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Huntington's Disease - A Breakthrough in Treatment using CRISPR/Cas9

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Abstract

Huntington's disease is classified as a rare neurodegenerative disease that affects patients' motor skills. The disease is passed on from generation to generation due to it being a dominant genetic disease. Some symptoms of Huntington's disease include chorea and cognitive decline. Although there is no current cure, scientists believe that gene editing methods such as CRISPR/Cas9 could be a possible way to combat Huntington's. A couple of methods scientists used to research treatments for Huntington's, include using hiPSCs with CRISPR/Cas9 and personalizing the CRISPR/Cas9 treatment using specific PAM sites. These methods have not been tested clinically yet, and there is still more research to be done on whether this is entirely safe, however, there is still hope that this technique could become a cure in the future.

Keywords: Huntington's Disease, Gene Editing, CRISPR, Biology, Genetics

1. Introduction

Huntington's disease (HD) is a dominant, genetic disorder that causes neural degeneration. This disease is relatively rare, occurring in around 1 in every 10,000 people in the United States (Huntington's Disease Overview, Incidence and Prevalence of HD, n.d.). Many patients usually start developing symptoms between the age of 30 and 50, however it can begin as early as the age of two (Roos, 2010). HD is characterized by unwanted choreatic movement as well as motor, cognitive, and psychiatric disturbances (Roos, 2010). Chorea is classified as jerky, involuntary movement that initially takes place in the fingers or toes and eventually moves up to facial muscles and other parts of the body. In addition to the chorea, patients experience a decline in motor skills such as slurred speech or balance issues (What is Huntington's Disease?, n.d.). Day-to-day activities become harder, and patients have a difficult time walking or standing. In addition to the many physical symptoms, HD patients also experience significant cognitive issues such as depression, apathy, or dementia (What is Huntington's Disease?, n.d.).

HD is caused by a DNA error in the huntingtin gene which is important since it makes proteins that help brain development before birth and plays a role in synaptic function (Roos, 2010). It also has an anti-apoptotic function which is crucial so that a cell can get rid of itself in the case of abnormalities such as improper cell division. Normally, the gene has less than 26 CAG letter repeats, but the faulty gene consists of more than 40 CAG repeats, which cause the protein to be too long (UC Davis Health, n.d.). Over time, the mutant protein misfolds and forms clumps in neurons, eventually leading to cell death by stopping necessary functions (Finkbeiner, 2011). The reason why many treatments or cures have not been developed so far, is because it is difficult to

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treat the mutation without completely removing the gene. As the huntingtin gene is essential to have in the body, a patient cannot afford to lose it, making this option unviable.

A Huntington patient's life is divided into 2 stages, at-risk preclinical and clinical (Roos, 2010). If someone has a parent with Huntington, doctors have enough information about genetic inheritance to know that the child could potentially develop the disease, labeling them as at-risk preclinical. People in this stage do not show any symptoms and have not manifested the disease yet, so it is part of preventative care to let the patient and doctor know to look out for signs (Roos, 2010). This stage comes to an end once they confirm that the patient begins to display symptoms and carry the extra CAG repeats on the huntingtin gene (Roos, 2010). As a person approaches the clinical stage, they start developing more symptoms and signs associated with the disease.

As of now, there are no cures for Huntington's, however there are treatments to help patients. Genetic counselors can aid both patients and families with the mental health symptoms and help explain what exactly the patient is going through (Roos, 2010). Additionally, physical and occupational therapists work with patients and families to ease symptoms; there are also medicines to provide relief. For example, dopamine receptor blocking, or depleting agents are used to treat chorea (Roos, 2010). Additionally, many antidepressants are used to help with patients' depression as a result of the disease. However, scientists are currently doing intensive research to look for cures for Huntington's and believe that the answer lies in gene editing with tools like CRISPR/Cas9.

