The Application of CRISPR/Cas9 Technology in Neurological Diseases

Hao Ning [1, *], Yushan Wu [2]

[1] School of stomatology, Southern Medical University, Shenzhen, China

[2] Shenzhen Xin'an Middle School, Shenzhen, China

* Corresponding Author Email: 20203473@stu.hebmu.edu.cn

Abstract. Neurological disease refers to the functional disorders of the central nervous system, peripheral nervous system or plant nervous system caused by a variety of causes such as bacterial infection or genetic. Patients usually show problems such as conscious disorder, sensory disorders, and motor disorder. Common neurological diseases such as Huntington, and Alzheimer's disease, have always been one of the main diseases that endanger human life because of their complex mechanisms and treatment. Gene therapy has always been considered a way to treat diseases. The discovery of Clustered Regularly Interspaced Short Palindromic Repeats(CRISPR) has improved gene editing technology and realized the application of various biological fields. CRISPR uses sgRNA to identify target sequences, so that CAS9 protein cuts DNA, and combines the guided RNA with the recognized DNA. Then the nucleic acid-cutting enzyme breaks the DNA dual chain structure, and the host cells were repaired through homologous or non-source mechanisms. CRISPR/Cas9 technology can better perform genetic treatment of neuropathy, making it possible for the development and new treatment methods of human neurodegenerative diseases. This review summarizes the basic principles of CRISPR/Cas9 technology and its application in Huntington's and Alzheimer's disease, as well as discusses the feasibility of CRISPR technology in neurological diseases in the future.

Keywords: Neurological disease; CRISPR/Cas9 system; Huntington's disease; Alzheimer's disease.

1. Introduction

Neurological diseases have always been a problem that all human beings focus on and are committed to solving. The main causes of neurological diseases were included in the year of disability adjustment in 2015, and they were also considered the second largest death group [1]. It is estimated that Alzheimer's disease (AD) affects millions of Americans. The number of people in AD may increase to 13.8 million in the middle of this century. The US government counted 121499 AD fatalities in total in 2019. Because AD is a very deadly disease, it represents a serious threat to Americans 65 years of age and older, demonstrating the harm that neurological disorders may do to people [2]. Recurrent and refractory nature are the characteristics of neuropathy, and it is often impossible to treat relying on traditional methods. A developing technique for gene editing is CRISPR/Cas9. Mojica et al. discovered repetitions in the bacterial genomes of other microbes that had an unclear purpose; they dubbed these repeats CRISPR [3]. It was ultimately found that the CRISPR sequence functions as an immune response system that can adapt to bacteria by destroying invaders' DNA using nucleases, so stopping the virus from infecting the host [4]. The primary benefits of the CRISPR system are its high efficiency, broad flexibility, and simplicity of use. Based on this system, genetic treatment, gene correction, etc. The CRISPR/Cas9 technology has been well applied in Huntington's disease (HD) [5-7], indicating that neurological diseases may have the opportunity to receive better treatment options. This article aims to introduce the application of CRISPR/Cas9 technology in neurological diseases such as HD and AD and discuss the feasibility of future CRISPR/Cas9 technology in treating neuropathy.

2. CRISPR/Cas9 technology

The CRISPR/Cas9 system includes Cas9 nucleic acid enzymes and the required single guide RNA (sgRNA). The function of sgRNA is to perform base complementary pairing with DNA, thereby navigating Cas9 to target genes. SgRNA binds to Cas9 and guides it together to the target DNA, causing the main DNA to be cleaved and double stranded (DSB), which is then repaired through homologous directed repair (HDR) or nonhomologous end junction (NHEJ) [8]. However, the precise sequence operation of the genomic DNA required for the therapy of Huntington's Dance Ethics, and the HDR mediated HDR mediated by CRISPR is limited to the editors in the split cell because these pathways are limited to the S and G2 phase of the cell cycle. Therefore, more accurate gene editing technology is needed.

