Cytogenet Genome Res 100:287–295 (2003)
DOI: 10.1159/000072864

Cytogenetic and Genome Research

Mechanisms of neuronal cell death in Huntington's disease

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Abstract. Huntington's disease (HD) is a genetically dominant neurodegenerative condition caused by an unique mutation in the disease gene *huntingtin.* Although the Huntington protein (Htt) is ubiquitously expressed, expansion of the polyglutamine tract in Htt leads to the progressive loss of specific neuronal subpopulations in HD brains. In this article, we will summarize the current understanding on mechanisms of how mutant Htt can elicit cytotoxicity, as well as how the selective sets of neuronal cell death occur in HD brains.

Huntington's disease (HD) is a genetically dominant neurodegenerative condition characterized by abnormal involuntary movements, including chorea and dystonia, and cognitive impairment (Huntington, 1872). HD is one of a number of diseases caused by an expansion of CAG repeats in the open reading frame of a disease gene, which in turn translates into a protein with an expanded polyglutamine tract (Ross, 2002). The disease gene for HD, *huntingtin*, was identified in 1993, and it encodes a large protein (350 kDa) with a polyglutamine stretch named Huntintin (Htt) (The Huntington's Disease Collaborative Research Group, 1993). Once the polyglutamine repeat becomes expanded over approximately 40, HD occurs. Htt is widely expressed in the whole body, but robust pathology occurs only in restricted areas, in particular, the caudate (Gutekunst et al., 1995; Sharp et al., 1995). In HD brains, medium spiny neurons in the caudate are selectively impaired. The extent of the polyglutamine expansion is correlated with the severity of symptoms, such as age of onset. The causal relationship between neuronal cell death and the onset of neurological dysfunction in HD is unclear; however, the progressive loss of specific neuronal subpopulations in HD coincides with disease progression.

Ten years have already passed since *huntingtin* and its unique mutation were identified. However, we still do not know the mechanisms of how the selective sets of neuronal cell death occur in HD brains.

In the first section, we will overview general understanding of neuronal cell death. In the second section, we will summarize current ideas on how mutant Htt can induce cytotoxicity. Finally, we will discuss possible mechanisms that may account for the selective neuronal cell death of HD brains.

Neuronal cell death

The classic definitions of necrosis and apoptosis were first proposed by intensive morphological examination using cellular systems outside the brain (Kerr et al., 1972; Kerr and Harmon, 1991) (Fig. 1). Apoptosis displays distinctly different morphological features from necrosis, including nuclear condensation and fragmentation, as well as convulsion of membrane. Apoptosis is also characterized by biochemical markers, such as internucleosomal DNA cleavage or DNA ladder formation. Apoptosis is a type of programmed cell death, in which cells participate actively toward their own demise. In contrast, necrotic cells dis-

Supported by grants from the Huntington's Disease Society of America, the Stanley Medical Research Institute, NARSAD, and the S-R Foundation (Washington, DC) (to A.S.).

Received 19 December 2002; revision accepted 21 January 2003.

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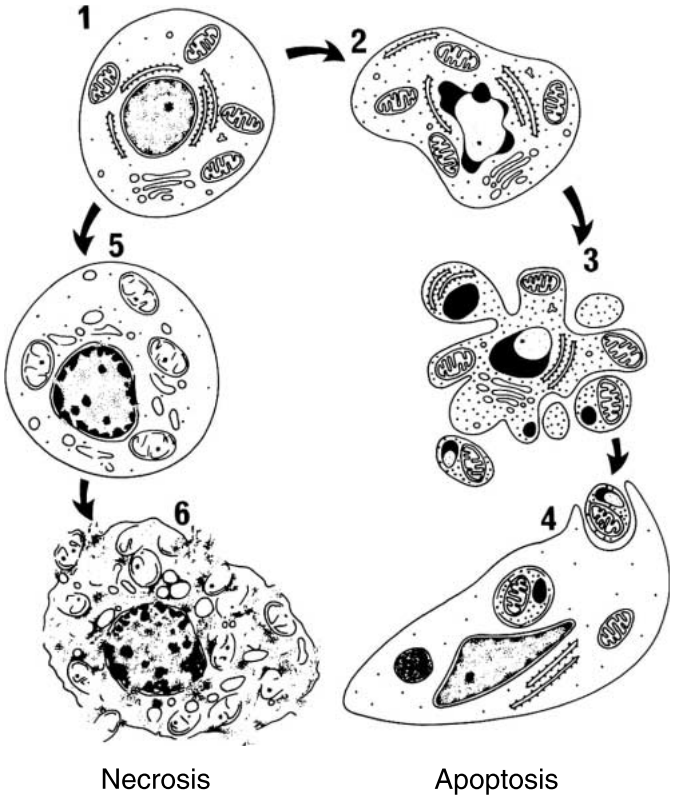

Fig. 1. Schematic description of necrosis and apoptosis. Modified, with permission, from Kerr and Harmon (1991).

play organelle swelling and subsequent cellular disintegration. Necrosis is frequently associated with cellular energy deficit and is regarded as passive cell damage and death.

