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Perspective

Huntington's Disease: Latest Research and Potential Treatments

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Abbreviations HD: Huntington's Disease; HTT: Huntingtin Gene; mHTT: Mutant Huntingtin Gene; Htt: Huntingtin Protein; mHtt: Mutant Huntingtin Protein; CAG or polyQ: Glutamine Repeats In Huntingtin; HDAC: Histone Deacetylase; iPSCs: Induced Pluripotent Cells; NSCs: Neuronal Stem Cells; NP: Nano Particles

Huntington's Disease (HD) is a genetically inherited autosomal dominant neurologic disorder that occurs when an expanded CAG repeat in the Huntingtin Gene (HTT) is passed down to offspring. The disease is characterized by cognitive, behavioral, motor and psychiatric problems. No effective treatment currently exists for HD despite the fact that many clinical trials have been performed on human patients and various studies have been done on animal models. Although the function of HTT and the corresponding Huntingtin protein (Htt) have previously been studied. In this short communication we summarize several original studies published within the last year that describe the use of new technology and techniques to gain a more in depth understanding of the pathogenesis of the disease and to develop potential treatments. These studies include the development and application of new inhibitors of various aspects of Htt pathogenesis, the use of patient-derived induced Pluripotent Cells (iPSCs) and Neuronal Stem Cells (NSCs), the nano particles, and CRISPR-Cas9, a new technology for gene editing.

The Huntingtin Gene and its Product

HTT encodes for a large protein, Huntingtin (Htt), whose exact function has not yet been characterized. HTT is thought to play a critical function since when it is completely knocked out of mice, the homozygote off spring do not survive [1]. When a Cre-loxP system was used to conditionally inactivate the HTT in adult mice at different times, the animals showed behavioral and neuropathological deficits [2]. These abnormalities included gait abnormalities, tremors, decreased movement and brain atrophy. The animals also showed a decreased life span. Overall, this study shows that long term decreases of Htt levels can result in significant complications [2].

Although Mutant Huntingtin (mHtt) is expressed throughout the brain, the most striking neuropathology occurs within the striatum and the cortex [3]. Within the striatum, loss of medium spiny neurons leads to the gross atrophy of the caudate and putamen, and provides a basis for grading the severity of HD pathology (from grade 0: no detectable gross or histological pathology, to grade 4: gross decrease of caudate nucleus). Marked neuronal loss is also seen in the deep layers of the cerebral cortex. Other regions, including the globus pallidus, thalamus, subthalamic nucleus, substantia nigra, and cerebellum, all show varying degrees of pathology depending on the grade.

Amino terminal fragments of Htt form intracellular amyloid-like fibrils also referred to as aggregates or inclusions which have become a key signature of HD pathology. Htt inclusions are found throughout the brain and the role they might play in the development of HD has been debated. Early studies focused on the potential toxicity of Htt aggregates presenting evidence of a proteotoxic effect of soluble oligomers of Htt fragments as well as adverse effect of aggregates on cellular trafficking including nuclear-cytoplasmic transport. Others have attributed their toxic effect on the sequestration of critical proteins including growth and trophic factors, chaperones and transcription factors. Later studies have suggested an adaptive role of aggregates whereby they sequester the toxic Htt fragments dampening their toxic effect. In a recent report, researchers used a previously developed biosensor able to distinguish monomeric and aggregated forms of exon1 of Htt (Httex1) peptide to study the effect of Httex1 conformation on mechanisms of cell death. Their findings that soluble mutant Httex1 can trigger apoptotic events, whereas Httex1 inclusions blocks apoptotic signals but activate delayed necrosis, led them to formulate a revised model reconciling the two paradoxical views previously proposed [4,5]. Another recent study that used poly (tretralose) nanoparticles was able to decrease polyglutamine aggregation in a mouse model of HD [6]. Nanoparticles have been specifically engineered to prevent protein fibril nucleation, or degrade mature protein fibrils. To facilitate their interaction with specific aggregated proteins, nanoparticles can be coated with a variety of molecules from synthesized peptides to plant extract. In this study, nanoparticles were coated with a poly- form of trehalose, a sugar which had been shown to alleviate polyglutamine-mediated pathology in HD mouse. When injected intraperinoteally into adult R6/2 mice expressing Exon 1 of mhtt, these nanotrehalose particles blocked mhtt aggregation in cortex, striatum and hippocampus [6]. Compared with molecular trehalose, the designed nanotrehalose particles work at much lower concentrations making it more practical for *in vivo* treatment.