Base Editing can install mutations directly in the cell DNA without inducing DSB. Base Editing and Prime Editing use cell mismatch repair mechanisms, which can be used to divide cells and end - differential cells. Nowadays, different types of base editors have been widely used, such as cytosine base editors (CBE) and adenine base editors (ABE) (Figure 1). ABE-dCas specifically targets DNA, catalyzes the conversion of adenine to inosine in the deoxyadenosine deaminase domain, and guides RNA programming. When DNA is copied, inosine is recognized by the body as guanine. The initial I-T base pairs may eventually become G-C base pairs, thereby achieving mutations in the target sequence. Although CBE and ABE have the powerful ability to edit DNA, Base Editing lack the ability to accurately edit the base outside the four transition mutations. Scientists have discovered Prime Editing to improve these shortcomings. The Prime Editing uses a prime-editing guide RNA (pegRNA) to guide the RNA, and pegRNA plays an important role in the system function. PegRNA contains a complementary sequence--to guide nCas9 to its target sequence target location, as well as the additional sequence of additional sequences --can be reversed and programmable. PegRNA's 5 ′ binding to the primer binding site (PBS) area on DNA reveal the non-complementary chain. In order to create a primer for reversing enzymes (RT) connected to nCas9, a PAM chain guides Cas9 to cut the unblocked DNA. RT subsequently expands the PAM chain through the interior of pegRNA as a template, thereby realizing programming modifications to the target area. This step generates 3 ′ and primitive unbound unless edited 5′PAM DNA petals edited by pegRNA [10]. The Prime Editing is not limited by the PAM sequence, which is more common, more accurate Base Editing, and more efficient than HDR. The Prime Editing expands the range of DNA editing to not only genetic conversion and horizontal mutations, but also precise insertion and deleting mutation genes.

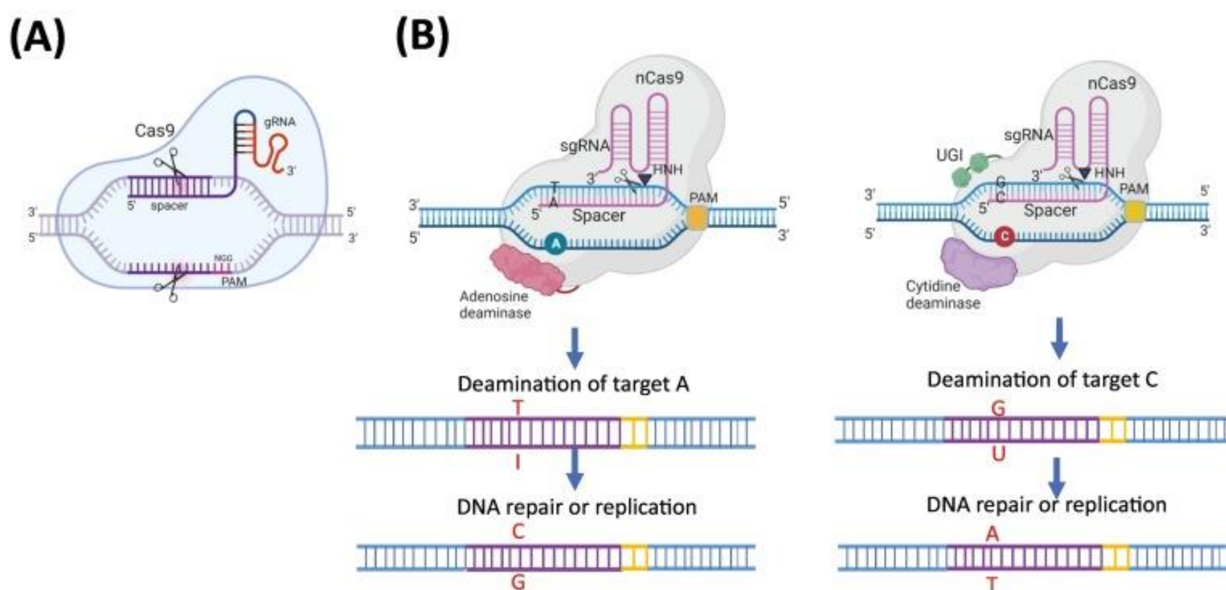

Fig. 1 CRISPR/Cas9 and based editing [3]