Robust neuronal cell death is observed in both physiological brain development and in pathological conditions of adult brains, such as neurodegeneration (Server and Mobley, 1991). Although the simple classification of cell death into apoptosis and necrosis is widely accepted in most biological contexts, it has become clearer that neuronal cell death is not easily classified into these two categories, but has more variety of cell death modes (Fig. 2).

Clarke (1990) extensively reviewed the morphological characteristics of neurons dying during development. He classified dying neurons based on morphological aspects into three types. Type 1 cell death is observed among developing thoracic spinal neurons, cervical visceromotor neurons, and lateral motor neurons of the lumbar spinal cord of the chick embryo. In mammals, this type of neuronal cell death was reported in the postnatal rat superior colliculus and retina. Type 1 cell death is characteristic of nuclear condensation and fragmentation, as well as convulsion of membrane. Cell shrinkage is seen during this type of cell death. These features are compatible with the classical definition of apoptosis. Type 2 cell death is characterized mainly by the existence of numerous autophagic vacuoles. Autophagy is a process whereby cells remove cytosolic proteins and organelles and degrade themselves from within. Type 3 cell death is seen in several developmental contexts, including embryonic chick ciliary ganglion cells and lumber motor neurons. This type of cell death, also called cytoplasmic cell death, is characterized by initial dilation of the endoplasmic reticulum (ER), as well as the nuclear envelope and Golgi apparatus. In some cases, mitochondrial dilation also occurs.

In the past decade, detailed molecular cascades for apoptosis, or type 1 cell death by Clarke, have been intensively clarified. Most of apoptosis includes a chain of activation of cysteinyl aspartic-specific proteases, called caspases (Thornberry and Lazebnik, 1998). Two major pathways for apoptosis via caspases exist: the intrinsic pathway, originating from mitochondrial release of cytochrome C and associated activation of caspase-9, and the extrinsic pathway, originating from the activation of cell-surface death receptors, such as Fas, and resulting in the activation of caspase-8 (Ashkenazi and Dixit, 1998; Green and Reed, 1998). Damages to genome DNA activate p53, which activates the intrinsic pathway via mitochondria (Vogelstein et al., 2000). Caspase-independent apoptosis also exists. Apoptosis-inducing factor (AIF), a mitochondrial intermembrane flavoprotein, is released from the mitochondria to the nucleus in response to specific death signals (Susin et al., 1999). AIF causes high-molecular-weight DNA fragmentation and chromatin condensation in cells and isolated nuclei in a caspase-independent manner. Neuronal cell death mediated by p53 reportedly occurs in both a caspase-dependent and an AIF-dependent manner, with different kinetics (Cregan et al., 2002). PML, a main component of a nuclear matrix-associated structure, is another factor that mediates caspase-independent apoptosis (Quignon et al., 1998). Roles of PML in neuronal apoptosis remain elusive; however, PML is necessary for toxic actions of p53 (Zhong et al., 2000).

Molecular mechanisms for autophagic cell death (type 2 cell death) still remain elusive. Recently, *beclin 1*, the mammalian ortholog of a yeast autophagy gene *(Apg6/Vps30)*, was identified (Liang et al., 1999). Interestingly, Beclin 1 protein binds to Bcl-2, a key pro-apoptotic gene product, and inhibits tumorigenesis. Moreover, in Purkinje cell death of *lurcher* mutant mice, the causative mutation in GluRδ2 can activate protein signaling to Beclin 1 (Yue et al., 2002). These findings suggest that autophagic cell death, like apoptosis, is programmed via intracellular molecular signaling.

Cytoplasmic cell death (type 3 cell death) has not yet been characterized at the molecular level. Bredesen and colleagues (Sperandio et al., 2000) recently reported a unique programmed cell death (paraptosis) distinct from classic apoptosis. In 293T cells and fibroblasts, expression of insulin-like growth factor I receptor intracellular domain (IGFIR-IC) can induce cell death characterized by cytoplasmic vacuolation and resistance to well-known apoptosis inhibitors. In this setting, caspase-9 is activated independently of Apaf-1. Valosin containing protein (VCP)/p97 is a member of the AAA+ family of ATPase proteins. Expression of VCP/p97 with a mutation in the second ATP binding domain causes cytoplasmic vacuolation followed by cell death (Hirabayashi et al., 2001). These two observations may not be directly linked to the molecular mechanisms for cytoplasmic cell death (type 3 cell death); their existence, however, suggests interesting connotations.

