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REVIEW

Therapeutic Advances in the Management of Huntington's Disease

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Trinucleotide repeat disorders are a set of genetic disorders characterized by the expansion of certain genes of a segment of DNA that contains a repeat of three nucleotides, thus exceeding the normal stable threshold. These repeats in the DNA cause repeats of a specific amino acid in the protein sequence, and it is the repeated amino acid that results in a defective protein. Huntington's disease is a well-known genetic disorder associated with trinucleotide repeat expansions. Patients first present clinically in midlife and manifest a typical phenotype of sporadic, rapid, and involuntary control of limb movement; stiffness of limbs; impaired cognition; severe psychiatric disturbances; and ultimately, death. There have been a number of therapeutic advances in the treatment of Huntington's disease, such as foetal neural transplantation, RNA interference, and transglutaminase inhibitor. Although there is intensive research into Huntington's disease and recent findings seem promising, effective therapeutic strategies may not be developed until the next few decades.

INTRODUCTION

Trinucleotide repeat disorders are a set of genetic disorders characterized by the expansion of certain genes of a segment of DNA that contains a repeat of three nucleotides, thus exceeding the normal stable threshold [1]. There is usually an increase in the number of triplet repeats as the gene is passed from generation to generation, resulting in abnormalities in gene expression and function. Examples of diseases that are caused by triplet repeat expansion are Fragile X syndrome, Myotonic dystrophy, Friedreich ataxia, and Huntington's disease. It is important to understand the mechanism

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†Abbreviations: DM, myotonic dystrophy; PolyQ, polyglutamine; HD, Huntington's disease; BDNF, Brain Derived Neurotrophic Factor; FDA, U.S. Food and Drug Administration; VMAT, monoamine transporter; RNAi, RNA interference; siRNAs, small interfering RNAs; Ago, argonaute; RISC, RNA-induced silencing complex; cc-siRNA, cholesterol-conjugated small interfering RNA; Htt, huntingtin; TGase, transglutaminase; UBL, ubiquitin-like; UBA, ubiquitin-associated; GFP, green fluorescent protein

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of trinucleotide repeat disorders because many of the recent successes in their therapies have been based on the understanding of their pathophysiology. This paper is aimed at explaining the genetic basis of trinucleotide repeat disorders and will also discuss the cellular/molecular mechanism that underlie recent advances in the therapy of a named triplet repeat expansion disorder: Huntington's disease. While there is active ongoing research into several potential therapies, this paper would highlight five specific therapies from various experiments.

TRINUCLEOTIDE ("TRIPLET") REPEAT DISORDERS

Triplet expansion is caused by slippage during DNA replication [2]. As a result of the repetitive nature of DNA sequence in these regions, there may be formation of "loop out" structures during DNA replication while maintaining complementary base pairing between the parent strand and daughter strand being synthesized. There is an increase in the number of repeats if the loop out structure is formed from the sequence on the daughter strand, while there is a decrease if the loop out structure is formed from the parent strand. There appears to be a clear bias toward continued expansion of the size of the repeat unit array, and it is the repeated amino acids that result in defective proteins.

Although approximately 30 disorders have now been identified as being caused by trinucleotide repeats, this unusual type of mutation was first discovered in humans in the medical condition known as Fragile X syndrome in 1991 [3]. Many of these disorders are rare, but mostly affect the central nervous system. While they are all caused by the dynamic mutations of triplet repeats, there are significant differences among these disorders. Among the differences are the base sequence of the repeat; the location of the repeat within genes; the pathogenesis of the disease; the size of the repeat in unaffected and affected individuals; and parent-of-origin effects on repeat expansion [4].

Despite several researches, according to Turnpenny and Ellard, the precise mechanisms by which disease is caused by triplet repeat expansions are still unknown [5]. Dynamic trinucleotide repeats may be within coding or non-coding regions of genes, which may be a contributory factor to the variation in their pathogenic mechanisms.

Research has shown that there is a strong correlation between the number of repeats and the severity of the disease. For example, in the case of myotonic dystrophy (DM†), normal individuals usually have five to 37 copies of CTG repeats, while those with 50 to 80 copies could be mildly affected or have no symptoms at all; however, those with more than 80 copies to several thousand copies of the repeat sequence usually present with full-blown myotonic dystrophy [6]. The CTG repeats in myotonic dystrophy occur in the 3' untranslated region of a DM candidate gene [7].