The increased number of glutamines produced from the CAG repeats has been hypothesized to abnormally affect protein interactions and transcription. One consequence is the formation of aggregates of huntingtin with the histone acetyl transferase enzyme. When genes are under- or hypo-acetylated, their transcription can be reduced. Some of these genes play a role in memory. Histone deacetylases are the enzymes that cause genes to be hypo-acetylated. Histone deacetylase 3 (HDAC3) has been reported to play a role in expanding the number of CAG repeats that a person has, which is one underlying mechanism found to be a predictor for HD and plays a role in the pathogenesis of the disease. When an inhibitor of HDAC3 (RGFP966) was given to HdhQ111 knock-in mice early in their lives, memory impairments and motor learning deficits were prevented. Additionally, there was a decrease in striatal CAG expansions [7]. Another area of study is the conformation of Htt. A number of neurodegenerative diseases, including HD, are characterized by abnormal folding of proteins. The increased repeat length of the polyglutamine in HD has been found to contribute to the abnormal conformation of the protein. When fragments of the amino terminal region of Huntingtin form, they can associate with each other and form aggregates. The phosphorylation state of the mHtt affects its toxicity suggesting a possible area of therapeutic exploration. The conformational studies were based on TR-FRET assays of peripheral tissues, HD brain tissue and tissues from animal models. This assay is important in developing new treatments for HD and for monitoring their effectiveness [8].

Patient-derived Induced Pluripotentcells (iPSCs) and Neuronal Stem Cells (NSCs) have become useful tools to study mechanisms of neurodegeneration and to test the effect of particular therapies at the in vivo molecular level. These cells carry the disease specific mutation as well as accompanying molecular alterations and provide another mean of study to animal model derived cells. HD patients-derived iPSCs have been used to examine the effect of silencing the HTT gene. In lines derived from HD patients and controls, continuous expression of HTT shRNA suppresses both normal and mutant allele [5]. HD relevant signaling pathways can becompared between Htt depleted iPSCs and their respective parent cells carrying the same homogenous genetic background. HTT silencing on iPSCs derived from a mouse model rescued the cells from the HD p53 down regulation phenotype [5]. This was, however, not the case in the patient-derived iPSCs suggesting different mechanisms between model and disease [5].

Treatments

A number of drug trials in animal models and humans have been undertaken to try to treat HD. However, to date, there is still no cure for HD. Laquinimod is an anti-inflammatory drug that has been tested for Multiple Sclerosis. Current investigations are analyzing the effects on specific immune parameters including dendritic cells, monocytes and the Nf-kb pathway. It is presently in clinical development for both Multiple Sclerosis (MS) and HD. The effectiveness and safety of the drug are currently being evaluated [9]. A recent study tested laquinimod in the R6/2 animal model of HD [10]. Although it did not improve survival or decrease the weight loss in the animals, it did improve motor coordination and balance as indicated by improvements in rotarod performance at 12 weeks of age. There was also an increase in BDNF in the striatum and a decrease in mutant Htt and iNOS in both the striatum and cortex. BNDF is a growth factor that is important for neuronal survival and protects from neurodegeneration. A decrease in Htt aggregates was found by staining with EM48 and ubiquitin antibodies. Although laquinimod does not completely cure the HD symptoms, it does have some positive effects and hence should continue to be evaluated as a potential treatment [10].