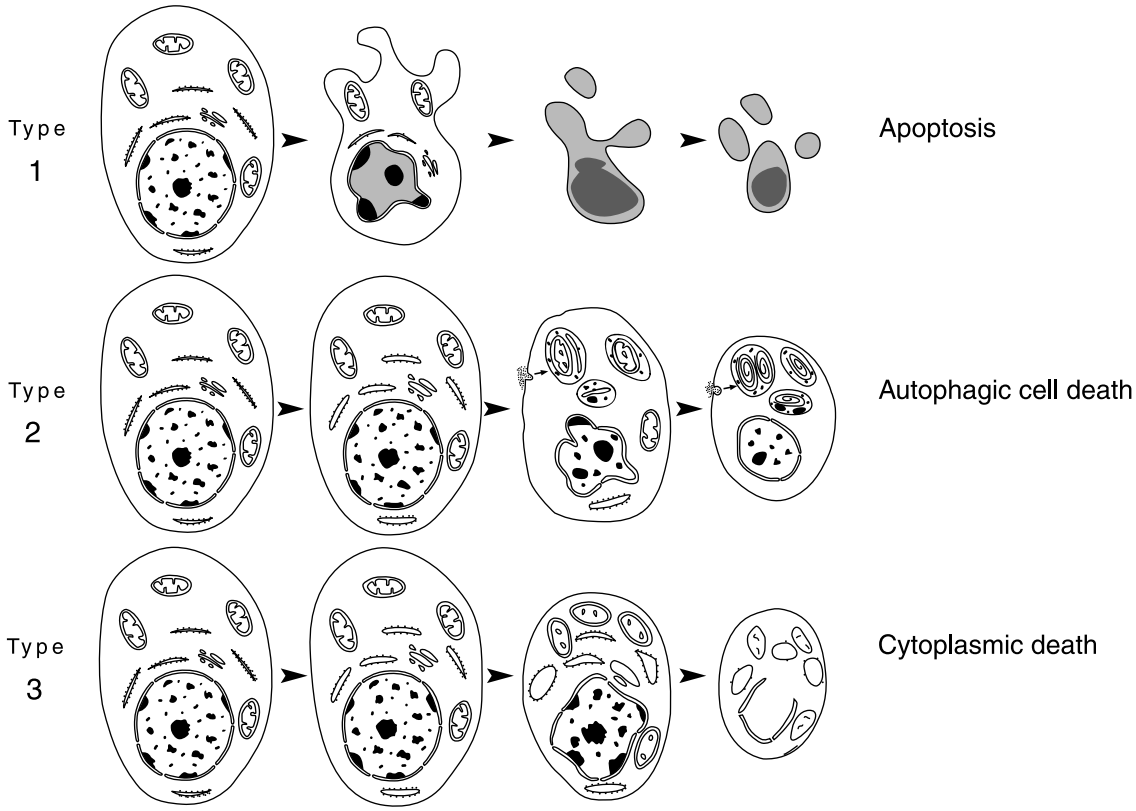

Fig. 2. Schematic description of Clarke's classification for neuronal cell death. Modified, with permission, from Server and Mobley (1991) and Clarke (1990).

Question 1: How can mutant Htt induce cytotoxicity? (Fig. 3)

HD occurs in an autosomal dominant fashion. The dominant model can include both "gain of function" and "loss of function" (haploinsufficiency). After the identification of Htt, its toxic mechanisms were initially addressed as "gain of function" for the following reasons:

First, the overexpression of mutant Htt, especially the *N*-terminal short fragment including the polyglutamine stretch, causes cytotoxicity as well as inclusion formation that is characteristic of HD brains (Cooper et al., 1998; Hackam et al., 1998; Wellington et al., 2000). Analogously, transgenic mice with overexpression of the *N*-terminal fragment with expanded polyglutamine display neurological deficits similar to HD, as well as early death and the inclusion formation (Mangiarini et al., 1996; Schilling et al., 1999).

Second, several disease genes for other types of neurodegenerative conditions, such as spinocerebellar ataxia (SCA) and dentatorubral and pallidoluysian atrophy (DRPLA), also possess a similar type of mutation, expansion of polyglutamine in the disease gene products. The genes that cause these disorders have no homology with each other except for the polyglutamine stretch itself. The other portions of the disease proteins may serve as regulatory units. Therefore, people have reasonably grouped these diseases as polyglutamine diseases (Ross, 2002). Transgenic mice expressing expanded polyglutamine in an unrelated gene also display neurological deficit (Ordway et al., 1997). This reinforces the notion of the polyglutamine disease.

