Silencing Huntington’s chorea: 
Is RNA Interference a Potential Cure?

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Huntington’s chorea is a genetic disorder characterized by cognitive, motor and psychiatric impairments. It is caused by a dominant mutation on chromosome 4 featuring the multiplication of a portion of the gene in which the codon CAG occurs. CAG codes for the amino acid, glutamine and so the Huntingtin protein (htt) features an excessive expression of glutamine. The abnormal protein htt is prone to accumulate in neurons eventually resulting in brain cell death and the consequent progressive symptoms of the disease. Therapies for Huntington’s chorea include targeting the symptoms, the progression, and the cause of the disease, but no treatment has as yet been found to be completely effective. After describing the symptoms, genetic and proteomic basis of Huntington’s chorea, this paper discusses a new approach to treatment of Huntington’s chorea, RNA interference. The problems, limitations, and the benefits of RNA interference will be examined, along with avenues for overcoming the limitations of gene therapy with a transposition system, such as Sleeping Beauty. This review suggests that although substantial amount of research is still necessary before RNA interference treatment is successful, success will open new avenues for treating other degenerative nervous system diseases.

Key words: Huntington’s chorea; Symptoms; Treatments; CAG repeat; Gene Therapy; RNA interference; Sleeping Beauty transposition system.

Abbreviations: CAG—Trinucleotide repeat; DNA—Deoxyribonucleic acid; dsRNA—Double stranded ribonucleic acid; htt—Huntingtin protein; mRNA—Messenger ribonucleic acid; RNA—Ribonucleic acid; RNAi—Ribonucleic acid interference

Introduction

At the age of twenty-two, George Huntington proposed that a peculiar dysfunction characterized by bizarre movements could be the result of an inherited nervous system disorder. The disorder is now identified as Huntington’s chorea, in his honor. His description stemmed from observing his father’s patients while he accompanied him on rounds (Huntington, 1872; Lanska, 2000). Before Huntington’s identification of the condition, so peculiar were the disease symptoms that some people suffering from the disease were identified as witches and were persecuted for witchcraft (Lanska, 2000). Currently, there are approximately 5 to 10 people per 100,000 that are affected by Huntington’s chorea each year (Kandel et al., 1991).

The purpose of this paper is to describe disease symptoms and the proposed cellular processes in the brain that are involved in Huntington’s chorea. The paper will then summarize the current and proposed experimental therapies for Huntington’s chorea. Special attention will be directed to how RNA interference (RNAi) has potential as a therapy and a cure the disease. This paper will also feature a discussion of how adjacent therapies can enhance RNAi treatment. The following sections will successively describe: (1) symptoms (2) the genetic and proteomic basis, and (3) application of RNAi therapy to Huntington’s chorea.
Symptoms

Huntington’s chorea is characterized by cognitive, motor and psychiatric symptoms. Because the disease is progressive, symptoms are initially very mild and then over years they become so severe that they result in death. Some of the major features of each of these symptoms are discussed below.

Cognitive Symptoms

The earliest indicator of functional decline in Huntington’s chorea is cognitive (Hayden, 1981). Among the first symptoms of cognitive impairment is memory loss (Hayden, 1981). The first symptoms of memory loss could include forgetting where items are left and/or forgetting names. These are events that everyone experiences at some time, but as the disease progresses the memory impairment becomes so severe as to pose a handicap. Eventually memory loss could become so severe that family members may not be recognized and well-practiced skills may be forgotten. The memory loss in Huntington’s chorea is similar to that found in individuals with Alzheimer’s disease (Rosser et al., 2002). Alzheimer’s disease is a degenerative disease that leads to confusion, disorientation and memory failure (Kolb and Whishaw, 2001). Alzheimer’s disease is distinguished from Huntington’s chorea by a later onset in life and the absence of a clear genetic origin. Thus, an earlier age of onset and the presence of immediate family members who have had the disease distinguish the conditions (Rosser et al., 2002).

Other cognitive impairments that are displayed by individuals affected with Huntington’s chorea include slowed thinking and being and difficulty in articulating ideas (Marshall, 2004). In addition, individuals can have slowness and/or hesitancy in speech (Hayden, 1981). During the terminal stages of the disease speech often becomes disorganized and there is a reduced capacity of conceptual thought. There is also an overall decline of executive function, that ability used to organize and perform daily tasks (Hayden, 1981; Marshall, 2004).

