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Dysfunction of Wild-Type Huntingtin in Huntington disease

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Huntington is the protein involved in Huntington disease (HD), an inherited neurodegenerative disease. Research activities have focused on the abnormal functions of mutant huntingtin. However, recent results indicate that wild-type huntingtin has important activities in brain neurons, suggesting that loss of these activities may play a role in HD.

Huntingtin is a 348-kDa cytoplasmic protein that is widely expressed in the body (15). Interest in this protein stems from the demonstration that an abnormally expanded repetition of a CAG nucleotide triplet into the huntingtin gene causes Huntington disease (HD), a neurodegenerative disease that hits at a mean age of 35 years. HD is greatly disabling and progressive, leading to death within 15–20 years (7).

Despite the wide pattern of expression of wild-type and mutant huntingtin, selective degeneration of the medium-sized spiny neurons of the striatum and, at later stages of the disease, of the neurons of the cerebral cortex represents the typical neuropathological hallmark of the disease. Due to this peculiar pattern of neurodegeneration, HD is characterized primarily by motor dysfunctions (“chorea,” which in Greek means “dance”) and cognitive and behavioral disturbances. These neuropathological and clinical manifestations are evident when the number of CAG repetitions in the huntingtin gene exceeds the limit of 35 that are present in the normal population (15). Repeats above 36 define an HD allele. CAG repeats (15). Repeats above 36 define an HD allele. CAG repeats number and age of disease onset; the greater the number of CAG repeats, the earlier the age of onset, with cases of juvenile onset of HD characterized by expansions of >60 repeats (15). Age of disease onset is, however, not only influenced by the number of CAG repeats but also by other modulating factors. For example, variations in the kainate-specific glutamate receptor GluR6 genotype have been shown to account for some of the fluctuations in age of onset of HD not accounted for by CAG repeat length (14).

The gained toxic activity of the mutant huntingtin protein

The current hypothesis in HD is that the disease arises from a gained toxic activity of the mutant huntingtin protein. The first evidence against a simple loss of normal huntingtin function in HD was derived from the demonstration that deletion of a large portion of chromosome 4, which includes the IT15 locus, does not cause HD (1). Yet most patients with this deletion do not survive long enough to develop HD. Additional evidence obtained in animal models indicates that expression of mutant huntingtin in the background of a mouse having two normal copies of the wild-type huntingtin gene does cause neurological dysfunction and aggregate formation (13, 14). Although these observations support the notion that mutant huntingtin, or the CAG per se, initiates the disease, they do not completely rule out the possibility of a loss of normal huntingtin activity in HD (1).

Since the cloning of the HD gene in 1993 (7), most of the efforts toward understanding the pathogenic mechanisms were driven by the gain-of-function hypothesis and focused on the analyses of the mutant protein. A striking feature shared by mutant huntingtin and other proteins involved in other CAG repeat diseases, is their tendency to form insoluble aggregates either through “polar zippers” or by transglutaminase-mediated events (15). Such aggregates, composed of NH₂-terminal mutant huntingtin, accumulate in striatal and cortical neurons of HD patients both in the nucleus and in dystrophic neurites (15). Aggregates of mutant huntingtin are also common to many transgenic mouse models of HD although description of their nuclear localization in striatal neurons varies greatly depending on the mouse model (15). The role of these aggregates in the disease is still debated. Indeed, cell death seems to occur in mice and cultured cells in the absence of visible aggregates, and conversely the presence of aggregates does not always cause cell death. Therefore toxicity of mutant huntingtin is not conclusively shown to be mediated by its aggregation, and a deeper understanding of what mutant huntingtin inclusions do inside the cells remains crucial.