In addition to the "gain of function" theory, studies of functions of normal (wild-type) Htt have disclosed the importance of Htt for cell survival and implicated its "loss of function" in HD pathology (Cattaneo et al., 2001). The normal form of full-length Htt has several important roles, such as endocytosis and up-regulation of brain-derived neurotrophic factor (BDNF), a pro-survival factor (Zuccato et al., 2001). In cell models, normal Htt protects from apoptosis (Rigamonti et al., 2000). This observation was confirmed by animal models in which inactivation of the mouse *huntingtin* gene in the brain and testis results in progressive neurodegeneration and sterility (Dragatsis et al., 2000). In addition to simple loss of normal beneficial functions of Htt by expanded polyglutamine, mutant Htt may recruit wild-type Htt into insoluble aggregates, which results in an accelerated depletion of wild-type Htt.

Cell death in HD cellular models

The condition of "gain of function" can be mimicked in cellular models by overexpressing mutant Htt. Overexpression of Htt, especially the *N*-terminal fragment of Htt, with expanded polyglutamine kills many types of cell lines and increases cellular susceptibility to death (Cooper et al., 1998; Hackam et al., 1998; Wellington et al., 2000). Under these conditions, robust

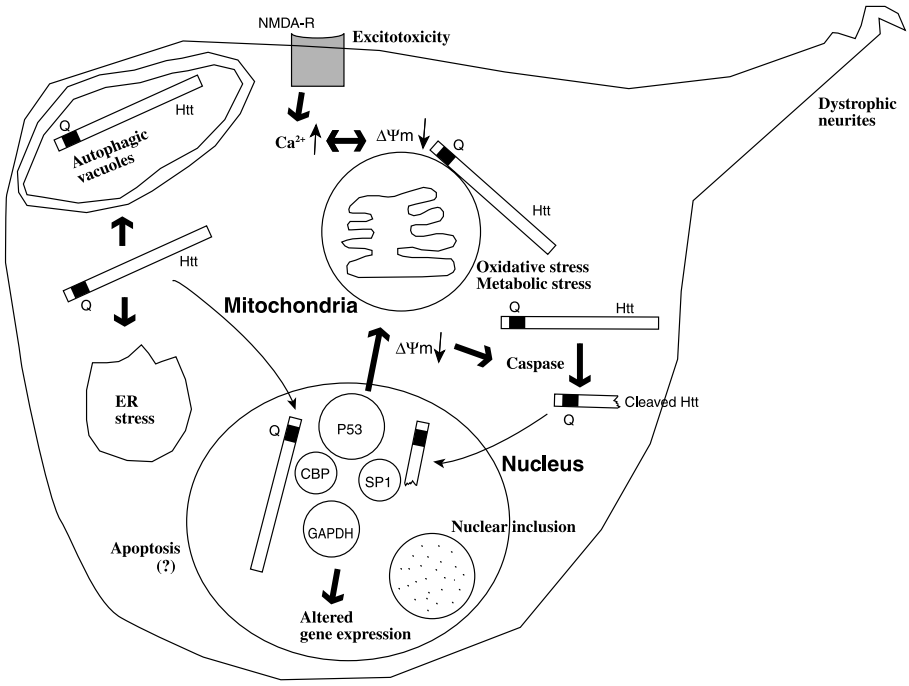

Fig. 3. Schematic description of possible pathological mechanisms induced by mutant Htt. Mutant Htt seems to display cytotoxicity via multiple pathways. Mutant Htt can induce formation of autophagic vacuoles and stress on the endoplasmic reticulum. Mutant Htt influences mitochondrial functions by several mechanisms, inducing oxidative and metabolic stress. Increased susceptibility to mitochondrial membrane depolarization can also occur in this setting, followed by increased levels of cytosolic calcium and a caspase activation chain. Increased level of calcium can be associated with vulnerability to excitotoxicity. The activated caspase can cleave Htt. Mutant Htt, especially cleaved Htt, can translocate to the nucleus and disturb gene transcription via interaction with CREB binding protein (CBP), Sp1, and p53, while p53 regulates cell death by modulating many mitochondrial proteins and proteins associated with oxidative stress. In the nucleus, nuclear inclusion stained with Htt also occurs, but its pathophysiological significance is not clear. Dystrophic neurites occur in the presence of mutant Htt.

inclusion body formation inside both the nucleus and cytoplasm, followed by cell death, is observed. The pathological significance of inclusion body formation for cell death is unclear. The cells dying from mutant Htt frequently manifest several apoptotic features, including nuclear changes and caspase-3 activation.

