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Application and Prospect of CRISPR System in Alzheimer's Disease Treatment Research

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Abstract: Alzheimer's disease (abbreviated as AD), commonly known as senile dementia. As its name implies, it is the most common degenerative disease of the central nervous system that occurs in the elderly population. At present, there is still no effective treatment method. At present, CRISPR system-based research continues to advance in the field of AD therapy, such as CRISPR Cas9 for the construction of cell and animal models and gene knockout therapy, CRISPR Cas13 for the development of new detection technologies, CRISPRi has made progress in exploring related gene regulatory mechanisms, and CRISPRa can achieve gene transcriptional activation and functional characterization. However, these studies are still in the exploratory stage and have not yet been fully translated into effective clinical treatments. In this paper, we analyze the application of different CRISPR systems in AD treatment research, including model construction, gene editing, and exploration of regulatory mechanisms, and demonstrate their potential in AD treatment research. It provides a comprehensive reference for follow-up research, and can further optimize the technology to improve efficiency in the future, and focus on solving problems in clinical translation, so as to promote the development of AD treatment.

Keywords: Alzheimer's disease, CRISPR Cas9, AD therapeutic strategies.

1. Introduction

The primary cause of dementia and one of the biggest healthcare issues of the twenty-first century is AD, a progressive neurodegenerative disease [1]. Dementia is thought to affect 40 million people globally, the majority of whom are over 60. At least until 2050, this number is predicted to double every 20 years [2]. The reasons of AD are now being explained by a number of ideas, including as the neurovascular hypothesis, tau protein hypothesis, and β-amyloid cascade hypothesis. The most traditional explanation for the pathophysiology of AD is the β-amyloid cascade hypothesis, which holds that excessive Aβ deposition in senile plaques is the first step toward the pathophysiological changes linked to AD. Tau protein hyperphosphorylation, neuronal loss, and synaptic damage are among the pathological changes that result from a series of cascading reactions set off by Aβ deposition. Currently, mutations in one of three genes—amyloid precursor protein (APP), presenilin 1 (PSEN1), or presenilin 2 (PSEN2)—are thought to be the primary causes of AD. These genes either contribute to or have an impact on the production of Aβ.

At present, the main strategy of many companies to develop AD therapeutics is to inhibit the formation of Aβ oligomers, or promote the depolymerization of Aβ oligomers. It is based on the

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amyloid cascade hypothesis, which suggests that oligomerization of Aβ can be toxic, eventually leading to damage to nerve tissue and subsequent disease [3]. For example, first, anti-Aβ active immunotherapy, that is, exogenous Aβ is used as an antigen to stimulate the body to produce corresponding antibodies, and the antibodies then combine with endogenous Aβ to form antigen-antibody complexes, which are finally eliminated by phagocytic cells [4]. secondly, passive immunotherapy for anti-Aβ, in which monoclonal or polyclonal humanized anti-Aβ antibodies are injected into the body, bind to endogenous Aβ, and directly induce the breakdown of Aβ oligomers or their removal by phagocytes; In addition to this, β secretase inhibitors can also be used [5].

The traditional pharmacological treatment for Alzheimer's disease primarily utilizes cholinesterase inhibitors, glutamate receptor antagonists, cognitive enhancers, antidepressants, mood stabilizers, anxiolytics, and medications that improve cerebral blood circulation. In recent years, there have been some advancements in the research and development of drugs for Alzheimer's disease treatment, with several new medications having been approved for market release. For example, aducanumab, mannitol sodium capsules, and lecanemab.

The intrinsic potential of CRISPR technology for multiplex genome engineering is one of its primary benefits. Large expression constructs and multiple selection markers had to be repeatedly delivered in order to use previous gene editing methods. Without the requirement to create particular DNA-targeting domains, the Cas9 complex can be directed to multiple locations at once with the introduction of sgRNAs. Additionally, the experimental difficulties of duplicate construct delivery and selection are circumvented by using a "all-in-one" vector that carries numerous sgRNA expression cassettes on a single plasmid. This tool's strength is its high-throughput capability of identifying the functional effects of genetic variation. Therefore, analyzing the etiology of complex disorders like AD requires the use of CRISPR-Cas9 technology [6].

Clustered regularly spaced palindromic repeats (CRISPR) and CRISPR-associated protein tools can play a positive role in the development of AD treatment by correcting defective genes. CRISPR systems with different Cas enzymes play a huge role in exploring the gene targets of Alzheimer's disease and generating in vitro and in vivo models of AD treatment. This article focuses on summarizing the research status and prospects of CRISPR/Cas9, CRISPR/Cas13, CRISPRi, and CRISPRa for the treatment of AD.

2. Various CRISPR Systems

2.1. CRISPR Cas9

The primary application of CRISPR Cas9 in AD lies in the construction of cellular and animal models, and utilizing it to knock out AD-related genes represents a novel strategy for treating AD.

