CRISPR-cas9 Gene-Editing Implications on Huntington's Disease

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Abstract

Several studies have examined the efficacy of the CRISPR/Cas9 gene-editing tool in remedying the toxic effects of Huntington's disease (HD) as a potential therapy for preventing the neurodegeneration that is observed in HD. Huntington's disease is caused by an abnormality in the huntingtin (HTT) gene, which leads to the encoding of the mutant huntingtin protein (mHTT). The accumulation of mHTT protein in neurons has been found to cause oxidative stress and inflammatory pathways. Additionally, the abnormality in the HTT gene has been attributed to the abnormal expansion of a CAG trinucleotide repeat sequence. Therefore, some studies used CRISPR/Cas9 to target the mHTT gene, along with genes that play a role in redox regulation, including Nrf2 and SOD2. The results of this study showed a decrease in oxidative stress and greater mitochondrial function. A second study builds on these findings in recognizing the dangers involved with removing the CAG repeat in the gene. This study tested the use of a single gRNA to selectively remove particular CAG repeats in the mutated HTT gene at the site of transcription. This allele-specific approach changes genetic expression permanently.

Introduction to Huntington's Disease and CRISPR

Huntington's disease is a neurodegenerative disease that affects brain function and gradually breaks down the neurons in the brain, causing those suffering from the disease to slowly lose motor and cognitive function. Specifically, Huntington's disease targets a part of the basal ganglia, which is the major neural system in the brain that receives cortical inputs, processes them, and delivers the inputs and processed information to the cerebral cortex through connections in the midbrain and the thalamus (Charles R. Gerfen et al., 2007). Cortical inputs, specifically, are the inputs and brain activity having to do with the cerebral cortex, where higher processes of the brain occur, such as thinking, processing emotions, learning, memory, and

reasoning. The basal ganglia itself primarily controls motor controls (movement, writing, speaking, etc.), as well as learning and behavior (José L. Lanciego et al., 2012).

Unfortunately, there is currently no cure for Huntington's disease. Someone born with Huntington's disease will have it their whole life, though typically it does not appear or begin to show symptoms until ages 30-50. Huntington's disease is fatal primarily because it promotes the degeneration and death of neurons (or brain cells). These cells do not regenerate and extensive damage to these brain cells can lead to a loss of function.

Scientists are currently working on using CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats), a new technique that involves cutting out, replacing, or inactivating a gene causing a problem in a particular DNA sequence. In Huntington's disease, the issue stems from an issue in the gene for the protein huntingtin, causing the DNA building blocks to be repeated more times than necessary. CRISPR is a good tool for this type of disease because rather than focusing on prevention or reversal of effects (through therapies, etc.), which are oftentimes not helpful in a genetic disease, CRISPR actually targets the root of the problem, which is the genes themself.

Mitochondrial and Redox Modifications in HTT Gene

Introduction

The huntingtin (HTT) gene provides instructions for making the huntingtin protein, which is important for normal development and function of the brain. In people with Huntington's disease (HD), there is an abnormality in the HTT gene, where a CAG trinucleotide repeat sequence is abnormally expanded. This results in the production of a mutant huntingtin protein (mHTT) that is longer and more toxic than the normal huntingtin protein. The abnormal expansion of CAG repeats in the HTT gene leads to the production of mHTT, which is prone to

misfolding and aggregation. The accumulation of mHTT protein in neurons is believed to disrupt cellular processes, leading to neuronal dysfunction and eventual cell death, particularly in the striatum and cortex regions of the brain (Alkanli et al., 2023). The toxic effects of mHTT are thought to be due to a range of mechanisms, including impaired protein clearance, mitochondrial dysfunction, altered transcriptional regulation, and disrupted vesicular transport. These mechanisms ultimately lead to the accumulation of oxidative stress and the activation of inflammatory pathways, which can contribute to the neurodegeneration observed in HD (Monteys et al., 2017). Therefore, targeting the mHTT gene to reduce the levels of toxic protein has been a major focus of research in the development of treatments for Huntington's disease.

Discussion

Mitochondrial dysfunction and oxidative stress have been implicated in the pathogenesis of Huntington's disease (HD). Recent studies have suggested that CRISPR/Cas9-mediated gene editing may be a promising approach for reducing the levels of mutant huntingtin protein (mHTT) and improving mitochondrial function in HD models. In one study, CRISPR/Cas9 was used to target the mHTT gene in neurons derived from HD patient iPSCs, resulting in a significant reduction in mHTT levels and an improvement in mitochondrial function. Additionally, the use of CRISPR/Cas9 to target genes involved in redox regulation, such as Nrf2 and SOD2, has been shown to reduce oxidative stress and improve mitochondrial function in HD models (Monteys et al., 2017). These findings suggest that the CRISPR/Cas9 gene-editing method may effectively target and reduce mHTT protein while also modulating mitochondrial and redox pathways in HD cellular and animal models, providing a promising avenue for the development of new therapies for HD (Alkanli et al., 2023).