In cultured striatal neurons, the *N*-terminal Htt fragments with expanded polyglutamine induce apoptosis with the formation of intracellular inclusions (Saudou et al., 1998). Apoptotic cell death was characterized by nuclear condensation in this study. Anti-apoptotic compounds and neurotrophic factors such as CNTF and BDNF can prevent the apoptosis induced by mutant Htt. The Htt-induced neural degeneration was not correlated with the formation of intranuclear inclusions in this study.

Mutant Htt can augment susceptibility to apoptotic stress. Lymphoblasts from HD patients, but not control subjects display susceptibility to mitochondrial membrane depolarization, an initial event for cell death, upon receiving low doses of cyanide or staurosporine (STS) (Sawa et al., 1999). After STS stress, caspase-9 and caspase-3, downstream components of the apoptotic cascade from mitochondria, are sequentially overactivated in HD lymphoblasts compared to normal lymphoblasts. This leads to accelerated apoptosis in HD cells. Apoptosis in this cell system is characterized by nuclear fragmentation and condensation, as well as DNA ladder formation.

In contrast to frequent reports of apoptotic death by mutant Htt in HD cell models, Johnson and colleagues (Moulder et al., 1999) reported an instance of expanded polyglutamine-induced cell death resistant to z-VAD-fmk, a potent general apoptosis inhibitor. As the polyglutamine-induced cell death shares, at least in part, the molecular mechanism with mutant-Htt-induced cell death, this report may shed some light on our overall understanding of Htt-induced cell death.

In some settings, vacuole formation is also observed in Htt-induced cell death. Most of the vacuoles in these systems have features of autophagy.

Exogenous overexpression of mutant Htt in clonal striatal neurons leads to Htt accumulation in cytoplasmic vacuoles with cell shrinkage (Kegel et al., 2000; Petersen et al., 2001). The Htt-containing vacuoles look similar to autophagosomes. Autophagic mechanisms are believed to play a role in the degradation of Htt, especially the *N*-terminal cleaved fragments with expanded polyglutamine, for the following reasons (Ravikumar et al., 2002): Htt tends to accumulate more when cells are treated with inhibitors to the autophagy-lysosome pathway. In contrast, rapamycin, which stimulates autophagy, enhances the clearance of aggregate prone mutant Htt. It is not yet clear what triggers the autophagic cell response and what signaling bridges from mutant Htt to autophagosome formation. Moreover, we observe the existence of large vacuoles in HD lymphoblasts, but not in control subjects (Nagata et al., manuscript submitted for publication). The vacuoles are stained with anti-Htt antibody, as well as with an antibody against cathepsin B, a marker for lysosome. The vacuoles in HD lymphoblasts are even more enlarged upon an apoptotic stress, such as STS addition. It remains elusive whether the autophagic vacuoles seen in HD cell models and brains are cytotoxic or cytoprotective.

As described above, a mutation in VCP/p97 causes cell death with associated cytoplasmic vacuoles (Hirabayashi et al., 2001). VCP/p97 binds and co-localizes to polyglutamine tracts. Interestingly, *Drosophila* VCP is reported as a modulator of polyglutamine-induced neurodegeneration (Higashiyama et al., 2002). The relationship between VCP/p97-induced vacuoles and autophagic vacuoles is still unclear.

Frequent observation of apoptosis and autophagy in HD cell models may imply certain molecular mechanisms by which mutant Htt can induce both apoptosis and an autophagic cell

response. However, Htt-induced cell death in previous studies usually occurred by overexpression of the *N*-terminal fragments of Htt or upon several apoptotic stimuli, so caution when interpreting the data is necessary.

Neuronal cell death in HD animal models

Both drug-induced and genetically engineered animals are currently available for HD models.

Chronic, systemic administration of 3-nitropropionic acid (3-NPA), a mitochondrial toxin, to rats (Beal et al., 1993) or primates (Brouillet et al., 1995) can cause a selective loss of medium spiny neurons in the striatum. Their pathology is similar to HD neuropathology, such as selective loss of medium spiny neurons and some sparing of large neurons. Both freeze-clamp measurements and chemical shift magnetic resonance spectroscopy show that 3-NPA impairs energy metabolism (Beal et al., 1993). Deficit of energy metabolism in neurons is frequently associated with necrotic cell death. Dysfunction of mitochondria is, in some contexts, associated with apoptotic cell death.