Triplet repeat expansions usually occur over a number of generations within a family, and the number of repeats often increases with succeeding generations. Therefore, symptoms of the disease usually manifest at an earlier age in the offspring than in the parents, and the severity of the disease increases in subsequent generations [8]. This phenomenon is known as **anticipation**. Although this phenomenon was controversial among scientists for several years, recent discovery in molecular genetics has shown that anticipation does have a biological basis.

POLYGLUTAMINE (POLYQ) DISEASES

In many of the trinucleotide repeat disorders, the repeated codon is CAG, which codes for glutamine in a coding region, resulting in a polyglutamine tract. These disorders are commonly referred to as polyglutamine (PolyQ) diseases. The abnormal protein conformation resulting from PolyQ expansion seems to be central to pathogenesis [9]. It has been suggested that triplet expansion increases the probability that PolyQ will adopt a novel abnormal conformation [10]. Studies have been conducted to support the concept of altered conforma-

tion. It has been shown in studies that some antibodies preferentially bind expanded PolyQ, while in some other studies in disease tissue, transfected cells and animal models have revealed that expanded PolyQ proteins form insoluble intracellular inclusions [10]. However, it is still unclear how the altered conformation leads to neurodegeneration.

In the remaining disorders, the repeated codons do not code for glutamine and are referred to as non-polyglutamine diseases. Huntington's disease belongs to the subset of polyglutamine diseases. Other diseases in this group include dentatorubropallidoluysian atrophy, spinobulbar muscle atrophy or Kennedy disease, and Spinocerebellar ataxia Types 1, 2, 3, 6, 7, and 17.

HUNTINGTON'S DISEASE

Huntington's disease (HD) is a well-known genetic disorder associated with trinucleotide repeat expansions. It was first described by an American physician, George Huntington, in 1872 after he studied several affected individuals and also noted observations made by his father and grandfather who were also physicians [11]. Its mode of inheritance is autosomal dominant, and its prevalence is about 5 in 100,000 worldwide [12]. Penetrance is almost 100 percent as individuals with the dominant allele eventually develop the disease. The average age of onset is 38 years, though the timing ranges from 25 to 70 years. However, recent studies claim that approximately 5 percent of HD cases have presented before 20 years of age [5]. Patients first present clinically in midlife and manifest a typical phenotype of sporadic, rapid, and involuntary control of limb movement; stiffness of limbs; impaired cognition; severe psychiatric disturbances; and ultimately, death.

MOLECULAR BIOLOGY OF HUNTINGTON'S DISEASE

HD is a single gene disease with autosomal dominant inheritance pattern. Although the disease locus of HD was mapped to chromosome 4p16 by the G8 marker in the early 1980s, the HD gene was not cloned until 1993 [13]. It has been suggested that HD is caused by the mutation of the gene IT15, which contains 67 exons and encodes a 3144-amino-acid protein called "huntingtin" [6]. The function of huntingtin is unclear. Nasir and colleagues suggest that it is essential for development and that absence of huntingtin is lethal in mice [14]. They concluded in their study that the HD gene is essential for postimplantation development and that it may play a significant role in the normal functioning of the basal ganglia. It has also been claimed that wild-type huntingtin up-regulates the expression of Brain Derived Neurotrophic Factor (BDNF) at the transcription level; however, the mechanism by which huntingtin regulates gene expression has not been determined [15].

The normal repeat number of CAG is 10-26. CAG encodes the amino acid glutamine within the huntingtin gene on chromosome 4, and it is not toxic in itself as it is present in all humans. However, it has been suggested that expansion of polyglutamine tract results in aggregate formation that may become toxic and could be one of the factors responsible for HD as aggregates are never observed in the brains of unaffected individuals [16,17]. It has been suggested that individuals with more than 39 will almost always show manifestations of HD [18], with the largest expansion observed being 121 trinucleotides [19]. Conscientious studies of people with HD have shown that the cause may be heterogeneous. According to Lutz, studies among HD patients have revealed that approximately 70 percent of the variation in the age of onset of the disease is linked to the size of the CAG repeats, while 13 percent of variation in the onset has been attributed to polymorphism in the GRIK2 gene, whose product forms part of the subunit of the excitatory glutamate receptor [20]. Therefore, there are other factors that can affect the onset, severity, and outcome of HD.

