Histone Hypo-acetylation: An Underlying Mechanism of Transcriptional Dysregulation in Huntington’s Disease

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Outline

– Huntington’s disease overview

– Transcriptional dysregulation underlying pathogenic mechanism

– Histone modifications: histone acetylation

– Results
  • R6/2 mouse model of HD
  • ChIP
  • Genone-wide location analysis
  • HDAC inhibitor treatment (phenylbutyrate)

– Summary & conclusions
Huntington’s disease (HD)

- Inherited autosomal dominant genetic disorder
  - *When inheritance of only one mutant allele on any non-X or non-Y chromosome results in a disease*
- 1 in 10,000 people are afflicted
- 150,000 people in the USA are at risk
- Occurs in young adult life (35-40 years)
- Problems with movement, cognition, behavioral functioning, and weight loss.
- Medium spiny projection neurons of striatum are the most affected cells. Lesser involvement of other brain regions: cortex, subthalamic nucleus and thalamus.
- Invariably fatal, no effective treatments
HD is caused by a mutation in **Huntingtin** (Htt) Protein

CAG: cysteine-adenosine-guanine encodes glutamine (Q)

DNA:

\[5' - \text{xxxCAGCAGCAG...CAG} \ldots -3'\]

Chromosome 4, 67 exons

protein:

\[\text{QQQ...Qn} \quad \text{H}_2\text{N-} \quad \text{COOH}\]

3144 amino acids,
350kD

mutant protein:

\[\text{QQQQQQQQQQQQQ...Qn} \quad \text{H}_2\text{N-} \quad \text{COOH}\]

\(\text{polyQ} > 35\)
HD pathogenic mechanisms

- Proteolytic cleavage
- Mutant Htt
- Altered vesicular transport
- Altered protein interactions
- Mitochondrial dysfunction
- Neuritic aggregates
- Proteasome dysfunction
- Nuclear translocation
- Altered transcription
- Gene expression changes

Excitotoxicity
Mitochondrial dysfunction
Caspase activation
Apoptosis
Nuclear localization
Aggregation
Synaptic dysfunction
Autophagy
Transcriptional dysregulation
Proteasomal dysfunction
Calcium homeostasis
HD pathogenic mechanisms

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- Transcriptional dysregulation
- Proteasomal dysfunction
- Calcium homeostasis
Transcriptional dysregulation is an early event in HD

- Patients with early HD have decreased expression of cannabinoid receptor 1 (CB1), dopamine D2 receptor (D2) and adenosine A2a receptors (A2a) (Glass et al., 2000).

- Positron emission tomography (PET) studies of gene-positive/clinically asymptomatic patients in early stages of HD demonstrates a decrease in expression of D1, D2 receptors (Weeks et al., 1996; Augood et al., 1997)

- There is a problem with gene expression in HD that precedes the onset of abnormal symptoms

(Glass et al., 2000)
R6/2 mouse model of HD

• Transgene protein: exon1 of the human Htt gene with 150 CAG repeats.

• Transgene is expressed in all tissues.

• As in HD patients, R6/2 HD mice lose body and brain weight, starting at age 4 weeks

• Death occurs usually between 12 to 16 weeks

• Useful for revealing underlying pathogenic mechanisms as well as for testing therapeutics.

Phenotype Progression in the R6/2 Mice

Neurodegeneration

Motor Dysfunction

Transcriptional dysfunction (mRNA & Protein)

Huntingtin aggregation

Age in weeks
Decrease in mRNA and protein levels in R6/2 brain

**Receptor binding:**
Decrease in neurotransmitter receptors (8-12 week old)

**In situ hybridization:**
Decrease in corresponding mRNA species (4 week old)

Changes occur before onset of symptoms

**Expression Profiling:**
- Decrease in mRNA species (2-4%)
- Similar to changes in human HD encoding:
  - Neurotransmitter receptors
  - Calcium signaling
  - Retinoid signaling
- Changes start early (6 weeks)

Cha et al., *PNAS*, 1998

Transcription: A problem in HD

• Previous work suggests that transcription is a major problem in the HD brain

• Selective decreases in certain mRNA reflected in decreased levels of key proteins

• Alteration of mRNA occurs early in the disease process and get progressively worse

• Downregulated genes especially important to the functioning of neurons

• The mechanism of transcriptional dysregulation in HD is unknown.
Possible roles of mutant Htt in transcriptional dysregulation

