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Increased mitochondrial fission and neuronal dysfunction in Huntington's disease: implications for molecular inhibitors of excessive mitochondrial fission

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Huntington's disease (HD) is a fatal, progressive neurodegenerative disease with an autosomal dominant inheritance, characterized by chorea, involuntary movements of the limbs and cognitive impairments. Since identification of the HD gene in 1993, tremendous progress has been made in identifying underlying mechanisms involved in HD pathogenesis and progression, and in developing and testing molecular therapeutic targets, using cell and animal models of HD. Recent studies have found that mutant Huntingtin (mHtt) interacts with Dynamin-related protein 1 (Drp1), causing excessive fragmentation of mitochondria, leading to abnormal mitochondrial dynamics and neuronal damage in HD-affected neurons. Some progress has been made in developing molecules that can reduce excessive mitochondrial fission while maintaining both the normal balance between mitochondrial fusion and fission, and normal mitochondrial function in diseases in which excessive mitochondrial fission has been implicated. In this article, we highlight investigations that are determining the involvement of excessive mitochondrial fission in HD pathogenesis, and that are developing inhibitors of excessive mitochondrial fission for potential therapeutic applications.

HD is a fatal, progressive neurodegenerative disease, characterized by involuntary movements, chorea, dystonia, cognitive decline, intellectual impairment and emotional disturbances [1–4]. HD is a midlife disease and mainly found in individuals of Caucasian origin. The prevalence ranges from approximately four to ten individuals in 100,000 [5]. A progressive loss of body weight is a major factor in disease progression in patients with HD [6]. Reduced volume of frontal and temporal cortical lobes and an atrophy of striatum were found in HD brains [7,8]. A marked decrease in glucose utilization in the striatum was shown to correlate with several scores in performance-task difficulties in patients with HD, including immediate recall memory, verbal associative learning and executive functions, suggesting that cerebral glucose metabolism is relevant to HD [9,10].

Histopathological examination of brains from patients with HD revealed that several regions of the brain are affected, including caudate and putamen of the striatum, cerebral cortex, hippocampus hypothalamus and subthalamus. Genetic mutation that causes HD has been identified as an expanded polyglutamine-encoding repeat (or CAG repeat). This mutation is located in exon 1 of the HD gene. In unaffected individuals, polyglutamine repeats are highly polymorphic, whereas in patients with HD, the CAG repeat length ranges from 36 to 120 [5]. The CAG repeat length was found to increase in every generation of male patients with HD who inherited the CAG repeats. This phenomenon, referred to as 'genetic anticipation' [5] and CAG repeats, correlates inversely with disease progression in patients with HD.

Htt, a 350-kDa protein, is ubiquitously expressed in the brain and peripheral tissues of patients with HD. Htt has been typically a cytosolic protein. However, a small portion of mHtt as been found in several subcellular organelles, including the nucleus, plasma membrane, mitochondria, lysosomes and endoplasmic reticulum; and the translocated Htt has been found to impair organelle function [11–15]. In addition, mHtt protein aggregates were found in the brains of patients with HD and brain specimens from HD mouse models, mainly in the sites of pathology.

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The mechanisms underlying neuronal damage in patients with HD are not well understood. However, the following cellular changes and pathways have been proposed to explain these underlying mechanisms, including: transcriptional dysregulation, expanded polyglutamine repeat protein interactions, calcium dyshomeostasis, defects in axonal trafficking and abnormal mitochondrial dynamics.

Recent studies of HD pathogenesis [16–21] have focused on elucidating impaired mitochondrial dynamics, particularly excessive fragmentation of mitochondria and the subsequent mitochondrial dysfunction, and defective axonal trafficking and synaptic damage in HD-affected neurons. Several groups [17,18] have recently found mHtt interacting with the mitochondrial fission protein Drp1, elevated levels of GTPase Drp1, enzymatic activity, and increased fission and reduced fusion in HD-affected neurons. Furthermore, some progress has been made in identifying molecules that are capable of reducing excessive mitochondrial fission and consequently maintaining healthy mitochondria and neuronal function in HD neurons.

In this article, we highlight recent developments in HD research, with a particular focus on mitochondria and mHtt. We also discuss recent advances in developing therapeutic molecules that inhibit excessive mitochondrial fission.

