Huntington's disease: Molecular basis of pathology and status of current therapeutic approaches (Review)

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Abstract. Huntington's disease (HD) is a frequent and incurable hereditary neurodegenerative disorder that impairs motor and cognitive functions. Mutations in huntingtin (HTT) protein, which is essential for neuronal development, lead to the development of HD. An increase in the number of CAG repeats within the *HTT* gene, which lead to an expansion of polyglutamine tract in the resulting mutated HTT protein, which is toxic, is the causative factor of HD. Although the molecular basis of HD is known, there is no known cure for this disease other than symptomatic relief treatment approaches. The toxicity of mutHTT appears to be more detrimental to striatal medium spiny neurons, which degenerate in this disease. Therapeutic strategies addressing a reduction in the mutHTT content at the transcriptional level using zinc finger proteins and at the translational level with RNA interference and antisense oligonucleotides or promoting the proteosomal degradation of mutHTT are being studied extensively in preclinical models and also to a limited extent in clinical trials. The post-translational modification of mutHTT is another possibility that is currently being investigated for drug development. In addition to the pharmacological approaches, several lines of evidence suggested the potential therapeutic use of stem cell therapy, in particular using the patient-derived induced pluripotent stem cells, to replace the lost striatal neurons. The multi-pronged clinical investigations currently underway may identify therapies and potentially improve the quality of life for the HD patients in future.

Contents

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1. Introduction

Huntington's disease (HD) is a lethal autosomal dominant and progressive neurodegenerative disorder, that is characterized by motor, cognitive, and behavioral impairment (1). HD incidence is approximately 5-10 in 100,000 individuals worldwide (2) and encompasses psychiatric symptoms (e.g., affective disorders, suicide tendency, mania, apathy, and schizophrenia-like symptoms), cognitive defects (e.g., organizational deficit, lack of attention and motor skill learning deficits), motor impairment (e.g., chorea, rigidity, gait abnormalities, and bradykinesia), sleep disturbance, and weight loss (3).

Despite the identification of the gene that is critical for the pathogenesis of HD as huntingtin (HTT), located in the short arm of chromosome 4, >20 years ago (4), the development of effective therapies for HD are is proving to be formidable. Currently, there are no disease-modifying treatments available other than some approaches to address certain specific symptoms of HD. Onset of HD symptoms emerges usually at 35-45 years of age and varies considerably (5). HD leads to severe brain atrophy and death, with a clinical course that spans >15-20 years (1). Specifically, striatal medium spiny neurons (MSNs) of brain appear to be vulnerable in HD, although potentially other regions of brain can also be affected (6-8). MSNs are GABAergic neurons, are predominant in the striatum (9), and project to the substantia nigra (striatonigral) and globus pallidus (striatopallidal) (10). It has been reported that there is a significant loss of approximately 88% striatal neurons in HD patients as compared to healthy individuals, even though the precise reasons for this selective vulnerability and loss of striatal MSNs is not known (11,12).

2. *HTT* and mutated *HTT*

It has been recognized that the gene encoding huntingtin (*HTT*), present on chromosome 4 is mutated in the exon-1 region with the resultant expansion of a CAG repeat region in this exon. This leads to enlargement of the polyglutamine (polyQ) domain within the HTT protein's N-terminus in HD patients (4). Thus, while the normal *HTT* gene has

approximately 35 CAG repeats, mutations that increase this to ≥40 CAGs, lead to the development of HD with full penetrance, and individuals with 36-39 CAG repeats in *HTT* exhibit variability in the appearance of HD (13). Although HD can be inherited in an autosomal dominant pattern, because of the instability of the number of CAG repeats in *HTT* gene, this number can be different between a parent and child (14,15), with a tendency to increase in the next generation. Such instability of the CAG repeats has also been noted within the same patient's brain and sperm cells, resulting in mosaicism (16). These elevated number of CAG repeats appear to cause either 'gain-of-function' or loss of function of the wild-type HTT with toxic effects, including HD-related cardiac dysfunction (17) and skeletal muscle wasting (18). Most of the mutations in muHTT disrupt its normal function, and promote several pathological protein-protein interactions resulting in neuronal loss and dysfunction in the striatum, cortex and other parts of the brain (19). Thus, mutHTT interferes with several intracellular activities through aberrant interactions as well as the accumulation of mutHTT aggregates, particularly in the cell nucleus and neurophil of the affected neurons, ultimately disrupting several cell processes, including protein degradation, mitochondrial respiration and transcription, leading to neuronal malfunction and cell death (20). Apparently the increase in CAG repeats can be as high as 1,000 in certain subsets of striatal neurons while in other brain regions the increase is much lower (21). Recent genome-wide single nucleotide polymorphism (SNP) association studies showed that MLH1 (MutL homolog 1, a DNA mismatch repair gene) and an SNP within a nuclear factor-κB binding site in the HTT promoter may play a role in the altered onset of HD (5,22).

