Huntington’s disease pathology

Huntington’s disease (HD) is an inherited, neurodegenerative disorder that usually presents between the ages of 35 to 50 and for which there is currently no cure. HD is caused by an increase in the length of a CAG tri-nucleotide repeat array within the HD gene, leading to expansion of a polyglutamine tract within the huntingtin protein (htt). The mutant huntingtin protein (mhtt) is known to cause selective degeneration of neurons in the striatum resulting in movement and cognitive dysfunction. The molecular basis of mhtt-dependent HD pathology is unclear, but a recent study has shown that inhibiting cleavage of mhtt by the protease caspase-6 is sufficient to halt death of striatal neurons and behavioral dysfunction in a rodent model of HD. In this report, current hypotheses concerning the molecular basis of HD, and existing and experimental therapies for treating HD will be reviewed with particular emphasis on the potential development of anti-caspase-6 drugs that may provide the most promising breakthrough yet for treating this devastating disorder.

There are currently five predominant hypotheses to explain how a genetic abnormality in the HD allele results in neuronal dysfunction and the clinical manifestations of HD (Figure 1). First, HD aggregates become highly ubiquinated in an attempt by the ubiquitin-proteasome system (UPS) to degrade and clear them from the cell. Ultimately their abundance overwhelms this cellular response, leading to dysregulation of the UPS and the accumulation of toxic intracellular fragments. Second, aggregation of mhtt in the nucleus has been shown to sequester transcription factors, such as p53, CAMP response element binding protein (CREB-binding protein), specificity protein 1 and TATA-binding protein. Their sequestration leads to a large disruption of gene expression, which impairs normal cellular functioning. Third, excitotoxicity has been shown to contribute to HD pathogenesis. Excitotoxicity is caused by the overstimulation of N-methyl-D-aspartic acid (NMDA)

Figure 1. The major pathological mechanisms involved with Huntington’s Disease: Impairment of the ubiquitin-proteasome system (1), transcription dysregulation (2), excitotoxicity (3), mitochondrial toxicity and oxidative stress (4), and apoptosis (5). Figure adapted from Landle and Bates, 2004.
and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors by glutamate. Excitotoxicity results in a large influx of intracellular calcium, the generation of reactive oxygen species, the release of cytochrome c from the mitochondria and the initiation of apoptosis. Post-mortem analyses of the brains of HD patients reveals a significant reduction in the expression of NMDA receptors, implying that the cells expressing these receptors have already died due to excitotoxicity. In animal models there is additional evidence for the involvement of excitotoxicity in HD pathology. It has been shown that there is decreased expression of the major astroglial glutamate transporter (GLT1) in the striatum of a rodent model of HD, which leads to decreased glutamate uptake from the synapse. In addition, it has been shown that there is an increased release of glutamate in the striatum of HD mice. Both of these events lead to an increase in synaptic glutamate concentration, which results in excitotoxicity of striatal neurons. Fourth, pathological analyses of the brains of HD patients reveals increased accumulation of lipofuscin, a marker of lipid peroxidation, and increased levels of 8-hydroxy-2-deoxyguanosine, a molecular marker for oxidative damage to DNA. Both of these markers indicate the involvement of oxidative stress in HD.

The emerging picture of the underlying pathophysiological mechanism preceding HD is very complex, consisting of many interconnected pathways. In spite of the growing body of knowledge surrounding HD pathology, few compounds have been developed to prevent neurodegeneration. The following section will present current therapies used in the treatment of HD, highlighting the fact that few of them have proven useful in slowing disease progression.

Current therapies for Huntington’s disease

To date, most therapeutic strategies for treating HD have focused solely on addressing the associated symptoms including chorea, dystonia, dyskinesia, depression and psychosis. Neuroleptics, such as pimozide, olanzapine, fluphenazine and haloperidol, function by antagonizing the effect of dopamine in the central nervous system and have been used widely in the treatment of chorea. High doses of neuroleptics, however, have been associated with unwanted side effects including impairment of oculomotor function, orolingual function, swallowing and fine motor tasks. NMDA receptor antagonists, such as amantadine and riluzole, have also been shown to reduce chorea in HD patients. In addition to their symptomatic benefit, these compounds may also prove useful in slowing HD progression by blocking excitotoxicity. Few clinical studies have been done to investigate the efficacy of compounds at reducing dystonia in HD patients, but generally anticholinergic drugs, diazepam or botulinum toxins have been prescribed. Traditional antidepressants, such as selective serotonin reuptake inhibitors, tricyclic antidepressants and sedatives, have been used in the treatment of depression caused by HD. Interestingly, although 40% of HD patients report experiencing depression, only 2% of these patients receive treatment for it, indicating that it is a feature of HD that commonly goes undiagnosed or under treated. A more complete overview of the treatment options available for HD are outlined in Figure 2.