2.1.1. The Construction of Cell Models

With benefits like inexpensive expenses and comparatively quick experimental cycles, cell models are frequently utilized in AD research. Numerous in vitro cell models of AD have been developed in recent decades. In order to develop the pathophysiology of AD and medications resulting from PSEN2 dysfunction, Yu et al. created cell models by introducing the N141I mutation into the PSEN2 gene and using CRISPR/Cas9 gene editing technology to differentiate human-induced pluripotent stem cells into neural stem cells and brain organoids [7].

2.1.2. Construction of Animal Models

The development of appropriate cell lines for the identification of genetic markers and possible genomic targets for AD treatment, the assessment of viral vectors to introduce mutations, the use of

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safe non-viral vectors for gene editing manipulation, and finally the creation of appropriate in vivo models are all made possible by in vitro experimental AD models based on the CRISPR-Cas9 gene tool [8]. Pang, et al. used the Single guide RNA to introduce target mutations into ZDHHC21 genes using CRISPR-Cas9 technology, specifically target ZDHHC21 genes through the CRISPR-Cas9 system, and construct a mouse model of ZDHHC21 gene mutations to be used as a model for AD models to study the pathogenesis, development, or screening of AD therapeutics [9]. Ma, et al used chimeric mouse/human amyloid APP and mutant PSEN1 gene to target central nervous system (CNS) neurons under the control of mouse prion promoters to construct APP/PS1 dual transgenic AD model mice, systematically knocked out hepcidin gene by using CRISPR Cas9 technology technology, and hybridized to construct APPswe/PS1dE9+HAMP-/-(+) triple transgenic animal model [10]. Chen, et al. microinjected Cas9 mRNA, gRNA target sequence pairs, and homologous recombinant vectors into the zygotes of C57BL/6J mice, transplanted the zygotes into wild-type mice, in which the offspring of the mice were F0 mice, and the positive F0 mice were identified by PCR amplification, and the positive F0 mice were mated with wild-type C57BL/6J mice to breed F1 mice, and finally ABCA7-Floxp mice with stable genetics were obtained. The ABCA7-Floxp mouse model can be used as an animal model to study the relationship between the tissue-specific function of the ABCA7 gene and/or ABCA7 and the pathogenesis of AD [11].

2.1.3. CRISPR Cas9 for the Treatment of AD

By injecting adeno-associated virus (AAV) vectors with APP-specific guide RNA and the Cas9 coding sequence into the hippocampus of transgenic mice, György et al. demonstrated that the APP gene was somewhat disrupted, mostly in the form of single base pair insertions, which decreased the production of pathogenic Aβ [12]. By modifying the extreme C-terminus of APP and reversing the amyloid process, Sun et al. reduced APP β-cleavage and Aβ formation while increasing neuroprotective APP α-cleavage in cellular and animal models. There was no discernible effect on in vitro neurophysiology, and the compensatory APP homologs and APP N-terminus stayed intact [13]. In the study by Evangelos Konstantinidis, et al., it was found that the CRISPR-Cas9 system could selectively disrupt alleles in PSEN1 human fibroblasts, resulting in the destruction of over half of the mutant alleles and a reduced extracellular Aβ42/40 ratio. The results indicate the effectiveness of CRISPR-Cas9 in selectively targeting the PSEN1 alleles and mitigating AD-related phenotypes. Wang, et al. successfully knocked out ADAM10 in SHSY5Y cells using the CRISPR/Cas9 editing system, constructing an AD cellular model that significantly enhanced the interaction between PTBP1 and Tau, improved neuronal differentiation capacity, increased neuronal length, and significantly enhanced intercellular information exchange [14]. Therefore, these systems utilizing CRISPR/Cas9 to knock out corresponding genes could develop into a therapeutic strategy.

2.2. CRISPR Cas13

Lei R and Liu X developed a new gRNA typing probe capable of selecting AD-related single nucleotide polymorphism (SNP) loci from rs671, rs7412, rs429358, and rs7649121, utilizing CRISPR/Cas13 nucleic acid detection technology. This probe accurately types a series of important SNPs, enhances classification discrimination ability by introducing additional mutated bases, and simplifies the operational process by replacing PCR with RP nucleic acid isothermal amplification technology for early target molecule amplification, thereby improving detection sensitivity.

Scientists debated whether CRISPR-Cas13 targeting Bace1 RNA could enhance memory problems in AD patients at the recent 27th Annual Meeting of the American Society of Gene and Cell Therapy (ASGCT), offering fresh perspectives on AD therapeutic approaches.

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2.3. CRISPRi

CRISPRi (CRISPR interference) uses dCas9 to inhibit the transcription of genes by precisely binding to the promoter region or coding sequence of the gene of interest through guidance with sgRNA, hindering the binding of transcription factors and the function of RNA polymerases. Researchers can use CRISPRi technology to reduce the expression of specific genes without having to modify their DNA sequences.