One important set of data indicates that mutant huntingtin impairs gene transcription, either by intranuclear aggregate formation and/or by sequestration of important transcription factors (2). Various authors demonstrated that transcription factors such as CBP [cAMP-response element binding protein (CREB) binding protein], TATA binding protein, and Sin3A can be recruited into the intranuclear aggregates, thus reinforcing the hypothesis of a role for transcriptional dysregulation in HD (15). Cha and other colleagues (9, 10, 16, 19) were the first to suggest this possibility and initiate more through investigations aimed at examining the early changes in gene expression in mice and cells by gene chip analyses. This large body of evidence indicates that the presence of mutant huntingtin is associated with a reduced transcription of several important genes.
In addition, it was shown that the poly-Gln-containing domain of huntingtin directly binds to two distinct proteins, CBP and p300/CBP-associated factor (P/CAF) and inhibits their acetyltransferase activity, anticipating the possibility of a reduced histone acetylation as demonstrated in vitro (18). Importantly, this reduction could be reversed by administration of inhibitors of histone deacetylase (18). Sequestration of CBP by elongated poly-Gln tracts leading to altered gene transcription and cell toxicity is also invoked in another beautiful study by Nucifora (10).

Additional observations in favor of the gain-of-function hypothesis indicate that huntingtin is a substrate for caspases. Huntingtin cleavage by caspases occurs in a poly-Gln-dependent manner, i.e., the longer the poly-Gln in huntingtin, the more efficient is the cleavage, which then generates NH₂-terminal fragments of mutant huntingtin endowed with cell toxicity (6, 15). An alternative hypothesis is suggested by a recent study demonstrating that mutant huntingtin from human brain, transgenic animals, and cells is more resistant to proteolysis than normal huntingtin (4). This study proposes that inhibition of mutant huntingtin proteolysis leads to aggregation and toxicity through sequestration of important targets, including normal huntingtin (4).

Most of these data indicate that mutant huntingtin toxicity in HD occurs via the formation of NH₂-terminal fragments that aggregate. However, it is unclear how these events lead to striatal vulnerability. One study has compared the patterns of inclusion formation in HD transgenic mice expressing only exon 1-containing expanded repeats with those in knock-in mice expressing full-length mutant huntingtin. Although the former develop inclusions only in striatum (8). Although inclusion formation is not a direct measure of cell dysfunction or death, these data seem to indicate that tissue-specific proteolysis of mutant huntingtin could determine which cell population may be affected. Yet these data indicating a preferential cleavage of mutant huntingtin are in contrast with data by Dyer (4) showing preferential cleavage of wild-type huntingtin.

**Physiology of wild-type huntingtin**

In a research context mostly focused on the analyses of mutant huntingtin toxicity, interest in the function of the normal protein has been very scant. However, new investigations, some of which were performed in our laboratory, indicate that wild-type huntingtin has important roles in striatal neurons, implying that loss of normal huntingtin activity may occur in the disease, leading to striatal vulnerability (20).

Distribution of wild-type huntingtin has been analyzed by various groups that demonstrated its presence in all brain neurons. These studies also showed the lack of any correlation between huntingtin expression and neuronal vulnerability (15). More recently, variable expression of huntingtin in brain regions was reported. In particular, one study showed low expression of wild-type huntingtin in the striatal neurons. In contrast, cortical neurons were found to be rich in huntingtin protein and mRNA (15). Huntingtin is mostly cytoplasmic, although some reports indicate its presence in the nucleus (15). Of note, whereas the human huntingtin gene carries a normal polymorphic CAG stretch ranging from 9 to 35 repeats, in all investigated species so far the number of CAG found was equal or inferior to the normal range of human IT.
alleles. A longer uninterrupted CAG stretch was found in the pig and consists of 21 CAG, whereas rat and mouse genes have eight and seven repeats, respectively, the pufferfish has only four repeats, and Drosophila does not have any (1, 11). It is possible that increased CAG length during evolution has endowed the normal protein with specific functions.