Mitochondrial abnormalities

Recent research has revealed multiple alterations in mitochondria, in HD progression and pathogenesis, including: (i) reduced enzymatic activity in several components of oxidative phosphorylation, including complexes II, III and IV of the electron transport chain, in HD postmortem brains and HD mouse models [22–24], suggesting that mitochondria are involved in HD pathogenesis; (ii) low mitochondrial ATP and decreased mitochondrial ADP uptake in HD knock-in striatal cells and lymphoblasts from patients with HD [25]; (iii) defective calcium-induced mitochondrial permeability in HD cell lines and HD mice (reviewed in [26]); (iv) mHtt-induced defective mitochondrial trafficking in HD primary neurons [15,17,18,27]; (v) age-dependent mitochondrial (mt)DNA damage and mtDNA deletions in HD-affected neurons [28,29]; and (vi) biochemical, confocal and electron microscopy studies revealed structurally damaged mitochondria with broken cristae, and small and round mitochondria in HD-affected neurons [18–21] (Fig. 1).

Abnormal mitochondrial dynamics

Mounting evidence suggests that structural and functional abnormalities in mitochondria are involved in HD pathogenesis [16,18–21]. In neurons that express mHtt, an imbalance between fission and fusion was found to lead to abnormalities in mitochondrial structure and function, and to damaged neurons. Several studies have reported such abnormal mitochondrial dynamics in patients with HD [16–20], HD mouse models [17,18], damaged HD lymphoblasts, HD cell lines and primary neurons that express mHtt [17–19,21].

Recently, we studied abnormal mitochondrial dynamics in tissues from postmortem brains of patients with HD3 (symptomatic with 80% neuronal loss [1]) or HD4 (advanced stage with over 90% neuronal loss [1]) [16] and primary neurons from BACHD transgenic mice [18]. In the 2011 study of postmortem HD brains, we found increased levels of Drp1 and Mitochondrial fission 1 (Fis1) in HD4 rather than in HD3, in HD-affected brain regions but not in HD-unaffected brain regions [16]. We also found reduced expressions of Mitofusin-1 and 2 (Mfn1 and Mfn2) and optic atrophy 4 (Opa1) in HD4 rather than in HD3, in HD-affected brain regions but not in HD-unaffected brain regions. Taken together, these findings suggest that abnormal mitochondrial dynamics are related to HD [16].

Using BACHD mice that express the full-length human Htt gene with 97 CAA and CAG mixed repeats, we studied mHtt, and mitochondrial and synaptic genes [17]. We found significantly increased mRNA levels of Drp1, Fis1 and Cyclophilin D (CypD), and decreased levels of Mfn1 and Mfn2 in 2-month-old BACHD mice relative to age-matched wild type mice, suggesting that abnormal mitochondrial dynamics is an early event in HD progression [17].

Furthermore, to determine whether Drp1 interacts with mutant Htt, we performed co-immunoprecipitation (IP) of Drp1 and of mHtt from HD postmortem brains [17]. We found an 82-kDa and a 40-kDa mHtt protein in IP elutes from patients with HD3 or HD4. We also found Drp1 interacting with wild type Htt in brain specimens from the patients with HD3 or HD4 patients, but to lesser extent than in the control subjects, suggesting that mHtt, interacting with Drp1, is specific and is related to HD progression. Using cortical protein lysates from BACHD mice and wild type mice, we also conducted co-IP analysis. Our results were similar to results from our IP analysis of Drp1 from HD brains. We found Drp1 interacting with mHtt in HD neurons, suggesting that Drp1 participates in mitochondrial fission and impaired mitochondrial biogenesis. We also measured GTPase Drp1 enzymatic activity in brain specimens from patients with HD and from BACHD mice to determine whether increased Drp1, which interacts with mHtt, enhances GTPase activity and results in excessive mitochondrial fission. We found increased levels of Dp1 enzymatic activity in the cortex but not in the cerebellum of patients with HD3 or HD4 relative to Drp1 enzymatic activity in control subjects. We also found elevated levels of Drp1 enzymatic activity in the cerebral cortex and striatum from the BACHD mice relative to the levels of Drp1 enzymatic activity in the cerebral cortex and striatum specimens from wild type mice. Furthermore, using primary neurons from BACHD mice and wild type mice, live-cell imaging techniques and DsRed-mito transfections, we studied mitochondrial transport along the axonal projections of primary neurons from BACHD and wild type mice. We found significantly decreased anterograde movement of the mitochondria in the primary neurons from the BACHD mice relative to the wild type neurons [17].

Song and colleagues [18] studied the effects of mHtt on the fission–fusion balance in mitochondria in HD pathogenesis. They found that mHtt triggers mitochondrial fission *in vitro*, in rat neurons and in fibroblasts from patients with HD; and *in vivo*, in a mouse model of HD. These events occurred before the development of neurological deficits and mHtt aggregates. mHtt interacted abnormally with Drp1 in mice and in humans with HD, and this interaction, in turn, stimulated the enzymatic activity of GTPase Drp1. Furthermore, a reduction of Drp1 GTPase activity, in association with the dominant-negative Drp1 K38A mutant, appeared to rescue mHtt-mediated mitochondrial fission, defects

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