**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. 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.

**Cell Death and Disease** (2011) 2, e228; doi:10.1038/cddis.2011.112; published online 10 November 2011

**Subject Category:** Neuroscience

Huntington’s disease (HD) is an inherited autosomal dominant neurodegenerative disorder characterized by adult-onset of motor dysfunctions, psychiatric disturbances and intellectual decline. 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. 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. 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.

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 complex (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. 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. 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. HTT is ubiquitously expressed during embryonic development and at high levels in testis and in mature postmitotic neurons in adult human brain.

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, while heterozygous knockout animals exhibit severe cognitive deficits as a consequence of increased neuronal loss in the subthalamic nucleus of the basal ganglia. Similarly, postnatal neuronal-specific inactivation of Htt is accompanied by progressive apoptotic neuronal degeneration, 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 binding as well as to the activation of prosurvival pathways controlled by the serine/threonine kinase Akt. 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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3Keywords: ageing; autophagy; calpains; excitotoxicity; Huntington’s disease mitochondria; neurodegeneration
4Abbreviations: 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

Received 27.9.11; accepted 27.9.11; Edited by G Melino
This physiological function may be related to the N-terminal polyglutamine region, as it can form polar zipper structure able to bind transcription factors. 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. 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. 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, immuno-histochemical analyses of postmortem brain tissue has demonstrated the presence of intracellular inclusions, which are mainly associated with the selective loss of medium spiny neurons in the striatum. 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. Even though some mouse models expressing N-terminal truncated mutant HTT exhibit abnormal behavioral and neurological phenotypes, 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. Thus, as the full-length HTT is indispensable for manifestation of neuropathology clearly analogous to human HD, the deposition of proteolytic products is not sufficient to initiate a toxic cycle leading to extensive neuronal damage in the striatum. In AD and PD, the deposition of proteolytic products is not sufficient to initiate a toxic cycle leading to extensive neuronal damage in the striatum. In AD and PD, reviewed in refs Douglas and Dillin; McCormack and DiMonte (reviewed in refs Douglas and Dillin; McCormack and DiMonte) 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. 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. 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. Caspases are highly conserved cysteine-aspartic proteases associated primarily with apoptotic cell death and essential for the processing of a large number of substrates. Proteolytic fragments processed by caspases are detectable in brains of HD patients and HD mice before the loss of neurons in the striatum, with the cleavage efficiency dependent on the polyQ tract length. Blocking HTT cleavage by site-directed mutagenesis or by pharmacological approaches reduces cytotoxicity in cultured cells. 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. 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. 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 products that promote NMDA-R-mediated excitotoxicity. 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.

Another RNAi screening study has also shown that small HTT fragments can be generated by the proteolytic activity of some matrix metalloproteinases (MMPs). 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*. 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. 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. 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
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 death68–70 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 phosphoinositol 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 Ca2+ concentration and organelle dysfunctions.
**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.⁹⁷ 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.⁹⁸ 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 1¹⁰⁰,¹⁰¹ 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).⁵⁰,¹⁰³,¹⁰⁴ 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-1α, 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.¹⁰⁹ 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. Inflammation is a critical process that affects neuronal survival during pathological conditions. It has been shown that mutant HTT can lower the expression and release of glial chemokine, which can enhances neuronal excitotoxicity. Inflammation is a critical buffering capacity by altering the expression of the glutamate for neuroprotective intervention. HD patients might provide new efficient and beneficial targets abnormal accumulation of unfolded proteins, represent an pharmacological target due to its myriad biological functions, the pathology. As mutant HTT is not considered to be an ideal mechanism 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.

Acknowledgements. We thank Dr. Sarah Jewell, Dr. Sybille Krauss, Professor Ina Vorberg and Professor Gerry Melino for their useful comments.