Possible roles of mutant Htt in transcriptional dysregulation

Histones
Histone acetylation promotes gene transcription by de-condensing chromatin

repressed chromatin

Core histones

DNA
Histone acetylation promotes gene transcription by de-condensing chromatin.
Histone acetylation promotes gene transcription by de-condensing chromatin.
Alterations in histone acetylation in HD

Studies in HD models have demonstrated a potential therapeutic role for HDAC inhibitors
(Steffan et al., 2001; Ferrante et al., 2003; Hockley et al., 2003; Gardian et al., 2005; Bates et al., 2006; Oliviera et al., 2006)

Unknown:
- Improved locomotor activity
- Increased survival
- Increased body weight
- Decreased neurodegeneration
- Decreased ventricle size

Ferrante et al., 2003
Histone H3 is hyper-acetylated in R6/2 Brain

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Chromatin immunoprecipitation (ChIP)

Measure of protein-DNA association in native chromatin

Allows for gene by gene resolution

COMPARE:
Genes known to be **downregulated**: Enkephalin, dopamine D2 receptor

Genes known to be **expressed at normal levels**: β-actin, NMDA receptor subunit NR1
Progressive decrease in AcH3 association with genes in R6/2 striatum

Decreased association AcH3 with SELECT group of downregulated genes in HD models

- Unbiased approach.

- Can we measure changes in AcH3 association with genes across the whole genome?
ChIP-chip/Genome-wide location analysis

- ChIP product and inputs are labeled with Cy5 and Cy3

- Hybridized to Agilent 244K promoter arrays
  - 18,925 genes
  - 244,000 probes
  - Average 25 probes/gene
  - 60-mer oligonucleotide
  - Span -5.5 kb to +2.5 kb from transcriptional start site
Decreased AcH3 binding in Tg R6/2 striatum

Unpublished data, Courtesy of Karen McFarland, PhD
Genome-wide AcH3 binding sites in R6/2 Tg striatum

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Genome-wide AcH3 binding sites in R6/2 TG striatum

Unpublished data, Courtesy of Karen McFarland, PhD
There is differential histone acetylation at specific loci in Wt and Tg striatum

• Used composite score to rank genes.
  – Composite score calculated by using:
    • Number of probes bound
    • Signal intensity of each probe

• List of top 30 hyper- and hypo- acetylated genes
  – Zinc finger protein 469
  – Period homolog 1
  – PI3-kinase subunit (p85 alpha)
  – Homer homolog 1
  – Chimerin
  – Voltage-dependent calcium channel (alpha 2/delta subunit 3)

• Confirm findings using single gene ChIP
Single gene ChIP (ChIP-qPCR) confirmation of ChIP-chip findings

Unpublished data, Courtesy of Karen McFarland, PhD
Does promoter acetylation predict gene expression?

• Compare

Affymetrix gene expression profiles (22,000 genes) to
Agilent AcH3 ChIP-chip binding events (19,000 genes)

13,000 genes

• Ongoing analysis…
AcH3 binding predicts gene expression

Chromosome 1

WT

TG
• Decreased AcH3 association with downregulated genes in Tg striatum

• Are changes in histone acetylation necessary to cause changes in gene expression in HD?
  – Can phenylbutyrate (PB), a non-selective HDACi, change AcH3 levels?
  – Can PB improve gene expression and reverse mRNA abnormalities?
Inhibiting HDAC activity leads to increased histone acetylation.
PB treatment increases AcH3 levels in R6/2 brain & liver

**Liver**

![Liver AcH3 and Coomassie blots](image1)

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**Cerebellum**

![Cerebellum AcH3 and Coomassie blots](image2)

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PB treatment increases AcH3 association with downregulated genes

mRNA Levels are increased following PB treatment

Summary & Conclusion

• Transcriptional dysregulation in HD.

• Potential underlying mechanism: genome-wide decrease in AcH3 binding to gene promoters in the Tg R6/2 striatum.

• Correction of histone modification abnormalities using HDAC inhibitors improves transcription in R6/2 model of HD.
  – Reverses hypo-acetylation of histones (single gene ChIP)
  – Corrects mRNA abnormalities

• Development of specific HDAC inhibitors proves to be of therapeutic benefit for the treatment of HD.
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