Surprisingly, despite its genetic cause and characteristic progression, the precise nature cognitive changes in Huntington’s chorea can be quite variable from person to person in both onset and severity (Hayden, 1981; Marshall, 2004).

Motor Symptoms

The motor symptoms of Huntington’s chorea include three types of involuntary movements. The first and most distinctive is referred to as “dance mania” and this symptom is the origin of the term chorea, which in Latin means dance. During the initial stages of the disease, the chorea is minimal perhaps including slight twitches in a digit or hand. As the disease progresses, jerking and twisting movements begin to occur in many of different parts of the body. For example, an individual may display writhing movements in a limb or even hopping movements while walking (Hayden, 1981).

The second involuntary movement is known as dyskinesia (meaning changed movement), which is abnormality in the way that normal movements are performed. An affected individual may display pouting of the lips together with twitching of the cheeks and irregular elevation of the eyebrows. They might also show an irregular motion of hands and legs while sitting. Although the movements in some ways resemble normal movements, dyskinetic movements characteristically occur repeatedly (Hayden, 1981).

The third motor symptom is dystonia (Hayden, 1981), which is a change in muscle tone. A part of the body may involuntarily adopt an abnormal, sometimes painful, posture. Dystonia can affect the arms and legs, trunk, neck, eyelids, face, or vocal cords. As the disease progresses patients potentially become bradykinetic (movements are slowed) and akinetic (movements stop altogether) leaving an individual almost rigid. Rigidity, when severe and enduring, abolishes chorea and dyskinesia. In the terminal stages of the disease symptoms extend to a difficulty in chewing and swallowing (Marshall, 2004; Hayden, 1981).

Psychiatric Symptoms

Psychiatric symptoms include changes in mood, changes in engagement with others, and changes in thought processes. One third of
the affected individuals develop either depressive or explosive disorders during the course of the disease (Branwald et al., 2001). Manic-depressive episodes, in which mood is elevated and then depressed, may increase as the disease progresses (Hayden, 1981; Branwald et al., 2001; Marshall, 2004). The possibility that an individual will commit suicide increases. The tendency to suicide is influenced by the degree of functional capacity retained, the level of insight remaining to the individual, and the extent of social support available. In addition, apathy and extreme social withdrawal are common (Hayden, 1981; Marshall, 2004).

Cell Death is Progressive

The cellular changes in the brain associated with Huntington’s chorea always include portions of the basal ganglia. The basal ganglia are a collection of brain structures in the forebrain, located beneath the frontal cortex, which have extensive connections with the neocortex (the thinking part of the brain) and with the brainstem (the part of the brain that serves regulatory and motor functions). The precise contributions of the basal ganglia to behavior have not been completely worked out, but they are invariably considered to be associated with motor functions, including the production of movement and motor memory. For example, the basal ganglia may be involved in ensuring that the force and speed of movements are appropriate for a desired action. They may also be involved in ensuring that movements occur at the appropriate time and place. Not surprisingly, many of the motor and memory symptoms of Huntington’s chorea can be characterized as failures of movements to have appropriate force and failures of movements to occur at appropriate times (Hersch et al., 2001).

The actual changes in the neurons of the basal ganglia include selective and progressive loss of one type of cell. Histological studies at autopsy have shown that the medium spiny GABAergic neurons undergo changes. As the disease progresses the number of processes that leave the cell body of the GABAergic neurons become reduced and the number of GABAergic cells that are found are also reduced. Figure 1a illustrates a normal GABAergic neuron, one in the process of losing processes, and one in which the cell body is also undergoing change (Hersch et al., 2001). GABA is the major inhibitory neurotransmitter in the brain (Kolb and Whishaw, 2001). The malfunction and death of GABA neurons in the basal ganglia leaves other basal ganglia cells functioning but without the inhibitory influence of the GABA neurons. Without inhibitory control from GABA neurons, remaining systems can become hyperactive thus

![Figure 1](image1.png)

Figure 1. Pictorial representation of cell death in Huntington’s chorea. (A) GABAergic neurons lose their processes, shrink, and eventually die as the disease progresses. (B) Cell death starts in the medial striatum and spreads to lateral and ventral areas of the striatum. Cell death in the neocortex is patchy and variable.
producing the abnormalities that characterize the disease (Kolb and Whishaw, 2003).