Transgenic mice overexpressing exon 1 of the *huntingtin* gene are widely used as HD animal models, with HD-relevant manifestations in neurological phenotype as well as neuropathology including neuronal intranuclear inclusions and dystrophic neurites (Mangiarini et al., 1996; Schilling et al., 1999). In more detailed examinations, dying neurons with neuronal intranuclear inclusions display condensation of both the cytoplasm and nucleus, as well as ruffling of the plasma membrane, while maintaining ultrastructural preservation of cellular organelles. These cells do not develop blebbing of the nucleus or cytoplasm, apoptotic bodies, or fragmentation of DNA. Taking these observations together, Davies and colleagues (Turmaine et al., 2000) propose that neuronal cell death in the mice model might be neither apoptosis not necrosis. In contrast, Tagle's group (Reddy et al., 1998) reports TdT-mediated dUTP-biotin nick end labeling (TUNEL) positive dying neurons in mice expressing the full length of mutant Htt, suggesting the existence of apoptotic cell death. Mitochondrial abnormalities, especially a deficit of energy metabolism in brains, have been reported in Htt transgenic mice (Tabrizi et al., 2000).

Although it is still controversial in the existence of apoptosis in HD animal brains by neuropathological access, genetic intervention of an apoptotic cascade can ameliorate the pathology and phenotype of HD mice (Ona et al., 1999). Cross-breeding of the R6/2 line of Htt transgenic with mice expressing a dominant negative form of caspase-1 leads to extended survival, delay in appearance of neuronal inclusions, and neurotransmitter receptor alternations, as well as the onset of symptoms. Involvement of caspases in HD pathology is also suggested by a YAC transgenic mice expressing full-length Htt (Hodgson et al., 1999, Wellington et al., 2002). The YAC mice expressing mutant Htt, but not wild-type Htt, show neuronal degeneration and accumulation of *N*-terminal fragments of Htt that have occurred by caspase cleavage. This observation in YAC mice fits well with the cytotoxicity related to overexpression of the *N*-terminal fragment of mutant Htt in HD cell models described above (Cooper et al., 1998; Hackam et al., 1998; Wellington et al., 2000).

Dystrophic neurites are remarkable features of dying neurons in HD animal models. Li and associates (Li et al., 2001) report neuropil aggregates and dystrophic neurites prior to intranuclear accumulation of Htt, suggesting that such axonal degeneration is an early pathological event in HD mice.

Neuronal cell death in HD patient brains

Gross examination of coronal sections reveals bilateral, symmetric atrophy of the striatum in 95% of HD brains. In later stages of the disease, atrophy of the cerebral cortex becomes pronounced (Vonsattel, 2000).

Immunohistochemical analysis using antibodies against the *N*-terminal portions of Htt clarified the localization of Htt to neuronal intranuclear inclusions and dystrophic neurites in the HD striatum and cortex (DiFiglia et al., 1997). The prevalence of dystrophic neurites in deep layers of the cortex correlates with greater neurodegeneration in these layers, and their appearance in a presymptomatic adult suggests that they precede clinical onset. Dystrophic neurites are associated with neurofilament-positive axonal fibers, which agrees with evidence that dystrophic neurites are distended axon terminals. In addition, perinuclear and nuclear staining of Htt is observed with an antibody against the *N*-terminal Htt (Sapp et al., 1997).

There is some evidence using TUNEL assays for detecting apoptotic neurons to support the existence of apoptosis in HD brains (Dragunow et al., 1995; Portera-Cailliau et al., 1995; Thomas et al., 1995). However, it is still controversial whether the major form of neuronal cell death in HD patient brains is apoptotic.

Fitting with the observation of autophagic vacuoles in some HD cell models, endosomal- and lysosomal-like organelles and tubulovesicular structures with Htt immunoreactivity are more prominent in HD brains than in brains of control subjects (Sapp et al., 1997).

Biochemical analysis of HD brains has characterized mitochondrial abnormalities (Browne et al., 1997; Gu et al., 1996; Schapira, 1997; Tabrizi et al., 1999). The enzymatic activities of several mitochondrial proteins involved in oxidative phosphorylation, such as complex II, III, and IV, are decreased in HD brains. This type of deficit can link to necrosis, as well as apoptotic cell death. These enzymatic abnormalities are consistent with the findings in drug-induced and Htt transgenic mouse models.

Intracellular signaling

Modes of cell death by mutant Htt are, as described, very complex. However, at least two organelles play important roles for its cytotoxicity: the mitochondria and the nucleus (Sawa, 2001).

