

Review

Neurodegenerative processes in Huntington's disease

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Huntington's disease (HD) is a complex and severe disorder characterized by the gradual and the progressive loss of neurons, predominantly in the striatum, which leads to the typical motor and cognitive impairments associated with this pathology. HD is caused by a highly polymorphic CAG trinucleotide repeat expansion in the exon-1 of the gene encoding for huntingtin protein. Since the first discovery of the huntingtin gene, investigations with a consistent number of *in-vitro* and *in-vivo* models have provided insights into the toxic events related to the expression of the mutant protein. In this review, we will summarize the progress made in characterizing the signaling pathways that contribute to neuronal degeneration in HD. We will highlight the age-dependent loss of proteostasis that is primarily responsible for the formation of aggregates observed in HD patients. The most promising molecular targets for the development of pharmacological interventions will also be discussed.

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Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disorder characterized by adult-onset of motor dysfunctions, psychiatric disturbances and intellectual decline.¹ As revealed by postmortem analysis of tissues from HD patients, the neuropathological changes are predominantly detected in the striatum, although marked alterations have also been observed in other areas of the brain, including the cerebellar cortex, thalamus and cerebellum.^{2,3} HD is associated with an unstable CAG expansion in the huntingtin gene (*HTT*) on chromosome 4. In humans, the exon-1 of *HTT* gene normally contains between 6 and 35 CAG repeats, whereas in patients affected by HD more than 40 trinucleotides have been described.⁴ In most cases, an intermediate number (36–40) of CAG repeats leads to a slower progression of the pathology as a result of the incomplete penetrance of the mutant allele. Importantly, the onset and severity of the pathology is directly correlated with the number of CAG repeats, although the actual function of the trinucleotide stretch remains unknown.^{5,6} As reported by recent findings, the length of the CAG repeats might be relevant in the translation of the *HTT* mRNA transcript, as a result of binding with a ribosome-containing complex⁷ (Krauss S., unpublished data). The *HTT* gene encodes for an approximately 350 kDa protein composed of several subdomains. At the N-terminus, the polyglutamine (polyQ) stretch encoded by the CAG repeats functions as potential membrane association signal.⁸ In mammals, the polyQ-containing domain is followed by a polyproline sequence that stabilizes protein conformation. The N-terminal portion of HTT is followed by three main clusters of HEAT repeats, which are essential for the binding with interacting proteins. In addition

to these motifs, HTT contains a range of consensus sites for posttranslational modifications, including proteolytic cleavage, phosphorylation and sumoylation. Within cells, HTT has been detected in the nucleus, mitochondria, Golgi and endoplasmic reticulum and can be found in the neuronal body, dendrites and synapses.^{9,10} At the molecular level, there is evidence that HTT can interact with a variety of proteins, including some transcriptional factors, synaptic complexes, plasma membrane and cytoskeleton proteins.¹¹ HTT is ubiquitously expressed during embryonic development and at high levels in testis and in mature postmitotic neurons in adult human brain.¹²

Although the physiological role of HTT has not been fully defined, analysis of transgenic mice with a targeted deletion of the *Htt* gene has demonstrated its role in mammalian development. Complete suppression of *Htt* expression in mice leads to embryonic lethality as a result of increased apoptosis,^{13,14} while heterozygous knockout animals exhibit severe cognitive deficits as a consequence of increased neuronal loss in the subthalamic nucleus of the basal ganglia.¹³ Similarly, postnatal neuronal-specific inactivation of *Htt* is accompanied by progressive apoptotic neuronal degeneration,¹⁵ which suggests an essential function of the protein in the neuronal maintenance and activity. The antiapoptotic effect is likely due to the both inhibition of caspase-3 activity by its direct binding¹⁶ as well as to the activation of prosurvival pathways controlled by the serine/threonine kinase Akt.¹⁷ This pattern strongly supports the idea that HD pathogenesis results from a combination of increased gain-of-function of the mutant HTT together with the decreased wild-type HTT physiological function.