Not only do cells of the basal ganglia die, there is a progressive pattern to their death. As is illustrated by the arrows on a portion of the basal ganglia called the striatum in Figure 1b, cell death first occurs in the medial region of the basal ganglia and then progresses to its ventral and lateral areas. Both the extent of cell death and the progress of cell death are related to the number of CAG repeats on the Huntington gene (see below) With more CAG repeats there is an earlier onset of cell loss and cell loss is more severe (Hersch et al., 2001).

Neuro-imaging techniques, such as magnetic resonance imaging (MRI) have shown that cell death also occurs in other areas of the brain. As is also illustrated in Figure 1b, the neocortex displays patches of cell death, but the location and extent of cell death is individually variable and patchy. It is very likely that the progress and the variability in the clinical symptoms of Huntington's chorea are closely related to the process, pattern, and location of neuronal disease and death (Hersch et al., 2001).

**Summarizing the Symptoms and Cell Death**

In summary, Huntington’s chorea causes cognitive, motor and psychiatric impairments. The cognitive impairments are often the first indicator of the disease and they progress to produce severe mental deficiencies. Motor and psychiatric impairments follow cognitive symptoms. Motor impairments include a variety of changes in movement, including both increases in involuntary movements and the eventual loss of movement. Psychiatric impairments include increased depression, suicide and personality changes.

The cell death associated with Huntington's chorea is distinctive (Hayden, 1981). Cell death affects the spiny GABAergic cells within the striatum and other brain regions. There is a pattern of cell death within the striatum while cell death in the neocortex is patchy and variable. Individual variability in both the progress and the severity of behavioral symptoms and the extent and pattern of cell death in Huntington’s chorea patients are likely closely related.

**Genetic and Proteomic Basis of Huntington’s chorea**

Huntington’s chorea is hereditary and caused by a dominant and autosomal mutation on chromosome 4. Therefore, if one parent carries the allele for the disease their offspring have a fifty percent chance (two out of four offspring) of inheriting the defective gene and then of developing Huntington’s chorea (Huntington, 1872; Fawcett et al., 2001).

As is well-known, each gene is responsible for making one protein, and any change (mutation) on a gene produces a protein that is accordingly different. A modified protein can be nonfunctional or detrimental in its actions. As we will describe below, the abnormality of the Huntington gene can vary extensively and the extent of the abnormality is directly related to the extent of the pathology produced by the gene (Fawcett et al., 2001)

![Figure 2. A. Huntington’s chorea gene (IT15), on chromosome 4 showing normal individual and Huntington’s chorea (HT) individual. Note that the abnormality is the expanded section of CAG repeats beyond the normal range of 10-20 repeats.](image-url)

The mutation in Huntington’s chorea consists of a change in the IT15 gene, which is responsible for making the Huntingtin (htt) protein (Fawcett et al., 2001). In 1993, a group of researchers identified a CAG expansion as the cause of the abnormal htt protein (The Huntington’s Disease Collaborative Research, 1993). A portion of the IT15 is characterized by a number of repeats of the bases CAG. An increase in the number of the CAG repeats is directly related to the pathological consequences of the resultant protein. Figure 2 illustrates the abnormality by showing two copies of the IT15 gene, the first normal and the second abnormal. A normal gene has approximately 10-20 CAG repeats on one end. When this region of the gene expands beyond 20 CAG repeats the gene becomes progressively more abnormal (The
Huntington’s Disease Collaborative Research, 1993).

The length of the CAG repeat is directly correlated with the age of disease onset (Hayden, 1981). Therefore, when there are a large number of CAG repeats, the onset of Huntington’s chorea occurs in childhood. With fewer numbers of CAG repeats the onset of Huntington’s chorea is later in life (Hayden, 1981). The number of CAG repeats that an offspring inherits is influenced by the parent who contributes the abnormal gene. If Huntington’s chorea is inherited from the father the expanded areas of the CAG repeat may increase in transmission. Thus, paternal inheritance can cause juvenile onset and a general increase in severity of the disease. About 5% of all Huntington’s chorea cases are juvenile (Kolb and Whishaw, 2003). Since very early onset will limit reproduction, an onset after the age of potential reproduction (i.e. age 40) is common. There is no expansion or reduction in the number of CAG repeats when maternal inheritance occurs, and so offspring with a maternal gene have a similar disease profile as the mother (Martin, 1995). For adult onset Huntington’s chorea, death often occurs 15 to 20 years after the appearance of the first symptoms (Kandel et al., 1991).

