Huntington’s disease

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Abstract
The most recent findings in the elucidation of the molecular pathology of Huntington’s disease are reviewed. Particular interest has been paid to the role of huntingtin and its associated proteins in excitotoxicity mediated via NMDA and kainate receptors.

Keywords: Huntington’s disease; neurodegenerative disorder; Huntington; NMDA receptors; kainate receptors

This short review sets out to consider the most recent findings in the search to elucidate the pathological mechanisms underlying the fatal, neurodegenerative disorder known as Huntington’s disease (HD), first described by George Huntington in 1872. HD involves the extrapyramidal motor system and is characterised by chorea, a dementia which is progressive, and other psychiatric symptoms. In terms of brain pathology, GABAergic medium spiny striatal neurones are most affected, resulting in atrophy of the caudate nucleus, putamen, and globus pallidus; there is also pronounced atrophy of the cerebral cortex. The neuronal loss in the striatum is accompanied by pronounced gliosis. The vulnerable striatal neurones contain enkephalin, dynorphin, and substance P; and primarily innervate the substantia nigra and globus pallidus. Medium sized spiny neurones containing somatostatin, neuropeptide Y, and NADPH diaphorase, in addition to cholinergic interneurones and parvalbumin, in the striatum is accompanied by pronounced gliosis. The excitotoxicity hypothesis plays a central role in the pathogenesis of HD. Nevertheless, other pathways—oxidative stress, impaired energy metabolism, apoptosis, and abnormal protein–protein interactions—which in turn may cause transcriptional dysregulation and altered gene expression, have also attracted interest.

Table 1 Receptor changes in Huntington’s disease

<table>
<thead>
<tr>
<th>Decreased</th>
<th>Increased</th>
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<tr>
<td>Receptor</td>
<td>Regions</td>
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<tr>
<td>Early clinical phase</td>
<td></td>
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<tr>
<td>Cannabinoid CB (1)</td>
<td>CN, P, GPE</td>
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<tr>
<td>Dopamine D2</td>
<td>CN, P, GPE</td>
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<tr>
<td>Adenosine A (2a)</td>
<td>CN, P, GPE</td>
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<tr>
<td>Intermediate clinical phases</td>
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<tr>
<td>Dopamine D1</td>
<td>CN, P, SN</td>
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<tr>
<td>Final clinical phase</td>
<td></td>
</tr>
<tr>
<td>Dopamine D1</td>
<td>CN, P, GPI</td>
</tr>
</tbody>
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CN, caudate nucleus; GPE, globus pallidus externum; GPI, globus pallidus internum; P, putamen; SN, substantia nigra.
much interest. Central to all these have been attempts to understand the role of huntingtin in normal physiology and in HD.

The role of huntingtin in HD

Huntingtin has no great structural homology with other human proteins, so determination of its normal function has proved difficult. Changes seen in embryonic stem cells of huntingtin knockout mice indicate that it is essential for normal nuclear and perinuclear organelles and that one factor regulating its synthesis is iron. The protein (∼349 kDa) is composed of approximately 3150 amino acids (depending on the size of the polyQ tract). As with many other large proteins, huntingtin is made up of repeated structures. The polyQ tract starts at residue 18. In addition, there are 10 Huntington elongation factor 3 (a subunit of protein phosphatase 2A-TORI (HEAT)) repeats, each of which is a loosely conserved repeating segment of approximately 40 amino acids, forming two hydrophobic α helices and a polymorphic polyproline region. The protein is widely distributed throughout the central nervous system (CNS), and this has posed the question of how its altered functioning gives rise to selective neuronal loss in HD. Initially, the most likely explanation was thought to be that the huntingtin gene mutations confer a “deleterious gain of function”, with the concept of loss of function gaining ground more recently, although how the variant huntingtins go on to cause neurodegeneration has remained a mystery.