1. Martin JB, Gusella JF. Huntington’s disease. Pathogenesis and management. N Engl J Med 1986; 315: 1267–1275.
2. Reiner A, Albin RL, Anderson KD, D’Amato CJ, Penney JB, Young AB. Differential loss of striatal projection neurons in Huntington disease. Proc Natl Acad Sci USA 1988; 85: 5733–5737.
3. Rosas HD, Koroshetz WJ, Chen Yi, Sikeuse C, Vangel M, Cudkowicz ME et al. Evidence for more widespread central pathology in early HD: an MRI-based morphometric analysis. Neurology 2003; 60: 1615–1620.
4. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. The Huntington’s Disease Collaborative Research Group. Cell 1993; 72: 971–983.
5. Andrew SE, Goldberg YP, Kremer B, Teiinuov H, Thelmann J, Adam S et al. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington’s disease. Nat Genet 1993; 4: 398–403.
6. Rubinstein DC, Barton DE, Davison BC, Ferguson-Smith MA. Analysis of the huntingtin gene reveals a trinucleotide-length polymorphism in the region of the gene that contains two CCG-rich stretches and a correlation between decreased age of onset of Huntington’s disease and CAG repeat number. Hum Mol Genet 1993; 2: 1713–1715.
7. Aranda-Orgilles B, Agner J, Kunath M, Luz R, Schneider R, Schweiger S. Active transport of the ubiquitin ligase MID1 along the microtubules is regulated by protein phosphatase 2A. PLoS One 2008; 3: e3507.
8. Atwal RS, Xia J, Pinchev D, Taylor J, Epand RM, Truant R. Huntington has a membrane association signal that can modulate huntingtin aggregation, nuclear entry and toxicity. Hum Mol Genet 2007; 16: 2600–2615.
9. DiFaglia M, Sapp E, Chase K, Schwarz C, Meloni A, Young C et al. Huntington is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron 1994; 14: 1075–1081.
10. Trottier Y, Devey D, Imerb G, Sadaou F, An I, Lutz Y et al. Cellular localization of the Huntington’s disease protein and discrimination of the normal and mutant form. Nat Genet 1995; 10: 104–110.
11. Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutic targets in Huntington’s disease. Physiol Rev 2010; 90: 905–981.
12. Strong TV, Tagle DA, Valdes JM, Elmer LW, Boehm K, Swaroop M et al. Widespread expression of the human and rat Huntington’s disease gene in brain and nonneural tissues. Nat Genet 1993; 5: 259–285.
13. Nardaccio SB, Oksyfu JS, Dewett VM, Richman JM, Zelisier J et al. Targeted disruption of the Huntington’s disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell 1995; 81: 811–823.
14. Zettin S, Liu JP, Chapman DL, Papaioannou VE, Elfratadiatia A. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington’s disease gene. Nat Genet 1995; 10: 155–161.
15. Dragatis I, Levine MS, Zettin S. Inactivation of Hdh in the brain and tests results in progressive neurodegeneration and sterility in mice. Nat Genet 2000; 26: 300–306.
16. Zhang Y, Li M, Drozza M, Chen M, Ren S, Mejia Sanchez RD et al. Depletion of wild-type huntingtin in mouse models of neurologic diseases. J Neurochem 2003; 87: 101–106.
17. Humbert S, Bryson EA, Cordovesi FP, Concors NC, Datta SR, Finkbeiner S et al. The GF-1/4Kt pathway is neuroprotective in Huntington’s disease and involves Huntington phosphorylation by Akt. Dev Cell 2002; 2: 831–837.
18. Perutz MF, Johnson T, Suzuki M, Finch TJ. Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. Proc Natl Acad Sci USA 1994; 91: 5355–5358.
19. Clabough EB, Zettin SO. Deletion of the triplet repeat encoding polyglutamine within the mouse Huntington’s disease gene results in sublethal behavioral/motor phenotypes in vivo and elevated levels of ATP with cellular senescence in vitro. Hum Mol Genet 2006; 15: 607–623.
20. Zhang S, Clabough EB, Sarker S, Futter M, Rubinstein DC, Zettin SO. Deletion of the huntingtin polyglutamine stretch enhances neuronal autophagy and longevity in mice. PLoS Genet 2010; 6: e1000838.
21. DiFaglia M, Sapp E, Chase KD, Davies SW, Bates GP, Vonsattel JP et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997; 277: 1990–1993.
22. Martindale D, Hackam A, Wieczorek A, Eleryr L, Wellington C, McCutcheon K et al. Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. Nat Genet 1998; 18: 150–154.
23. Sauer F, Finkbeiner S, Devey D, Greenberg MB. Huntington acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 1998; 95: 55–66.
24. Thakur AK, Jayaraman M, Mishra R, Thakur M, Chellgren MV, Byeon UJ et al. Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. Nat Struct Mol Biol 2009; 16: 380–383.
25. Zudner T, Brundin P. Mutant huntingtin can paradoxically protect neurons from death. Cell Death Differ 2008; 15: 435–442.
26. Davies SW, Turmaine M, Cozens BA, DiFaglia M, Sharp AH, Ross CA et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 1997; 90: 537–548.
27. Slow EJ, Graham RK, Osman AP, Devon RS, Lu G, Deng Y et al. Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin inclusions. Proc Natl Acad Sci USA 2005; 102: 11402–11407.
28. Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y et al. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet 2003; 12: 1555–1567.
29. Hodgson JG, Apponyan N, Gutekunst CA, Leavitt BR, LePlaine F, Singhara R et al. A YAC mouse model for Huntington’s disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron 1999; 23: 181–192.
30. Civardinelli D, Silvestri E, Visco B, Swaroop M, Gregoric DD, Moreno M et al. Alterations of brain and cerebellar proteomes linked to Abeta and tau pathology in a female triple-mutant mouse model of Alzheimer’s disease. Cell Death Dis 2010; 1: e90.
31. Lee MH, Lin SR, Chang YJ, Schultz L, Heath J, Hsu L et al. TGF-beta induces TIAF1 self-aggregation via type II receptor-independent signaling that leads to generation of amyloid beta plaques in Alzheimer’s disease. Cell Death Dis 2010; 1: e110.
32. Sivanathan SN, Lee AW, Goodger CG, LeBlanc AC. Familial amyloid precursor protein mutants cause caspase-6-dependent but amyloid beta-peptide-independent neuronal degeneration in primary human neuron cultures. Cell Death Dis 2010; 1: e106.
33. Yacoubian TA, Stone SR, Harrington AJ, Hamamichi S, Schieltz JM, Caldwell KA et al. Differential neuroprotective effects of 14-3-3 proteins in models of Parkinson’s disease. Cell Death Dis 2010; 1: e2.
34. Douglas PM, Dillin A. Protein homeostasis and aging in neurodegeneration. J Cell Biol 2010; 190: 719–729.
35. McCormack AL, Di Monte DA. Allostereic alpha-synuclein expression in human neurodegenerative diseases: pathogenic and therapeutic implications. Curr Protein Pept Sci 2009; 10: 476–482.