2. Gene Editing

Gene editing has become a beneficial tool for scientists over the past few decades. Specifically, a tool called CRISPR/Cas9 has become popular in recent years as it is simpler, faster, and more efficient to use compared to other gene editing tools. CRISPR, also known as clustered regularly interspaced short palindromic repeats, is a fairly new technology that allows scientists to precisely and accurately edit the DNA of an organism to remove mutations or create new beneficial ones. CRISPR is based on the use of very specific and programmable nucleases that produce changes in regions of interest in genomes using double-strand breaks (DSBs). CRISPR directs a nuclease called Cas9 to a specific location to create the DSB. Later, the cell repairs the DSB by its natural mechanism and corrects or adds new sequences in the DNA strand. (Schmidt, et al., 2021) Another important component of the CRISPR technology is the guide RNA (gRNA) that guides the Cas9 nuclease to the target site in order to edit the genome. gRNA is made up of two parts: crispr RNA (crRNA) and tracr RNA. Once these components join and guide Cas9 to the site, they perform a sequence-specific cleavage by recognizing the base pairings and target sequence. ("Full Stack Genome Engineering," n.d.)

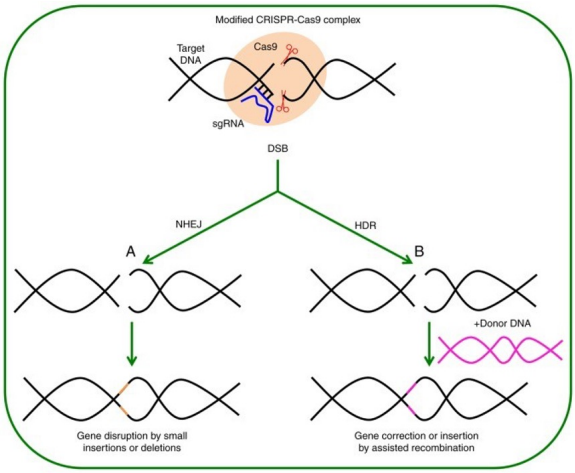

Figure 1: This figure shows the general steps associated with CRISPR/Cas9. Once the Cas9 enzyme creates a DSB in the DNA strand, new nucleotide bases are either added or removed, and finally repaired by cellular mechanisms such as NHEJ and HDR. NHEJ is a mechanism that simply joins the two ends of the strand together, while HDR repairs the DNA strand by matching the nucleotide pairs. This results in an addition or deletion of certain genes. (Rodríguez-Rodríguez, et al., 2019)

In addition to gRNA, CRISPR requires protospacer adjacent motif, or PAM, sites to accurately find the intended target site. PAM sequences are groups of around three to five

nucleotides that are close to the target which help Cas9 identify where to bind to. The length of the PAM sequence helps determine the frequency of the target sites; longer sequences are found less frequently in the genome than shorter ones, while shorter sequences are more frequent. The length also helps figure out how persistent unintended off-target cuts will be. If a PAM site is long, there are less chances for there to be off-target cuts since the more specific the sequence is, the less likely it is for Cas9 to mess up while reading the nucleotides. However, if a PAM site is short, that means the sequences occur more frequently on the genome so the Cas9 might pick one that is not intended. Although a lot of research has been done on CRISPR, scientists are still not sure about the full effects off-target cuts might have on organisms. Nevertheless, there are many methods that are used to perfect gRNA design as much as possible to limit these cuts. One way the gRNA design is enhanced is to use the mutant version of Cas9 called nSpCas9. This mutant enzyme requires two nSpCas9 to make a DSB, each with its own sgRNA. Off-target activity is reduced by 50 to even 1,500 times using this method (Roos, 2010; Rodríguez-Rodríguez, et al., 2019).