Several neuroprotective compounds, such as coenzyme Q10, remacemide, alpha-tocopherol, lamotrigine and idebenone, have undergone clinical trials in hopes of slowing HD progression; however, none of these compounds have provided significant improvements compared to control groups. Clinical studies investigating the utility of the anti-apoptotic antibiotic minocycline at slowing HD progression have provided encouraging results, although reproducibility has been an issue. Preclinical studies in a HD rodent model and in Drosophila indicate that histone deactylase inhibitors...
Inhibition of caspase-6: A potential therapy for Huntington’s Disease

Inhibition of caspase-6 by selective mutation in the caspase-6 cleavage site in mhtt and site-directed mutagenesis of all five caspase cleavage sites demonstrated that mutating the amino acid sequence of mhtt may have caused a conformational change in the protein, leading to a reduction in its toxic effects. The authors argue that this explanation is unlikely for two reasons. First, both htt and mhtt have been shown to possess anti-apoptotic effects during embryonic development. Mice that do not express htt do not survive past embryonic day 7.5. Caspase-6 resistant HD mice were able to undergo normal embryonic development, which demonstrates that caspase-6 resistant mhtt retained its anti-apoptotic effects. Second, caspase-6 resistant HD mice were resistant to excitotoxicity, which is consistent with the observation that htt is protective against excitotoxic insults. Both of these observations demonstrate that mutating the caspase-6 cleavage site in mhtt did not affect its physiological function and presumably its three dimensional configuration.

Conclusion

HD is a devastating, inherited, neurodegenerative disorder. Currently, no cure exists for HD and modern therapies have minimal benefit, attempting solely to decrease HD symptoms. In spite of an increasingly complex pathological network underlying HD, caspase-6 cleavage of mhtt represents an early event triggering disease progression. The caspase-6 study suggests that inhibition of caspase-6 might be a novel therapeutic target in preventing HD or slowing its progression.

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References

5. Huntington’s Disease Collaborative Research Group. A novel gene containing HDAC are able to attenuate the toxic effects of mhtt by normalizing the transcriptional dysregulation associated with HD. Recently, the HDAC phenylbutyrate has been shown to normalize mRNA levels in 10 out of 12 HD patients in an ongoing phase II clinical trial, indicating its possible usefulness as a therapeutic intervention. Due to the interaction of many cellular pathways, it appears likely that a combined neuroprotective strategy will be necessary in order to successfully slow HD progression. In spite of the complex pathophysiology underlying HD, a recent study has identified an exciting new molecular event leading to the onset of HD in a rodent model.

Cleavage of mhtt by caspase-6 is necessary for the initiation of Huntington’s disease

Whatever the ultimate cause of HD, involvement of the caspase-mediated apoptotic pathway is a likely contributor to HD pathogenesis. Many cleavage sites for proteases, calpains, caspases and endopeptidases have been identified near the amino-terminus of mhtt. Cleavage of mhtt by caspase-3 and caspase-6 occurs in vitro, and caspase cleavage fragments are present early on in HD patients. Selective site-directed mutagenesis of all five caspase cleavage sites in mhtt inhibits cytotoxicity in a cellular model of HD. Interestingly, although transgenic mice expressing a truncated amino-terminal fragment of mhtt display intracellular inclusions and behavioral deficits, they do not display neurodegeneration and HD pathology. This suggests that it is a specific proteolytic cleavage fragment of mhtt that directly results in cellular pathology and not any arbitrary amino-terminal mhtt fragment.

Development of a caspase-6 resistant HD mouse demonstrated that cleavage of mhtt by caspase-6 is necessary for the initiation of striatal neurodegeneration and behavioral pathology in a rodent model of HD. These mice have a selective mutation in the caspase-6 cleavage site in mhtt and thus the mutant protein cannot be cleaved by caspase-6. The results of this study showed that mice containing a wild-type allele and mice containing a caspase-6 resistant HD allele were indistinguishable in terms of brain weight and striatal volume, whereas mice with a caspase-6 sensitive mutant HD gene exhibited a significant decrease in striatal volume and striatal neuronal cell counts. The characteristic formation of intranuclear inclusions was delayed in the caspase-6 resistant mice compared to the caspase-6 sensitive HD mice. Also, caspase-6 resistant HD mice exhibited increased tolerance to NMDA-receptor stimulation compared to HD mice. This is consistent with the observation that excitotoxicity plays an important role in HD pathogenesis and shows that the caspase-6 cleavage fragment of mhtt increases susceptibility to excitotoxic stress. Caspase-6 resistant HD mice did not show any behavioral deficits on the rotarod test compared to wild-type animals up to 10 months, whereas caspase-6 sensitive HD mice showed a severe deficit beginning at 2 months. Also, caspase-6 resistant HD mice were indistinguishable from wild-type mice when assessed in an open-field trial, whereas caspase-6 sensitive HD mice exhibited a hypokinetic behavior.

The authors of this study hypothesized that the inability of caspase-6 resistant HD mice to generate the caspase-6 cleavage fragment of mhtt was the reason for their delayed HD onset. An alternative hypothesis suggests that mutating the amino acid sequence of mhtt may have caused a conformational change in the protein, leading to a reduction in its toxic effects. The authors argue that this explanation is unlikely for two reasons. First, both htt and mhtt have been shown to possess anti-apoptotic effects during embryonic development. Mice that do not express htt do not survive past embryonic day 7.5. Caspase-6 resistant HD mice were able to undergo normal embryonic development, which demonstrates that caspase-6 resistant mhtt retained its anti-apoptotic effects. Second, caspase-6 resistant HD mice were resistant to excitotoxicity, which is consistent with the observation that htt is protective against excitotoxic insults. Both of these observations demonstrate that mutating the caspase-6 cleavage site in mhtt did not affect its physiological function and presumably its three dimensional configuration.