3. CRISPR/Cas9 technology mediated genetic therapy in the application of neuropathy

3.1. CRISPR/Cas9 technology mediated gene therapy in the application of Hunington's disease

Huntington's disease (HD) is a neurodegenerative disease in which autosomal abnormalities are often detected in patients and are usually dominant mutations caused by base changes. Patients have cognitive impairment of exercise and mental illness, showing unwillingness, emotional fluctuations and personality changes in their limbs. The pathological principle of HD is the repetitive expansion of the Huntington's protein gene (HTT) internal cytosine-adenine-guanine (CAG) [11]. The caudal nucleus and putamen form the new striatum, which can be damaged by toxicity. The abnormal accumulation of Huntington's protein (mHTT) destroys its normal structure. It is believed that HD damages a variety of neurons, of which the most easily affected and harmful to the body is the middle spine neuron (MSN). In order to study the pathogenesis of HD, researchers have developed a variety of animal models. In related studies, scientists reduced the expression of mHTT through various methods, so that the disease of animals could be alleviated [12]. Therefore, targeting mHTT expression using CRISPR/CaS9 may be a way to alleviate HD symptoms in humans. The idea is based on previous research showing that RNA interference, applied to HD mouse models, enhanced the activity of the basal neuron-mediated indirect pathway, which mitigated abnormal behavior in mice [13]. Moreover, the lack of normal HTT in the brain of adult mice will not affect the normal growth of animals or survival of neurons [14]. Shin et al. engineered two Sgrnas for mHTT that selectively cut ~44kb of DNA, thereby achieving gene silencing. They excised the promoter region, the transcription starts site and the repeated expansion of CAG in the mutated Huntington gene, which inactivated all the mutated alleles while not affecting the normal gene, preventing the production of mRNA and protein of the mutated mHTT [15]. Open reading frame (UORF) and exon-1 intron boundaries are key regions for mRNA transcription. Using CRISPR/CAS9 technology, Kolly et al. designed Cas9 to act as a plasmid vector that cuts DNA in the upper region, thereby preventing transcription and translation of the Huntington gene. Accumulated stem cells (MSCS) exist in the bone marrow and have HD mutated gene, which has a good study effect. The plasmid was applied to MSCS from YAC128 mice, the results showed that CRISPR-Cas9 had some negative effects on the translation of mHTT, such as destroying UORF's normal translation of mHTT and exon-1 intron boundaries [16]. YANG et al. used CRSPR/ Cas9-mediated mHTT to establish a mouse model of HD140Q-Ki. They constructed AAV-HTT-gRNA and AAV-CMV-Cas9 and transferred them into the new striatum of mice and found that the accumulation of mHTT in the new striatum was reduced, while conducting exercise tests on mice and finding that HD140Q-Ki mice performed significantly better in these tests and their disease slowed down. The study showed that even when the mutant protein was suppressed and expressed, old neurons were still able to clearly accumulate mHTT and repair it. In short, mHTT expression may be the key to CRISPR/ Cas9-mediated therapy, and it also provides ideas for addressing other neuropathies [17].

3.2. Application of CRISPR/Cas9 technology-mediated gene therapy in Alzheimer's disease

Alzheimer's disease (AD) is an irreversible neurodegenerative disease. It is characterized by cognitive dysfunction, behavioral abnormalities, social deficits, and ultimately an inability to perform everyday tasks.

Important pathological features of Alzheimer's disease are brain plaques filled with beta-amyloid peptide (Aβ) and neurofibrillary tangles prominent in the structure of the medial temporal lobe [18]. Mutations that occur in early Alzheimer's disease include mutations in presenilin 1 (PSEN1) and presenilin 2 (PSEN2) as well as If the APP protein or gene products involved in APP generation to form beta-amyloid protein are mutated, the production of beta-amyloid protein is increased [19, 20]. We can use CRISPR/Cas9 technology to regulate Aβ expression. When APP allele was knocked out using CRISPR/Cas9 technology, Aβ protein expression was decreased [21]. In recent years, several

studies have analyzed the possibility of using CRISPR technology to correct similar mutations. CRISPR/Cas9 was used to modify the autosomal mutation in PSEN2 in ipSC-derived neurons. In this study, in this study, 65-80% of AD patients had at least one APOE4 allele, where the CRISPR/Cas9 system works to turn APOE4 into APOE2 or apoe3 [22]. Therefore, a feasible way to use CRISPR/Cas9 is to change the structure and function of apoE4 to apoE3 or apoE2 to treat AD patients carrying apoE4 [23].

4. Conclusion

Targeting efficiency of CRISPR/Cas9 is higher than several other methods such as basic editing and lead editing. CRISPR/Cas9 technology has a broad and effective application prospect in nervous system diseases. With the development of this technology in recent years, it also has a certain feasibility for mental diseases such as Alzheimer. The regulation of gene expression levels and the specificity of targets are great challenges for future research. The regulation of gene expression levels and the specificity of targets are great challenges for future research. HD is the repeated expansion of a large number of CAG, and a system capable of multi-repeat base editing needs to be improved. In general, the technology holds great potential for treating other neurological disorders as well.

Author contributions

All the authors contributed equally, and their names were listed in alphabetical order.

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