RNAi for Neurological Diseases

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Repeat expansion diseases

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- Polyglutamine (polyQ) Repeat Diseases
  - Due to an expanded CAG repeat that encodes glutamine in the disease protein

Normal: ..nnCAGCAGCAGnn..

Disease: ..nnCAGCAGCAGCAGCAGCAGCAGCAGnn..

..XQQQX.. XXQQQQQQQQ QQQQQQQQQX..
**Huntington’s disease**
- 1872: First described by Dr. George Huntington
- Huntington noted peculiarities of the disease:
  - Dominant heritability
  - Adult onset – commonly affects people in their 30’s and 40’s
  - Chorea – involuntary, dance-like movements
  - Drastic personality changes
  - Insanity & suicide
  - Gradual progression leading to death
- 1983: HD mutation localized to short arm of chromosome 4
- 1993: HD gene identified (called IT15)
  - Gives rise to huntingtin protein
  - Mutation: Polyglutamine repeat expansion (CAG codon)

**Huntington’s disease (2)**

**RNAi**
- Transcriptional Dysregulation
- Apoptosis/Pro-death Pathway Inhibitors
  - Trafficking defects
  - Oxidative Damage
  - Mitochondrial Dysfunction
- Excitotoxicity
- HTT aggregation
- HTT proteolysis
- Transglutaminase inhibitors
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Reducing mutant protein expression reverses disease

- Striatal and cortical aggregates
- Progressive behavioral phenotypes
  - Tremors
  - Hypoactivity
  - Motor deficits
- Aggregates resolve
  - Gene "Off" – 1% of neurons
  - Gene "On" – 60% of neurons
- Significant reversal of behavioral abnormalities

Add Dox to water
Add Dox at 18 weeks to turn Gene "Off"
Wait 16 more weeks

Vectors for delivering genetic material to the brain - AAVs

Adeno Associated Virus
- Small (20 nm), nonintegrating
- Can be completely gutted, expresses no viral genes and does not replicate
- In mammals – lasts out to 10 years (last time point tested)
- Currently in trials for PD and storage diseases
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AAVs for delivering RNAi

- ITR
- RNAi expression cassette
- CMV hrGFP
- ITR

- Inhibitory RNA
- Evaluate transduced cells

- Provides for persistent expression without toxicity
- Can address important questions without dealing with re-dosing and delivery issues

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Distribution of cells expressing reporter (GFP)

- Nose
- Tail


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Types of cells expressing the reporter (GFP)

- Oligos
- Astrocytes
- Neurons


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Preclinical rodent data

- N171-82Q mouse
  - Striatal inclusions present after 20-22 wk
  - Motor deficit apparent after 10 wk
  - Gait deficits

RNAi reduces target gene expression in vivo

- Relative expression of target (human htt) RNA mRNA

RNAi improves rotarod performance in HD mice

Harper et al., PNAS 102(16): 5820-5, 2005
Untreated or Control treated HD mice
shHD2.1-treated HD mice
shHD2.1-treated WT mice
Untreated, Control or shHD2.1-treated WT mice
Testing the efficacy of RNAi

- RNAi improved HD-like symptoms in mice models
  (similar results in mouse model of SCA1)
  - Reduction of target
  - Improvement of symptoms (behavior and pathology)
    with 60% reduction

Xia et al., Nature Medicine 10(8): 816-820, 2004
Harper et al., PNAS 102(10): 5820-5, 2005

Allele-specific, proof-of-principle studies
- 4-5 SNPs may be useful in 75% of HD patients;
  what about the other 25%?