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Abbreviations: HD, Huntington's disease; HTT, huntingtin; polyQ, polyglutamine; AD, Alzheimer's disease; PD, Parkinson's disease; NMDA-R, N-methyl-D-aspartate receptor; MMP, matrix metalloproteinases; TOR, target of rapamycin; PI3K, phosphoinositol 3-kinase; IGF-1, insulin-like growth factor 1

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This physiological function may be related to the N-terminal polyglutamine region, as it can form polar zipper structure able to bind transcription factors.¹⁸ Importantly, the physiological role of the polyQ-repeated expansion in higher organisms has been recently explored in mice carrying only seven CAG repeats within the murine *Htt* gene. These animals revealed subtle memory and learning deficits, with an altered energy status caused by changes in mitochondrial function.¹⁹ In a knock-in mouse model for HD, overexpression of the full-length *Htt* lacking the polyQ specifically stimulates the catabolic process of autophagy, significantly reduces mutant *Htt*-containing aggregates and, as a result, extends the lifespan in comparison with HD mice.²⁰ Taken together, this evidence suggests the presence of an evolutionary positive selection favouring the expansion of the repetitive element as modulator of the protein activity itself.

HD is characterized by protein aggregates that accumulate within cells in a manner similar to that seen in various forms of spinocerebellar ataxia, as well as in other neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). In human patients affected by HD, immunohistochemical analyses of postmortem brain tissue has demonstrated the presence of intracellular inclusions,²¹ which are mainly associated with the selective loss of medium spiny neurons in the striatum.²² These aggregates are enriched in truncated polyglutamine containing-fragments generated by several proteases, however the precise mechanisms responsible for the toxicity of these proteolytic products remain elusive.^{20,23–25} Even though some mouse models expressing N-terminal truncated mutant *HTT* exhibit abnormal behavioral and neurological phenotypes,²⁶ other transgenic lines present widespread intracellular inclusion formation without any functional neuronal deficits. For example, the 'shortstop' is a mouse line expressing the first two exons of *HTT* with an expanded CAG repeat. In these transgenic mice, there is no evident neurodegenerative phenotype, and neurons are less susceptible to excitotoxic cell death compared with other HD mouse models.²⁷ Thus, as the full-length *HTT* is indispensable for manifestation of neuropathology clearly analogous to human HD,^{28,29} the deposition of proteolytic products is not sufficient to initiate a toxic cycle leading to extensive neuronal damage in the striatum. In AD^{30–32} and in PD,³³ (reviewed in refs Douglas and Dillin³⁴; McCormack and DiMonte³⁵) inclusions do trigger neurotoxicity. In HD, in a limited number of conditions, intracellular aggregates can also sequester toxic soluble fragment and therefore have beneficial effect.³⁶ Nevertheless, the majority of evidences indicates that any mechanism promoting maintenance of the correct protein folding conformation or that enhances the clearance of huntingtin-containing aggregates represents a powerful therapeutic approach in HD.^{37,38} In the next section, some of the key molecular mechanisms that influence proteostasis will be outlined and their relevance in the progression of HD will be discussed.

Proteolytic Cleavage of HTT

HTT is susceptible to proteolysis by a number of proteases (Figure 1). Historically, *HTT* was initially identified as a caspase substrate and it was the first example of a protein

associated with a neurodegenerative disorder cleaved during apoptosis.³⁹ Caspases are highly conserved cysteine-aspartic proteases associated primarily with apoptotic cell death and essential for the processing of a large number of substrates.⁴⁰ Proteolytic fragments processed by caspases are detectable in brains of HD patients and HD mice before the loss of neurons in the striatum,⁴¹ with the cleavage efficiency dependent on the polyQ tract length.³⁹ Blocking *HTT* cleavage by site-directed mutagenesis or by pharmacological approaches reduces cytotoxicity in cultured cells.⁴² In line with these findings, mice overexpressing a caspase-6 non-cleavable mutant *HTT* have milder neuropathological defects and are protected against excitotoxic stimulation compared with mice carrying the cleavable mutant *HTT*.⁴³ This strongly suggests that caspase-dependent proteolytic cleavage of the aberrant protein might be a key step in the toxic events during HD, and that *HTT* functions as prosurvival factor.