Each CAG codon codes for the amino acid glutamine and so the Huntington protein htt contains a portion on which the glutamines is directly proportional to the number of CAGs of the gene (Fawcett et al., 2001). The exact function of the htt protein is not known. Thus, the molecular pathways by which an abnormal htt protein mediating the neuropathology of Huntington’s chorea is also poorly understood (Thomas et al., 2004). Nevertheless, an abnormal htt protein can lead to the death of brain cells. At present, it is not known why certain brain cells are more susceptible to death or why cell death takes so long to occur. Possibly, the Huntington gene is more active in making the htt protein in some cells than in others, or susceptible cells have a poorer clearance mechanism for the abnormal protein than do other cells (Fawcett et al., 2001).

Therapies and Therapy Targets for Huntington’s chorea

Three factors can be targeted for the development of therapies for Huntington’s chorea; symptoms, progression, and cause (Norflus et al., 2004).

Some of the current symptom-specific therapies include the administration of a number of medications that help control emotional and motor problems associated with Huntington’s chorea. Dopamine blockers (anti-psychotic drugs) like haloperidol and clozapine help manage chorea and also to help control hallucinations, delusions and violent outbursts. Nevertheless, these drugs of themselves produce a slowing of cognitive and motor behavior and also often produce side effects including increased bradykinesia and dystonia (Branwald et al., 2001). Thus, as the disease progresses, this drug treatment may actually aggravate the condition.

For depression associated with Huntington’s chorea, anti-depressant drugs like fluoxetine, sertraline and nortriptyline are prescribed. Tranquilizers such as Valium can help control anxiety. Lithium may be prescribed to combat pathological excitement and severe mood swings (Wood & Morton, 2003; Norflus et al., 2004; Zucker et al., 2004; National Institutes of Neurological disorders and stroke, 2006).

Drug therapy can target the progression of the disease, but at present there are no known drug therapies that slow disease progression. More invasive and exotic therapies include use of cell transplants and deep brain stimulation (Rosser et al., 2002; Dunnett & Rosser, 2004; McBride et al., 2004). Cell transplantation involves taking basal ganglia brain cells from a donor, often a fetus but possibly the patient’s own neural stem cells, and putting them into the affected areas of the brain (Rosser et al., 2002; Dunnett & Rosser, 2004). The objective is to have the transplanted cells mature and replace lost brain cells (Rosser et al., 2002; McBride et al., 2004; Peschanski et al., 2004). Rosser et al. (2002) find that cell transplantation in affected human Huntington’s chorea patients is both safe and feasible; however, the efficacy requires a
longer follow up, as well as a larger number of treated subjects.

Deep brain stimulation is a procedure in which a wire electrode is implanted in the brain and electrical stimulation is delivered to the targeted region of the brain (Moro et al., 2004). The treatment is based on the idea that with the death of some brain cells, the functions of remaining brain cells in a circuit show increases in activity that is responsible for producing the abnormal movements that characterize the disease. By stimulating the remaining functional cells electrically, their activity is depressed and thus results in a reduction of abnormal movements. Thus, deep brain stimulation is thought to restore the balance between excitatory and inhibitory regions of the basal ganglia and neocortex (Moro et al., 2004). Moro et al. (2004) do report that deep brain stimulation decreased the symptoms of chorea. Again, however, because the disease is progressive at the latter stages of the disease this therapy may be less helpful.

Lastly, therapies can target the cause of the disease. This is the optimal approach to the disease in that a successful therapy would be a cure. First, because the disease is genetic, it could, in principle, be cured by blocking the expression of the abnormal gene. Because each cell contains two alleles of the Huntington gene, if one is abnormal, the remaining normal pair will still produce required normal protein. Thus, blocking the abnormal gene in a heterozygous individual still leaves that individual with normal *htt* protein. Second, gene therapy could be directed toward replacing the abnormal gene with a normal gene or modifying the abnormal gene so that the abnormality is no longer expressed. Third, the normal cellular mechanisms that clear *htt* protein from the cell could be boosted. Because the first of these three approaches is currently the most feasible, the following section we will consider the possibility of using RNAi techniques to block or slow the production of the abnormal *htt* protein. Because this therapy is being pioneered in a mouse model of Huntington’s chorea, a description of this mouse model will be presented first (Martin, 1995).

