Huntington's disease: progress toward effective disease-modifying treatments and a cure

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Huntington's disease (HD) is caused by a dominant mutation in *HTT*, the HD gene. This discovery opens possibilities for treatment based on silencing of the disease-causing allele or with compounds that reduce the production of disease-causing mRNA and/or protein. Although additional developments are needed related to the delivery of gene silencing and discovery and development of drugs that reduce disease-causing gene products, these treatments are predicted to be effective since they act by reducing the source of toxicity. The identification of therapies that act by blocking toxicity is conceptually more complicated, as this requires an accurate understanding of the cellular location and the specific molecular dysfunctions that cause the phenotypes of HD, which is not yet available. Though challenges remain, significant progress has been made. Effective disease-modifying treatments will soon be tested and may lead to disease-altering therapies.

INTRODUCTION

A disease is a condition of the body associated with a defining set of deleterious symptoms, the disease phenotype. The rational identification of treatments and cures for human diseases is generally predicated upon an understanding of the disruption(s) in cellular and molecular pathways that cause the disease phenotype. This understanding is usually obtained from the observation of behavioral phenotypes and studies of abnormalities in the structure of organs, tissues and cells from diseased individuals.

Identification of disease-causing gene mutations for monogenic disorders can provide insight about the underlying functional disruption and, even in the absence of such insight, opens the way for the construction of disease models in experimentally tractable organisms. The study of such models facilitates the discovery of potential causes of disease phenotypes. They also provide platforms for validating these causes and for testing therapeutic approaches designed to obviate their deleterious effects.

Among the more common neurodegenerative diseases, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD), HD is unique in that most (~99%) individuals presenting with the HD phenotype have a mutation in the same gene. Although mutations that cause Alzheimer's, Parkinson's or ALS have been identified, there are several genes involved for each, and only 5–15% of patients carry a specific causative mutation.

As a very nearly monogenic disease with clear, defining phenotypes, HD is an attractive model in which to attempt to unravel the mechanism of disease causation as an approach to identifying therapeutic interventions capable of modifying the course of the disease and/or preventing its onset. In this article, we note some recent results impacting the status of this effort, summarize our view of the currently most promising approaches to treatments and a cure and comment on the research required to accomplish this goal.

HD PHENOTYPES

The defining phenotype of HD is generally considered to be chorea, involuntary movements, some dance-like, that occur in many parts of the body. Individuals with the disease frequently also display psychiatric symptoms, most commonly depression and irritability, as well as declines in cognitive abilities. It seems likely that people with HD have a variety of still-being-characterized behavioral changes; for example, recently, the occurrence of abnormal sleep patterns was

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documented in the laboratory, although patients and families usually complain of this symptom as well (1).

Post-mortem analyses of HD brains show massive degeneration of the striatum as well as the cortex with lesser, but still significant, changes in the hippocampus, hypothalamus and in other brain structures. Non-invasive imaging studies are consistent with this picture and further indicate that changes usually begin as far as 15 years prior to the onset of overt disease symptoms. In addition, recent results, using diffusion tensor imaging, emphasize the early occurrence of changes in white matter (2–4), and both positron emission tomography and biochemical studies demonstrate the presence of neuroinflammation in the HD brain (5–7). Understanding the causes of these changes requires consideration of the roles of endogenous functional differences in the constituents of the brain. These include the roles of various glial cells (oligodendrocytes, astrocytes, microglia), endothelia and perivascular cells as well as potential influences from the periphery (8). Efforts to integrate this information comprise a significant research effort, using mostly HD model mice.

Biochemical studies of mRNA, protein and metabolite content in tissues from people with HD and in HD mouse models have long demonstrated the presence of many changes within the CNS. Recent results have emphasized changes beyond the striatum and cortex (9). Many alterations are also found in peripheral tissues including muscle (10), adipose tissue (11) and peripheral inflammatory cells (5). The presence of widespread dysregulation of gene expression, and changes in protein and metabolite content, emphasizes the difficulty inherent in establishing the molecular and cellular basis of HD phenotypes.

THE CAUSE OF HD

HD is a dominantly inherited disease caused, in most cases, by an expansion of a CAG repeat located in the DNA encoding the first exon of *HTT*. Most individuals with HD carry only one disease-causing (mutant) allele.