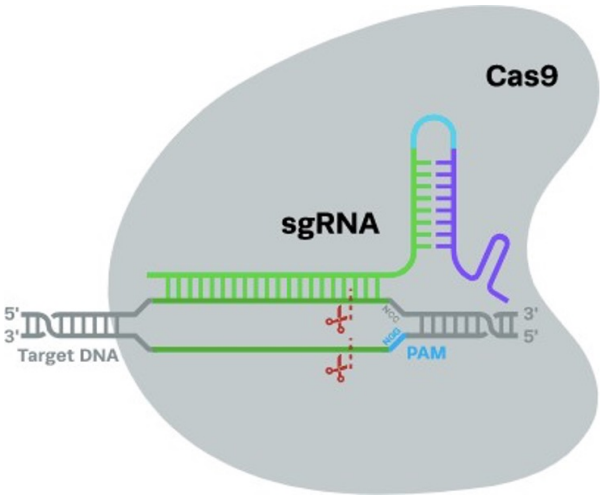

Figure 2: Here, the sgRNA, modeled with the green, guides the Cas9 molecule to the target site near the PAM sites in blue and creates a DSB in the strand. (Full Stack Genome Engineering, n.d.)

Even though CRISPR has a lot of potential, it is important to be aware of its risks as well. In a study done at Columbia University, researchers created 40 embryos with a mutation responsible for blindness ("Lab Tests Show Risks of Using CRISPR Gene Editing on Embryos," 2020). They edited these embryos to get rid of the mutation, however they found that instead of editing the mutation, the chromosome with the mutation disappeared altogether. This change is extremely fatal for embryos and can alter many important functions. Furthermore, there is not enough evidence from research showing any long-term effects that CRISPR could have on a genome or person ("Lab Tests Show Risks of Using CRISPR Gene Editing on Embryos," 2020).

Figure 3: This figure shows the huntingtin gene on chromosome 4. The blue sequences model the pam sites and the orange triangles are the cut site. The green guide RNA is guiding the Cas9 to the specific target site to correct the mutation. (Reproduced from "Full Stack Genome Engineering," n.d.)

Despite its risks, CRISPR could still be extremely useful and promising. Many therapies and treatments for diseases such as sickle-cell, cancer, down syndrome, and Huntington's include the use of CRISPR. Although these therapies are still being researched, some are going through clinical trials and might become widespread treatments. Recently, some scientists conducted experiments with CRISPR to see if they could suppress the mutation in Huntington's disease and found promising results.

3. New Potential Therapies

As Huntington's disease does not have any cures right now, scientists are doing all they can to research potential ones. However, there are not any clinical trials right now for HD because of the limitations of CRISPR. Currently, CRISPR is mostly used for blood disorders such as sickle cell anemia or thalassemia

because of how accessible and easy it is to treat blood cells. But, since this disorder mainly affects the brain and neurons, it is much more difficult to do treatments with gene editing in the brain due to its inaccessibility. Additionally, there is the issue of getting the CRISPR treatment to every affected cell in the human body. There are millions of cells in a person, and current technology is not equipped to deliver the treatment to every single cell yet. Nonetheless, there is still research going on in vitro related to treatments for Huntington.

An example is an experiment that a couple of scientists conducted in 2017 where they used human induced pluripotent stem cells (hiPSCs) and edited their DNA using CRISPR/Cas9. hiPSCs are special types of stem cells that are taken from a person and treated with different transcription factors that maintain their pluripotency, which is the ability of a stem cell to make other types of cells in the body (Xu, et al., 2017). hiPSCs are particularly useful since they are easy to culture in a lab and can become any desired cell type. Since Huntington affects neurons and muscle cells, scientists can use hiPSCs to create these different types of cells while having them remain genetically identical.

Because these cells are being cultured outside of a natural environment there are chances that they can pick up different types of undesirable mutations or changes, so remaining genetically identical is crucial to get accurate results throughout the study. After creating their gRNA, the scientists made different types of selections to see which cells received the correct CRISPR editing, did not receive it, received a mutant version, or received it and died (Xu, et al., 2017). This marker is useful when they go back to study the population of cells that CRISPR successfully altered. Once the CRISPR/Cas9 reached the cells, the researchers concluded that it successfully corrected the HD mutation in the cell population. They compared the corrected cells with normal ones and reported that the corrected ones were functioning normally (Xu, et al., 2017).

