Final Degree Project
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GENE THERAPY AS A TREATMENT FOR HUNTINGTON'S DISEASE

Huntington's disease (HD) is a progressive, neurodegenerative disease that affects about 5-10 out of 100,000 individuals and for which there is no cure. The disease is due to an excessive number of CAG repeats (>35) on the *IT15* (or *HD*) gene, which result in an expanded polyglutamine (polyQ) region on the Huntingtin protein (HTT) sequence. The mutant form of Huntingtin (mHTT) acquires toxic function through altering several processes within neurons, especially in the striatum and cortex, leading to cell death. The only drug that has been approved by the FDA is Tetrabenzazine, used to suppress the Chorea syndrome associated to these patients. Currently, multiple strategies are being tested for the treatment of HD, being gene therapy the most promising one, since it makes possible to stop all the downstream effects caused by mutant Huntingtin.

OBJECTIVES

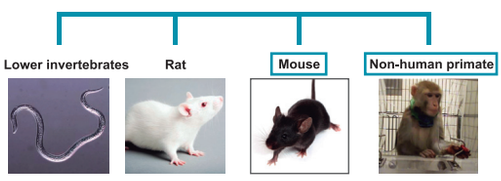
- To understand the genetic and molecular mechanisms that lead to Huntington's disease phenotype.
- To catch up with the most relevant treatment strategies that are currently being tested, and the experimental models used.
- To gain insight on the role of gene therapy in the field and on the different approaches that are being studied.
- To get the opinion of experts in the field on the research status, and the possibility of obtaining a cure soon.
- To learn how to obtain information from various sources, and how to manage time to organize and process this information.

METHODOLOGY

1. Acquisition of a background on de field: search of websites with general information.
2. Deepening on the different subjects of the project: search of actualized papers, reviews and conferences.
3. Getting experts' opinion: Interviews to active researchers.

RESULTS

GENETIC ANIMAL MODELS



GENETIC/NON-GENETIC MODELS

Since the identification of the *IT15* gene in 1993, it has been possible to generate genetic animal models that express the human mutant Huntingtin. Non-genetic models induced cell death by excitotoxic mechanisms (quinolinic acid, kainic acid) or by disruption of mitochondrial machinery (3-Nitropropionic acid).

MICE-BASED MODELS

Type	Characteristics	Advantages	Disadvantages
Transgenic	5' fragment of human *HD* gene + 2 copies of the endogenous gene. HTT overexpression.	They manifest most of symptoms. Fast progression of the disease: shorter experiments.	No reproduction of human HD.
Konck-in	1 full length human *HD* gene + 1 endogenous gene. Physiological HTT levels.	No overexpression, full human mHTT gene, slower progression: better reproduction of human HD.	Longer experiments.

2013: Generation of a fully humanized mouse model that expresses human wild type and mutant Huntingtin.

Table 1. Most used animal models

CURRENT PROMISING APROACHES

- **Pharmacological therapy**
  - HDAC inhibitors: ↑ Gene expression
  - KMO inhibitors: ↓ Excitotoxicity
  - PDE inhibitors: ↑ Neuroprotection
- **Cellular therapy**
- **Gene therapy**
  - *In vivo*
    - Non-selective
    - Allele-selective
  - *Ex vivo* — BDNF: ↑ Neuroprotection

PHARMACOLOGICAL THERAPY

Mutant Huntingtin affects signaling within neurons, neurotransmitters release, energy production, gene expression, protein refolding and protein degradation.
Symptomatic treatments are based on the use of small drugs to inhibit or compensate of one or more altered pathways.

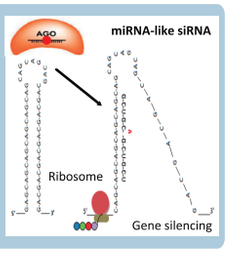
IN VIVO GENE THERAPY FOR HUNTINGTON'S DISEASE

POTENTIAL TARGETS

- **Non-selective silencing** → **Homologous regions:** Most patients are heterozygous. What are de consequences of silencing wild-type Huntingtin?
- **Allele-selective silencing** →
  - **SNPs:** There isn't a common SNP present in all patients
  - **(CAG)n:** Present in all patients but at different length. More than 200 mRNAs contain CAG repeats (off-target effects)

ALLELE SPECIFIC SILENCING

- The chosen target must be present in heterozygosity.
- This strategy is only being tested in vitro due to the lack of a fully humanized in vivo model.
- **miRNA-like siRNAs:** siRNAs (from shRNA, artificial miRNA, or synthetic siRNA) that contain mismatches. Selectivity of siRNAs that target the CAG sequence increases when complementarity isn't full. These siRNAs mimic the endogenous miRNA pathway.