HTT is also a substrate of calcium-activated proteases, that is, calpains. Calpains belong to the family of cysteine proteases typically activated by the elevation of intracellular Ca^{2+} levels, either in response to plasma membrane depolarization or in response to Ca^{2+} release from the intracellular stores.⁴⁴ In mice overexpressing mutant *HTT*, increased glutamate release from afferent neurons enhances NMDA-R activity. This leads to an intracellular Ca^{2+} increase and therefore activation of calpains, which in turn cleave the *HTT* protein into a series of proteolytic products⁴⁵ that promote NMDA-R-mediated excitotoxicity.⁴⁶ Moreover, calpains can modulate *HTT* homeostasis via the catabolic process of autophagy. As shown by recent RNAi and chemical compound screenings in cultured cells, inhibition of calpains likely stimulates the lysosome-mediated degradation of intracellular aggregates.^{47,48} Another RNAi screening study has also shown that small *HTT* fragments can be generated by the proteolytic activity of some matrix metalloproteinases (MMPs).⁴⁸ The activation of the MMPs and the resulting cleavage of *HTT* were confirmed in samples from HD mouse models. Reduced MMP activity, especially MMP-10 and MMP-14, correlates with lower amount of proteolytic fragments and, as a result, suppression of neuronal degeneration induced by mutant *HTT* in cellular model systems as well as in *Drosophila*.⁴⁸ Collectively, these findings suggest that protease inhibition might be a beneficial therapeutic approach for HD as it delays the formation of *HTT*-containing intracellular aggregates.

Autophagy

Autophagy is a cellular catabolic process that seems to have an important role in the pathogenesis of cancer as well as in neurodegenerative disorders.^{49–57} The process of autophagy involves the formation of a double-membrane structure (autophagosome) that then encloses a portion of cytosol and delivers its cargo content to the lysosomes for digestion.^{58–60} This nonspecific bulk degradation pathway is highly conserved from yeast to mammals. Autophagy occurs at constant low levels in all cells as part of ongoing cellular protein quality control and organelle turnover. However, it also has a primary role in the response to nutrient deprivation as it

