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Transcriptional dysregulation in Huntington's disease: a failure of adaptive transcriptional homeostasis

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Huntington's disease (HD) is a signature polyglutamine disorder. An enduring theory of HD pathogenesis has involved dysregulation of transcription. Indeed, transcriptional regulatory proteins can be modulated to overcome cardinal features of HD-modeled mice, and efforts to move these into human studies are ongoing. Here, we discuss a unifying hypothesis emerging from these studies, which is that HD represents the pathological disruption of evolutionarily conserved adaptive gene programs to counteract oxidative stress, mitochondrial dysfunction and accumulation of misfolded proteins. Transcriptional dyshomeostasis of adaptive genes is further exacerbated by repression of genes involved in normal synaptic activity or growth factor signaling.

HD is a neuromotor disorder, characterized by progressive decline in muscle coordination, cognition and psychiatric dysfunction leading inexorably to death. The underlying pathophysiology involves the selective loss of medium spiny projection neurons, sparing interneurons present within the striatum [1]. HD is an autosomal dominant disease attributable to a toxic gain of function of a mutated huntingtin gene (*mHtt*) with specifically an expanded stretch of CAG repeats within the exon 1 coding region. Elegant protein mapping studies revealed several proteins with microsatellite repeats, including polyglutamine stretches, and that most of these proteins were transcription factors [2]. Accordingly, several groups, including the Cha, Neri, Thompson, Schaffner and Jones labs, provided converging evidence supporting transcriptional dysregulation as a central feature of HD. Here, we review some of these data and provide a unifying theme for how transcriptional dysregulation creates vulnerability to subsets of neurons in HD.

Huntingtin with a toxic gain of function

The *mHtt* gains a toxic function by unstable expansion of in-frame CAG triplet repeats. The mutant gene encodes a full-length protein that is initially cytosolic. The proteolytic cleavage of mHtt generates N-terminal fragments that are preferentially translocated to the nucleus, where nuclear aggregates form over time [3]. Caspase-6 cleaves mHtt at amino acid 558 into these toxic N-terminal fragments capable of trafficking to the nucleus [4]. Ser-16 phosphorylation can also regulate N-terminal cleavage of mHtt and its nuclear translocation [5]. Recently, Zheng *et al.* [6] showed that the N-terminal region of Htt itself functions as a nuclear export signal (NES) and mutation of any of the nucleotides in this sequence leads to its enhanced nuclear accumulation. This finding indicates that increases in the polyQ stretch beyond 37–40 repeats in exon-1 of Htt could disrupt the htt NES sequence, resulting in its enhanced nuclear localization. Blocking the nuclear localization of mHtt suppresses neuronal cell death [7], whereas specific targeting of mHtt by including a nuclear localization signal was sufficient for initiation and progression of transcriptional dysregulation and pathogenic behavioral symptoms in a transgenic mouse model of HD [8,9]. Collectively, these data suggest that nuclear localization of N-terminal fragments of mhtt is necessary and sufficient to induce the dysfunction and death of neurons.

mHtt-induced early molecular changes in HD

Signs of transcriptional dysregulation were found early in the R6/2 model of HD and were coincident with initial behavioral changes but only after visible signs of intranuclear inclusions related to

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1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2014.03.016

increased caspase-6 activity were observed [10,11]. Accordingly, mRNA levels of different genes (growth factor ligands, neurotransmitter receptors and growth factor receptors) with important neuronal functions have been reported to be downregulated both in a HD mouse model as well as patients with HD [12,13]. Given that progressive changes in protein levels and signs of neuronal cell death were evident only after changes in mRNA levels of affected genes [14], the aggregate of evidence suggests that nuclear events are necessary for at least some features of clinical HD.

Mechanisms of transcriptional dysregulation

Mechanisms invoked for transcriptional dysregulation in HD are protean. The toxic N-terminal fragments of mHtt can modulate the transcriptional process by having an aberrant protein–protein interaction with the transcriptional machinery, by either modifying chromatin, or through a direct interaction with genomic DNA (Fig. 1).

