Application of CRISPR/Cas9 Gene Editing Technology in Targeted Therapy of Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is a neurodegenerative disease related to many factors, such as genes and environment. It is characterized by memory loss and cognitive impairment, and the symptoms are irreversible. At present, there is no drug that can reverse the disease. Moreover, in recent years, the number of patients with AD has increased sharply, and it is urgent to find a new treatment. CRISPR/Cas9 is the third generation Gene manipulation based on the principle of DNA recombination and repair, which shows great potential in the research of neurodegenerative diseases. This paper analyzes the research and application progress of this technology in the construction of pathological model of AD, screening of pathogenic risk factors, finding therapeutic targets and targeted therapy, and obtains a new idea for treating AD, hoping to play a reference role for researchers in related fields. However, how to efficiently and pertinently deliver the CRISPR/Cas9 system to the cells needed in vivo is still the biggest bottleneck in the development of gene editing somatic cell therapy, and future research can focus on this direction.

Keywords: CRISPR/Cas9; Alzheimer's disease; Gene editing technology.

1. Introduction

AD is a degenerative disease of the central nervous system, which is the most common, irreversible and progressive dementia, accounting for about 60% ~ 70% of dementia patients. The behavioral manifestations of AD patients are cognitive decline, memory loss and reduced ability of self-care of daily life. According to World Health Organization, there will be about 139 million dementia patients in 2050 [1]. Dementia is the main cause of disability and dependence in the world, and the total social cost of dementia in the world is estimated at $1.3 trillion. A cross-sectional study shows that the total prevalence of dementia in China is 6.0%, and there are 15.07 million dementia patients among people aged 60 and over, including 9.83 million AD patients [2].

At present, the prevention, early identification and intervention of AD are gradually on the right track. The potential pathogenesis of AD includes: the deposition of β -amyloid (Aβ) to form senile plaques (SPS) in the brain, and the hyperphosphorylation of tau protein to form neurofibrillary tangles, NFTs), oxidative stress injury, cholinergic neuron degeneration, Neuroinflammation-induced inflammatory factor release, brain metal ion disorder, intestinal flora imbalance and so on. Therefore, the pathogenesis of AD is not a linear process, but the result of many factors. However, the treatment of AD is still limited. The drugs listed in China mainly include cholinesterase inhibitors and N- methyl -D- aspartate (NMDA) receptor antagonists. These drugs can only slow down the progress of the disease in a limited way [3], so it is necessary to introduce new research methods to obtain more accurate and effective research and treatment methods. With the development of gene editing technology, especially the appearance of CRISPR/Cas9 technology, gene therapy is no longer out of reach. This technology not only provides a new method for the cure of genetic diseases, but also provides a new idea for the research and treatment of complex idiopathic diseases.

2. Overview of CRISPR/Cas9

2.1. Composition and Mechanism of CRISPR/cas9 System

CRISPR (clustered regularly-interspaced short palindromic repeats) is a short palindrome repeating sequence with regular intervals. CRISPR/Cas9 system is an adaptive immune system of

bacteria and archaea. By cutting foreign DNA and integrating it into the host chromosome near CRISPR, it can protect itself from viruses and plasmids, so that it can detect specific sequences and silence foreign nucleic acids [4-5]. By designing guide RNA, specific target gene sequences can be targeted and cut, so CRISPR/Cas's system is an efficient, universal and programmable genome editing tool. CRISPR/Cas's system can be roughly divided into two categories, the first category is that CRISPR/Cas's system uses multi-protein effect complex, and the second category is that CRISPR/Cas's system uses single protein effect complex.

At present, the most widely used CRISPR/Cas9 system belongs to the second category, which is Cas9 nuclease (SpCas9) from Streptococcus pyogenes. It can not only cut double-stranded DNA in vitro, but also realize genome editing in bacteria, mammals and human cells. Clinical trials using CRISPR/Cas9 technology have been carried out, including infectious diseases such as human immunodeficiency virus, hematological diseases such as β thalassemia, sickle cell anemia and multiple myeloma, and immunotherapy for various solid tumors such as non-small cell lung cancer and esophageal cancer [6].

CRISPR/Cas9 is a low-cost, fast, efficient and extensible tool for manipulating genome sequences, and its potential for disease treatment is constantly being tapped, and more and more researches are exploring new strategies for gene therapy [7-9].

2.2. Advantages of CRISPR/Cas9

Modern gene editing technology is based on the repair mechanism of broken DNA by organisms. By introducing targeted DSB, the genome DNA sequence of animals, plants and human beings can be edited effectively and accurately. In the traditional gene editing technology, it is very difficult to target the endogenous genome, and it is even more difficult to introduce the target genome of cells and organisms into specific sites for modification. Therefore, the development and design of DNA cleaving enzymes has become a hot spot in this field]. At present, four nucleases that have been applied in gene editing technology include: zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (transcription activator-like effector nucleases), TALEN), ENG-inered meganucleases (MNS) and RNA-dependent CRISPR/Cas9 system. Among them, although the miss rate of MNs is low, it is seldom used because of its high price.

Although CRISPR/Cas9 has some limitations at present, such as the need to rely on PAM sequences and delivery problems, compared with the previous two generations of gene editing technology, CRISPR/Cas9 has the characteristics of lower price, shorter experimental period, simple operation and lower off-target rate. CRISPR/Cas9 has been widely used in human gene therapy research [10], rice crop genotype improvement [11], etc. At the same time, the field of CRISPR is developing at an incredible speed, and the gene editing technology is found in NCBI, CNKI and other databases. In recent years, articles all use CRISPR/Cas9 as editing technology, so this technology has broad application prospects.

