Gene Targeting Techniques for Huntington's Disease

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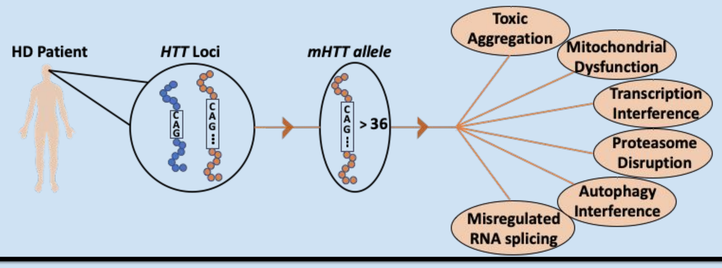
Introduction

Huntington's disease (HD) is an autosomal neurodegenerative disorder caused by extended trinucleotide CAG repetition in the *HTT* gene.
This mutation is mostly associated with both neurological and physical symptoms. The main cause of HD symptoms is due to aggregation and accumulation of mutant HTT (mHTT) proteins in neurons. In this poster, we review multiple approaches targeting DNA and RNA to reduce mHTT expression. These approaches are categorized into non-allele-specific silencing and allele-specific-silencing using SNPs and haplogroup analysis, and the possible limitations of targeting mHTT is also discussed.



Methods

RNA targeting

- siRNA based
  - Targets specific mRNA sequence
  - About 20-24 base pair specificity
- miRNA based
  - Targets 3'-UTR
  - About 22 base pair specificity
- ASO based
  - DNA molecule that targets RNA sequence
  - About 18-30 base pair specificity

DNA targeting

- CRISPR based
  - Edits DNA sequence
  - Requires gRNA and PAM sequence match (~20 base pairs and 3 base pairs)

Allele specificity

- Target endogenous SNPs
- Established HD haplogroups

Disease models

- Fibroblast (skin) cells
- Neural stem cells
- TruHD immortalized fibroblast cell line

Results

RNA targeting

- Artificial viral-mediated miRNA designed by Boudreau et al. resulted in improved behavior and survival in mice, also finding mammals can tolerate up to 75% decrease in wild type HTT expression
- Another approach using an AAV that degraded HTT with siRNA saw reduction in levels by 40% and improved behavior (Stanek et al., 2014)
- ASO approach by Datson et. al targeting CAG repeats resulted in a reduction of mHTT transcripts by 83% and wild-type transcripts by 43%
  - Other CAG repeat genes had reduced transcription, displaying off targeting
  - However, neurological function was restored in mice

DNA targeting

Non allele-specific (Xu, 2017)

- HD-patient derived iPSCs were transfected with a vector containing the sgRNA and cas9 variant
- Normal karyotype and retained pluripotency
- Differentiated into functional GABAergic neurons that had no evidence of HTT aggregates
- Rescued ability of hiPSCs from HD to form neural rosettes and functional mitochondria

Allele specific (Shin et al 2016)

- Excised the promoter, transcriptional start site, and CAG repeats while leaving the healthy allele unaffected
- 6 SNPs upstream of HTT exon 1 were identified that created or destroyed a PAM sequence on mutant allele
- 80% (n=11) of HTT fibroblasts tested were heterozygous for rs2857935, an SNP in the promoter region that destroys a PAM sequence
- Effectively used as a target to delete promoter on mutant allele while leaving healthy allele unaffected

Conclusion

The gene targeting techniques showed notable promise for the reduction of mHTT in cells. Both RNA targeting and DNA targeting methods were able to demonstrate improved behavior in non-human animal models. RNA targeting approaches have been more thoroughly researched, but with the more recent advances with CRISPR technology, DNA targeting approaches may become more advantageous for their ability to permanently edit the expression of mHTT. The risk of these techniques largely is in the potential for off targeting, which presently is not negligible. Some approaches have directly targeted the CAG repeat, but with the presence of other genes with CAG repeats in the genome, this is likely risky. Targeting of SNPs have been shown to allow for a large degree of allele specificity so as to avoid targeting the healthy allele. Although SNP haplogroups have been established, individual screening of SNPs for patients would be needed with this approach.

Future Directions

Our future direction seeks to utilize novel gene editing technique, prime editing, to target the *mHTT* gene by inserting stop codons that will prematurely terminate the production of mHTT protein, which may effectively reduce the symptoms of Huntington's. This technique only nicks one DNA site to insert its desired sequence rather than the two sites that CRISPR-Cas9 requires, which significantly reduces off-targeting effects associated with CRISPR and potentially increases gene targeting efficiency as compared to already existing methods (Anzalone et al., 2019). This approach would ideally be combined with SNPs in order to achieve allele specificity, but with limited time and funds, the approach may instead be applied in a non-allele specific manner. In terms of general Huntington's research, there are a number of ongoing clinical trials for the methods discussed so far and the results of those trials are being awaited. There are numerous studies in various stages of clinical trials and are available at clinicaltrials.gov.