### Transgenic Mouse Model of Huntington’s chorea

Because the modification of genes is a new technology that can have unexpected consequences, scientists cannot directly experiment with human Huntington’s patients. Therefore, since the discovery of the CAG repeat as a causative factor in the disease in 1993, scientists have been working to develop an animal model of the disease (Stack et al., 2005). They have been successful in developing a transgenic model (transgenic means that a gene or a portion of a gene is transferred from one place to another) of Huntington’s chorea. In this mouse transgenic model, mice receive the human gene or a portion of that gene responsible for human Huntington’s chorea. Scientists have successfully inserted modified genes that contain more CAG repeats from human genome into the mouse genome. For example the N171-82Q transgenic mouse contains 82 CAG repeats, whereas the R6/1 and R6/T contain 150 CAG repeats (Stack et al., 2005; Kilvenyi et al., 2006).

Researchers have found that at eight weeks of age the N171-82Q transgenic mice fail to gain weight and by week twelve of life they exhibit irregular and uncoordinated movements, such as abnormal gait and tremors, and by week twenty-four they die. In the second mouse model, the R6/1 and R6/2 mice, there are similar behavioural symptoms that are related to the differences in the percentage of *htt* expression (Stack et al., 2005; Kilvenyi et al., 2006). The R6/1 model has a thirty-one percent *htt* expression, whereas R6/2 model has a seventy-five percent expression. During week three of life these mice develop motor symptoms, which include locomotor hyperactivity. In addition, learning and memory impairments have been observed. Mice are good swimmers, and when placed in water they will learn to escape to an island refuge in the water. They can learn the location of the refuge by noting its relation to room cues and so shorten the time required to escape form the water. This test, called the Morris water maze test, can be used to measures cognitive ability by measuring how many trials the mice require to most quickly locate the refuge. Thus, the task not only measures
cognitive function by measuring the time taken to learn, but also measures motor function by measuring how well the mice swim. By week seven, R6/1 and R6/2 animals begin making involuntary movements and display memory impairments and changes in movement (Stack et al., 2005; Kilvenyi et al., 2006).

In other tests, motor function is tested by placing the mice on a slowly rotating drum, a task referred to as the rotorod task. Normal mice are able to walk on the drum without falling, while mice with motor impairments tend to fall onto a cushion placed below the drum. The usual measure of motor ability is the time to stay up on the drum. By week twelve transgenic mice with the abnormal Huntington gene mice are severely impaired on this task (Li et al., 2005; Stack et al., 2005).

In summary, psychiatric impairments have not been well studied in transgenic mice models of Huntington’s chorea. Additionally, psychiatric impairments in a mouse might be difficult to define and measure. Nevertheless, the convincing deficits in movement and learning suggest that the transgenic models approximate a human Huntington’s condition. As a demonstration of the utility of the model, a recent study (Mcbride et al., 2004) finds that there was functional recovery in the mice when they received transplanted brain tissue taken from the basal ganglia of normal fetal mice. Thus, the transgenic mouse model can be used to investigate novel therapies such as RNAi therapy, as is described in the following section.

RNAi Therapy

RNAi, also referred to as RNA silencing, was first discovered as a natural occurring phenomenon. When RNAi is inserted into a cell it inactivates specific genes by preventing their translation.

RNAi is a tool that makes it possible to regulate gene expression after transcription. As shown in Figure 3, messenger RNA (mRNA) is transcribed from DNA and carries the “message” or the code which is then translated into the appropriate protein. RNAi can target the mRNA and modify or silence it, therefore decreasing or completely blocking protein production. Note that the abnormal gene still exists - it still makes abnormal mRNA - but the abnormal mRNA can no longer be translated into the abnormal protein.

Initial work with blocking protein production using RNAi has been effective in a variety of species including, the fruit fly (Drosophila), a round worm (Caenorhabditis elegans), a number of plants, and recently, in mammalian cell cultures (Ren et al., 2006; Harper et al., 2005).