CURRENT MANAGEMENT

At this time, there is no cure for HD. The aim of current treatment strategies is to

manage the symptoms and improve the quality of life of the patients. It is important to determine whether patients require treatment when they present. In the early stages, the chorea may not be interfering with their lifestyle and so may not require treatment. However, if the symptoms begin to affect their lifestyle such as in walking, writing, and eating, then intervention becomes a necessity. Management of HD patients should be multidisciplinary. Hence, if they are falling at home, for example, as a result of their progressive symptoms, it may be important to involve the occupational therapists who would assess the facilities in the patient's home and then suggest modifications that would suit the patient's needs. The physiotherapists can also help optimize mobility and maintain independence. If the non-drug management strategies fail, then drugs such as tetrabenazine could be tried. Tetrabenazine is efficient in the management of HD and has been shown to reduce chorea. This is, in fact, the only drug approved by the U.S. Food and Drug Administration (FDA) for HD [21]. Tetrabenazine is a drug used for the symptomatic treatment of hyperkinetic movement disorder. Tetrabenazine works mainly as a vesicular monoamine transporter (VMAT) inhibitor, therefore promoting the early metabolic degradation of monoamines, especially the neurotransmitter dopamine. In the central nervous system, VMAT 2 is the only transporter that transports cytoplasmic dopamine into synaptic vesicles for storage and subsequent exocytotic release [22].

It is also important to assess patients for depression and suicidal thoughts so as to prescribe antidepressants if necessary.

THERAPEUTIC ADVANCES

Several studies have been carried out to demonstrate the abnormalities in the brain of individuals with HD, in anticipation that this could possibly help in its treatment. According to Agamanolis, for example, the brain of individuals with HD shows atrophy of the caudate nucleus and putamen and dilatation of the anterior horns of the lateral ventricles on gross examination [23]. Simmons and colleagues also state that radiological investigations of four patients with HD reveal that the caudate nucleus and corpus striatum were atrophic [24].

Biochemical study of neurotransmitter changes in the brain in HD has shown that there is a significant reduction in the enzymes producing gamma-aminobutyric acid and acetylcholine in the striatum, indicating the extensive loss of GABA-ergic and cholinergic striatal neurones [25]. Although most therapeutic advances in publication have only been tested on animal models, they offer insight into possible treatment of HD in the near future.

FOETAL NEURAL TRANSPLANTATION

As the striatum commonly degenerates in HD, resulting in loss of motor and cognitive functions, efforts have been made to restore these functions by transplanting foetal striatal neuroblasts into the striata of HD patients. However, this therapy has not been very successful in the long term. In a study by Bachoud-Lévi and colleagues in which five patients were grafted with human foetal neuroblasts, there was an increase in metabolic activity in various subnuclei of the striatum in three of the five patients, although there was a progressive deterioration in the two other patients 2 years after surgery [26]. There was also an improvement in motor and cognitive functions in the same three patients [26]. However, 4 to 6 years after surgery, clinical improvement initially observed in the three patients began to decline and dystonia deteriorated consistently, while the two patients who did not benefit from the transplantation continued to deteriorate in a comparable way to non-grafted HD patients [27]. Therefore, although neuronal transplantation may provide improvement and stability initially, it is not a permanent cure for HD.