Goal: Test non-allele specific RNAi for HD

Testing inhibitory RNAs that reduce expression of both alleles in vivo

AAVmiHD-GFP

miR-based shuttles better for the brain:
- Boudreau et al., RNA 14(9): 1834-44, 2008
- Boudreau et al., Mol Ther 17: 169-175, 2009
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Testing miHD for efficacy
- Short term test in the N171-82Q mouse
- Experimental design
  - Inject at 7 wks of age
  - Behavior at 10, 14 and 18 wks of age

miHD silencing targets human and mouse huntingtin

miHD improves the rotarod phenotype
Rotarod data at 14 and 18 weeks relative to week 10

miHD treated mice show improved survival

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miHD reduces wildtype and mutant htt mRNA levels

QPCR for human and mouse htt at 20 weeks of age

Artificial miRNAs
shRNAs

Adapted from: Nature. 2004 Sep 16; 431(7006): 343

RNA Expression Pattern

Artificial miRNA Transient

Target mRNA Coexpression

Seed Complement Frequencies

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How to make them safer?

- Alter the seed region in a manner that will not affect silencing, but reduces probability of off targeting

Moving RNAi towards the clinic as a therapy for HD

- miHD1 exhibits sequence homology to mouse, rhesus macaque and human HTT

MRI-guided stereotaxic delivery of AAV constructs into the non-human primate putamen

- T1 weighted MRI to obtain surgical coordinates
- Anti-GFP staining in the rhesus putamen
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In vivo assessment of AAV.miHDS1 efficacy in rhesus brain

- AAV2/1-miSAFE (control miRNA)
- AAV2/1-miHDS1 (active miRNA)
- Sacrificed 4 weeks post-surgery

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Non-human primate and huntingtin

- Will reduction of HTT
  - Induce behavioral deficits
  - Induce loss of neurons
  - Elicit an inflammatory response
    - Cellular, humoral

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Study design

- Pre-surgical MRI
- HD mRNA construct AAV1-miHD1
- Control for injection AAV1-GFP
- Control mRNA construct AAV1-control
  - n=4
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Suppression of HTT in the putamen does not alter day or night home cage behavior

- Animals fit with nylon collar with an Actical Accelerometer mounted on a nylon frame
- Increased speed, distance or change in direction registers an activity count
- Data reflects gross body movements, overall home cage activity and circadian behavior

Motor rating scale developed for non-human primates, adapted from the Unified Huntington’s Disease Rating Scale

Weekly homecage rating:
- Ocular pursuit
- Forelimb use
- Ability to bear weight (hindlimbs)
- Posture
- Balance
- Startle response
- Dystonia
- Bradykinesia
- Chorea

Ratings ranged from 0-3
- 0 = normal behavioral phenotype
- 3 = severe abnormal phenotype

Lifesaver test - assess manual dexterity and fine motor skills

- Time taken to remove treat from the metal rod is recorded (60 seconds maximum time limit)
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Reduction of HTT expression in the putamen does not alter fine motor skill performance

Three weeks of daily training in all animals prior to collecting baseline data

HTT suppression in the putamen does not alter the ability to perform the Question Mark Task

No training or prior exposure to question mark test prior to surgery:
- Assessed the ability to learn and perform a novel and more complex sequence of motor actions (procedural learning)

HTT levels are reduced

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Building a convergence of evidence

Is RNAi Effective & Safe?

- Peripheral immune response? No
- Local immune response? No
- Loss of neurons? No
- Motor rating scale? No
- Manual dexterity? No
- Body weight? No
- Procedure learning? No
- General activity? No

Summary of pre-clinical proof-of-principle RNAi studies in rodent models of Huntington's disease

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Vector and RNAi construct</th>
<th>Allele targeted</th>
<th>Region injected</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>N171-82Q transgenic mice</td>
<td>AAV1-shRNA Mutant</td>
<td>Striatum and cerebellum</td>
<td>Harper et al., 2005</td>
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<tr>
<td>R6/1 transgenic mice</td>
<td>AAV5-shRNA Mutant</td>
<td>Striatum</td>
<td>Rodriguez Lebron et al., 2005</td>
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<tr>
<td>Truncated Htt-GFP transgenic mice</td>
<td>AAV2-shRNA Mutant</td>
<td>Striatum</td>
<td>Machida et al., 2006</td>
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<tr>
<td>N171-82Q transgenic mice</td>
<td>AAV1-miRNA Both</td>
<td>Striatum</td>
<td>Bouthenel, McBride et al., 2009</td>
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<tr>
<td>Poly-Gln rat</td>
<td>LV-creRNA Both</td>
<td>Striatum</td>
<td>Dresel et al., 2009</td>
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<tr>
<td>Yac128 and R6/1 mice</td>
<td>ASOs Both Whole brain</td>
<td>Striatum</td>
<td>Konradi et al., 2012</td>
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Summary I

- Inhibitory RNAs are a promising therapeutic platform for dominantly inherited neurodegenerative diseases
What about other gain-of-function diseases?

