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CRISPR/Cas9 gene editing: A promising approach towards Huntington's Disease

I Im Liu

University High School, California, 92614, America

Liuamy316@gmail.com

Abstract. Huntington's Disease (HD) is an incurable neurodegenerative condition marked by the gradual decline of motor abilities, cognitive capabilities, and emotional stability. It results from a mutation in the Huntingtin gene (HTT), which triggers the generation of a harmful variant of the Huntingtin protein known as mutant Huntingtin (mHTT). Despite significant advancements in understanding the disease's molecular basis, effective treatments to halt or reverse its progression remain elusive. Over the past few years, the groundbreaking genetic modification technique called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has risen as a hopeful tool in the realm of genetic investigation and treatment. CRISPR has the potential to precisely target and modify specific genes, offering new possibilities for the treatment of Huntington's Disease. This paper aims to provide an overview of Huntington's Disease, the CRISPR technology, and its potential applications in addressing the underlying genetic causes of HD. By exploring the fundamental aspects of both HD and CRISPR, this paper hopes to provide a clearer picture to the therapeutic potential of CRISPR in mitigating the effects of this neurodegenerative disorder.

Keywords: CRISPR Cas9, Huntington's Disease, gene editing, neurodegenerative disorder.

1. Introduction

Huntington's Disease (HD) is a rare autosomal dominant neurodegenerative disorder that involves involuntary muscle movements, poor coordination, problems swallowing, speaking, and breathing, and mood swings and mental illnesses [1]. This disease is related to an expansion of a CAG trinucleotide repeat within the first exon of the Huntingtin (HTT) gene. It occurs worldwide but is most commonly seen in Europe and countries of Europe origin, such as the United States and Australia. Individuals with HD could begin to show symptoms anytime from age 1 to 90 [1]. However, before then, patients will be healthy and have mostly no detectable abnormalities. Each individual possesses a gene responsible for encoding the huntingtin protein. Near the start of this gene, there exists a sequence of three codons, C-A-G, which is repeated around 10 to 35 times. Each occurrence of the C-A-G sequence corresponds to the amino acid glutamine. When an individual's glutamine repeats surpass 35, the probability of contracting HD escalates, and once the glutamine repeats reach 41, the disease is fully penetrant [1, 2].

CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is a revolutionary gene-editing technology that allows scientists to make precise modifications to DNA [3-6]. It originated as a natural defense mechanism found in bacteria and archaea, serving as their adaptive immune system against viral infections. CRISPR systems consist of two primary elements: the CRISPR

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array and the Cas proteins. The CRISPR array consists of short, recurring DNA sequences, combined with unique spacer sequences derived from viruses or external genetic material that are inserted between these repetitions. Cas proteins, particularly Cas9, act as molecular scissors that can recognize and cut DNA at specific target sequences guided by a small RNA molecule called the guide RNA (gRNA) [5, 6]. Many CRISPR systems, such as CRISPR/Cas9 and CRISPR/Cas12, must be followed with a protospacer adjacent motif (PAM) to perform gene editing because gRNA alone is not sufficient to determine the specific site for Cas9 binding. PAM is a short DNA sequence that is essential for guiding the Cas9 enzyme to the current location in the genome for gene editing. The Cas9 protein recognizes the PAM sequence, and once it finds the PAM adjacent to the target DNA, it positions itself to initiate the gene editing process. Researchers have the ability to introduce fresh genetic material, eliminate present DNA sequences, or enact precise modifications to the DNA sequence by providing a repair template.

The simplicity and efficiency of CRISPR-Cas9 have revolutionized genetic research and opened up a wide range of applications. It has greatly accelerated the study of gene function, allowing researchers to investigate the roles of specific genes in various biological processes and diseases. CRISPR-Cas9 also holds immense promise for the development of new treatments for genetic disorders, like Huntington's Disease, as it offers the potential to correct disease-causing mutations in human cells.

This paper aims to provide an overview of Huntington's Disease, the CRISPR technology, and its potential applications in addressing the underlying genetic causes of HD. By exploring the fundamental aspects of the methods used to apply this revolutionary gene editing technique onto this neurodegenerative disorder, this paper hopes to shed light on the therapeutic potential of CRISPR in mitigating the effects of HD.

2. Mechanisms of action

2.1. Current therapeutic approaches towards Huntington's Disease

Therapeutic approaches for HD can be categorized into several groups. One approach aims to decrease the amount of mutated HTT protein by targeting its transcription and translation levels, as well as inducing its degradation through the proteasomal pathway [7]. Examples of this include RNA interference (RNAi) and antisense oligonucleotides (ASOs). Another strategy involves modifying the mutated HTT protein through post-translational modifications, which is being explored as a pharmacological approach [7]. This involves ubiquitination, phosphorylation, and other modifications. The more popular methods for treating HD would be using RNAi and ASO. RNAi has gained significant attention and have been extensively investigated in preclinical studies and clinical studies. Many companies have also produced RNAi-based drugs targeting the mutated HTT genes, with some even advancing into clinical trials. However, these methods induce partial degradation. Moreover, they require long term and continuous therapy [7]. HTT gene editing can therefore become a potential therapeutic approach as it permanently inactivates/repairs the HTT gene.

