The Abuse of Neurodegeneration Disease and How CRISPR-Cas9 Could Help with The Disease

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Abstract. Neurodegenerative disease has been discovered for more than a hundred years. This disease gradually damages the brain neuron activity and leads to death. There is no way to entirely heal the disease currently but scientists are still looking for some methods to slow it down and heal from it. More hope appears after the third generation of gene editing technology-CRISPR-Cas9- is discovered. This is a natural gene editing technology by CRISPR gene and Cas9 protein in the body with more precision and less cost than the previous two generations. CRISPR-Cas9 technology is based on the natural body system that detects and prevents virus RNA, and targets a precise sequence of genes to accomplish gene editing. Researchers believe that the damage of neurons by the disease could be slowed down or even stopped by knocking out the neurodegeneration related gene and knocking in desired healthy genes. Huntington disease, one of the most popular diseases in neurodegenerative disease, has been discovered relatively more. This research review focuses on the history of CRISPR-Cas9 gene editing technology and how it helps with neurodegenerative disease, especially with Huntington Disease.

Keywords: Neurodegenerative Disease, CRISPR-Cas9, Huntington Disease.

1. Introduction

Neurodegeneration disease is characterized by the gradual loss of neuronal function and structure leading to cognitive impairment. It is caused in part by neuronal death and glial homeostasis. In most of the cases, the neuron in the brain starts to die accidentally and gradually becomes a neurodegenerative disease. Aging is the most isogenous risk factor for having a neurodegenerative disorder especially after the age of 65 [1], but scientists are still looking for the trigger that causes the beginning of neuron death. Neurodegenerative disease is a brain disorder in which the neuron or spinal cord is partially lost and thus causing cognitive dysfunction [2]. Because the disease starts with the death of one or few neurons, the patients easily consider themselves as getting old or depressed. As there are more and more unworking neurons, the patients start to realize that they forget more and more things or the dememorization starts to affect surrounding people. They would start to consider whether they themselves have neurodegenerative disease at this point. Some well-known neurodegenerative diseases include Alzheimer's disease, Huntington disease, and Parkinson's disease. The symptoms of neurodegenerative diseases include memory loss, moodiness, anxiety, depression, agitation and sleeping disorder.

The incidence rate of neurodegenerative diseases in general is about 2/1,000,000 around the world [3]. Although it is much less common than other popular diseases like gastric cancer or lung cancer, scientists still consider this disease seriously, and more research is needed for development of deeper clinic technology. South-East Asia has lower incidence rate than the Western pacific regions and there is less rate as medical technology is developing recently [4]. Although neurodegeneration disease is a newer disease that is related to the brain, many scientists started to focus on it since it became popular rapidly. This disease could affect the patients themselves by losing their ability to take care of themselves. It could also affect the surrounding people of the patients mentally. The death of neurons makes individuals not remember the name and identity of people who love them and take care of them, thus almost every individual who is involved in this disease could feel insecure and unsafe. The lack of healing possibility makes the situation even worse. As neurodegenerative disease is a worldwide disease that affects many families, scientists are starting to have interest in looking for

more risk factors and methods to slow down the progress of the disease and reduce the appearance of symptoms or even heal the disease. Unfortunately, current clinic technology cannot heal neurodegenerative disease, and it would be a lifelong journey to fight with the disease to limit its harm to the patient physically and mentally. The patient loses the ability to self-care gradually and requires people to take care of as time passes by. The family of the patient could be seriously affected until hopeless for not seeing any possibility of healing the disease.

2. Brief History of Crispr-cas9 System

With the rapid development of gene-editing technology, research on neurodegenerative diseases has been significantly advanced. Clustered Regularly Interspaced Short Palindromic Repeats - associated protein 9 (CRISPR-cas9) is the third generation of gene editing technology after zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs), with its high efficiency, low cost, and easy design. CRISPR-cas9 is not only used to delete some target genes to slow down progression, but also used to discover more risk factors caused by genes in many neurodegenerative diseases [5].

It is not an easy path for scientists to finally know how to use CRISPR-Cas9 technology. It took a long time for scientists to first find and then learn to use the technology. In the year of 1987, Dr. Ishino, a Japanese microbiologist, first found that there are abnormal repeating gene segments in cells which were later named as CRISPR [6]. Scientists haven't realized the function of CRISPR for a long period of time until the year of 2007 that CRISPR-Cas9 has been proven to be a part of the cell immune system that CRISPR keeps the viral gene and Cas9 protein cut the infection gene once the same viral gene been realized again in the cell [7]. After the discovery of this immune system, scientists are looking for a method to control Cas9 protein as gene scissors that they could cut some precise gene as they need to. In the year 2020, Professor Charpentier and Professor Doudna announced that trans-activating RNA can be used as a tool to make Cas9 protein cut precisely located genes by targeting specific genes. After Charpentier and Doudna got the Nobel Prize award for this technique, it started to be widely used in laboratories all over the world for more and more research. Recently, CRISPR-Cas9 technology has become the most popular gene editing technology in research for it is the easiest, cheapest, and most efficient method.