The HD gene is located on the distal arm of chromosome 4 at 4p16.3. In most populations of European descent, the prevalence of HD is 5–10 per 100 000, and >99% of those diagnosed with the disease are found to have an expanded CAG repeat in *HTT* on one allele, hence the dominant inheritance. A small percentage of the 1% without a mutation in the HD gene have alterations in other recently recognized genes (12), whereas the remainder are idiopathic. The prevalence of HD in populations of non-European descent is generally >10-fold lower, and, in some of these, mutations in a gene other than the HD gene are more frequent (13).

Recently published data show that most disease-causing alleles of the HD gene occur on a common haplotype that is relatively rare in individuals of European descent and absent from several populations of Asian and African origins (14). Though the explanation for this finding is controversial (15), the conclusion is of immense importance for programs designed to develop disease allele-specific gene silencing as a treatment for HD (see what follows).

HOW DOES THE MUTANT HD GENE CAUSE HD?

The HD gene encodes a large, 3144 amino acid long protein called 'Huntingtin', with roles in a variety of cellular functions, prominent among which are vesicle trafficking, energy production and transcription. The CAG repeat in the HD gene encodes a stretch of glutamine residues in the N-terminus of Huntingtin, beginning after the 17th amino acid. In control populations, the CAG repeat averages between 17 and 20 units long. Individuals with repeats of ≥40 units are at risk for HD and can expect to develop disease phenotypes if they live a normal-length life, i.e. the penetrance of such mutations is considered to be 100%. It is also known that longer repeats confer, on the average, earlier ages of symptom onset. Individuals with 36–39 CAG repeats are at risk for developing HD though the penetrance is reduced.

The mechanism by which the expressed expanded CAG repeat in *HTT* causes HD is only poorly understood. Though most investigators consider the mutant Huntingtin protein with its expanded glutamine stretch the prime culprit, toxicity resulting from triplet repeat-containing RNAs and/or dysregulation of antisense transcripts has not been fully explored. The expression of long glutamine tracts either alone, in the context of an N-terminal fragment or full-length Huntingtin protein, or inserted into other proteins has been shown to disrupt a wide variety of biological functions in cells and model organisms. Nonetheless, which disruptions in which cells lead to the phenotypes of HD remains unsettled.

HD THERAPY: REDUCING TOXICITY BY REDUCING MUTANT HUNTINGTIN

As the vast majority of HD is caused by mutation of a single gene, an attractive approach to therapy, which sidesteps the need to understand the mechanism by which expression of the mutant gene causes the disease, is to silence the expression of the mutant allele (reviewed in 16–18). Efforts to accomplish this using antisense oligonucleotides or siRNAs in a number of forms are being actively pursued by multiple investigators and in collaborations with academic and industrial partners (19–23).

Early studies tested the effects of allele-specific silencing, in which the inhibitory RNA was directed against the mutant human HTT fragment in a transgenic model of HD (19). Silencing of the mutant allele reduced disease progression and improved disease-linked behaviors (19). However, silencing both normal and mutant alleles naturally raises concerns about toxicity resulting from loss of normal Huntingtin functions. Complete loss of expression of the mouse HD gene causes an embryonic lethal phenotype, suggesting that non-allele-specific knockdown of HD gene expression will have serious side effects. However, the embryonic lethal phenotype in the mouse has been traced to lack of expression in extra-embryonic tissues. Although knockout of *HTT* in the brain perinatally induces subtle neurological phenotypes, there is a delayed onset. Interestingly, homozygous knockout ES cells survive and appear normal in culture and *in vivo* in chimeras formed with wild-type ES cells. Further, complete loss of the *Drosophila* ortholog of *HTT* has only subtle phenotypes (24), and the genomes of some animals, such as

Caenorhabditis elegans, appear to lack a homolog entirely. These data support the prediction that a significant amount of non-allele-specific HD gene silencing in adults may be tolerated. Indeed, two recent studies in rodent models support the safety of non-allele-specific silencing (20,23). Further development and testing of non-allele-specific gene silencing as a treatment for HD thus appears warranted.

On the other hand, as noted earlier, recent publications show that most disease-causing HD alleles are carried on a common haplotype that is rare in the population (14). The mRNA of the mutant *HTT* allele that is expressed from this haplotype has a number of sequence differences, mostly single-nucleotide polymorphisms, which are not present in mRNAs from many other haplotypes. These findings provide an opportunity to develop reagents that selectively silence the disease-causing allele in a substantial proportion of people destined to develop HD (14,18,25). It will be interesting and important to test the safety and utility of sequences targeting these disease-allele-linked SNPs.