2.2. Modify mutant HTT

The primary goal is to target and modify the mutant HTT allele while leaving the wild-type allele unaffected. Two main approaches will be discussed: global silencing of both alleles and allele-specific targeting [7]. For global silencing, both alleles of the HTT gene are targeted using CRISPR/Cas9. To accomplish this, the sgRNA needs to target a core region within the promoter or the beginning of the coding sequence to interrupt HTT transcription or translation [8, 9]. This technique leads to a significant reduction or complete inactivation of HTT expression. However, long-term effects and potential side-effects of global silencing need to be carefully considered and analyzed.

Alternatively, allele-specific strategies can be considered. In this approach, therapeutic agents are designed to precisely target and suppress only the mutated allele, while leaving the healthy, non-mutated allele unaffected. This strategy offers several advantages over general silencing methods, such as improved specificity and effectiveness, as well as a reduction in adverse effects. The analysis of a group of individuals with HD using advanced sequencing tools has provided insights into specific variations

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associated with expanded HTT alleles. Certain genetic variations, known as single nucleotide polymorphisms (SNPs), show a stronger connection to the expanded allele in families with a history of HD. Successful allele-specific suppression has been achieved using technologies like shRNA and ASOs with specific SNPs. However, when it comes to gene editing, it's crucial to target a region near the mutation, which limits the pool of usable SNPs for editing compared to simple suppression.

One approach to allele-specific targeting involves using a sgRNA to target a central region of the HTT promoter, disrupting its transcription. SNPs with high heterozygosity frequency in the HTT promoter among HD patients could be suitable for this method. The CRISPR/Cas9 system would differentiate between the two alleles and specifically edit the mutant HTT gene, leaving the normal allele untouched. Furthermore, CRISPR/Cas9 has the capability to simultaneously target multiple genes through a technique called multiplexing, using different sgRNAs. In the context of treating HD, a combination of two sgRNAs can be employed to selectively delete exon 1 of the mutant HTT allele. By eliminating exon 1, the detrimental effects of the mutated HTT gene can be addressed, offering potential therapeutic advantages for individuals with HD.

2.3. Designing specific gRNAs

To utilize CRISPR/Cas9 for HD, researchers need to first design specific gRNAs and predict theon/off target efficiencies. These gRNAs comprise a sequence of 20-23 nucleotides that can attach to the target gene, along with a PAM sequence of 3-6 nucleotides situated adjacent to and downstream of the targeted sequence. These elements are crucial for the binding of the gRNA with the DNA and for the activation of the gRNA [7, 11]. Crafting gRNAs for HTT allele-specific editing poses greater complexity, necessitating the targeting of a genetic sequence with a heterozygous SNP [10, 12-13]. One strategy for gRNA design entails incorporating the SNP near the PAM sequence [14]. The efficiency of cleavage within the wild-type HTT allele might be notably inferior to that within the mutant allele due to the decreased effectiveness of Cas9 nucleases when encountering mismatches in this region [15]. For optimal implementation of this approach, placing the SNP near the 3' end of the sequence is vital, given that discrepancies are better tolerated at the 5' end [16]. Another approach to specifically target the mutant HTT gene involves situating the SNP within the PAM of the mutant allele while excluding the wild-type allele, thereby enabling allele-specific editing [7, 17]. A successful demonstration of this strategy was achieved by Beverly Davidson's research group, who employed two gRNAs to excise exon 1 of the mutant HTT allele.

It's also crucial to consider the various combinations of wild-type and mutant allele haplotypes present in their genomes. Focusing on the most common diplotype covers only a small percentage of the population, meaning multiple SNP-specific sgRNAs will be required to edit the HTT gene specifically for each individual's genetic makeup. This personalized approach is necessary to address the diverse genetic factors involved in HD [7].

2.4. Expression and cloning methods

For the expression and cloning methods of the Cas9 nuclease and sgRNA, the Cas9 is expressed from a Polymerase II promoter, while sgRNAs are controlled by Polymerase III promoters such as H1, U6, and 7sk [18]. Different promoters like EFS-NS, CMV, CAG, and CBh are used to control Cas9 expression. Various techniques are used to clone sgRNAs into vectors. To prepare sgRNA can be ordered as a double-stranded DNA fragment or two complementary oligonucleotides. Once obtained, the sgRNA is cloned into a plasmid. For optimal transcriptional efficiency, it is recommended to have a G nucleotide at the 5' end of the sgRNA sequence, especially when using the U6 promoter, which drives the transcription of the sgRNA.