Redox modification refers to the process of changing the redox state, or the balance between oxidizing and reducing agents, within cells. This process is crucial for maintaining cellular homeostasis, as redox reactions are involved in a wide range of biological processes, including energy metabolism, signaling, and gene expression. However, excessive oxidative stress resulting from an imbalance between oxidizing and reducing agents can lead to cellular damage and contribute to the pathogenesis of many diseases, including Huntington's disease (HD). In HD, there is evidence of increased oxidative stress and altered redox signaling, which can lead to neuronal dysfunction and death. One important redox pathway that is affected in HD is the Nrf2-Keap1 signaling pathway, which regulates the expression of genes involved in the antioxidant response. In HD, the Nrf2-Keap1 pathway is impaired, leading to reduced expression of antioxidant genes and increased susceptibility to oxidative stress. Recent studies have suggested that targeting redox pathways using gene-editing tools such as CRISPR/Cas9 may provide a promising approach for reducing oxidative stress and improving cellular function in HD models. For example, one study used CRISPR/Cas9 to target the SOD2 gene, which encodes a mitochondrial antioxidant enzyme, resulting in improved mitochondrial function and reduced oxidative stress in HD models.

Conclusion

Overall, redox modification represents an important area of research in the development of new therapies for HD and other neurodegenerative diseases, and gene-editing tools such as CRISPR/Cas9 may play a critical role in modulating these pathways to improve cellular function and reduce oxidative stress.

Polyglutamine Repeats in the Huntington's Gene

Introduction

The HTT gene produces the huntingtin protein, found all over the body, but most concentrated in the brain. Huntington's disease as a neurodegenerative disorder is caused by the misfolding of this protein due to the presence of polyglutamine repeats which produce a mutant Huntingtin gene (mHTT). eventually cell death of impacted neurons. Allele-specific targeting of mHTT as a focus of suppression has been investigated, however, there have been negative consequences. In mice, embryonic lethality has occurred when there is a loss of HTT and it is unclear whether suppression of any allele is safe.

Various studies have addressed this issue and have found that targeting polyglutamine expansions, which cause the disease, may provide safer therapies. These studies have shown that Using CRISPR/CAS-9 mediated gene editing can extinguish polyglutamine expansion-mediated neuronal toxicity in the adult brain (Yang et. al). Most studies have investigated this in striatal neurons due to HD having a profound effect on this brain region.

Discussion

In order to target mutant HTT using the polyglutamine repeats as a target, researchers first designed guide RNAs (gRNA) that would be delivered via viral vectors such as adeno-associated viruses (AAVs) along with CAS-9. AAVs can deliver the payloads but do not cause disease themselves. These loaded AAVs were injected into the mice striatum. Gene editing efficiency was analyzed by examining Striatal neuronal DNA and mHTT levels were observed through Western blot analysis and immunohistochemistry.

This method of gene editing has been demonstrated in multiple studies (Yang et. al., and Ekman et. al.) to improve motor functions, cognitive function, and neuronal survival. Results indicate that mice injected with the loaded AAVs had decreased mHTT proteins by about 50% in

the whole striatal lysate as compared to the control animals. This problem of using CRISPER/CAS-9 to combat HD has been approached in different ways, some have tried eliminating the polyglutamine trinucleotide repeats, some have tried to introduce mutations that permanently disable mutant gene function, and others have tried gene editing in less effective ways. The research is indicative that gene editing can provide viable solutions to the HD problem. However, one of the concerns is that the CAS-9 system may target other cells with similar polyglutamine repeats. Refining the precision of the cuts and the specificity of CRISPR/CAS-9 should be examined. Although studies have shown improvement in various ways with this new therapeutic, not all the side effects are known in humans, and despite promising murid trials, more studies should confirm the results already obtained and investigate potential unintended consequences of this gene editing procedure. Finally, these therapies have been shown in mice to be effective in stopping the effects of HD and even increasing survival and motor function, however, these solutions do not replace lost cells. Research is needed in order to investigate whether CRISPR/CAS-9 gene editing therapy can be used with cell replacement therapy to bring forth even better recovery.