36. Bodner RA, Outeiro TF, Altman S, Maxwell MM, Cho SH, Hyman BT et al. Pharmacological promotion of inclusion formation: a therapeutic approach for Huntington's and Parkinson's diseases. Proc Natl Acad Sci USA 2006; 103: 4245–4249.

37. Krainc D. Clearance of mutant proteins as a therapeutic target in neurodegenerative diseases. Arch Neurol 2010; 67: 388–392.

38. Munoz-Sanjuan I, Bates GP. The importance of integrating basic and clinical research toward the development of new therapies for Huntington disease. J Clin Invest 2011; 121: 478–483.

39. Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, Graham RK et al. Cleavage of huntingtin by aoppan, a proapoptotic cytosine protease, is modulated by the polyglutamate tract. Nat Genet 1996; 13: 422–449.

40. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003; 4: 552–565.

41. Wellington CL, Ellerby LM, Gutekunst CA, Rogers D, Warby S, Graham RK et al. Caspase cleavage of mutant huntingtin precedes neurodegeneration in Huntington's disease. J Neurosci 2002; 22: 7982–7987.

42. Cowan CM, Fan MM, Fan J, Shehadeh J, Zhang LY, Graham RK et al. Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. J Biol Chem 2000; 275: 19831–19838.

43. Heidari N, Hicks MA, Koide HB, Graham RK et al. GX15-070 (obatoclax) overcomes glucocorticoid resistance in acute lymphoblastic leukemia through induction of apoptosis and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. Cell Death Differ 2010; 1: e18.

