Huntington’s Disease: Toxic Conformations that Mediate Multiple Molecular Mechanisms

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Mutant Huntingtin Disrupts Numerous & Diverse Intracellular Processes

Mutant Htt Affects Multiple Cell Types & Communication Between Cells

Support For Role of Cell Death in Pathogenesis

• Extent of postmortem striatal atrophy (Vonsattel score) strongly correlates with functional disability prior to death

• Extent of MRI striatal atrophy in life strongly correlates with functional disability

• Progression of atrophy correlates with progression of motor and cognitive symptoms and disability
Support For Role of Neuronal Dysfunction in HD

- Mouse models have relatively little cell death, does not correlate to behavior.
- Behavioral abnormalities and inclusion body formation is reversible in one inducible model of HD.
- Rare individuals with early HD die of other causes and at postmortem exam appear to have little neuronal cell death (Vonsattel score 0).
- Golgi study of postmortem HD brain indicates extensive “plastic” appearing morphologic changes in surviving neurons.

Slide adapted from Steve Finkbeiner
Homicide or Suicide?

Evidence in favor of suicide:

• Expanded polyglutamine is capable of causing cell-autonomous toxicity

• Among published mouse models that use cell-selective promoters, toxicity primarily appears in the cells in which the polyQ-expanded protein is expressed

Evidence in favor of homicide:

• Striatum is richly innervated by potentially excitotoxic glutamatergic and dopaminergic terminals

• The best model of HD prior to the discovery of the gene was intrastriatal injection of the excitotoxin, quinolinic acid

• Papers using conditional expression of mutant huntingtin in discrete cell types consistent with cell-cell interactions being critical

Slide adapted from Steve Finkbeiner
Transcriptional Dysregulation: Molecular Mechanisms

- Nuclear targets
  - CBP
  - p53
  - TAF130
  - BDNF gene

- Cytoplasmic targets
  - NMDA receptor
  - Proteasome

- Postulated Mechanisms
  - Gain of function
  - Alteration of normal function
Evidence for Transcription Dysregulation

- Androgen receptor—known transcriptional regulator
- Nuclear Localization of polyQ proteins in disease
  - Intranuclear inclusions and nuclear accumulation
  - Nuclear interacting proteins
  - Toxicity in the nucleus
- Alterations in gene expression patterns in disease models (and modifiers of phenotype in invertebrate models)
- Candidate mechanism: direct interference with transcriptional activation, eg CBP-mediated HAT activity (possible mediators could include BDNF)
- Inhibitors of HDACs support relevance to pathogenesis
Decreased Expression of Many Genes in HD Transgenic Mice

- Gene expression screen using oligonucleotide arrays

- About 1% of transcripts decreased, far fewer increased
  - Signaling related
  - Neurotransmitter related, especially glutamate
  - Calcium related
  - Others

- Confirmation by Northerns and in situ hybridization

- Possible relation to CBP mediated transcription

CBP in Transcriptional Activation

- CBP contains a short polyglutamine stretch
- It is critical for neuronal survival signaling
- CBP brings together specific transcription factors with general gene transcription machinery
- One mechanism is activation of HAT activity—opposed by HDAC activity

Goodman Genes Devel. 14 1553-77, 2000

Slide adapted from Steve Finkbeiner
Interference by Huntingtin with CBP Leads to Transcriptional Dysregulation

- Mutant huntingtin sequesters CBP from its normal site of action in cells, mouse models and human postmortem patient material

- Mutant huntingtin interferes with CBP-mediated transcription

- Toxicity caused by mutant huntingtin can be blocked by over-expressing CBPΔQ

Nucifora et al Science 291 2423-28, 2001

Slide adapted from Steve Finkbeiner
Sequestration of CBP *in vivo*

- In control mouse brain CBP is diffuse in the nucleus

- In HD transgenic brain CBP is sequestered away from its normal location into inclusions

- In control human post mortem brain CBP is diffuse in the nucleus

- In HD post mortem brain CBP is sequestered away from its normal location into inclusions

Nucifora et al 2001

Slide adapted from Steve Finkbeiner
CBP Activated HAT Activity is Opposed by HDAC

- Histone acetylation promotes DNA accessibility to transcriptional activators
- Histone deacetylation opposes and leads to transcriptional repression
- Family of histone deacetylase enzymes (HDACs)
- HDAC inhibitors are available and might compensate for HAT inhibition. They include sodium butyrate, trichostatin-A and SAHA

Slide adapted from Steve Finkbeiner
Inhibition of HDACs Ameliorates PolyQ Toxicity in Yeast, Flies & Mice

- McCampbell et al HMG 2001
  - SAHA (HDAC inhibitor) decreases mutant AR induced cell death
- Steffan et al Nature 2001
- Hockly et al PNAS 2003
  - SAHA protective in fly & mice models of HD

Slide adapted from Steve Finkbeiner
Problems with the Transcription Dysregulation Hypothesis?

- People who are hemizygous for CBP get Rubenstein Tabei syndrome, not HD
- Animals that lack CREB or CBP die during early development
- Cell specificity in polyQ diseases difficult to explain by actions on a ubiquitous transcription factor
- Complex partial loss of function?

Slide adapted from Steve Finkbeiner
HD Pathogenic Hypotheses… a few of them anyhow

• Based on toxic models
  – Excitotoxicity (eg quinolinic acid)
  – Metabolic toxicity (eg 3-nitropropionic acid or 3-NPA)
  – Free radical toxicity

• Based on genetic models
  – Protein aggregation (toxicity uncertain)
  – Altered conformation
  – Proteasome inhibition (Kopito)
  – Abnormal gene transcription
  – Loss of trophic support
  – Possible involvement of proteolytic processing
Conclusions

• Though a mutation in a single just causes HD, the downstream effects are quite complicated

• Many diverse cellular pathways have been implicated, suggesting the pathology of the disease may be due to the effects of many cellular dysfunctions

• Unbiased genetic approaches may be ideally suited to dissect some aspects of pathogenesis

• Effective therapy for HD will likely require a “cocktail” of drugs designed to hit several specific targets