RNA INTERFERENCE

RNA interference is a biological process currently being studied as a poten-

tial therapy for Huntington's disease. RNA interference (RNAi) is a natural, selective method of turning off genes, which can be induced by the production of small interfering RNAs (siRNAs) formed by a guide strand and a passenger strand. The discovery of RNA interference emerged from work on the genetic modification of plants in the late 1980s. In an attempt to deepen the violet hue of petunias by expressing higher levels of an enzyme involved in the synthesis of the pigment, many white flowers appeared following the introduction of extra copies of the gene. For unknown reasons at the time, the introduction of additional copies of the gene had resulted in the suppression of gene expression rather than the expected increase in expression [28,29]. The phenomenon was later observed in the filamentous fungus *Neurospora crassa* and the nematode worm *Caenorhabditis elegans*. Initial attempts to induce RNA interference in human cells were unsuccessful as the introduction of double-stranded RNA into mammalian cells resulted in the inhibition of all gene expression and rapid cell death. However, the breakthrough came when short, double-stranded RNA molecules were shown not to induce the interferon response, which usually cause the inhibition of all gene expression [30]. RNA interference is now frequently used in biological and biomedical research to study the effect of blocking the expression of a particular gene. Proteins such as Drosha, DGCR8, and argonaute (Ago) participate in RNA processing. Drosha and DGCR8 form a complex that initiates the maturation of micro-RNA by precise cleavage of the stem loops that are embedded in primary transcripts (pri-miRNAs) [31]. Furthermore, Drosha facilitates the overall production rate of miRNA by generating the 3' protruding ends, which are recognized by Exp5 and Dicer [32,33,34]. Neither DGCR8 nor Drosha alone are active in pri-miRNA processing, but combining the two proteins restores this activity, suggesting that both proteins play crucial roles in pri-miRNA processing [35,36]. The processing of primRNA is a critical step in miRNA biogenesis because it defines the miRNA sequences embedded in long pri-miRNAs by generating one end of the molecule [31]. The resulting pre-miRNAs, following the initial processing, are exported by the nuclear factor, Exp5 [34,37,38]. Dicer, a cytoplasmic RNase III type protein, then dices the transported pre-miRNAs to generate miRNA duplexes [39,40,41]. Ago 2 is an essential component of RNA-induced silencing complex (RISC) that cleaves siRNA and mRNA target when they are bound together by complementary base pairing. The miRNAs then bind to their target mRNAs to induce translational repression and inhibition of protein synthesis. It has been demonstrated that a sole injection of cholesterol-conjugated small interfering RNA duplexes (cc-siRNA) targeting huntingtin (Htt) gene into the adult striatum of a viral transgenic mouse model of HD silences mutant Htt, attenuates neuronal pathology, and delays the unusual behavioral phenotype observed in the mouse. In a study by DiFiglia and colleagues, for example, an adeno-associated virus containing either wild-type (18 CAG) or expanded (100 CAG) Htt cDNA encoding Htt 1-400 and siRNA were injected into a mouse striata [42]. It was observed that treatment of the mice that had the mutant Htt with cc-siRNA-Htt prolonged survival of striatal neurones, lowered neuropil aggregates, and diminished inclusion size. siRNA reduces the production of mutant Htt protein and silences its expression via RNA interference.

TRANSGLUTAMINASE INHIBITOR

Transglutaminases are a family of enzymes that catalyze the formation of a covalent bond between a free amino group and the gamma-carboxamid group of protein- or peptide-bound glutamine. They form extensively cross-linked, generally insoluble protein polymers and have been implicated in a number of medical conditions such as celiac disease [43], Parkinson's [44], and Huntington's disease [45]. Recent research suggests that people suffering from Parkinson's and Huntington's diseases may have an unusu-

ally high level of a type of transglutaminase — tissue transglutaminase.

Karpuj and colleagues demonstrated that inhibition of transglutaminase (TGase) could provide a new treatment approach to HD [46]. It has been suggested that TGase could be significant to the pathogenesis of HD, via cross-linking huntingtin. Cross-linking is the formation of bonds to link one polymer chain to another. It is thought that this promotes the formation of the protein aggregates that cause HD. When the competitive inhibitor of TGase, cystamine, is administered to transgenic mice expressing exon-1 of huntingtin containing an expanded repeat of polyglutamine, it is observed that the course of their HD-like disease is distorted. It is known that cystamine inactivates TGase via a disulfide-exchange reaction — a process of activation or inactivation of proteins by the folding and unfolding of the amino acids that produce the proteins [47]. Furthermore, it is a primary amine, thus it is a good substrate for TGase and serves as a TGase competitor by obstructing access to the enzyme's active site for the glutamine residues in proteins like huntingtin, therefore limiting TGase activity. When cystamine is administered intraperitoneally, it enters the brain, where it inhibits TGase activity. Karpuj and associates state that when treatment started following the manifestation of abnormal movements, cystamine prolonged survival, lowered associated tremor and irregular movements, and improved weight loss [46]. Therefore, inhibition of TGase could be useful in the treatment of HD in humans, although with experiments still in animal models, trials in humans would not be expected in the next few years. Furthermore, the fact that it appears promising in animal models does not necessarily imply that it would be successful in humans.