44. Orrenius S, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003; 4: 731–801.

45. Gahr J, Ellerby LM. Calpain activation in Huntington's disease. J Neurosci 2002; 22: 4843–4849.

46. Cowan CM, Singaraja R, Ellerby L, Savill J, Roy S, Leavitt B et al. Calpain-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. Cell Death Differ 2010; 16: 21–30.

47. Irving S, Krauel K, Harada H. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003; 4: 552–565.

48. Russell R, Barlocci L, Adornetto A, Varano GP, Cavaliere F, Nucoli C et al. Calpain-mediated cleavage of Beclin-1 and apoptosis deregulation following retinal ischemic injury in vivo. Cell Death Differ 2011; 1: e144.

49. Youssouf S, Perrozzi R, Schindl I, Ziemiecki A, Schaffner T, Scapozza L et al. Calpain-mediated cleavage of Atg5 switches apoptosis to autophagy. Nat Cell Biol 2006; 8: 1124–1132.

50. Xia H, Zhang L, Chen G, Zhang T, Liu J, Jin M et al. Control of basal apoptosis by calpain1 mediated cleavage of ATGs. Autophagy 2010; 6: 61–66.

51. He C, Bartholomew CR, Zhou W, Klionsky DJ. Assaying autophagic activity in transgenic GFP-LC3 and GFP-Gabarap zebrafish embryos. Autophagy 2009; 5: 520–526.

52. Martinez-Monzat C, Tailoczy Z, Wong E, Tang G, Koga H, Kaurisch S et al. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci 2010; 13: 567–576.

53. Steffen JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL et al. Histone deacetylases inhibit autophagy-dependent neurodegeneration in Drosophila. Nature 2001; 413: 739–743.

54. Jeong H, Then F, Melia Jr TJ, Mazzulli JR, Cui L, Savas JN et al. Huntington's disease. Cell 2009; 137: 60–72.

55. Piao L, Bodai L, Lukasovich T, Purcell JM, Steffen JS, Thompson LM et al. Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a Drosophila model of Huntington's disease. Hum Mol Genet 2006; 15: 3776–3777.

56. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG et al. Small molecule activators of sirtuins extend lifespan. Nature 2003; 425: 191–196.

57. Morselli E, Markaki M, Megalou E, Pasparaki A, Palikaras K et al. Caloric restriction and resveratrol promote longevity by activating the Sir2-dependent induction of autophagy. Cell Death Differ 2010; 1: e10.

58. Blagosklonny MV. Linking calorie restriction to longevity through sirtuins and autophagy: any role for TOR? Cell Death Differ 2010; 16: 121–132.

59. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 2007; 8: 101–112.

60. Caughey B, Lansbury PT. Procaspase9, pors, biftis, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. Annu Rev Neurosci 2006; 29: 267–298.

61. Goedt M, Spillantini MG. A century of Alzheimer's disease. Science 2006; 314: 777–781.

62. Cohen E, Bieschke J, Pericheck RM, Kelly JW, Dillin A. Opposing activities protect against age-onset proteotoxicity. Science 2006; 313: 1604–1610.

63. Cohen E, Paulsson JF, Blinder P, Burstyn-Cohen T, Du D, Estepa G et al. Small molecule activators of sirtuins extend lifespan. Nature 2003; 425: 191–196.

64. Williams A, Jahreiss L, Sarkar S, Saks S, Menzies FM, Ravikumar B et al. Aggregate-prone proteins are cleared from the cytosol by autophagy: therapeutic implications. Curr Top Dev Biol 2006; 152: 89–101.

65. Sarkar S, Ravikumar B, Floto RA, Rubinsztein DC. Rapamycin and mTOR-independent autophagy induces ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. Cell Death Differ 2009; 16: 68–69.

66. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008; 451: 1068–1075.

