Prevention and Treatment of Alzheimer’s Disease Through CRISPR/Cas Technology

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Abstract. Neuronal loss and the stereotyped deposition of misfolded proteins are hallmarks of Alzheimer’s disease (AD), a neurodegenerative disease related to age that is common, progressive, and fatal. The formation of amyloid β oligomers may contribute to the pathogenesis of multiple diseases, including the development of tau neurofibrillary tangles and oxidative stress. Between 20% and 40% of persons with AD go on to have severe dementia or pass away within three years. The only things that the current generation of Alzheimer’s medications can do are postpone the onset of the illness and make a maximal difference in the lives of those who use them. Early diagnosis, management, and treatment of individuals with neurodegenerative disorders are crucial. But there are currently no relevant treatment options for the accumulation of amyloid beta and neurofibrillary tangles. CRISPR has been widely used in molecular biology research because it is fast, effective, cheap, and easy to operate compared to traditional gene editing methods, and thus has quickly become one of the most popular biological research tools. This article intends to use the gene editing CRISPR technology, which has the advantages of simple design, time period, and low cost, to knock out or edit the genes corresponding to Alzheimer’s disease to achieve targeted treatment of this accumulation phenomenon.

Keywords: Alzheimer's disease; CRISPR/Cas technology; treatment.

1. Introduction

Alzheimer disease (AD) is the most common form of dementia, which is a progressive neurodegenerative disorder characterized by global cognitive decline involving memory, orientation, judgment, and reasoning. As the global aging process accelerates, the incidence of AD has increased in recent years. According to the latest statistics released by the World Health Organization (WHO), by the middle of this century, the proportion of the world’s population over the age of 60 will rise by more than 10% from about 11% in 2019 [1]. Alzheimer’s disease worldwide the number of people suffering from Alzheimer's disease will increase from the current 50 million to 152 million [2]. As to the findings of China's seventh nationwide census conducted in 2020, China's elderly population, that is, the population over 60 years old, is 26,000, accounting for more than 18% of the country's total population (of which 190,000 are 65 years old and over, accounting for 19% of the total population 13.50% of the Department). Compared with the last census, the proportion of the elderly population in my country has increased by more than 5% [3]. As of 2020, the number of Alzheimer's patients in my country has exceeded 10 million, ranking first in the world. With more than 300,000 new cases growing rapidly every year, it has become a major public health issue affecting my country's socioeconomic development [4].

Of the several pathogenic theories about the development of Alzheimer's disease, the creation of amyloid Aβ protein and metabolic problems are the ones with the most influence. Glee and Wong identified and isolated the Aβ protein from abnormal plaques in AD patients' brains in 1984. Additionally, the protein's amino acid sequence was established. Amyloid precursor protein (APP), which produces Alzheimer's disease through proteolysis by β- and β-secretase, contains 39 to 43 amino acids [5]. Among them, the two main types of soluble Aβ proteins are soluble Aβ40 and insoluble Aβ42 [6]. The patient's brain becomes heavily deposited with Aβ protein because to irregularities in the generation and clearance rates of the protein. At present, the Aβ protein is thought to be a common route that causes Alzheimer's disease due to a number of factors, and it plays a significant role in the illness's development [6].
The CRISPR and Cas protein systems are natural acquired immune systems discovered while studying bacteria. Through clustered, regular, interspaced short palindromic repeat sequences, they inhibit plasmid and viral invasion. The CRISPR /Cas system used to fight viruses can be used as a simple and flexible genome editing tool [7]. After being infected by viruses such as bacteriophages, bacteria can obtain part of their DNA fragments and integrate them into the genome. When the virus invades again, the CRISPR clusters are first transcribed into long CRISPR RNA (crRNA) precursors and then into small mature crRNAs. In this instance, base pairing between crRNA and tracrRNA results in double-stranded RNA. The Cas9 protein is directed by this binary complex to snip the invasive virus's DNA at a particular location, preventing the virus from replicating in bacteria [8,9]. CRISPR/Cas9 technology utilizes this principle and designs a guide sgRNA that can recognize Cas9 protein and guide Cas9 protein to bind to the target site based on the structural characteristics of the tracrRNA-crRNA complex. This results in DNA breakage, initiating the intracellular genome repair mechanism, enabling gene knockout, introduction or repair of specific mutations, site-directed transgenes, etc [10,11]. CRISPR/Cas technology has great potential for disease treatment due to its advantages of simple design, time period, and low cost. Therefore, applying CRISPR/Cas technology to Alzheimer's disease is expected to achieve improvement or treatment effects (Fig.1)

![Fig. 1 Mechanism of CRISPR/Cas9 system](image)