For complex constructs, DNA assembly or recombination strategies like Golden Gate, Gibson, and Gateway can be used [19]. LVs and AAVs are commonly used to deliver Cas9 and sgRNA in gene editing. LVs have a larger capacity and can carry both Cas9 and sgRNA in a single vector, while AAVs require shorter Cas9 variants and use two vectors [19, 20]. Plasmids like lenti-CRISPRv2 are available

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for these viral vectors, enabling efficient gene delivery [21]. DNA extraction is performed using various kits based on phenol-chloroform separation or affinity column purification.

2.5. Choosing animal models

Selecting animal models for validating sgRNAs relies on the region in the HTT gene where it is targeted and the specific aspects being measured. Knock-in mice are suitable for targeting human HTT Exon 1, while transgenic HD mice are used to assess editing efficacy but not selectivity. HD mouse models expressing HTT under the human HTT promoter, like YAC or BACHD mice, are required for targeting the HTT promoter region. Fully humanized HD mice with two human HTT alleles have been developed for testing allele-specific HTT editing strategies, offering more accurate representations of human genetic backgrounds.

2.6. Yang S. et al study on KI mice

In recent studies led by Yang S. and colleagues, it was found that reducing the levels of normal HTT in the brains of adult mice does not affect the survival of the animals, their growth, or the health of their neurons. Additionally, tests conducted on knock-in mice expressing mutant N-terminal HTT demonstrated that the initial embryonic development does not rely on the presence of the N-terminal portion of HTT, indicating that focusing on eliminating this region might offer a viable therapeutic approach for HD. Using CRISPR/Cas9 technology, the researchers designed four gRNAs to target specific DNA regions flanking the CAG repeat in exon 1 of the human HTT gene. In cellular experiments, a combination of two gRNAs (T1 and T3) effectively reduced mHTT protein levels. Subsequently, they tested this approach in HD mice expressing full-length mHTT with 140 CAG repeats. Injecting two gRNAs (T1 and T3) into the mice's striatum using AAV vectors along with a separate AAV vector expressing Cas9 resulted in significant reductions in mHTT levels, nuclear accumulation, and aggregation of mHTT, while attenuating reactive astrocytes, an early neuropathology in HD. These discoveries highlight the promise of using CRISPR/Cas9 to reduce HTT levels as a potentially effective path for treating Huntington's disease.

3. Advantages and disadvantages of CRISPR Cas9

CRISPR CAS9 is a powerful tool that might become possible to help with various disorders because of its simplicity and specificity. It is believed that this gene editing tool would help fasten the curing process of this disease, ultimately coming up with a way to solve this neurodegenerative disorder forever. There have been many other gene editing tools that have come before CRISPR such as TALEN and zinc-finger nuclease. However, CRISPR CAS 9 shows many advantages in front of them. (1) Ease of use: It is relatively simpler to design and implement compared to TALEN and zinc-finger nucleases. The CRISPR system utilizes a sgRNA that can be easily programmed to target specific DNA sequences, making it more user-friendly. (2) Target specificity: CRISPR-Cas9 provides higher target specificity, resulting in fewer off-target effects. TALEN and zinc-finger nucleases may have a higher risk of causing unintended changes in the genome due to their larger recognition domains. (3) Efficiency: CRISPR-Cas9 is generally more efficient in inducing gene modifications. It can efficiently create insertions, deletions, or substitutions in the DNA sequence of interest, making it a preferred choice for genetic editing experiments.

The central issue of CRISPR CAS 9 is its off-target problem, which causes a multitude of problems, such as genomic toxicity, genome instability, epigenetic disruptions, and gene functional disruption. Thus, further studies should aim at looking for ways to limit the off-target effects. Currently, there are approximately 3 approaches to this problem: the first approach is to conduct large-scale sequencing of the modified genome [23]. Secondly, a technique called BLESS could be used [24]. Keith J. and the team also discovered another technique called The Guide-Seq, which is more accurate, sensitive, and cost-effective [25]. It relies on capturing short DNA sequences into DSBs and then specifically amplifying the regions of DNA that contain these tags.

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4. Conclusion

CRISPR-Cas9 is undeniably a revolutionary gene editing technique with vast potential for transforming the field of medicine and genetic research. Despite the social and ethical concerns surrounding its application, continuous research and responsible exploration of CRISPR's capabilities hold the promise of groundbreaking advancements in healthcare. By using the power of this technique, people have the potential to overcome previously insurmountable challenges and find effective treatments for devastating diseases like Huntington's disease. As we navigate the complexities of gene editing, it is crucial to maintain rigorous scientific scrutiny, ethical considerations, and open dialogue to ensure that CRISPR's benefits are maximized while its potential risks are minimized. With a commitment to responsible research, CRISPR-Cas9 offers hope for a future where we can address previously untreatable conditions and improve the lives of countless individuals.

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