Conclusion

Using CRISPR/CAS-9 technologies to target polyglutamine expansion sequences is promising as a way to combat neurodegenerative diseases caused by these repeats like Huntingtins disease. Whether this is through eliminating Polyglutamine repeats or by causing mutations nullifying the mHTT gene, research has shown improvements in motor function, decreased levels of mHTT, and higher survival rates in mice. Studies should be conducted to investigate how gene editing technologies can be paired with cell replacement therapy to initiate even more dramatic recoveries.

Allele-Specific CRISPR Treatments

Introduction

Although the CAG trinucleotide repeats in the HTT gene have been correlated to the onset of Huntington's disease, treatment is hindered by the complex biology of the HD disease. Regardless, the advancement of CRISPR/ cas9 technology has allowed the silencing of mutant HTT genes. Despite identifying the CAG repeat in the HTT gene, removing this region raises the possibility of potentially inactivating the normal HTT gene and other genes that contain CAG repeats, thus threatening delicate genetic structures (Cattaneo et al., 2005). Additionally, the absence of an NGG protospacer adjacent motif (PAM) sequence in CAG repeat impedes CRISPR's ability to perform endonuclease. Thus, CRISPR is most effectively utilized to modify specific alleles rather than common CAG repeats in HTT genes. A strategic selection of targets within the HTT haplotype containing CAG repeats is possible using PAM sites dependent on SNPs (Monteys et al., 2017). This RNA approach selectively removes certain CAG repeats at the site of transcription in the mutated HTT gene. Ultimately, permitting the production of normal HTT. The usage of a single gRNA is preferred to avoid off-targeting to improve selectivity (Shin et al., 2022). It is known that the allele-specific approach permanently alters genetic expression. Concerns may arise regarding the timing of treatment. Moreover, it is recommended that the mutation be treated prior to symptoms to prevent adverse effects of such mutation. In the study conducted by Shin and other researchers (2022) the allele-specific treatment was only applicable in certain PAS sites that are present in only 20% of HD patients with European ancestry. This limitation may hinder the accessibility of such a treatment to various demographic and overall patient populations. Limitations of the CRISPR technology do not allow the removal of the

known CAG repeat but allele-specific treatment is an advancement towards a cure for Huntington's disease.

Discussion

To remedy the lack of inclusivity in treatment, 8,543 European subjects carrying one mutant HTT gene with 40-55 repeats of CAG had their genotypes analyzed (Shin et al., 2022). When treatment was applied, it was found that different sites of PAS must be targeted in order to efficiently excise CAG-producing alleles in mutant and normal HTT haplotypes. 16 common haplotypes within the HD gene were identified in patients and serve as a foundation for the development of genomic treatment and medicine (Shin et al., 2022). CRISPR targeting technologies are most effective on patients with common haplotypes present within various sites of the mutant HTT gene. Through further trial, 3 PAS sites were found to be most effective by maintaining on target. Clinical trials revealed a lack of regeneration and thus treatment must be regarded as a means to protect surviving cells while deleting compromised genes. The use of treatment was significantly insignificant amongst patients in various stages of Huntington's Disease. It is proposed that the early application of treatment may block the pathogenesis of HD and possibly delay the loss of neurons (Hadded et al., 2016). In any case, the application of early treatment poses certain challenges. In mice with complete HTT knockout, there were mice lethalities as well as developmental problems (Nasir et al., 1995). Yet, proper application of treatment has created functional HTT genes that do not cause HD or other developmental problems.

Conclusion

In the final analysis, allele-specific genome editing is highly specific and brings about permanent modifications. Such treatment must undergo further testing to improve efficiency and

applicability amongst larger populations of patients with HD. The identification of more PAS sites across genetically diverse populations will greatly improve the inclusivity of treatment and expand study sample sizes. In the end, current technologies provide mild protection from further cellular degradation and allow for more extensive investigations regarding the application of CRISPR gene-editing technology in aiding Huntington's Disease.

Conclusion

The first study involving CRISPR/Cas9 therapy focused on targeting the mutant huntingtin protein (mHTT) gene, along with the genes involved in redox regulation, including Nrf2 and SOD2. The mutant huntingtin protein (mHTT) in individuals suffering from HD is known to cause the accumulation of oxidative stress and activation of inflammatory mechanisms. The results of this study showed a decrease in oxidative stress, along with a greater mitochondrial function, to counteract the effects of the mHTT protein. The second study used CRISPR/Cas9 techniques to use a single gRNA to remove specific CAG repeats in the mutated HTT gene at the site of transcription. Allele-specific genome editing leads to permanent changes in gene expression and should undergo additional testing to ensure applicability to a wider population of HD patients. Both CRISPR/Cas9 approaches target specific aspects of the gene and yield potential therapies for alleviating the symptoms of Huntington's disease.

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