Cellular mechanisms that promote the clearance of mHtt are of therapeutic interest as they can lower the level of mutant Htt and its toxic aggregation suggesting that HD may be reversible. mHtt degradation is mediated by two main pathways, the ubiquitin-proteosome system (UPS) and autophagy. Polyl-isomerase (PIN1) catalyzes the cis-trans isomerization of phosphorylated Ser/Thr-Pro sites inducing a conformational changeultimately leading to UPS degradation targeting. Genetic Pin1 deletion increases aggregate load in HdhQ111: : Pin1-/- mouse striatum. Furthermore, co-expression of Pin1 and mHtt in HEK293 cells and neuroblastomas reduces the number of cells containing mHtt aggregates independently of the length of the PolyQ tract [11]. Pin1 does not appear to directlyinteractwith Htt, but reduces its half-life by stimulating its degradation through the UPS possibly by regulating its phosphorylation-dependent ubiquitination [11]. Therefore, it is proposed that pharmacological agents that can alter the level of activity of Pin1 could represent a promising therapeutic avenue for HD [11].

Htt associates with the repressor element-1 silencing transcription factor, REST, preventing its nuclear translocation. In HD, the cytosolic sequestration of REST is impaired and its excessive nuclear accumulation leads to the silencing of neuronal genes important for the maintenance and function of specific neurons. REST undergoes alternative splicing with more than 45 variants already identified. REST exon 3 contains a ZF-5 domain that is critical for its nuclear location and is predicted to behave as a modulator of REST activity that could be targeted to treat HD. Antisense oligonucleotides designed to decrease REST splicing variants containing exon 3 lead to reduced REST nuclear translocation and rescue neuronal gene expression in a cellular model of HD [12] suggesting that targeting REST activity using shRNA gene therapy may provide some benefit for HD [12].

Novel disease-modifying strategies aimed at down regulating the expression of HTT are being evaluated as a potential treatment for HD. A tat-independent third-generation lentiviral (pCCL) based therapy expressing short hairpin (sh) RNA targeting exon 3 and 4 of HTT (named shHTT6) was recently tested in striatal neurons derived from HD patient iPSCs and in the striatum of HD transgenic mice. In both models, pCCL-shHTT6 induced astrong silencing of both normal and mutant Htt. Silencing was accompanied by a reduction in the loss of medium spiny neurons and less ubiquitin positive aggregates, two pathological characteristics of HD [13]. The lenti viral pCCL-shHTT6 did not induce an inflammatory response in the brain and was properly processed by the cell showing encouraging bio safety requirements necessary for potential clinical application [13]. In another study, micro (mi) RNAs engineered to induce either total or allele specific HTT knock-down were tested in an acute HD rat model showing local formation of mutant Htt aggregates followed by severe neuronal dysfunction. Adeno associated viral vectors expressing these constructs suppressed mutant HTT mRNA resulting in the dramatic decrease of mHtt aggregate formation as well as

reduce accompanying neuronal dysfunction. These data provide evidence for the efficacy of this new line of therapy [14].

Finally, a new tool based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) has generated significant enthusiasm in the field of genome editing. A couple of studies show the effectiveness of CRISPR/Cas9 mediated silencing of the mHTT in *in vitro* models of HD [15] and in patient derived induced pluripotent stem cells [16]. Permanent suppression of endogenous mHTT expression in the striatum of mHTT-expressing mice (HD140Q-knockin mice) using CRISPR/Cas9-mediated inactivation has also been shown to effectively eliminate HTT aggregates and attenuated early neuropathology as well as lessened motor deficits [17]. Although promising results have been obtained using this unique technology, there remains many safety concerns regarding the potential off-target effects of this therapy.

This report has discussed numerous features associated with the HTT protein and hence with HD. Briefly, this includes the role of HTT, neuropathology and aggregate formation. Recent treatments were discussed including the use of drugs and other mechanisms to clear mutant HTT as well as decrease nuclear localization of the protein. Promising preclinical studies also suggest the possibility of a therapeutic window where suppressing total Htt may provide a treatment for all HD patients independent from their genotype. The discovery of the gene mutation that causes HD in 1993 led to an explosion of new avenues of research and a substantial increase in the number of laboratories focusing on finding a cure for HD. However, despite numerous animal and clinical drug trials and the discovery of various mechanisms involved with the disease, progress has been slow. Scientists are still searching for a cure in a similar manner to diseases such as HIV and cancer. With the advances in technology and the development of new techniques that were discussed in this review, it is hoped that a cure for HD can soon be realized.

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