2. Application of CRISPR/Cas9 in Alzheimer’s disease

2.1. Construction of Alzheimer’s disease model

CRISPR/Cas technology has applications in many areas. More model organisms, such as zebrafish, also use CRISPR/Cas technology for gene editing. Researchers at the National Science Foundation (NIH) performed tissue-specific gene knockout in zebrafish using a vector system based on CRISPR-Cas9 technology [12]. They used CRISPR/Cas9 technology to rapidly obtain gene knockouts in zebrafish through simple injections of guide RNA (sgRNA) and Cas9 mRNA at the single-cell
embryonic stage. This technology can accelerate the discovery of gene functions and the identification of genes related to human diseases. Researchers also used CRISPR/Cas9 technology to successfully breed two gene knockout minipig models: tyrosinase gene knockout and PARK2 and PINK1 double gene knockout. Knockouts of these genes establish models of human albinism and parkinsonism. The scientific community generally believes that "this is a key step towards xenogeneic organ transplantation" and opens up a new world of human organ transplantation. The above research further supports the application research of this system in mammals, because it helps people to construct disease-related animal models, thus promoting the study of disease pathogenesis [13]. A research team at the Governors Institute for Regenerative Medicine at Cedars-Sinai Medical Center in the United States is studying hereditary retinitis pigmentosa, a degenerative eye disease that can lead to blindness and for which there is currently no treatment. Researchers used CRISPR/Cas9 technology to remove a genetic mutation that could cause the eye to lose photoreceptor cells. When they injected the system into young rats in the laboratory, they simulated a model of inherited retinitis pigmentosa. As a result, these rats had improved vision compared with control animals [14].

Through the above research, Alzheimer's disease therapy can be approached with the same concept in mind. Dominant mutations in the genes producing presenilin 1 and presenilin, as well as the amyloid-beta precursor protein (APP) gene, are the pathophysiology of familial Alzheimer's disease. Extracellular amyloid plaques and intracellular neurofibrillary tangles in various brain areas are characterized by presenilins 1 and 2. In the Swedish mutant transgenic mouse model of the human APP, in the hippocampus-β After selective whole brain disruption using mutated APP alleles in relevant pathology, the association between mice and amyloid protein decreased for at least six months. By intravenous injection of CRISPR-Cas9 constructs, amyloid protein can improve malnutrition, neuritis, cognitive impairment, microglial cell proliferation, and Sedimentation caused by adeno-associated viruses capable of overcoming the blood-brain barrier [15].

2.2. Targeted therapy

Aberrant protein ubiquitination and degradation contribute to many diseases. The ubiquitinase-targeting chimera is a small molecule with two purposes [16]. To maintain the concentration of certain proteins that are ubiquitin-dependently degraded, it comprises of a deubiquitinase supplement coupled to a protein-targeting ligand. This will help in the treatment of many diseases. This bispecific antibody overcomes the shortcomings of previous antibodies, features like restricted tissue targeting, simplicity of development, and modularity. Additionally, it functions on extracellular and cell surface proteins. Moreover, it offers a genetically encoded, modular, selective, adaptable, and straightforward technique for triggering lysosomal transport to extracellular and cell surface targets with distribution unique to certain tissues.

However, due to misfolding of β-amyloid, its spatial conformation changes, making it difficult to identify and disassemble [17-21]. A unique antibody is needed that can recognize these beta amyloid proteins in the brain. First, we extracted and modified amyloid β(Aβ) peptide. We then used CRISPR technology to find amyloid-beta oligomers in Alzheimer's disease patients. Pfizer and Johnson & Johnson created the humanized monoclonal antibody known as bepineuzumab [22]. The antibody specifically targets the neurotoxic amyloid (A) peptide, which is a primary component of AD brain plaques and an early indication of Alzheimer's disease pathology. Bapineuzumab is a humanized analog of mouse antibody 3D6 (Fig.2), which acts on the amino terminus of Aβ and can recognize soluble and insoluble Aβ [22]. Based on this property, it can be modified and combined with a deubiquitinating enzyme-targeting chimera through CRISPR technology to design a drug specifically targeting β-amyloid. So that it will not be excessively deposited in the human body [16].
On the other hand, Elizabeth Riddell, Azu Patel, Joshua Putman, Du Siqi, and Daniel Armstrong studied amyloid protein-β. The impact of antibody binding peptide epimers/isomers, as well as their potential impact on immunotherapy and drug development [7]. In addition, they studied only unmodified Aβ. Peptide immunotherapy, i.e. wild-type (WT) all L-Aβ Peptides [9,10]. Does not include Aβ isomers and epimers. Thus, it was found that Bapineuzumab is not suitable for all Aβ, and it does not have a good target binding effect on its isomers and epimers [22].

Firstly, the immunoprecipitation program evaluates the antibody binding potency. After that, it was rebuilt in borate buffer and subjected to tandem mass spectrometry and liquid chromatography analysis. When the following MS/MS peak regions were integrated and their intensity areas were plotted, the results showed that the antibody binding affinities of Aβ1 D-(D23) and Aβ10 D-(S26) were greater than those of WT (All L) Aβ for all tested antibodies [23]. Thus, by using CRISPR/Cas9 technology, we may further investigate the antibodies of Aβ1 D-(D23) and Aβ10 D-(S26) to obtain their specific binding to β-amyloid, therefore, decreasing the misfolding of the amyloid-beta (Aβ) peptide, which causes an excess of Aβ to be produced and deposited in the brain. Because of the hyperphosphorylation of Tau protein, subsequent inflammatory responses, neuronal degeneration, neuronal death, and eventually dementia caused by excessive deposition. Therefore, by preventing its accumulation, it plays a role in treating or alleviating the symptoms of Alzheimer's patients.

3. Conclusion

This article mainly uses CRISPR/Cas9 technology to achieve the treatment of Alzheimer's disease through gene knockout and targeted therapy. Amyloid-β epimers and/or isomers, however, could have distinct spatial conformations and/or aggregation tendencies, which might hinder the antibody's ability to work. This will be a major difficulty in the process of targeted therapy. This will require more research to explore solutions or new approaches.

References