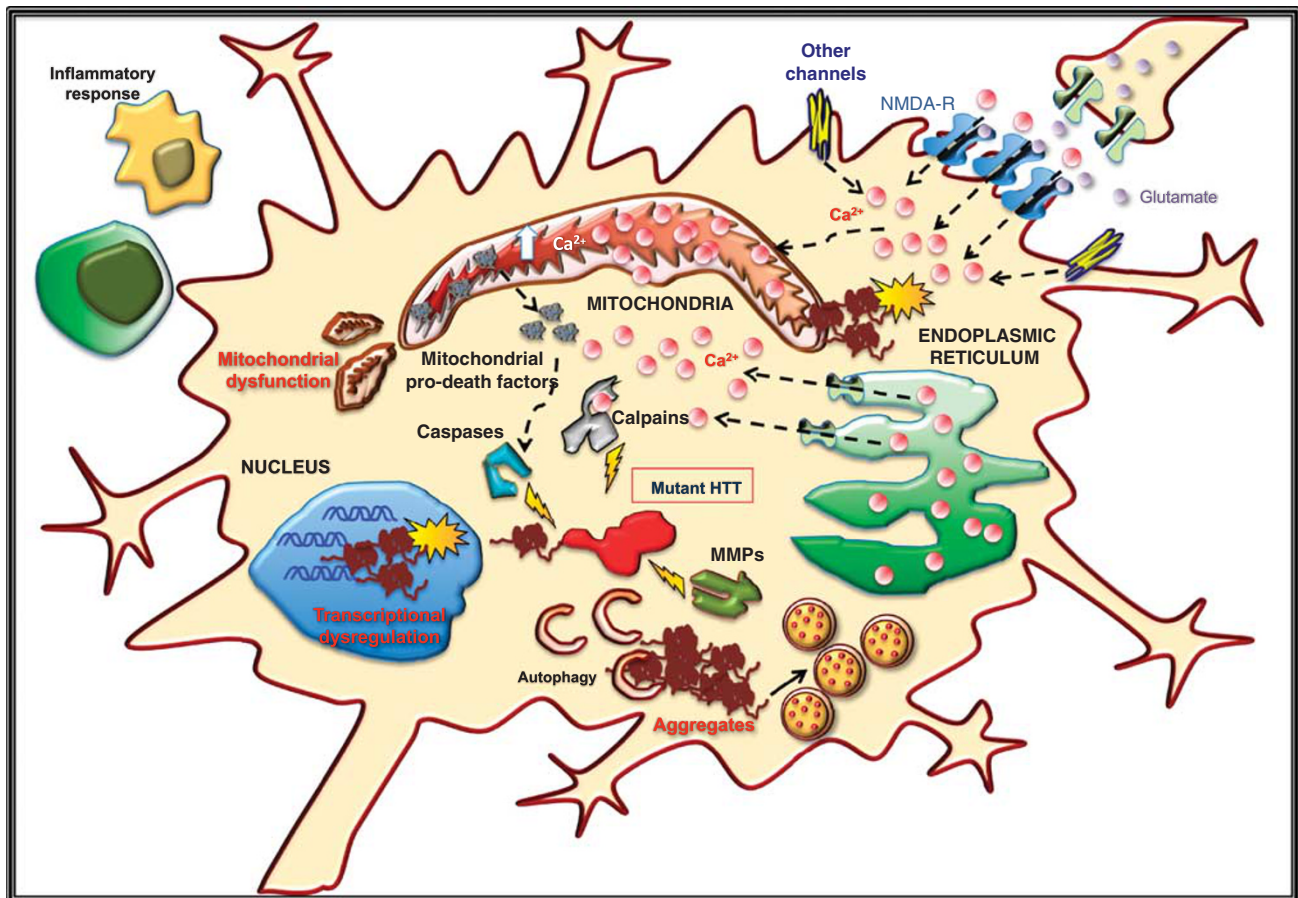


Figure 1 Representative intracellular events in neurons expressing mutant HTT. In HD, processing of mutant HTT by caspases, calpains and MMPs facilitates the formation of intracellular aggregates, which are mainly degraded by autophagy. Failure in the clearance of HTT proteolytic fragments eventually results in excessive cytosolic Ca^{2+} concentration and organelle dysfunctions

sustains metabolic functions by providing energy and metabolites to the cells. In different experimental settings, autophagy activation blocks detrimental processes and therefore facilitates cell stress resilience and survival.^{61–65} Furthermore, autophagy is one of the primary degradation pathways for various aggregate-prone proteins associated with neurodegenerative diseases.^{66,67} As the tight regulation of autophagy is essential for cellular homeostasis, it is not surprising that autophagic dysfunction can cause metabolic stress and cell death^{68–70} mainly through apoptosis resulting from mitochondrial deficiency or via cleavage of Atg proteins.⁷¹

Among several key regulators of autophagy, the ‘target of rapamycin’ (TOR) senses energy status and the availability of the nutrients within the cell through the upstream class I phosphoinositide 3-kinase (PI3K), the serine/threonine kinase Akt and the 5′-AMP-activated protein kinase (AMPK).⁷² Inhibition of the TOR complex promotes the recruitment of Beclin-1 and Atg proteins involved in the formation of the mature autophagosome. The modulation of autophagy is therapeutically promising in HD: the inhibition of TOR by rapamycin enhances the clearance of mutant HTT-containing aggregates via the autophagy-lysosome pathway (Figure 1).^{64,66} Similarly, drugs that block a rise in intracellular Ca^{2+} , such as

L-type Ca^{2+} channel antagonists, decrease the activity of calpains and result in the indirect activation of autophagy, likely by preventing the degradation of Beclin-1- and Atg-related proteins.^{73,74} Although calpain inhibition promotes autophagy *in-vitro*⁷⁵ and in zebrafish,⁷⁶ it still remains to be determined whether it can be effective against HD in *in-vivo* mammalian models or in clinical settings.