Notably, although mutations in *HTT* gene lead to the pathogenesis of HD, this is an essential gene for normal neuronal development as knockout of the HTT gene ($Hdh^{-/-}$) is lethal in embryonic mice (23,24). There is significant neurodegeneration and motor and cognitive abnormalities even with a single allele deletion ($Hdh^{+/-}$) in mice (23). Even conditional Hdh deletion in adult mice in the neurons of the forebrain and to a small extent in cerebellum leads to the development of progressive neurodegeneration (25). HTT is a 350-kDa protein with dynamic subcellular localization in nuclei, endoplasmic reticulum, Golgi apparatus and endosomes and is known to have functions in the regulation of cell cycle and cell division (26,27). HTT is also found in axonal processes and synapses in association with microtubules, caveolae and synaptosomes (28).

3. Post-translational modifications of HTT/mutHTT

Post-translational modifications of the HTT protein play an important role in the pathogenesis of HD (Fig. 1). For example, mutHTT is prone to aggregate in neurons, which is suspected to be part of the underlying causes of HD. Although mutHTT is ubiquitinylated, its clearance by the proteosomal system is impaired leading to accumulation of the aggregates (29). HTT is also likely modified by phosphorylation, SUMOylation, acetylation and palmitoylation and these post-translational modifications are important in proper protein-protein interactions of HTT, which can be significantly altered by mutations and polyQ additions (30). Histone acetyltransferase (HAT) enzymes CBP and PCAF were found to be inactivated by mutHTT through protein-protein interactions, leading to transcriptional and chromatin remodeling deregulation and contributing to the pathogenesis of HD (31). It has been suggested that post-translational modifications be exploited for therapeutic purposes to enhance the clearance of mutHTT. Thus, acetylation of the lysine residue K444 in mutHTT enhanced its clearance via autophagosomes (32), whereas the phosphorylation of mutHTT at serine 431 and 432 altered the toxicity and accumulation of mutHTT (33). Phosphorylation of serine residues 13 and 16 reduced its toxicity of mutHTT *in vivo* (34), whereas phosphorylation at serine 421 restored the ability of mutHTT to promote axonal vesicular transport and brain-derived neurotrophic factor release (35).

4. Therapeutic approaches

Therapies that address mutHTT modification and degradation. Due to the interaction-mediated inhibitory effects of mutHTT on HAT enzymes, certain inhibitors of histone deacetylases (HDAC), in particular HDAC4, have been examined for their protective effects in some models of HD. The findings showed that these inhibitors were able to reduce the aggregation of mutHTT and also rescue the neuronal and corticostriatal synaptic function (36,37). Notably, acetylation of mutHTT marks it for ubiquitinylation and subsequent proteosomal degradation and there is a general decline in chromosomal and protein acetylation in HD. Thus, inhibition of HDACs, which sustains an elevated level of protein acetylation, can lead to an increased acetylation status of mutHTT (38). Inhibitors of other deacetylase enzymes such as sirtuin 1, and selisistat are shown to curtail the mutHTT-induced pathology in several model systems (39) and proved to be safe and tolerable in recent phase 1B clinical trials (40). Promotion of the proteolytic breakdown of mHTT through activation of the ubiquitin- proteasome- autophagy system is another pharmacological approach that is being explored (41). Thus, promoting autophagy by inhibiting mTOR with rapamycin, was shown to improve phenotypes in HD models in Drosophila and mouse (38) and similar effects were observed with other autophagy-promoting agents (42). Thus, enhancing autophagy to degrade mutHTT is a viable and important strategy towards HD therapy (Fig. 2).

Considering that selective modulation of phosphorylation of serine residues can be exploited to modulate mutHTT activity, small molecule kinase inhibitors are being tested, even though their selectivity is being investigated (43). Inasmuch as improper folding and aggregation of mutHTT is central to the pathogenesis of HD, attempts are being made to devise cell-permeable chaperones, such as TCP1-ring complex and ApiCCT1 to selectively prevent the aggregation of mutHTT and associated toxicity in neuronal cells (44,45).