CRISPR-Cas9 is the combination of two subjects, CRISPR gene and Cas9 protein, to protect the cell. The virus infects the cell by inserting their DNA into the nucleus of the cell. CRISPR is the method that the cell defends against this infection by copying the DNA of the virus and keeping it as part of the cell DNA. Each part of the copying DNA is called the protospacer adjacent motif (PAM), and there are many PAMs in the long double-helix CRISPR gene as different viruses may infect the cell before. Once the same virus DNA with existing PAM is detected in the cell again, this gives Cas9 the signal to cut the viral gene. Cas9 is the protein that could cut viral DNA inserted to protect the cell. Once the existing virus DNA infects the cell and CRISPR alerts Cas9, Cas9 protein targets the viral DNA at two ends and cuts it to make it invalid. This was a naturally but complex process of the cell protection system. Researchers found that Cas9 protein could cut not only the viral DNA but any precise gene in the cell with the help of gRNA. A strand of guide RNA (gRNA) is produced by a cell to unlock and match the viral DNA during infection and it signals the Cas9 protein when a new coming viral DNA matches with this strand of gRNA. Cas9 receives the signal and cuts the viral DNA with the help of gRNA. Naturally, Cas9 would only cut the viral DNA. Scientists found out that gRNA would affect the location being cut and therefore can control where to cut the cell DNA by Cas9 in any part of the gene [8]. The cutting is similar to mutation, the cell is willing to replace cutting codons and repair the DNA. The gene may not be expressed with improper repair. But the cell itself makes some mistakes pretty often when repairing and this is where scientists could work on to insert healthy codon to make the cell be fixed. After cutting the DNA, a sequence of DNA would be inserted into the original DNA by homologous directed repair (HDR) or nonhomologous end joining (NHEJ) to fix the cutting DNA. The advantages of this sequence of DNA could be detected after

repair. Therefore, the unexpected DNA has been cut and a specific sequence of DNA is inserted which processes the gene editing. One important point here is that if the gene editing happens in somatic cells instead of germline cells, this gene will not be inherited, and the disease may appear again in the next generation. Accordingly, the fixing gene may be passed off if this gene editing is performed in germline cells (Figure 1).

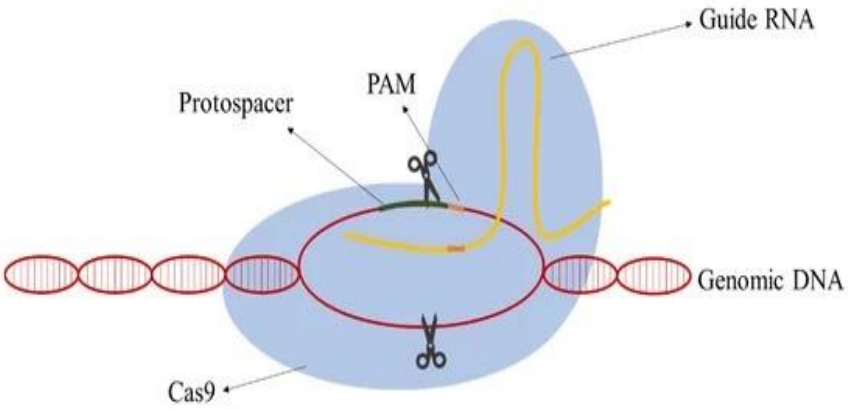

Figure 1. CRISPR-Cas9: A Powerful Tool to Efficiently Engineer Saccharomyces Cerevisiae [9]

3. Comparison between CRISPR, ZFN, and TALEN

CRISPR-Cas9 is the third generation of gene editing technology which has relatively large advantages over the past two gene editing methods. It has been a brilliant step while each of the methods has been detected. Previous two generations are zinc finger nucleases (ZFN), and transcription activator-like nucleases (TALEN). ZFN is an artificial genome editing method based on zinc-finger nucleases composed of sequence-specific DNA binding domains and non-specific DNA cleaving domains from the FokI restriction endonuclease to induce double-strand breaks in DNA [10]. Under the pleasure of discovering a gene editing method, scientists cannot increase the efficiency of ZFN. Endogenous correct rate is only 18% although a promise over 10% success is considered high [11]. While ZFN is the first generation of gene editing methods, its low-efficiency targeting makes scientists continue to look for more gene editing methods. TALEN, as the second generation of gene editing technology, is also based on double-strand DNA breaking. It uses transcription activator-like nucleases composed of sequence-specific DNA-binding domains working with transcription activator-like effectors (TALE) proteins to induce double-strand breaks in DNA [12]. It has a relatively higher efficiency than ZFN but spends too much time and cost in laboratory research. Both ZFN and TALEN are artificial to break double-strand DNA, while CRISPR is a natural tool to break single-strand RNA with defensive protein. Table 1 is to show the similarities and differences between three generations of gene editing technology.