The sequence of events for RNAi begins in a test tube making double stranded RNAs (dsRNAs). The dsRNAs are injected into specific cells of an organism. Injection of dsRNAs can occur in many ways, some of which include using pressure, or exposing the cell to a virus with dsRNAs. Since viruses replicate at a very high rate once they have entered a cell, this is useful for RNAi, because the dsRNAs can also be replicated quickly. Another method of inserting the dsRNAs is to use lipid molecules that can pass through the cell membrane easily. If they contain dsRNA they can easily release dsRNA into the cell (Tushi, 2001; Shuey et al., 2002).

Once in the cell, the dsRNA’s activate an enzyme referred to as a Dicer. The Dicer cleaves dsRNAs into strands of 21 to 23 nucleotides. After cleavage the dsRNA are referred to as small interfering RNAs. The small interfering RNAs bind to a nuclease complex to form a RNA induced complex. Once the RNA induced complex finds the complementary mRNA sequence cleavage will occur (Wall and Shi, 2003; Tuschi, 2001).

The cleavage mechanism still remains unclear (Constants, 2005; Tuschi, 2001). One possible explanation of the cleavage mechanism is that a ribonucleoprotein complex scans the...
mRNA cell content to degrade the corresponding mRNA target in a highly specific manner. The mRNA is degraded and protein production is turned off in a specific section (Constants, 2005; Tuschi, 2001).

In an exemplar study demonstrating the feasibility of RNAi, Zimmerman et al. (2006) describe the systemic administration of RNAi targeted to a specific gene in non-human primates. The primates were injected with a high dose of RNAi. Initially researchers found that there was an increase in liver enzymes during the first forty-eight hours after the administration of RNAi, but the number of enzymes diminished by day six. The gene silencing persisted for eleven days without any evidence of toxicity. The scientists ended the study after day eleven and expressed the view that the gene silencing far exceeded what they expected.

**Application of RNAi to Huntington’s chorea**

RNAi is a potential therapy for Huntington’s chorea. Because RNAi modulates gene expression, it can be used to inhibit production of the mutant \( htt \) protein. Any reduction in mutant \( htt \) production would slow the course of the disease and a complete blockade of the mutant \( htt \) protein would cure the disease. Even a reduction might be an effective cure because it might provide brain cells time to clear away accumulated abnormal \( htt \) protein.

In order to investigate the potential for using RNAi therapy for Huntington’s chorea, Harper and colleagues (2005) used a strain of mice in which human mutant \( htt \) gene had been inserted. These transgenic mice displayed both the pathological and behavioral features of Huntington’s chorea, including difficulty performing on the accelerating rotorod task. After RNAi treatment, both the pathology and abnormal behavior of the “disease” in the mice were attenuated. Thus, the study demonstrated that RNAi treatment can work in a transgenic mouse model, suggesting that it could similarly work in human Huntington’s chorea.

**Summarizing RNAi**

RNAi therapy is a treatment that reduces gene expression and could potentially be used to inhibit \( htt \) protein expression. RNAi works by preventing the RNA message carried from DNA from being translated into an abnormal protein. The therapy has been demonstrated to reduce cognitive and motor deficits in mice that have received and express the human abnormal gene causing Huntington’s chorea. Much more concerning the long term consequences of the treatment need to be investigated, but this gene silencing technique has been viewed as being very promising and potentially applicable to humans.

**Sleeping Beauty Transposons**

A major problem associated with using RNAi is that its’ effects are usually time constrained, because of the immune system responds to the foreign particles introduced by the procedure. When foreign genetic material in the form of RNAi is injected into the body, the immune system becomes active. (Chen et al., 2005). Specifically, any RNA that is longer than 30 nucleotides can activate a protein called kinase, which in turn activates interferons and cause the shutting down of translation, as well as dramatic alteration of cellular metabolism. In short, the immune system blocks the beneficial effects of RNAi therapy.

An alternative and more effective delivery of RNAi that evades the immune response might overcome this drawback. Transposons are one such evasive procedure. Transposons were discovered in corn by Barbara McClintock and in 1983 she was awarded the Nobel Prize for this discovery.