Therapies addressing signaling pathways. Another important HD pathology-associated change is in the cyclic AMP (cAMP) signaling (46) and aberrant transcription of genes regulated by the cAMP response element (CRE) (47). Inhibition of phosphodiesterase (PDE) 10A, which regulates cAMP and cyclic guanosine monophosphate signaling, and is mostly expressed in the MSNs of striatum (48) is shown to be beneficial against

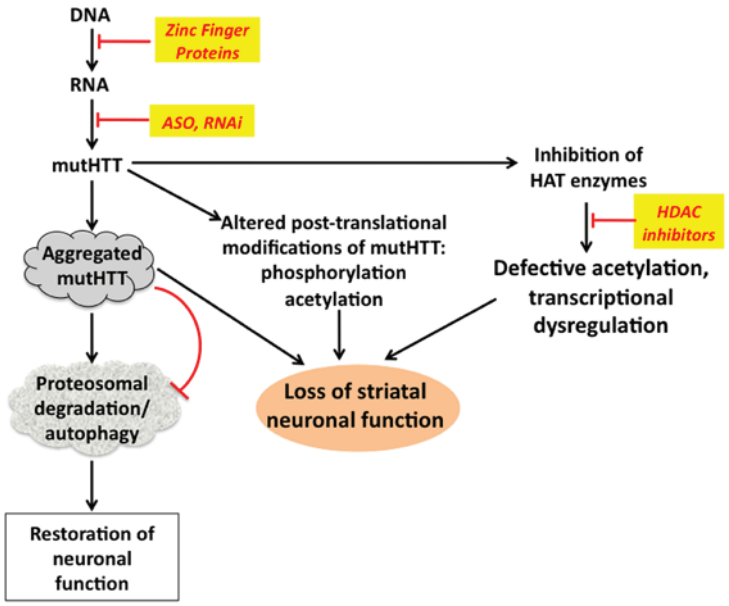

Figure 1. Potential pathological molecular events in Huntington's disease and possible therapeutic interventions. ASO, antisense oligonucleotides; HTT, huntingtin; HDAC, histone deacetylases; HAT, histone acetyltransferase.

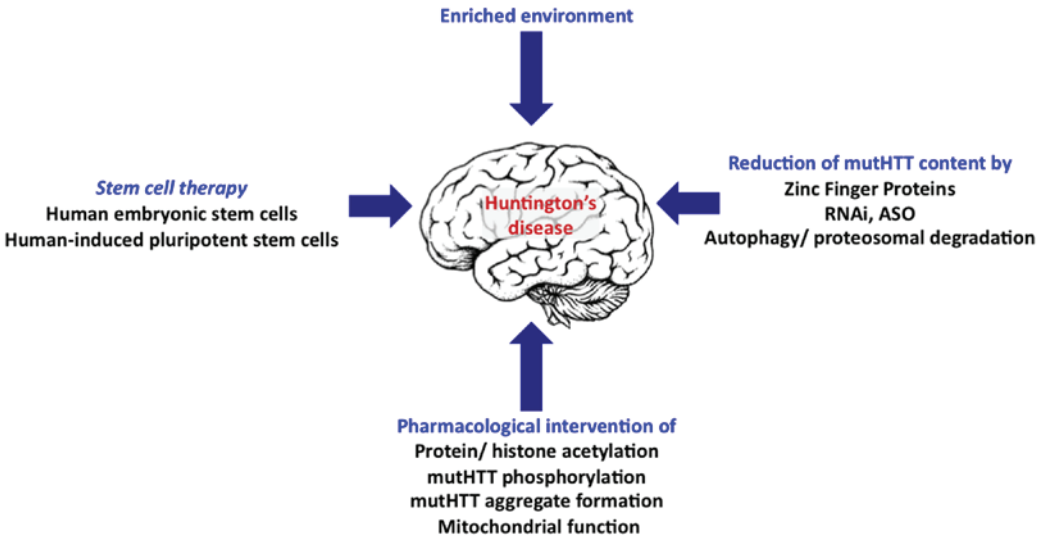

Figure 2. Emerging therapies for Huntington's disease. ASO, antisense oligonucleotides; HTT, huntingtin.

HD, via restoration of CRE-mediated gene expression (49). Speciffically, PDE10A inhibitor-based clinical trials in HD patients are currently addressing the efficacy and motor functional endpoints (50).

An important signaling pathway that is hyperactive and contributes to the pathology of HD is MAPK signaling (51). Specifically, overactive c-Jun N-terminal kinase likely leads to dysregulated axonal transport (52) and hyperactive p38 may cause NMDA receptor-mediated excitotoxicity (53). Thus, the overexpression of MKP-1, a negative modulator of MAPKs, was shown to prevent against mutHTT-mediated neuronal dysfunction in several models of HD (54). Similarly, inhibition of MLK-2 was retarded mutHTT mediated-toxicity (55). NMDA receptor-mediated excitotoxicity, has been suspected to be an important contributor to HD pathogenesis and quinolinic acid, an endogenous degradation product of tryptophan, is a known NMDA receptor agonist. In the pathway of tryptophan catabolism, kynurenine monooxygenase (KMO) activity determines the balance between the neuroprotective kynurenic acid and neurotoxic quinolinic acid. Post-mortem examination of brains from HD patients revealed that there is an increase in quinolinic acid and decrease in kynurenic acid. Treatment of HD animal models with an inhibitor of KMO led to elevated kynurenic acid, as well as improved survival and striatal neuron function. Recent studies reported an improved KMO inhibitor, CHDI-340246, which acts only peripherally and elevates kynurenine and kynurenic acid in HD rodent and non-human primate models, and protects from neuronal loss and dysfunction (50,56).