Autophagy induction clearly represents an appealing approach for HD treatment; however, the therapeutic window remains to be determined as mutant HTT has a negative effect on the sequestration of the autophagic cargo. Although the autophagosomes seem to form and fuse to the lysosomes efficiently, there is a failure in the recognition of targeting signals, such as p62 or polyubiquitin, that results in delayed engulfment of cytosolic macromolecules and damaged organelles.⁷⁷ Several strategies have been suggested to improve the clearance of HTT-containing aggregates by autophagy. One of them is based on the observation that histone deacetylase inhibitors block the polyQ protein-dependent neuronal degeneration in *Drosophila*.⁷⁸ In this case, the acetylation of mutant HTT facilitates the recruitment of the protein to the autophagosome and therefore increases the removal of toxic species within the cells.⁷⁹ More recent evidence demonstrates that HTT-mediated neuronal loss in

Drosophila can be suppressed by genetic or pharmacological inhibition of NAD⁺-dependent class III deacetylases sirtuins.⁸⁰ As pharmacological manipulation of sirtuins by resveratrol⁸¹ has been proposed to activate several pathways, including autophagy, these studies are of particular interest from a potential therapeutic standpoint.^{82,83}

Ageing Modifiers as Regulators of Proteostasis

Loss of proteostasis is a hallmark of several neurodegenerative disorders such as PD, AD and HD. In all of these disorders, aggregate-prone proteins trigger the formation of insoluble intracellular or extracellular aggregates as a result of environmental stress or metabolic changes. Whether the fibrillar protein aggregates are pathogenic or have protective roles, remains controversial.^{84–86} In nematodes and in mice, loss-of-function or decreased insulin/insulin-like growth factor 1 (IGF-1) signaling prevent the proteotoxicity caused by aggregate-prone peptides.^{87,88} The insulin/IGF-1 signaling pathway is an evolutionarily conserved process that stimulates cellular growth according to nutrient availability.^{89,90} The activation of the receptor leads to the potent activation of the downstream target PI3K and Akt, which coordinates multiple cellular processes such as proliferation, energy metabolism and survival. Together with TOR, Akt integrates the extracellular inputs with the intracellular status and tunes the cellular responses accordingly.

In *Caenorhabditis elegans*, loss-of-function mutations of the sole insulin/IGF-1 receptor *daf-2* extend the lifespan to more than twofold.⁹¹ Genetic studies in *C. elegans* have revealed that the shift of polyQ-containing proteins from the soluble to the aggregate form is time-dependent. Loss-of-function of the PI3K *age-1* not only extends the lifespan of nematodes but also significantly delays polyQ aggregation and toxicity.⁹² These protective effects are determined by increased expression of stress-response genes, such as heat shock proteins under the control of the transcription factors DAF-16 and HSF-1.⁹³ Interestingly, overexpression of full-length, but not of truncated, HTT lowers the expression of plasma IGF-1 levels and, as result, affects body weight in mice.⁹⁴ A decrease in IGF-1 expression has also been observed in different tissues of HD patients, which indicates that HTT loss-of-function can modulate IGF-1 signaling over time. In primary dissociated neurons expressing mutant HTT, treatment with IGF-1 induces specific activation of Akt and the direct phosphorylation of HTT, which results in a reduced number of HTT-containing intracellular inclusions and therefore neuroprotection.¹⁷ Thus, these findings suggest that IGF-1 signaling and HTT can apparently influence each other, although it still remains elusive whether this cross-talk potentiates or prevents detrimental cascades, including apoptosis.⁹⁵ Modification of proteostasis by the Insulin/IGF-1 signaling pathway is not the only process, which affects HTT homeostasis. Recent screenings in *C. elegans* identified the evolutionarily conserved protein MOAG-4/SERF1-2 as a modifier of protein aggregation during ageing. Loss-of-function or silencing of MOAG-4 suppress the formation of aggregates in animals carrying mutant huntingtin, α -synuclein or β -amyloid.⁹⁶ Whether MOAG-4/SERF1-2 and the interplay with other prosurvival pathways are relevant in HD remains to be

explored, nevertheless the modulation of proteostasis remains a promising approach for the treatment of neurodegenerative disorders.