Transposons, also referred to as jumping genes, are sequences of DNA or RNA that can move into or around a cells’ genome. Thus, they have the ability to increase or decrease the amount of DNA in a genome, move genes into a site where they have never existed before and even cause mutations, and some transposons can also move RNAi. They cannot exist independently, so they move via homologues pairing. Specifically, there is pairing between the transposon and the target DNA site (Campbell and Reece, 2002; Yusa et al., 2004). Furthermore, transposons act as vectors and help carry genetic information (i.e. DNA and RNA) into the cell. A vector is a sequence of genetic
material that can have DNA sequences inserted into it and then carry the insert genetic information to a living organism’s genome (Campbell and Reece, 2002; Chen et al., 2004; Yusa et al., 2004).

There are many individual transposons, so many in fact that they make up a large portion of an organism’s genome size. For example approximately forty-five percent of the human genome is composed of transposons. Yet, most exist in a form that is no longer active (Yusa et al., 2004).

Researchers interested in transposons because they allow manipulation of the DNA inside of a living organism (Yusa et al., 2004). Sleeping Beauty is an example of type of transposon. It has been shown to be the most efficient transposon in vertebrates and has been used as a tool for germ line mutagenesis and gene delivery. In addition it has been thought to have promising applications in gene therapy (Yusa et al., 2004).

Over the million years of evolution almost all transposons have become inactivated as a result of accumulating deleterious mutations. In 1997, a group of researchers from the University of Minnesota set out to make a transposon that was active. On the logic that when first formed a transposon was active, they needed to remove mutated portions in order to have only the active form. The researchers selected a specific nucleotide transposon sequence in fish. They compared this specific sequence across eight different fish species and weeded out common sequences in all eight species. What was common to all species was the original transposon. In other words, they woke up a transposon from over fifteen million years of sleep, hence the name Sleeping Beauty (Ivics et al., 1997).

What is advantageous about Sleeping Beauty (Figure 4) is that it can serve as a non-viral vector that is ignored by the immune system (Chen et al., 2004). The Sleeping Beauty system has been shown to be capable of transferring a segment of DNA to a new position on the same or another chromosome (Wilber et al., 2006). Studies now show that Sleeping Beauty transposition system can be successfully inserted into the genomes of cultured cells lines, mouse germ cells, and in vivo in adult mice and rats without causing an immune response (Chen et al., 2005).

Sleeping Beauty transposons is also capable of successful insertion when it is co-delivered with Sleeping Beauty transposase enzyme. The transposase enzyme is able to insert sequences into human cells more efficiently when compared to the Sleeping Beauty transposition system itself (Wilder et al., 2006).

Chen et al. (2005) has shown, in vivo, that the Sleeping Beauty transposons in combination with RNAi reduces long-term protein levels of the mutant htt protein by 90%, when compared to controls and just administration of RNAi. Therefore, the combination of RNAi with the Sleeping Beauty transposons and an animal model for Huntington’s chorea may permit the evaluation of this approach in preventing the pathogenesis associated with the expression of mutant htt protein.

Problems with Sleeping Beauty Transposition System

When RNA is the source of transposase there is a continued expression of transposase after the initial transposition event. This then raises the possibility of subsequent transposon excision and re-integration thus causing gene toxicity Wibler et al. (2006) show that mRNA can be used as an effective source of transposase for the Sleeping Beauty transposition system to mediated transposition in mammalian cells and tissues, however more work needs to be done to evaluate the effectiveness and safety of this procedure.
Summary of the Sleeping Beauty Transposons

Sleeping Beauty transposons can carry a piece of DNA and place it in a gene. Thus, in principle, transposons can carry a “good” piece of DNA to replace a “bad” piece of DNA. A study done by Chen and colleagues (2005) showed that the combined action of RNAi with the Sleeping Beauty transposons significantly reduces \textit{htt} mutant mouse protein levels when compared to controls. When transposons are co-delivered with the enzyme transposase successful insertion of RNAi can occur. However, transposase can potentially increase the risk of genotoxicity, therefore it is necessary to conduct more research on the role and safety of tranposase.

Problems and Benefits of RNAi

There are limitations associated with RNAi. One problem is that how RNAi actually works in mammalian cells is not known in detail. In order to promote understanding, the sophistication of the RNAi technique needs to be further developed and investigated. Specifically, the details of the concentration, delivery, and sequence need improvement (Tuschi, 2001).