Therapies that decrease mutHTT content. Reducing the content of mutHTT by inhibiting gene transcription, mRNA

translation or promoting the breakdown of mRNA coding for HTT, may reduce any associated downstream damaging effects of mutHTT, which otherwise lead to the pathogenesis of HD. However, considering that loss of HTT protein, even conditionally, led to neurodegeneration, caution must be exercised to employ procedures that suppress HTT completely. It is more prudent to selectively target *HTT* genes that harbor excessive CAG repeats, and not normal *HTT* gene (Figs. 1 and 2).

Inhibition of transcription by zinc finger proteins (ZFPs) were used to reduce the transcription from the HTT gene. ZFPs can be designed to allow specific binding to selected DNA sequences, and are fused to a transcriptional repressor domain, in order that the gene to which these ZFPs bind, is not expressed, and thus the corresponding protein production is blocked (57). Using ZFPs it has been observed that the proximity of the CAG repeat to the 5' end of the *HTT* gene confers selectivity over other genes containing poly-CAG sequences for targeting with viral vectors for delivering the ZFPs (58). Such as approach has been used successfully utilised in mouse model of HD with the resultant decease in pathological motor manifestations (59,60). ZFPs have been employed to deliver DNA nucleases to the target sequences, in a way that excessive CAG repeats are excised from *HTT* genes, thus raising the prospect of gene therapy for HD (61).

Another approach to lower the expression of mutHTT is to target the corresponding mRNA with specific antisense oligonucleotides (ASOs), which are single-stranded DNA oligonucleotides, and bind to complimentary mRNA sequences via base-pairing and lead to the degradation of the mRNA by RNAse H. Previous findings have shown that intraventricular infusion of mutHTT targeting chemically modified ASOs in three separate HD mouse models was successful in reducing HTT mRNA by 60% and HTT protein by >80% reduction, in a dose-dependent manner. These changes were accompanied by delayed mutHTT aggregation and improved motor performance on a rotarod test. This ASO-induced restoration of normal functionality was sustained even after the infused ASOs were removed, indicating that there was a restoration and recovery of the neurons rendered dysfunctional due to mutHTT (62-64). An advantage of use of ASO is its broad distribution into different brain regions following intraventricular infusion. Inasmuch as mutHTT synthesis is rather ubiquitous, a wider distribution of ASOs is useful in targeting mutHTT expression and thus curtailing its deleterious effects (62). Intrathecal infusion of ASOs for 21 days in non-human primates led to a sustained decrease by approximately 50% in mutHTT mRNA levels in frontal cortex, occipital cortex (68%), and spinal cord, indicating the possible application of ASOs for human situation (62).

Recent advancements in gene silencing efficiency as well as sustained long-term effects of RNAi agents have overtaken the ZFPs and ASOs, despite their therapeutic potential (65). RNAi techniques were successfully employed to reduce HTT mRNA and protein in *in vitro* models of HD (66). Subsequent, *in vivo* studies in HD transgenic mice employing AAV-based delivery of shRNA targeting HTT via single bilateral injections into the striatum, revealed a significant reduction in mutHTT mRNA and protein levels, mutHTT aggregates and marked improvement in behavioral and motor performance parameters (67). This study was followed by several other *in vivo* studies using RNAi approach with improvements in many other HD-associated pathologies (68). Although many of these initial studies employed pre-symptomatic animal models of HD, subsequent studies showed that RNAi approaches also reduced the number of mutHTT inclusions and significantly improved striatal functionality and motor performance (69). Despite the success of these gene expression approaches, clinical application of these methods is not yet feasible and much refinement needs to be attained in these technologies.

In addition to the abovementioned approaches, stem cell-based therapies, in particular, using the patient-specific iPSCs are being developed and are promising to combat HD (Fig. 2) (70).

5. Conclusions

HD is a hereditary neurodegenerative disorder that impairs motor and cognitive functions, by targeting striatal MSNs, with no known cure. MutHTT protein, with an expansion of polyQ tract is toxic to neurons and is the causative factor of HD. Therapeutic strategies addressing a reduction in the mutHTT content at the genome, mRNA or protein degradation level and post-tranlational modification of mutHTT are being studied in preclinical models and in clinical trials. Besides the pharmacological approaches, the use of stem cell therapy, to replace the lost striatal neurons, is also being examined. These multiple clinical investigations are promising to identify therapies that may improve the quality of life for HD patients in future.

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