In another study, a group of different scientists attempted to do the same thing, just using a different approach. Here, the researchers used a personalized CRISPR/Cas9 strategy based on SNPs to target specific CRISPR/Cas9 sites targeting the mutant huntingtin gene (Shin, et al., 2016). First, the researchers identified eight of the most frequent huntingtin gene haplotypes. Upon identification, they looked for pairs of PAM sequences that were present in the mutant chromosome but not from the normal chromosome. Afterwards, the scientists used CRISPR/Cas9 to target the PAM sites and eliminate the promoter region, transcription start site, and CAG repeats. Conclusions and results showed that performing these actions permanently inactivated the CAG repeats in the mutant Huntingtin gene, thus getting rid of the disease. Something unique about this approach is that it can be personalized for every patient which is important since one technique might work on someone's body but be rejected by another's. Therefore, this approach might be more favorable than the previous one (Shin, et al., 2016).

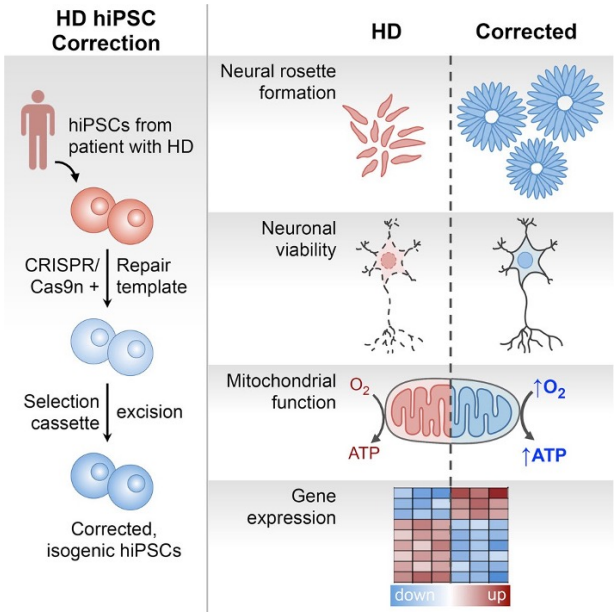

Figure 4: This figure shows the general steps that a group of scientists took to research potential Huntington cures. After taking out hiPSCs from patients, as shown on the left, they used CRISPR/Cas9 to correct the mutation. The right part of the figure shows the neural cells when they are affected with Huntington's disease compared to healthy neural cells. (Reproduced from Xu, et al., 2017)

4. Conclusion

Huntington's is a neurodegenerative disease that

mainly affects neurons. This disease is caused by the repeat of the nucleotide letters CAG in the huntingtin gene, causing the huntingtin protein to be abnormally large which eventually damages the cell. As of now there is no cure for the disease, but there are treatments available to ease symptoms of patients that are affected by it. In the search of a cure, scientists are researching gene editing methods to try to silence the mutation. The CRISPR/Cas9 system has been tested on cells and shows that it is possible to alter the gene and potentially cure the disease by using stem cells and targeting patient specific PAM sites. Clinical trials have not started yet since it is much more difficult to use CRISPR in patients due to the hardship of editing every cell in the body. However, there is hope that in the future this treatment could be used to help Huntington patients.

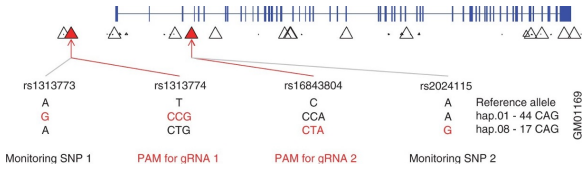

Figure 5: This figure shows the various PAM sites that were used in this study to narrow down the specific target sites. (Reproduced from Shin, et al., 2016)

Disclosure

S.S originated the research, did the literature searches, and wrote the paper with figures. M.K supervised the research and writing as well as project direction.

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