![Diagram showing 5' UTR, coding exon, and 3' UTR with various molecular patterns like (CAG), (CGG), and (CTG)].

What about other gain-of-function diseases? (2)

- Spinocerebellar ataxias:
  - Autosomal dominant SCAs
    - Late-onset, progressive neurodegenerative disorders which are often fatal
    - At least 29 SCAs have been described
    - Effective treatments not available

Polyglutamine-based SCAs

![Images of brain MRI scans labeled Ctrl, SCA3, and SCA6 showing different levels of polyglutamine expression].
Spinocerebellar Ataxia Type 1

- A fatal, autosomal dominant, late onset neurodegenerative disease
- Caused by CAG expansion in ataxin-1

WT ([CAG]6-[CAG]60) vs. Mutant ([CAG]6-[CAG]<40)

- Purkinje cell death and loss of brain stem neurons

Hypothesis: silencing of ataxin-1 by miRNA will rescue phenotypes in B05 transgenic SCA1 mice

Delivery strategy - targeted infusion for broad coverage of affected areas

AAV vectors Inject into DCN
**B05 transgenic mouse model (Orr lab)**

- Expresses human ATXN1 under Purkinje Cell specific promoter, Pcp2/L7
  - An expanded 82 CAG (82Q) pure repeat
- Rotarod deficit by 5-7 weeks
- PC dendritic loss at 16 weeks
- PC death at 24 weeks of age
- No decrease in life span

**Validation of AAV.miS1 expression and ataxin-1 silencing**

*In situ RNA analyses*

Keiser et al., *Neurobiol Dis* 56:613, 2013

**Experimental design**

Baseline Rotarod Injection

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>Baseline Rotarod</th>
<th>Rotarod</th>
<th>Hindlimb Clasping</th>
<th>Ledge Test</th>
<th>Euthanize</th>
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AAV.miS1 silencing of ataxin-1
35 weeks post-injection

QPCR: Human Ataxin-1

Keiser et al., Neurobiol Dis 56: 6F13, 2013

AAV.miS1 and AAV.HAtxn1L
improve motor phenotypes

Keiser et al., Neurobiol Dis 56: 6F13, 2013

Hindlimb clasping

Keiser et al., Neurobiol Dis 56: 6F13, 2013
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Ledge test

Less Agility, Balance, and Coordination

Greater Agility, Balance, and Coordination

Controls mSi1 B05 WT

Keiser et al., Neurobiol Dis 56: 6F13, 2013

AAV.miS1 and AAV.HAtxn1L improve Purkinje cell phenotypes

Wild Type B05 miC miS1

AAV.miS1 improves molecular layer thickness

Saline miC mSi1 WT

Keiser et al., Neurobiol Dis 56: 6F13, 2013

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Moving to the SCA1 knock-in mouse model and nonallele specific silencing of ataxin-1

- 154 expanded repeat knocked into the endogenous ataxin-1 locus
- In our colony, animals live over 1 year, with disease onset ~35 wks

Watase et al. Neuron. 2002; 34: 905-919

Experimental design

Expression and activity of miSCA1

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Expression and activity of miSCA1 (2)

miSCA1 improves motor phenotypes

miSCA1 preserves molecular layers
Summary II

- AAV.miSCA1 or miS1 silenced human or mouse ataxin-1 after DCN injection and:
  - Rescued or protected from transcriptional changes
  - Improved cerebellar pathology
  - Improved motor performance

Directed injections into the DCN may provide clinical benefit in SCA1, and possibly other SCAs with significant cerebellar PC and BS pathologies

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