Involvement of mitochondria in HD pathology has been originally supported by biochemical analyses of patient tissues (Browne et al., 1997; Gu et al., 1996; Schapira, 1997) and brains from HD animal models (Beal et al., 1993; Brouillet et al., 1995; Tabrizi et al., 1999). Other classic hypotheses for explaining HD pathogenesis, such as excitotoxicity, metabolic stress, and the involvement of free radicals, are also associated with mitochondrial dysfunction (Sawa, 2001). Increased levels of free radicals impair mitochondrial functions and energy pro-

duction, and metabolic inhibition predisposes neurons to excitotoxic damage.

In addition, our group (Sawa et al., 1999) reported increased vulnerability to mitochondrial membrane depolarization and apoptotic cell death in HD lymphoblasts. Greenamyre and associates (Panov et al., 2002) also observed abnormalities of mitochondrial membrane potentials. In their study, lymphoblast mitochondria from HD patients had a lower membrane potential and depolarized at lower calcium loads than did mitochondria from controls. In addition, transgenic mice expressing full-length mutant Htt display similar mitochondrial abnormalities that precede the onset of pathological or behavioral abnormalities.

The mitochondrial abnormalities found in HD patient tissues and model systems may underlie cytotoxic molecular signaling in HD. First, such susceptibility to mitochondrial depolarization can be directly associated with apoptotic signaling, which is initiated by release of cytochrome C or AIF (Green and Reed, 1998; van Loo et al., 2002). Released cytochrome C activates a chain of caspases via activation of Apaf-1. Secondly, such abnormalities impair mitochondrial buffering capacity of cytosolic calcium, which increases susceptibility to excitotoxicity. As a result, intracellular calcium levels are reportedly increased in the YAC-Htt transgenic mice (Hodgson et al., 1999) and PC12 cells expressing the *N*-terminal portion of mutant Htt (Bae, Snyder, and Sawa; unpublished observation). The elevated levels of cytosolic calcium can lead to several cellular dysfunctions, including over-activation of a calcium-dependent protease, calpain. Interestingly, cleavage of Htt by calpain has been reported in HD brains (Kim et al., 2001).

People have paid particular attention to the nucleus in HD pathology, since intranuclear inclusions with Htt immunoreactivity were found in brains of both HD patients and animal models of HD (Davies et al., 1997). Currently, the pathological significance of nuclear inclusions is controversial (Sisodia, 1998). However, the accumulation of Htt in the nucleus may play a role in the pathogenesis. In the YAC-Htt transgenic mice with expanded polyglutamine repeat, nuclear accumulation of Htt, particularly the *N*-terminal truncated form of Htt, was observed from earlier pathological stages (Hodgson et al., 1999). In Htt knock-in mice with the expanded polyglutamine tract, the nuclear accumulation of full-length Htt occurs prior to cleaved Htt and neuronal atrophy in striatal neurons (Wheeler et al., 2000).

Cytotoxicity by mutant Htt in the nucleus may be mediated via interactions with Htt-binding partners, such as CREB-binding protein (CBP) (Steffan et al., 2000), Sp1 (Dunah et al., 2002), TAFII130 (Dunah et al., 2002), glyceraldehydes-3-phosphate dehydrogenase (GAPDH) (Burke et al., 1996), and p53 (Steffan et al., 2000). CBP is depleted from its normal nuclear localization and is present in intranuclear inclusions in HD cell models, Htt transgenic mice brains, and HD autopsy brains (Nucifora et al., 2001). Such recruitment of CBP by mutant Htt decreases CBP-activated gene transcription, and overexpression of CBP rescues mutant-Htt-induced neuronal toxicity.

Htt interacts with the transcriptional activator Sp1 and coactivator TAFII130 (Dunah et al., 2002). Coexpression of Sp1 and TAFII130 in cultured striatal cells from Htt transgenic mice normalizes transcriptional inhibition and cytotoxicity caused by mutant Htt. The blockade of Sp1 function by mutant Htt may be due to a direct interaction of soluble mutant Htt with Sp1, which results in inhibition of Sp1 binding to DNA.

Htt binds to GAPDH, but the pathological significance of this is unclear (Burke et al., 1996). GAPDH translocates to the nucleus under oxidative stress, and nuclear GAPDH displays a gain-of-toxic function (Sawa et al., 1997). Nuclear translocation of Htt seems to be facilitated by GAPDH in some cellular models (Sawa and Snyder, unpublished observations). Thus, GAPDH together with Htt in the nucleus may, at least in part, mediate cytotoxicity of mutant Htt.