The major impediment to gene-silencing approaches to the treatment of HD is the requirement for effective delivery of the antisense or siRNA molecules into disease cells throughout the brain and periphery. Most investigators have focused on CNS delivery. Injection of inhibitory sequences once, or continually via implanted cannulas, has silenced the HD gene in mice and non-human primates. Whether a lifelong infusion of drugs into the brain can be tolerated remains unknown. A second approach for siRNA delivery is via viral vectors. In mice, direct intraparenchymal injection of recombinant adeno-associated viruses can achieve expression of recombinant inhibitory RNAs throughout the striatum and into the deep layers of the cortex (19,20,26). In non-human primates, infusion of AAV into the putamen and caudate nucleus transduces numerous neurons throughout the injected regions (27).

An alternative to silencing HD gene expression involves lowering the amount of mutant Huntingtin protein by either reducing its production or enhancing its clearance/degradation. Recently, considerable research has been devoted to developing better tools for characterizing and quantifying Huntingtin protein and fragments, oligomers and aggregates thereof within tissues from HD, HD mice and in cell-based models of the disease (28–32). These efforts are producing a better understanding of the metabolism of the protein, which may lead to the identification of specific toxic species and are expected to provide a platform upon which to select lead compounds that reduce mutant Huntingtin protein. A recent, related observation shows that the expression of an intracellularly retained antibody that increases turnover of mutant huntingtin improves disease readouts in HD mice (33).

HD THERAPY: BLOCKING MUTANT HUNTINGTIN-INDUCED TOXICITY

Much research on HD is focused on how mutant Huntingtin causes toxicity in cell-based and model organisms. Prominent among the mechanisms identified are increased oxidative stress, excitotoxicity, reductions in mitochondrial function, disruptions of vesicle trafficking including axonal transport, reduction in the synthesis and secretion of protective growth factors, changes in pre- and post-synaptic neurotransmitter-induced activities, alterations in transcription resultant from sequestration of transcription factors and specific interactions of mutant Huntingtin with a variety of transcription factors, cofactors and modulators and alteration of a bewildering variety of factors or pathways that directly or indirectly control cell survival and apoptosis.

The next step in the development of therapeutics designed to prevent these toxicities is validation that the mechanism contributes to the phenotypes of HD. Given the current status of the field, arguably the best available validation is achieved by a genetic manipulation or by alteration of the HD gene to mimic or inhibit the activity of a presumed therapeutic target or pathway *in vivo* in an HD model mouse. Recent data, for example, show that transgenic mice expressing a full-length HD gene encoding 97 glutamine residues with serines 13 and 16 changed to phospho-mimics (aspartate) does not result in disease: no detectable behavioral deficits, no neuropathology and no aggregates, an apparent complete amelioration of HD in the mouse (34). Control animals with an HD transgene encoding 97 glutamines and serine at positions 13 and 16 have readily detectable movement, psychiatric and cognitive behavioral phenotypes, brain atrophy including significant cell loss in striatum and cortex and accumulation of cytoplasmic and nuclear aggregates from an early age (35,36). Furthermore, animals expressing an HD transgene in which serines 13 and 16 were changed to alanines, which cannot be phosphorylated, had disease phenotypes comparable with the controls (34). This striking set of results was predicated on observations that conversion of serine 13 and 16 to aspartate in an N-terminal fragment of the HD gene with 97 glutamines alters its toxicity, aggregation and clearance in cell- and *Drosophila*-based models, though the change is not always in the same direction (37). These data show that the protective mechanism engaged by the conversion of serine 13 and 16 to aspartate could contribute significantly to HD pathogenesis, and provide strong encouragement for programs designed to understand this mechanism and develop drugs that emulate it.

CONCLUSIONS AND PROSPECTIVE

HD is a devastating disease caused, in the vast majority of cases, by a genetic mutation that is readily identified independent of the onset of disease symptoms. However, understanding the molecular and cellular pathogenic mechanisms that underlie the development of disease phenotypes remains a significant challenge, principally because it presumes an understanding of the molecular and cellular mechanisms that underlie normal functioning in humans, which, at the moment, is significantly incomplete. Nonetheless, efforts to develop therapies based on silencing the expression of the HD gene, though clearly a technical challenge, seem poised for success. Furthermore, identification of compounds that act to reduce the amount of mutant huntingtin protein in cell-based or model organism screens may also yield useful therapeutic leads.

Additional lines of research that are ostensibly designed to yield therapies for HD are likely to entail experiments that

provide validation of pathogenic mechanisms. In most cases, this will involve *in vivo* genetic experiments, such as those described earlier.

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