HUMAN SINGLE-CHAIN FV ANTIBODIES

A single-chain Fv antibody is a fusion protein of the variable regions of the heavy and light chains of immunoglobulins which is connected with a shorter linker peptide of about 10 to 25 amino acids. It involves the removal of the constant regions and the introduction of a linker, although the protein retains the specificity of the original immunoglobulins. Although recent works on neurodegenerative diseases have shown *in vitro* that precise engineered antibody species, peptides, or other general agents could restrain the formation of protein aggregates, Wolfgang and colleagues claim that when using a *Drosophila* model of HD, they succeeded in the intrabody-mediated *in vivo* suppression of the neuropathology and also prolonged life expectancy [48]. Intracellularly expressed single-chain Fv (sFv) binds with a distinctive HD protein, and it has been demonstrated that anti-N-terminal huntingtin intrabodies (C4 sFv) decrease aggregation and cellular toxicity in cells and tissue culture models of HD. Lecerf and colleagues claim that anti-huntingtin sFv fused with a nuclear localisation signal retargeted huntingtin analogues to cell nuclei, which provides further evidence of the anti-huntingtin sFv specificity [49]. It has been shown that when genes for sFVs are introduced into cells and expressed intracellularly, the intrabodies produced can bind stoichiometrically with the target protein and specifically block the lethal effects of pathogenic agents [50]. Therefore, the use of intrabodies could be a therapeutic approach to treating HD in humans in a distant future.

UBIQUILIN

As expanded polyglutamine (PolyQ) tracts have been implicated in protein aggregation and cytotoxicity in HD, ubiquilin has been discovered to reduce protein aggregation and toxicity induced by PolyQ in cells and animal models of HD. Ubiquilin is a ubiquitin-like (UBL) protein and has an N-terminal UBL domain and a C-terminal ubiquitin-associated (UBA) domain. Ubiquitin is a highly conserved 76 amino acid protein [51], and ubiquilin-1 is one of the four members of the ubiquilin protein family. Wang and associates state that overex-

pression of ubiquilin decreases the aggregation and toxicity of green fluorescent protein (GFP)–huntingtin fusion protein containing 74 polyQ repeats, while a decrease in the level of ubiquilin resulted in increased aggregation and cytotoxicity [52]. When ubiquilin-1 is overexpressed in cells and in a *Caenorhabditis elegans* animal model of HD, the co-expression of ubiquilin reduces the formation of aggregates and can both prevent and salvage the motility defect caused by GFP–Htt(Q55). Ubiqulin proteins belong to the family of UBL-UBA proteins, which regulate the ubiquinated-dependent proteasomal degradation of cellular proteins [53]. The UBA domain, which is a tight ubiquitin binder, mediates ubiquilin's interaction with polyubiquitins. It has been demonstrated in several researches that both *in vitro* and *in vivo*, ubiquilin proteins bind to and co-localize with polyQ aggregates [54], thus blocking the toxic effect of protein aggregates. Ubiquilin suppresses cell death induced by PolyQ in HeLa cells and primary cortical neurons. Ubiquilin could be particularly beneficial as it has the ability to differentially distinguish between toxic and non-toxic polyglutamine proteins, thus the inherited mutant copy of CAG allele in HD can be selectively eliminated without destroying the proteins encoded by non-expanded CAG allele.

CONCLUSION

The genetics and some therapeutic advances in the management of HD have been discussed. Most of these therapies focus on the development of neuroprotective strategies, with the aim of delaying the onset and slow the progression of HD. As the onset of neurodegenerative processes begin long before the clinical manifestations of HD, it is also important to develop laboratory methods of monitoring disease progression before the onset of clinical symptoms. Most of the advances discussed are still ongoing; therefore, it is hoped that the final outcome would become more apparent in the very near future. Although there is intensive research into Huntington's disease and recent findings seem promising, effective therapeutic strategies may not be developed until the next few decades. This is because many of the laboratory breakthroughs prove to be unsuccessful in humans for a variety of unknown reasons. It is difficult, at this stage, to suggest that one potential treatment is better than the other as most of them have not been tried in humans so as to evaluate their effect, hence the reason why all therapeutic options should be explored by researchers.

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