Table 1. Differences between three generations of gene editing technology

Differences	ZFN	TALEN	CRISPR
Components	Sequence-specific DNA-binding domains and non-specific DNA cleavage domain from the FokI restriction endonuclease with zinc-finger proteins	FokI cleavage domains and DNA-binding domains derived from TALE proteins	Trans-activating crRNA and a sgRNA with Cas's protein
Recognition sites	4-18 bp in mammalian cells	12-25 bp	1-5bp in PAM sequence
Disadvantages	Low efficiency target Time consuming design construct	Low efficiency target Expensive	Limited target selection by PAM sequence
Advantages	Cheap Fewer off-target effects	No genomic sequence target limitation Not time-consuming design construct	Cheap Highly efficient targeting of multiple sites Not time-consuming design construct

4. Gene Editing and Neurodegenerative Disease

Neurodegenerative disease has a relatively shorter history than other popular diseases like lung cancer because of the lack of technology and realization of human behavior in the old time. It attracts scientists quickly after its detection. The neurodegenerative disease is directed by the loss of specific sensitive neurons and the mutation of protein that function abnormally to toxic the brain. As the loss of neurons keeps going, the individual gradually loses the ability to recall their memory correctly and behave as normal. At the early time of the disease, most patients just consider the changes as getting old and ignore the possibility. This happens more at the early ages and when people do not have enough knowledge about neurodegenerative disease. Recently, after a number of researches, neurodegenerative disease is considered entirely genetic, which means that it is considered heritable. Its genetic manner requires researchers to edit many gene sequences related to neurodegenerative disease because there is hope. But the scientific field still lacks cases to prove the correct gene sequence. As a matter of common sense, the brain could not only affect emotional changes which affect both the patients and their surroundings, but also lead to actively bizarre, thus most neurodegenerative diseases have similar symptoms like losing memory, and acting weird. With further division, the neurons die with different diseases in each section of the brain, thus each subset of neurodegenerative disease is applied. Popular neurodegenerative disease includes but is not limited to Alzheimer disease, Parkinson disease, and Huntington disease. Huntington disease is most popular to work with CRISPR-Cas9 technology while Alzheimer disease is most well-known.

5. Huntington Disease

Huntington disease is a rare autosomal dominant neurodegenerative disease with abnormal limb movement, cognitive decline, sleep disorders, and complete loss of mobility. After mutation, the expanded CAG trinucleotide repeating in huntingtin protein would be more toxic and thus make the protein more brittle which lead to dysfunction and even death [13]. The breaking of protein in the brain would result in difficulty concentrating or depression at the early period and gradually develop into speaking disability and involuntary jerking or fidgety movements of the limbs and body. Like all other neurodegenerative diseases, Huntington disease needs time to process and has little hope to get healed.

After the discovery of CRISPR-Cas9 technology, scientists spend a long time working on it and start to apply this technology to help solve the problem. In the year of 2017, there has been a popular period that scientists all around the world are trying to apply CRISPR-Cas9 technology to block the expanded CAG trinucleotide repeating sequence that damages the normal protein. Kolli et al [56] constructed two plasmids by CRISPR/Cas9 technology, one plasmid can produce gaps in the untranslated region upstream of the open reading frame of the host DNA, and the other is produced at the boundary of DNA introns and exons. The two plasmids were introduced into HD model mouse bone marrow mesenchymal stem cells, and the results showed that the disruption of uORF inhibited the translation of mHTT, and the disruption of intron and exon boundaries inhibited the translation of mHTT to a lesser extent [14]. With the failure or decreasing rate of translation of mHTT, the normal neuron would be less affected by the toxic thus avoiding the pollution from the abnormal protein. This method may not entirely pause the mutation of mHTT but could be possible with some more precise technology. At this point, restraining the expression of HTT mutation became a popular method to deal with Huntington disease and many researchers started to work over this. Although CRISPR-Cas9 has been a high efficiency tool to edit genome sequences, it is still a challenge to entirely heal Huntington disease while CRISPR is not precise enough. Scientists are on their way looking for the hope to save many families all over the world.

6. Conclusion

Neurodegenerative disease has been a problem for both patients and surrounding individuals both physically and mentally. It starts with the death of neurons and gradually makes the patients lose their ability to react to the environment and take care of themselves. There is no trigger detected by the researchers, but the most possible risk factor is aging. Although neurodegenerative disease has been a problematic disease around the world for a long time, it is still a new disease in current ages that lacks exploration. The detection of the third generation of gene editing, CRISPR-Cas9, gives a big hope to scientists about healing the disease. CRISPR-Cas9 gene editing technology is based on the natural body anti-virus system by using CRISPR gene detecting the virus RNA sequence and cutting the sequence with Cas9 protein. Scientists use this natural protection system to knock in and knock out desired gene sequence to stop or slow down the disease. They recognize the possibility that CRISPR-Cas9 technology could help heal the disease although the technique is not mature enough since it is still a new technology that needs more time and research to be more discovered.

Although CRISPR-Cas9 is the newest generation of gene editing technology, there are still possibilities that ZFN and TALENs could all be applied to cure the disease. The scientific field not only needs more research over the technology application to clinics but also requires looking for a new generation of gene editing to help working on neurodegenerative disease.

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