HUNTINGTIN, EXCITOTOXICITY, AND APOPTOSIS

Although the above evidence supports the concept that aggregate formation is a causal agent in neuronal death, it is unclear whether this is the sole mechanism and how variant huntingtins relate to other features of the pathology, such as excitotoxicity. Much has been written about excitotoxicity via the NMDA and kainate receptors in HD (recent, extensive reviews on the structures and functions of these are by Chittajullu et al., Lerma et al., Cull-Candy et al., and Ravenscroft and Brodtie). Over the past two years, much of the intracellular signaling cascade underlying excitotoxicity in HD has been pieced together. Key players in this are the NMDA and kainate receptors, a protein with the formidable name of “post-synaptic density protein 95” (also known as synapse associated protein 90), abbreviated here to PSDP-95, mixed lineage kinase (MLK), and c-Jun-N-terminal kinase (JNK). Glutamate receptor stimulation was shown previously to be involved in MLK-1 activation, and in a separate study JNK activation was linked to apoptosis. Now the connection between these processes and the roles of huntingtin and PSDP-95 in excitotoxicity are becoming more clearly understood. PSDP-95 is a scaffold protein, which binds to several intracellular proteins and possesses guanylate cyclase activity. PSDP-95 binds via repeat units in its structure, termed PDZ domains, to the NMDA receptor. PSDP-95 plays a pivotal role in regulating synaptic plasticity and synaptogenesis. Specifically, PSDP-95 binds to the NR2 NMDA receptor and kainate receptor, GluR6, subunits. Variant huntingtins with expanded polyQ tracts interfere with the binding of PSD-95 to NMDA and kainate glutamate receptors, causing both receptors to become hypersensitive, thereby allowing increased Ca influx via specific uptake pathways. In turn, the MLK isoform, MLK-2, is activated, causing activation of MAP kinase kinases 4 and
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7 and stress signalling kinase, SEK-1, and consequently JNK-2 (dominant negative SEK-1 blocks JNK-2 activation). Activated JNK2 phosphorylates the N-terminal region of c-Jun, which is one half of the transcription factor AP-1, thus modulating gene transcription. Activated JNK also phosphorylates the C-terminal region of MLK-2. This step appears to be crucial in the triggering of apoptotic cell death. Cotransfection of a dominant negative MLK-2 blocks apoptotic cell death induced by variant huntingtins.

Interestingly, full length variant huntingtins, rather than N-terminal fragments, were found to be potent triggers of apoptosis in model cells expressing the NMDA receptor subtype prevalent in human striatal medium spiny neurones. Therefore, both full length variant huntingtins and nuclear N-terminal fragment aggregates cause cell death by different mechanisms. The actions of the full length variants may account for the observation that cellular dysfunction can be seen before aggregate formation in the nucleus.

MULTIPLE PATHWAYS LEADING TO CELL DEATH

There is continuing evidence that variant huntingtins affect multiple pathways, which potentially lead to cell death. One such pathway is the deprivation of neurotrophic support. Brain derived neurotrophic factor (BDNF) expression is reduced in the caudate and putamen of patients with HD. That enhanced expression of neurotrophic factors may mitigate the effects of neurotoxins and thus be a potential therapeutic strategy was explored in animal and cell models. Both BDNF, nerve growth factor 3 (NGF-3), and NGF4/5 support proved effective treatments against quinolinic acid treatment in the various models, with BDNF being the most effective, opening the possibility of treatment by implantation of neurotrophic factor releasing systems.

One potential cause of cell death that has received much interest in several neurodegenerative diseases, including HD, is oxidative stress. However, a detailed investigation of oxidative stress indices in the caudate nucleus, putamen, and frontal cortex of HD brains revealed no alterations in concentrations of thiobarbituric acid–malondialdehyde adducts and lipid peroxides (measures of lipid peroxidation), or of oxidative DNA and protein damage. These data strongly suggest that oxidative stress is not a major contributor to neurodegeneration in HD. However, evidence indicates that an alternative marker of oxidation—isoprostane concentration—is increased. This has been reviewed by Greco et al.

Reduced energy production as a consequence of defective mitochondrial complex II function is a well established feature of the late stages of HD. Garseth et al presented evidence of reduced lactate and citrate in the cerebrospinal fluid (CSF) of patients with HD, and this led them to suggest that the decreased citrate values were indicative of astrocytic and neuronal dysfunction. Whether the energy deficit is a cause or consequence of the disease is uncertain. Using a 31P nuclear magnetic resonance assay, Lodi et al found that the phosphocreatine to inorganic phosphate ratio was reduced in muscle tissue of both symptomatic and presymptomatic gene positive individuals, indicating that impaired energy production is an early feature of the disease. In contrast, Guidetti et al found no evidence of change in the functioning of mitochondrial complexes I to IV in the neonstriatum and cortex of presymptomatic individuals and patients with mild symptoms. Guidetti et al also found no change in the activities of complexes I to IV in the striatum and cortex of transgenic mice expressing full length polyQ expanded huntingtin before changes in the morphology of striatal and cortical neurons. They concluded that the changes in energy metabolism were a consequence rather than a cause of the disease.

