A Novel Therapeutic Approach for HD: Specific Gene Editing Strategies

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Overview

• Experimental Treatment options for Juvenile Huntington’s disease
  – Potential Targets for gene therapy
• Transcription Activator-like Effectors
  – Application to JHD
• Preliminary Findings
  – *Publication in Cell Transplantation*
• Future Directions
HTT gene → htt protein

Normal number: < 31 CAG
“Gray area”: 32-38 CAG
Huntington disease: > 38 CAG

Treatment Options

• Neuroprotection
  – Self regrowth of lost neurons

• Cell Replacement
  – Transplantation of cells that will grow into neurons

• Gene Modification/Correction
  – Silence the mutant gene
Neuroprotection

Ross et al, 2014
Cell Replacement

Ross et al, 2014
Gene Modification/Correction

Ross et al, 2014
Targets for Reducing mHtt

- Degrading the toxic protein and getting rid of it from the cell
- Disrupt mRNA so that it never gets translated into the toxic protein
- Silencing the mutant allele to prevent transcription of any mHtt mRNA or protein
Gene Therapy

• Traditionally thought of as the addition of a gene that is lacking in a specific disease.
  – Delivered via viral vectors

• Genetically reprogramming cells to a different fate for transplantation
  – Creation of pluripotent cells (iPSC) or induced neurons

• Correction or deletion of a gene
  – New technology Zinc Finger, Transcription Activator-like Effector, CRISPR/Cas9
Gene Therapy

AAV releases gene into cell

Target cell

Receptor protein

Patient

Cell therapy?

Advanced liver toxicity tests

Nature Reviews | Molecular Cell Biology
Transcription activator-like effectors

- TALE or TALEN (when paired with a nuclease)
- Derived from plant pathogenic bacteria from the genus Xanthomonas
- One of many DNA-targeting proteins
- Each repeat comprises 33-35 amino acids.
- Can be rapidly synthesized to target any base pair sequence
- Highly efficient and specific with minimal off-target effects
- Can be constructed with a variety of transcription factors (i.e., nucleases, activators, repressors)
Transcription activator-like effectors

A TALEN (FokI HETERODIMER) - Double-strand break and deletion

B TALE TRANSCRIPTIONAL ACTIVATOR - Turn on or enhance gene expression

C TALE TRANSCRIPTIONAL REPRESSOR - Block gene expression
How can we apply this to HD?

- We can Target unique sites in the mutant allele to silence only the mutant allele using gene repression.

Ross et al, 2014
Transfection Efficiency

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<th>GFP/SNP</th>
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Fink et al, Cell Transplantation, 2016
HD Fibro Panel

GM02151

GM02167

GM04781

GM04787

GM02123: Negative Control without T3y SNP
In vivo injection using TurboFect
Delivery Options for Gene Therapy

- Delivery of Recombinant Protein
  - Immune response, limited biodistribution

- Delivery via-direct *in vivo* transfection
  - TurboFect (DNA) – limited biodistribution, but observable knockdown
  - Invivofectamine (RNA)– Biodistribution? and possible immune response

- Delivery via viral vectors (AAV) – Fredric Manfredsson MSU
  Biodistribution in the degenerative brain
  - Immune response with repeated administration?

- Use of synthetic nanoparticles – Precision NanoSystems
  - Unknown biodistribution, immune response and uptake into neurons

- Use of Dendrimer – Julien Rossignol and Ajit Sharma
  - Unknown cellular uptake, biodistribution, and immunology

- Use of Mesenchymal Stem Cells as a delivery platform
  - May be able to delivery throughout the brain
  - May create “favorable” microenvironment via immune modulation
  - Able to delivery large proteins to cells of interest
  - Need to “Re-TALE”
Thank you

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