POTENTIAL THERAPEUTIC MOLECULES

Type of molecule	Delivery	Advantages	Drawbacks
shRNA	Into the cell nucleus via AAV (1 or 2), under U6 promoter.	Specific tropism of AAV.	Interference with endogenous siRNA processing machinery. Citotoxicity. Immunogenicity.
Artificial miRNA	Into the cell nucleus via AAV (1 or 2), under U6 promoter.	Specific tropism of AAV. Low citotoxicity.	Immunogenicity.
Synthetic siRNA	Into the cytoplasm via cationic liposomes/ cholesterol.	Low interference with endogenous machinery. Low immunogenicity.	Fast degraded.

iRNAs: Double-stranded RNAs that prevent mRNA translation, either through its degradation when there is full complementarity, or by blocking translation of these messengers when there are mismatches.

Antisense oligo-nucleotides (ASOs)	Naked DNA into the cytoplasm via endocytosis.	More potential targets than siRNA. Simpler synthesis and delivery.	Low stability and biodistribution: chemically modified and conjugated with other molecules.

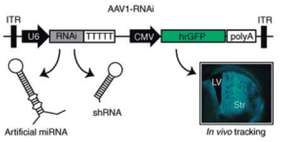
ASOs: Single-stranded DNA that can cause mRNA cleavage through RNase H, prevent mRNA translation or inhibit mRNA splicing.

Table 2. Therapeutic molecules tested in animal models or cell cultures.

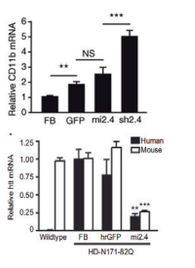
OUTSTANDING EXPERIMENTS

➡ 2009: siRNA-mediated Huntingtin suppression in adult mice for 4 months.

EXPERIMENT 1:
Intra striatum injection of a non-selective shRNA via AAV1.
Main results:
Potent gene silencing.
High immunogenicity.



EXPERIMENT 2:
Intra striatum injection of a non-selective artificial miRNA via AAV1.
Main results:
Lower immunogenicity.
Improvements on neurodegeneration, motor control and live expectance. Changes in gene expression detected.
No toxic effects observed.



Bordeau R L et al. (2009). *Nonallele-specific Silencing of Mutant and Wild-type Huntingtin Demonstrates Therapeutic Efficacy in Huntington's Disease Mice*. Molecular Therapy, 17 (6): 1053–1063.

➡ 2011: Preclinical safety testing of wild type Huntingtin suppression in primates for 6 weeks.
McBride J L et al. Molecular Therapy.

Experiment: Intra striatum injection of a non-selective miRNA via AAV1.
Main results: No toxic effects observed.

➡ 2012: Preclinical safety of wild type Huntingtin suppression in primates for 6 months.
Grondin G et al. Brain.

Experiment: Intra striatum injection of a non-selective shRNA via AAV2.
Main results: No toxic effects observed.

DISCUSSION: What are the main obstacles in the research?

Although gene therapy is giving positive results, there are several challenges that must be overcome. One of them is that the current animal models aren't completely reliable when it comes to extrapolate the results to humans. In addition, all functions carried out by normal and mutant HTT are still not known, making it more difficult to predict the consequences of inhibiting a certain pathway or the wild type HTT. Finally, iRNAs and ASOs must be optimized in order to improve efficacy, selectivity, and safety.

CONCLUSIONS: What are the future prospects regarding the development of a cure?

The real purpose of this project was to collect enough information in order to answer one question: how long before a cure for Huntington's disease? Nevertheless, the answer is uncertain.
Further research is still needed before any of the gene therapy strategies can enter clinical phases, but at the same time the required steps are being made.Since 2011 non-selective iRNAs are being tested in non-human primates for safety evaluation giving promising results, and recently there have been attempts to create better animal models, with apparent success. Gene therapy for HD might not be such a distant reality. Meanwhile, there is a relatively considerable activity regarding the use of small drugs, and probably the next approved treatments will still be symptomatic.