential of CD platforms to deliver ASO for the treatment of HD.

Acknowledgment and References

1. Svrzikapa N, Longo KA, Prasad N, Boyanapalli R, Brown JM, Dorset D, et al. Mol Ther Methods Clin Dev 2020; 19: 162-73

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