67. Vellai T. Autophagy genes and ageing. Cell Death Differ 2009; 16: 94–102.

68. Kouris N, Tavarakis N. Autophagy and cell death in model organisms. Cell Death Differ 2009; 16: 21–30.

69. van Ham TJ, Holmberg MA, van der Goot AT, Teuling E, Garcia-Arencibia M, Kim HE et al. Inhibition of caspase-3 and -7 ameliorates toxicity of polyglutamine-expanded huntingtin and related proteinopathies. Hum Mol Genet 2009; 18: e18.

70. Cohen E, Agustini M, Belin PS, Spillantini MG. Huntington's disease: two new ways to fight the disease. Nat Rev Neurol 2009; 5: 759–767.

71. Bano D, Agostini M, Melino G, Nicotera P. Ageing, neuronal connectivity and brain disorders: an unsolved riddle effect. Mol Neurobiol 2011; 43: 124–130.

72. Cohen E, Jiang C, Ginsch G, Rudner A, Tabrizian R, A C elegans mutant that lives twice as long as wild type. Nature 1993; 366: 461–464.

73. Morley JF, Brignull HR, Weyers JJ, Morimoto RI. The threshold for polyglutamine–expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in Caenorhabditis elegans. Mol Neurobiol 2010; 41: 1142–1145.

74. Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L et al. Huntington's disease. Mol Neurobiol 2010; 41: 1146–1153.

75. Poulaud MA, Xie Y, Sicotte NH, Ehrehofer DE, Graham PK, Kim JE et al. Full-length huntingtin levels modulate body weight by influencing insulin-like growth factor 1 expression. Hum Mol Genet 2010; 19: 1528–1538.

76. Mitchell GC, Fillingter JL, Sittadjojy S, Avila JL, Burd R, Limesand KH. IGFI activates cell cycle arrest following irradiation by reducing binding of Deltanp63 to the p21 promoter. Cell Death Differ 2010; 1: e50.

77. van Ham TJ, Holmberg MA, van der Goot AT, Teuling E, Garcia-Arencibia M, Kim HE et al. Identification of MOAG-4/SERF as a regulator of age-related proteotoxicity. Cell 2010; 142: 601–612.
97. Giacomello M, Drago I, Pizzo P, Pozzan T. Mitochondrial Ca2+ as a key regulator of cell life and death. Cell Death Differ 2007; 14: 1267–1274.

98. Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell 2004; 119: 873–887.

99. Young KW, Bampton ET, Pinon L, Bano D, Nicotera P. Mitochondrial Ca2+ signalling in hippocampal neurons. Cell Calcium 2008; 43: 296–306.

100. Costa V, Giacomello M, Hudec R, Lopreiato R, Ermak G, Lim D et al. Mitochondrial fission and cristae disruption increase the response of cell models of Huntington’s disease to apoptotic stimuli. EMBO Mol Med 2010; 2: 490–503.

101. Song W, Chen J, Petrilli A, Liot G, Klinglmayr E, Zhou Y et al. Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. Nat Med 2011; 17: 377–382.

102. Sassone J, Colciago C, Marchi P, Ascardi C, Alberti L, Di Pardo A et al. Mutant Huntingtin induces activation of the Bcl-2/adenovirus E1B 19-kDa interacting protein (BNip3). Cell Death Dis 2010; 1: e7.

103. Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K et al. Early and progressive accumulation of reactive microglia in the Huntington disease brain. J Neuropathol Exp Neurol 2001; 60: 161–172.

104. Ruiz A, Matute C, Alberdi E. Intracellular Ca2+ release through ryanodine receptors contributes to AMPA receptor-mediated mitochondrial dysfunction and ER stress in oligodendrocytes. Cell Death Dis 2010; 1: e54.

105. Glass CK, Sajo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell 2010; 140: 918–934.

106. Ruiz A, Matute C, Alberdi E. Intracellular Ca2+ release through ryanodine receptors contributes to AMPA receptor-mediated mitochondrial dysfunction and ER stress in oligodendrocytes. Cell Death Dis 2010; 1: e54.

107. Glass CK, Sajo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell 2010; 140: 918–934.

108. Ruiz A, Matute C, Alberdi E. Intracellular Ca2+ release through ryanodine receptors contributes to AMPA receptor-mediated mitochondrial dysfunction and ER stress in oligodendrocytes. Cell Death Dis 2010; 1: e54.