The protein p53 may have a particular importance in linking nuclear pathology and mitochondrial pathology. Many genes under the transcriptional control of p53 encode mitochondrial proteins that are directly associated with apoptosis and oxidative stress and include Bax, PUMA, proline oxidase, and PIG3 (Vogelstein et al., 2000). Mitochondrial dysfunction frequently occurs by oxidative stress. Since mutant Htt augments p53, it is conceivable that augmented p53 in the nucleus modifies mitochondrial functions, which may be associated with cell death signaling described above (Bae et al., manuscript submitted for publication).

Taken together, mutant Htt is expected to modulate functions of nuclear proteins, including important transcription factors. Thus, several groups have studied gene-expression changes mediated by mutant Htt, and one representative study reported diminished levels of mRNAs encoding components of the neurotransmitter, calcium, and retinoid signaling pathways in HD animal models (Luthi-Carter et al., 2000).

In addition to signaling cascades associated with the mitochondria and the nucleus, there are several other important death cascades that may play roles in HD pathology.

In neurons, the PI3K and Akt pathway functions as cytoprotective. In contrast, JNK and p38 play cytotoxic roles. Htt is reportedly phosphorylated directly by Akt, and the phosphorylation seems to be cytoprotective in striatal neurons with mutant Htt (Humbert et al., 2002). Apoptosis signal-regulating kinase 1 (ASK1) is activated under oxidative stress and ER stress that occurs in association with unfolded proteins (Matsuzawa and Ichijo, 2001). ASK1 is located upstream of JNK signaling. Essential roles of ASK1 in ER stress-induced neuronal cell death triggered by the expanded polyglutamine tract have been reported (Nishitoh et al., 2002).

Coexpression of human Hsp70 ameliorates polyglutamine toxicity, suppressing degeneration (Warrick et al., 1999). Despite the controversial role of Htt aggregate formations, unfolded and mis-folded proteins occurring in HD brains, as intermediate materials before forming aggregates, may have toxic properties. Molecular chaperones and heat-shock proteins in HD should have an important role in preventing these toxic cascades.

Question 2: How can mutant Htt cause selective neuronal cell death of HD brains?

Mutant Htt protein is widely expressed in both the central nervous system (CNS) and non-CNS tissues (Gutekunst et al., 1995; Sharp et al., 1995). Thus, it is still unclear as to why medium spiny striatal neurons selectively die in HD. One possible explanation attributes cell-type-specific interactions of Htt with other proteins that are associated with cell-death signaling. Htt interactors that are expressed exclusively in the striatum may play roles in HD pathology. As the cleavage of Htt by caspases and other enzymes is believed to mediate, or at least accelerate, HD pathology, cell-type preferential Htt cleavage in medium spiny striatal neuron can also be postulated.

An alternative possibility is an increase in the copy number of CAG repeats present in the mutant gene. Kennedy and Shelbourne (2000) investigated the mutation profile in somatic tissues derived from an accurate knock-in mouse model of HD. These mice, generated by inserting a 72–80 CAG repeat into the mouse counterpart of the human Htt gene, express full-length mutant Htt appropriately and display behavioral and pathological features reminiscent of early HD. These workers observed unexpectedly high levels of CAG repeat length variation in many tissues of the HD mice. The size of the mutations, particularly in some striatal cells, was much greater than previously seen in corresponding human tissue. Age-dependent and tissue-specific CAG repeat instability in Htt knock-in mice was reported by another group (Ishiguro et al., 2001).

As described above, much evidence suggests the involvement of excitotoxicity in HD (Beal, 1994). In medium spiny neurons, a selective combination of NMDA receptor subtypes, NR1A/NR2B is expressed. In HEK293 cells expressing mutant Htt, the co-expression of NR1A/NR2B-type NMDA receptors endows susceptibility to apoptotic cell death, but the co-expression of NR1A/NR2A does not (Zeron et al., 2001). Moreover, striatal neurons from YAC transgenic mice expressing full-length mutant Htt display a greater vulnerability to excitotoxic death, compared with striatal neurons from wild-type mice, and the NMDA-induced cell death is abolished by an NR2B subtype-specific antagonist (Zeron et al., 2002). These results suggest that the unique expression of a specific NMDA receptor subtypes in medium spiny neurons may, at least partly, contribute to their specific neuronal death in HD.

Conclusion

Mutant Htt seems to activate many death cascades. We should be cautious in considering their pathophysiological relevance to HD pathology. We will need efforts to integrate the cascades with hierarchy. Such organization may help to narrow down future therapeutic targets for HD.

Acknowledgements

We thank Ms. Y. Lema for preparation of the figures and the references. We thank Ms. J. Cheah and N. Zaller for their critical reading of the manuscript.

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