3. Application of CRISPR/Cas9 in AD Research

3.1. Construction of AD Model

Animal/cell line disease model is an important tool for studying the pathogenesis, disease progress and treatment methods. The development of CRISPR/Cas9 technology makes the establishment of mutant animal/cell line model cheaper, faster and more accurate, thus developing a model closer to the disease situation. Based on animal ethics issues, cell models are widely used in exploring the pathogenesis of AD and its related metabolic pathways [12], screening related therapeutic drugs [13] and preliminary verification of targeted therapy mechanism because of their advantages of large quantity, rapid reproduction and low cost [14].

At present, the cells commonly used in AD research mainly include human neuroblastoma cells SH-SY5Y and SK-N-SH, as well as mouse hippocampal neuron cell line HT22 and glial cell BV2.

3.2. Study on Pathogenesis of AD

At present, the understanding of the causes of AD is still limited. With the development of CRISPR/Cas9, gene editing can be realized more and more accurately, which can reduce the generation of non-target effects, thus contributing to the study of cell function and related signal pathways of specific genes. Recent genome-wide association studies shows that the risk of (sporadic Alzheimer disease, SAD) is also driven by genes to some extent [15]. Some studies used CRISPR/Cas9 to construct the SK-N-SH human-God-borne tumor cell AD model with apolipoprotein E (APOE) knocked out, in order to further study the endogenous function of APOE. Some studies have established the hiPSC cell line with risk gene knockout by CRISPR/Cas9 technology, and confirmed the importance of the AD risk gene SORL1/SORLA in regulating the processing of APP, and suggested that it may play a wider role in regulating the function of endosome network [16].

3.3. Screening of Risk Factors for AD

The formation of early familial AD is closely related to the mutation of APP and PS1/2 genes. In contrast, SAD is a multifactorial disease, which may be related not only to genetic factors such as allele variation of APOE and many other genes, but also to environment, age, and even some diseases including vascular diseases and diabetes. Mitochondrial dysfunction and infection are all risk factors for AD. Knupp et al. established an iPSC cell line sorting protein-related receptor L1 (SORL1) by using CRISPR/Cas9 technology. It was found that compared with the wild type, the deletion of SORL 1 gene led to the increase of endosome (in iPSC differentiated neurons), and the increase of endosome was not related to the processing pathway of amyloid. However, it will affect the transport of APP in neurons, which suggests that SORL1 may play a more extensive role in the regulation of the function of internal body network and the pathogenesis of AD [17].

3.4. Target Search and Targeted Therapy of AD-related Genes

Targeted gene therapy includes finding the target and carrying out specific targeted therapy in the body. For example, specific therapy targeting the disease-causing gene of AD can be carried out in the brain [18]. CRISPR/Cas9 technology has attracted some attention in constructing experimental models to explore targets and gene therapy.

In the aspect of finding targets, it can use CRISPR/Cas9 technology to study the role of suspicious genes in the body by knocking out or downregulating their expression [19], or construct an AD disease model based on a certain pathogenic mechanism to analyze some indicators. Compared with targeting mature transcripts by RNAi technology, CRISPR/Cas9 (CRISPRi) technology is more powerful in finding targets, which can target the whole transcription unit, including other splicing isoforms and embedded noncoding RNA, and realize gene knockout.

Park et al. combined with nanocarrier delivery technology, used CRISPR/Cas9 technology to target edit β-secretase 1 (BACE1) gene in mitotic neurons, and proved its therapeutic effect in 5×FAD and AD mouse models with APP gene knocked in [20]. Raikwar et al studied whether the editing of glia maturation factor, GMF) gene mediated by CRISPR/Cas led to the inhibition of GMF expression and microglia activation, and proved that GMF may be a new therapeutic target for AD [21]. György et al. produced the allele-specific deletion of APP Sweden mutation (Appswee) or APPWT with the help of CRISPR/Cas9 technology, and this system may be developed as a gene therapy tool for AD caused by other point mutations related to the increase of Appswee and β -amyloid (A β). Based on CRISPR/Cas9 strategy, Sun et al. intervened the amyloid formation pathway in cultured neurons, edited endogenous AP gene at the C-terminal, weakened the formation of Aβ, and promoted the non-amyloid cleavage pathway of APP, thus realizing the protection of nerves [22].

The potential of CRISPR/Cas9 technology in gene therapy should not be underestimated. In 2016, the first CRISPR/ Cas9 cancer treatment experiment was conducted in West China Hospital, in which the programmed cell death protein-1 (PD-1) gene in isolated T cells was knocked out by CRISPR/Cas9, and the T cells were amplified and then returned to patients to achieve the therapeutic

purpose. This experiment conceptually verified the application of CRISPR/Cas9in gene therapy. Targeted gene therapy is a therapeutic method with strong target specificity, but its risks are also great. The toxicity of the delivery medium and whether it can cross the blood-brain barrier (BBB) still need to be studied. Besides, the miss rate, effect and side effects of CRISPR/Cas9 are worth considering.

4. Conclusion

CRISPR/Cas9has the advantages of short cycle, low cost, high efficiency and expandability, which has shown great power in basic research and great potential in AD model construction, AD pathogenesis research and therapeutic target search. Although many achievements have shown its role in the research and treatment of AD, there are still many problems to be solved. It is feasible to find therapeutic targets in vitro by using CRISPR/Cas9. However, it is not feasible to apply this technology to targeted therapy of AD at present, and further research is needed in reducing off-target rate, design good delivery medium and reducing treatment risk. In addition, the application of gene editing technology should follow the constraints of morality, ethics and law.

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