The dysregulation of transposable elements (TE) is known to be associated with neuroinflammation in the brains of individuals with AD. However, the quantitative trait loci for TE (teQTL) have not been well characterized in the aging human brains of those with AD. Feng, et al. utilized large-scale bulk and single-cell RNA sequencing, whole genome sequencing (WGS), and xQTL from three human AD brain biobanks to characterize the dysregulation of TE expression, and employed CRISPRi to induce pluripotent stem cell (iPSC)-derived neurons to identify genome-wide significant TE expression QTL (teQTL). The analysis using CRISPRi determined that activated short dispersed nuclear elements exert a neuron-specific inhibitory effect on C1QTNF4 expression by reducing neuroinflammation in iPSC-derived neurons. Extensive TE dysregulation was found in the brains of AD patients, and teQTL provides a complementary analytical approach to identify potential risk genes for AD [15].

In tau biosensor cells, Polanco, Juan Carlos, et al. conducted a genome-wide CRISPRi screen and discovered that the most powerful regulators of tau aggregation triggered by exosomes and vesicle-free tau seeds were cellular regulators shared by two tau seeding mechanisms: ANKLE2, BANF1, NUSAP1, EIF1AD, and VPS18 [16].

The rapid generation of homogeneous neuron-glial spheroids, their characterization using immunohistochemistry and single-cell transcriptomics, and their combination with CRISPRi-based large-scale screening are all made possible by Li et al.'s 3D co-culture system, iAssembloids (Induced Multilineage Assemblies). This platform is also being applied to study the role of the AD risk variant, APOE-ε 4, in its effects on neuronal survival [17].

The most notable enrichment for AD heredity is seen in microglia's putative cis-regulatory elements (cCRE). Yang et al. prioritized previously unreported AD risk variant genes by combining genetic data with microglia-specific 3D epigenomic annotations. By screening single-cell CRISPRi in microglia, a functional connection between variant functionalities and target genes was further demonstrated. In order to advance genetic linkages with experimentally verified cell type-specific symptoms and processes, a methodical approach to identifying and characterizing variations relevant to AD was found [18].

2.4. CRISPRa

By catalytically deactivating Cas9 (dCas9) connected to transcriptional activators, CRISPRa, a recent addition to the gene overexpression technology, stimulates transcription at particular genomic locations. Schraubend et al. claim that CRISPRa employs certain activation mechanisms, like the dCas9-VPR protein, which is made up of three transcription factors (VP64, p65, and Rta) joined to dCas9 [19]. In order to assess the specificity of CRISPRa, multiplex gene expression activation of immediate early genes involved in neuronal function targeted a distinct upstream promoter region within the BDNF gene to modify particular mRNA transcription levels, confirming that this region is essential for various neuronal processes [20]. Michael et al. believe that this triple activator can be used to directly characterize the functional role of single mRNA transcripts by potentially initiating transcription upon being directed to the promoter region of the target gene. This would be a very powerful tool for studying transcriptional dysregulation of genes related to AD [6].

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Next-generation sequencing of sgRNA libraries, according to Michael et al., can aid in the investigation of how specific genes or epitopes affect cellular activity, which may prove to be a useful tool in the treatment of AD. A range of experimental approaches that are readily applicable to AD are made possible by the use of epigenetic modifiers for CRISPR hybrid screening [6]. Klann and colleagues developed the CRISPR-Cas9-based Epigenomic Regulatory Element Screening (CERES) technique, which uses CRISPRi and CRISPRa for gain-of-function and loss-of-function screening and effectively detects regulatory element activity in the native chromosomal context [21]. Applying this high-content functional annotation to AD could shed light on the regulatory components that control the disease's cellular manifestations.

3. Conclusion

This article demonstrates the multi-faceted application of CRISPR system in AD treatment research, and provides new tools and ideas for AD pathogenesis and treatment development. For example, the development and application of CRISPR Cas9 in cell animal models and the exploration of gene editing therapy provide the possibility of direct intervention in disease-causing genes. The study of gene regulatory mechanisms using CRISPRi and CRISPRa can help to deeply understand the molecular changes in the AD disease process and provide a theoretical basis for precision treatment. These studies provide an important reference for further optimizing and expanding the application of CRISPR technology in the treatment of AD. However, most of the current research focuses on the cell and animal model stage, and there is still a large gap from clinical application. There is a lack of large-scale, long-term clinical trial data to validate the safety and efficacy of these CRISPR-based treatments. There is insufficient research on the potential off-target effects and long-term effects of different CRISPR systems in complex in vivo environments. There are few studies on the synergies and joint applications of various CRISPR systems, and their overall therapeutic effects may not be fully utilized. Therefore, clinical trials should be strengthened in the future to evaluate the safety and efficacy of CRISPR technology in the treatment of AD and promote its translation into clinical practice. Gain insight into off-target effects and long-term effects to optimize CRISPR system design for precision and safety. Explore the combination of different CRISPR systems and synergies with existing therapeutics to develop more effective integrated treatment strategies. Future research will continue to explore new gene targets related to AD and further expand the application scope of CRISPR technology in AD treatment, which is expected to bring new breakthroughs to AD treatment.

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