However, another group using a different animal model—3-nitropropionic acid treatments—came to conclusions suggesting that energy impairment was at the root of the disease. Calabresi et al found that treatment with 3-nitropropionic acid, which is a specific inhibitor of complex II, produced long term NMDA mediated excitation in striatal medium spiny neurones. This effect was dopamine dependent, with dopamine acting via D2 receptors, whereas it was negatively regulated via D1 receptors, all of which mirrors the human disease. In a quinolinic acid treated rat model, energy metabolism, as measured by 2-deoxy-(18F)fluoro-D-glucose uptake was impaired within one week of lesioning, and this impairment deepened with time. However, D2 receptor expression did not simply follow the changes in glucose analogue uptake. One week after lesioning D2 receptor levels increased, after which there was a progressive decline. These last two sets of findings raise the question of the relation of animal models to the human condition. They clearly demonstrate changes that parallel pathological changes seen in humans but leave the question of the ultimate cause of HD unanswered.

Another potential cause of cell dysfunction in HD is disruption of gene transcription by binding of variant huntingtins to transcription factors and intracellular signalling proteins. Of particular interest have been the interactions with the cAMP response element (CRE) binding protein and p53. N-terminal huntingtin fragments were shown to colocalise with both of these proteins in nuclear aggregates in cell culture and transgenic mouse models. This colocalisation within nuclear aggregates occurs because of interaction between the polyQ repeats in huntingtin and a similar short repeat in the CRE binding protein.

Protein kinase C (PKC) is thought to be important in long term potentiation affecting brain plasticity and learning. In symptomatic transgenic mice expressing polyQ expanded huntingtin steady state levels of PKC beta II mRNAs were reduced in brain compared with control animals. This parallels the reduction of this mRNA in the caudate-putamen of human HD brain. It was postulated that the reduction

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in PKC may play a part in the reduced ability to store information in HD. The multiplicity of other pathways potentially affected in HD is illustrated by the number of proteins recognised to interact with huntingtin. These are known either as huntingtin associated proteins (HAPs), huntingtin interaction proteins (HIPs), or huntingtin–yeast partners (HYPs). Examples are HAP-1, HYP-J (α adaptin C), and HIP-3, which are associated with vesicular trafficking. HYP-A and HYP-I have nuclear functions, HIP-2 is a ubiquitin conjugating enzyme, and HYP-F is part of the 26S proteosome. New members of these categories are still being discovered and the physiological functions of recognised ones elucidated. Most recently, the functions of HIP-1 have been clarified. This molecule is encoded by a large gene of 32 exons, scanning 215 kb, which gives rise to two transcripts. The translation product is involved in D1 receptor trafficking, linking huntingtin and the endocytic protein, clathrin. In an immortalised striatal neuronal cell line, dopamine altered the subcellular localisation of huntingtin, resulting in an increase in membrane fragments containing HIP-1 and clathrin. Another recently described member of the HIP family (the gene located on 12q24) HIP-1R was also shown to be involved in endocytosis. The C-terminal, talin-like domain of this protein binds to F-actin, thus linking the endocytic machinery with the actin cytoskeleton of cells. HIP-1 is also a pro-apoptotic protein, the overexpression of which results in rapid caspase 3 dependent cell death via the intrinsic pathway of apoptosis. The association of huntingtin with HIP-1 attenuated the pro-apoptotic actions of HIP-1. Interestingly, wild-type huntingtin binds more strongly with HIP-1 than do the polyQ expanded forms, which thus represents another potential neurodegenerative mechanism in HD. The latest HAPs and HIPs to be identified are HAP40 and HIP-12. HAP40 is a 40 kDa protein encoded within intron 22 of the factor VIII gene. In the presence of normal huntingtin, HAP 40 is located in the cytosol, and in its absence it is actively transferred to the nucleus, although the consequences of this are not known. HIP-12 was discovered while elucidating the gene structure of HIP-1. HIP-12 has no detectable pro-apoptotic properties, does not bind to huntingtin, but does bind to HIP-1.

A model of cell death in neurodegenerative disease

Finally, in this brief review, mention should be made of an advance in the understanding of the rate of cell death in neurodegenerative diseases. Clarke et al studied photoreceptor decay in model systems and, with reference to HD, the uptake of 18F-glucose into the caudate nucleus of patients with HD, this being regarded as a measure of surviving neurones. The mathematical description that best fitted the data was that of a constant risk of cell death, with the qualification that an exponential decrease in the risk of cell death may also be a valid model. The constant risk model means that cell death would be random and that the death of a neurone would be independent of the fate of other neurones. This model proposes that the chance of cell death decreases as the number of surviving neurones decreases. They propose that the features of a neurodegenerative disease such as HD—genotype dependence, the random nature of cell death within a specific population, the constant risk or exponentially decreasing risk of cell death, and the late onset of the clinical signs and symptoms, which clearly means that most neurones survive for a considerable period of time—imply that the organism exists in a steady state. This condition, which term the mutant steady state, is different from the normal healthy state, and is achieved by adaptive changes in metabolism to cope with the effects of the mutant gene product.

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46 Garbett P, Gubellini P, Picconi B, et al. Inhibition of mito-