Mitochondrial Deficiency, Excitotoxicity and Inflammation

Energetic disturbances in HD is well described by post mortem, *in-vitro* and *in-vivo* evidences.¹¹ The high metabolic rate of excitable cells such as neurons makes them strongly reliant upon mitochondrial functions. Mitochondria are highly motile organelles that control dendritic spine formation and synaptic activity by buffering intracellular Ca²⁺ rise underneath the plasma membrane.^{97–99} Mutant HTT has been shown to affect mitochondrial morphology and the bioenergetic status by altering the balance between mitochondrial fusion and fission under the control of the dynamin-related protein 1^{100,101} or the interaction with other mitochondria-associated proteins.¹⁰² Alterations in mitochondria dynamics are reflected in deficits of the electron transport chain and of cellular respiration. The use of energy-related supplements, such as creatine, has been attempted in some clinical trials in order to correct mitochondrial defects in HD patients.³⁸ As a result of extensive mitochondrial depolarization, neurons exposed to prolonged Ca²⁺ rise become vulnerable to excitotoxic insults (Figure 1).^{40,103,104} In HD, mutant HTT affects glutamatergic signals as a result of altered neurotransmitter release and activity of the glutamate-ionotropic receptors at the plasma membrane (Figure 1). In addition, aberrant HTT with the expanded polyQ tract inhibits the expression of the transcriptional co-activator PGC-1 α , therefore compromising mitochondrial biogenesis and respiration.¹⁰⁵ Thus, the combination of the two effects – alteration of Ca²⁺ influx and diminished capability of Ca²⁺ clearance by mitochondria – seriously increases the susceptibility of striatal cells expressing mutant HTT to excitotoxic insults. For this reason, agents that can affect glutamatergic signaling (i.e. NMDA receptor antagonists-like memantine) have been undergoing clinical trials.³⁸ Similarly, other downstream targets that affect NMDA signaling and the excitotoxic neuronal demise might have some potential applications for the treatment of HD.¹⁰⁶

Mitochondrial dysfunction resulting from Ca²⁺ overload, prolonged membrane depolarization or impairment of the electron transfer chain is the main source of intracellular reactive oxidative species.^{107,108} Under certain circumstances, enhanced production of oxidative stress triggers neuroinflammatory responses by activation of the inflammasome in a cell-autonomous or non-autonomous manner.¹⁰⁷ Neuroinflammatory processes are key determinants of neurodegenerative disorders characterized by aggregate-prone proteins, as in the case of PD and AD.¹⁰⁹ Although the activation of inflammatory responses can be triggered by a variety of toxic species, the evidence indicates that most of the common neurodegenerative disorders have converging mechanisms that amplify the detrimental cascades. Remarkably, in the majority of the brain pathologies, neuroinflammation is a presymptomatic event and similar patterns have been shown in unrelated pathologies.^{110,111} In case of HD, the expression of mutant HTT in glial cells affects the

buffering capacity by altering the expression of the glutamate transporters, thus precluding the uptake of glutamate and enhancing neuronal excitotoxicity.¹¹² Inflammation is a critical process that affects neuronal survival during pathological conditions.¹¹¹ It has been shown that mutant HTT can lower the expression and release of glial chemokine,¹¹³ which can be neuroprotective under different circumstances.¹¹⁴ These data add additional complexity to the interaction between neurons and other brain cells. Whether targeting excessive activation of immune responses can be beneficial to HD remains to be determined, although it is tempting to consider it as a feasible possibility.¹¹⁵

Concluding Remarks

The identification of the *HTT* gene has contributed enormously to our understanding of the multiple pathogenic mechanisms involved in the onset of HD and in the selectively enhanced vulnerability of a subset of neurons to the mutant HTT. As discussed in this review, HD is a monogenic disease that results in a gain-of-function of the mutant form and in the loss-of-functions of the wild-type protein, which together severely compromise cellular homeostasis in a complex manner. To date, there is no cure for HD and most of the treatments available only help to alleviate some of the movement and psychiatric symptoms associated with the pathology. As mutant HTT is not considered to be an ideal pharmacological target due to its myriad biological functions, other biochemical pathways, such as those that prevent the abnormal accumulation of unfolded proteins, represent an encouraging alternative for the treatment of this neurodegenerative disorder. The identification and characterization of additional detrimental processes underlying cellular deficits in HD patients might provide new efficient and beneficial targets for neuroprotective intervention.

Conflict of Interest

The authors declare no conflict of interest.

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