One of the first pieces of evidence about the function of wild-type huntingtin was discovered in 1995, with the recognition that embryos of huntingtin homozygous knockout mice die during gestation (1). This evidence indicated that wild-type huntingtin is required for embryonic development. Of note, in one constitutive knockout mouse, much higher apoptotic cell death was observed in the embryonic ectoderm of null mutants with respect to normal (1). Huntingtin is also important in adulthood since heterozygous knockout mice displayed increased motor activity and significant neuronal loss in the subthalamic nucleus (1). One recently engineered mouse has allowed more rigorous testing of the role of wild-type huntingtin in postmitotic neurons results in neurological deficits and neurodegeneration, indicating a function for the wild type in the brain (3). Studies by our group (12) have provided the first direct evidence for a role of huntingtin in the survival of central nervous system cells. Central nervous system cells that were engineered to express wild-type huntingtin were resistant to the lethal effect of stresses such as serum deprivation, exposure to mitochondrial toxins, or transfection of proapoptotic Bcl-2 family proteins. In particular, we found that wild-type huntingtin acts as an antiapoptotic protein (12). We also reported that the antiapoptotic effect of wild-type huntingtin takes place upstream of caspase-3 activation. Specifically, in our experimental conditions wild-type huntingtin inhibited the conversion of procaspase-9 into active caspase-9, resulting in inhibition of the apoptotic cascade (1, 12, 15) (Fig. 1). Alternatively, the antiapoptotic effect of wild-type huntingtin may occur via sequestration of proapoptotic molecules like Hip1-Hippi, resulting in cell survival (5). Subsequent studies demonstrated that wild-type huntingtin also protects against apoptosis in the testis of mice expressing mutant huntingtin and that overexpression of wild-type huntingtin significantly reduces the cellular toxicity of mutant huntingtin in various cell types (1, 13, 15).

These data suggest that one of the roles of ubiquitously expressed wild-type huntingtin may include a protective mechanism from various apoptotic stimuli. Huntingtin also affects neuronal stability because its deletion in mice causes degeneration of axon fibers (3). Various evidence has converged to indicate that wild-type huntingtin may be involved in cellular vesicle trafficking, endocytosis, and synaptic function. Several proteins interacting with huntingtin are associated with membrane vesicles and interact with cytoskeletal proteins (15). Huntingtin also codistributes with intracellular and plasma membranes containing clathrins, and it appears to be associated with clathrin through huntingtin-interacting proteins (HIP-1) (15). Together these data argue in favor of the possibility that loss of wild-type huntingtin function may occur in HD, therefore contributing to neuronal death.

Despite this large body of evidence, a mechanism explaining the selective striatal vulnerability in HD is still elusive. It is worth mentioning that HD is one of eight inherited neurological diseases caused by the presence of a CAG expansion in genes encoding for different proteins of known [like for the androgen receptor involved in spinobulbar muscular atrophy (SBMA)] or unknown function and characterized by a different pattern of neurodegeneration. In SBMA, for example, the expanded CAG is found in the gene encoding for the androgen receptor and the degeneration specifically hits the motor neurons. It is therefore possible that although it is the CAG allele that evokes toxicity it is the context of the protein in which the poly-Gln expansion is present that defines which neurons will undergo cell death. In other words, we hypothesized that wild-type huntingtin could have important physiological functions for the striatal neurons that were lost in HD. Interestingly, we found that both full-length wild-type huntingtin and a 548-amino acid terminal (N548) were equally protective for cells, whereas a shorter NH2 terminal (of 63 amino acids) had no effect. This suggested that huntingtin may be organized into several functional domains (12).

In search of striatal-specific functions of wild-type huntingtin that, when lost, could account for the selective neurodegeneration observed in the disease, we recently reported that wild-type huntingtin upregulates the transcription of brain-derived neurotrophic factor (BDNF) (20), a prosurvival factor produced by cortical neurons and necessary for the survival of the striatal neurons in the brain. Cortical BDNF is delivered to striatum via the corticostriatal afferents, known to release glutamate and to control the striatal output. Our study demonstrated that this beneficial activity of wild-type huntingtin is lost when huntingtin is mutated, resulting in decreased production of cortical BDNF and leading to insufficient trophic support to the striatal neurons, which then die.

FIGURE 2. Wild-type huntingtin increases the transcription of the BDNF gene (left). BDNF is produced in cortex and anterogradely transported to the striatum via the corticostriatal afferents. Striatal neurons require cortical BDNF for their survival. In Huntington disease (right), loss of wild-type huntingtin results in a decreased production of cortical BDNF, leading to increased vulnerability of the striatal neurons.
We also reported that the ability of wild-type huntingtin to modulate BDNF gene transcription depends on a specific activity of the BDNF promoter (20).

In conclusion, analyses of the physiology of wild-type huntingtin has disclosed unexpected information about its activity and biology, also anticipating that strategies aimed at restoring wild-type huntingtin functions may represent one more strategy to interfere with the pathogenic mechanisms underlying HD.

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