Aberrant interaction between mHtt and transcription factors, coactivators or corepressors

The polyQ stretch at the N terminus of mHtt provides an appropriate motif for its interaction with glutamine-rich activation domains of different transcription factors, such as cAMP response element-binding protein (CREB), Sp1 and the transcriptional coactivator, CREB binding protein (CBP). Earlier models suggested that polyQ repeats of mHtt form insoluble aggregates [15,16], which sequester these transcription factors from the cellular pool [17], but Yu *et al.* [18] showed that the polyglutamine inclusions themselves are unable to deplete the cellular pool of glutamine-rich transcription factors, such as Sp1, significantly. However, the soluble form of mHtt is able to perturb the interaction of these transcription factors (e.g. Sp1) with their transcriptional coactivators [such as transcription initiation factor $(TAF)_{II}130$] as well as their target DNA [19]. Accordingly, mHtt reduces expression of Sp1 target genes, including the dopamine D2 receptor and nerve growth factor receptor [19,20]. The co-expression of Sp1 and $TAF_{II}130$ has been shown to prevent mHtt-mediated toxicity [19]. By contrast, other studies have suggested a pro-death, rather than a pro-survival role for Sp1 in HD [21]. Recent studies reconciled these apparently conflicting observations by demonstrating that agents such as the DNA-binding drug mithramycin that simultaneously inhibits subsets of Sp1-dependent genes (e.g. oncogenes) while activating other subsets (e.g. tumor suppressors) significantly extended lifespan in *Drosophila* and mice models of HD [22,23]. The role of Sp1 is highly gene dependent.

The expression of CREB target genes has been reported to be downregulated in both *in vitro* as well as *in vivo* models of HD [16,24]. The expression of one of these CREB target genes [peroxisome proliferator-activated receptor (PPAR) gamma coactivator (PGC)-1α], which has a crucial role in mitochondrial biogenesis, is reduced in HD mice as well as postmortem brains of patients with HD [25,26]. Other studies have shown that mHtt can disrupt not only nuclear CREB function, but also mitochondrial CREB function [27]. Together, these studies highlight the potential role of mHtt in repressing bioenergetic homeostasis.

CBP functions as a coactivator of many transcription factors, including CREB and Sp1. Aberrant interaction of mHtt with the glutamine-rich domain of CBP leads to perturbation of its coactivator function, which, in turn, disturbs the normal function of associated transcription factors. In addition to its coactivator function, the histone acetyl transferase (HAT) activity of CBP is also disturbed [28]. This causes the cognitive deficit observed in HD [29,30].

The transcriptional repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF) is a transcriptional corepressor that represses the expression of its target genes, such as brain-derived neurotrophic factor (*BDNF*), as well as miRNAs [31]. Wild type Htt, a cytosolic protein, interacts with REST and prevents its nuclear translocation and, thus, regulates the expression of its target genes in a positive manner via derepression. By contrast, the affinity of mHtt for REST is weaker, thus leading to its enhanced nuclear entry and, ultimately, to the repression of known REST target genes, such as *BDNF* and miRNAs [31,32]. Indeed, expression of BDNF has been found to be compromised in HD models [33–35].

Recently, Wang *et al.* [36] provided a new perspective on mHtt–transcription factor interactions by showing that Htt itself has putative sequences for different transcription factors, such as Sp1, CREB, signal transducer and activator of transcription (STAT), NRSF, p53, activator protein 1 (AP1), hypoxia-inducible factor (HIF) and nuclear factor (NF)-κB, in its promoter region and, thus, itself provides a regulatory hotspot to the transcription factors so as to play with its own expression. The authors showed that Sp1 can regulate the expression of Htt.

Aberrant interaction between mHtt and basal transcription machinery

mHtt has been shown to interact with many important components of core transcriptional machinery, such as the large subunit of RNA polymerase II, TATA binding protein (TBP), $TAF_{II}130$, TFIID and TFIIF [19,37–39], which favors model whereby mHtt disrupts the basic transcriptional machinery in HD subjects. However, it is unclear how this model would account for the regional specificity of transcriptional dysregulation in striatum and cortex despite the presence of mHtt inclusions throughout the brain. It raises a new question that, although mHtt has the potential to interact with many components of transcriptional machinery, we need to understand which of the specific mHtt-mediated interactions account for alterations of gene expression that can explain not only the selective vulnerability, but also the increased expression of some genes.

Histone modification

DNA is present within the nucleus in compact, repeating units called chromatin. The basic unit of chromatin, the nucleosome, comprises DNA packaged by histones and other associated proteins. The specific modification of histones and associated proteins determines the accessibility of the transcriptional machinery to the associated DNA and, thus, has a major role in transcriptional regulation. The post-translational modifications (PTMs) occurring mainly at histone tails are the key modifiers of histone structure and function. Some PTMs, such as acetylation, methylation, ubiquitylation and polyamination, are known to have functional relevance in HD pathobiology [26]. Among these, histone acetylation is the most well studied in HD. Acetylation correlates with increase in transcription, whereas deacetylation correlates with