A second limitation of RNAi is that RNAi may not work in later stages of mammalian development or in mammalian cells because RNAi can activate an interferon immune response. An interferon response is a nonspecific viral defense mechanism, thus making RNAi ineffective (Tuschi, 2001). A recent study done by Grimm et al. (2006) illustrates this problem. The researchers administered RNAi to the liver cells of the mice. The immune response to the administration of RNAi blocked microRNAs, tiny molecules within the cell that regulated vital functions, and the blocking of microRNAs lead to the death of the mice.

Other limitations are that RNAi is a type of gene therapy and the US Food and Drug Administration has not yet approved any human gene therapy products for sale because of their previous experiences with side effects of viral vectors and the immune system (Tuschi, 2001). Specific viruses are the carrier of choice for many gene therapies, but there are a variety of potential health risks for the patient. These risks include both immune and inflammatory reactions in response to foreign RNA (Tuschi, 2001).

In addition, a disorder like Huntington’s chorea may not just arise from one single gene mutation, there may be other genes and/or environmental interactions influencing its development (Zabel et al., 2006). In this case Huntington’s chorea would be especially difficult to treat by solely relying on one targeted gene therapy.

For current and future Huntington’s chorea patients, researchers and clinicians may be able to combine drug and RNAi treatments, in order to alleviate the symptoms of their condition. In case of a family history of Huntington’s chorea, genetic screening could be used to detect the \textit{htt} gene mutation and the RNAi could be used to correct for it (Zabel et al., 2006).

Discussion

Huntington’s chorea is one of the several neurodegenerative diseases that that can affect people as they age. Huntington’s chorea has a lot in common with other neurodegenerative diseases, like Parkinson’s disease, Alzheimer’s disease, and amyotrophic lateral sclerosis (ALS). These neurodegenerative diseases get worse as time progresses. They are associated with cell death that occurs in specific areas of the brain, and all are suspected to have a genetic component as their cause. Huntington’s chorea, however, is different from these other neurodegenerative diseases because genetic cause of the disease is known. Specifically, there is an increase in the number of CAG repeats, which produce an abnormal protein that leads to the onset of Huntington’s chorea. The purpose of this review paper was to describe the symptoms; genetic and proteomic cause of Huntington’s chorea and to consider the potential of RNAi therapy. In addition, this paper discussed the efficacy of administrating RNAi, and the conjoint use of the Sleeping Beauty transposition system.

Traditional therapies, like drugs have
targeted the symptoms of Huntington’s chorea. They have increased the quality of life for individuals suffering from the disease, however, cannot stop the disease from progressing and eventually killing the affected individual. Therapies that target CAG repeats, the cause of the disease, are a logical target for investigation, since their control could potentially stop the progression of the disease. An example of this kind of therapy that could be used is RNAi. RNAi can block the translation of excess CAG repeats into a protein containing excessive glutamine and thus prevent consequent cell degeneration and death that the protein causes.

Nevertheless, as has been noted above, administration of foreign RNA into mammalian bodies triggers an immune response and this natural and protective response poses a problem for the usage of RNAi in humans. The use of new technology, such as the Sleeping Beauty transposition system, may suppress or bypass immune reactions.

The use of viruses for the delivery of RNAi poses another problem. Because viruses can be dangerous to humans and cause disease, the administration of RNAi for disease conditions must be viewed with caution. A mutated virus could potentially spread from a treated individual into the general population.

Nevertheless, despite potential drawbacks, RNAi holds promise as an effective therapy. It might also become useful in other neurodegenerative diseases like familial Alzheimer’s disease and ALS (Martin, 1995). RNAi has also been hypothesized to possibly reduce viral induced replication and inflammation, as well as being an alternative to anti-retroviral drug regimes for HIV (Wall and Shi, 2003).

Conclusion

The present paper has reviewed the causes and symptoms of Huntington’s chorea. Because the genetic component of the disease is well characterized, it is feasible in principle to reverse the emergence of symptoms. There is promise that in the future RNAi could provide an effective cure for the disease. Although, RNAi looks like a promising therapy for Huntington’s chorea there is, however, a lot more future investigation and evaluation required in order for this therapy to be used on humans (Wall and Shi, 2003; Zimmerman et al., 2006). In addition the adverse effects of transposase involved in the Sleeping Beauty transposons need to be further understood. Despite drawbacks, treatment that targets gene expression has potential as a cure of Huntington’s chorea as well as other degenerative diseases of the nervous system.

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