Neurodegenerative Diseases Treatment Based on CRISPR

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Abstract. Parkinson's disease (PD) is a neurodegenerative disease that is difficult to study for humans. The etiology of induced diseases is closely related to genetics neuroinflammation, mitochondrial and lysosomal dysfunction, and synaptic transport problems. The early characteristics of Alzheimer's disease (AD) are cognitive decline, behavioral changes, and language deficits. Subsequently, the patient developed comprehensive amnesia and decreased motor function, with death typically occurring within 9 years after diagnosis. CRISPR Cas9 direct therapy has been used to repair pathogenic gene mutations and as a research object or carrier for disease simulation experiments. Neuroscientists want to edit target genes and their transcripts through CRISPR/Cas9. CRISPR technology can indirectly intervene in mitochondrial autophagy to determine the regulation of the etiology of PD. Targeted activation of SAM to produce dopamine (DA) to improve motor behavior in rats. It can also mediate the allele disruption of the Swedish APP used to suppress the initial symptoms of AD. And by weakening the app-β-splitting while enhancing apps with neuroprotective effects can reduce A β the generation and manipulation of starch protein pathways control AD. The Guangxi Bama miniature pig cultivated through CRISPR/Cas9 is expected to become a large-scale animal model for studying human neurological diseases. CRISPR technology can become a reliable tool for humans to solve mutated gene diseases.

Keywords: Neurodegenerative disease, CRISPR, Application.

1. Introduction

Bacteria and archaeal viruses (bacteriophages or bacteriophages) are an entity that is widely distributed on Earth and pose a constant threat to prokaryotes. Prokaryotes defend against bacteriophages through a variety of defense mechanisms. Clusters of regularly spaced short palindromic repeats (CRISPRs) and their associated proteins (Cas) integrate spacers that invade the genome into CRISPR sites to remember previous infections to demonstrate the prokaryotic adaptive immune system. Cas proteins use a repeating spacer as crRNA during double infection to targeted attacks on intruders' sequences. Through gene editing of cells, living organisms opens entirely new avenues about CRISPR-Cas9 could perform targeted scripting on target genes RNA. Over the past decade, the prokaryotic immune system CRISPR-Cas has attracted increasing attention from the scientific community due to its unique adaptability, as well as its therapeutic potential. The CRISPR-Cas genome editing system is derived from the ability of prokaryotes to achieve the effect of targeted editing of target genes in living cells of various species. CRISPR-Cas is easy to use and robust. This technology bridges gene editing in the scientific and medical neighborhoods, and the initial success of the experiment has led to an urgent need to discover new operational nucleic acid systems, most widely used being Cas9. To modulate Gene transcription and translation, as well as regulation of gene expression, can alter DNA and proteins on chromosomes through chemical modifications, thereby affecting gene expression, Interactions with chromatin, nuclease-free and RNA-targeted Cas proteins already fused with multitudinous effector proteins. Overall, these new gadgets are elevating our understanding and pushing observe the clinical trials of CRISPR-Cas technology on gene and cell therapy [1].

CRISPR/Cas9 gene editing technology is effective in treating neurological diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD). CRISPR/Cas9 can be a good medicine for some neurological diseases. Abnormal accumulation of extracellular amyloid-β (Aβ) and aberrant

hyperphosphorylation of intracellular tau protein are primarily markers of the emergence of AD is currently untreatable and cannot be medically prevented from getting worse. Traditional drug treatment requires long-term medication to curb the deterioration of the condition. However, as the disease worsens, the effects of drug therapy fall dramatically. The CRISPR/Cas9 strategy can effectively alleviate difference between symptoms and differentiation of AD patients. APP is a high-quality upstream protein in Aβ, and its expression directly affects the content of Aβ. Therefore, to reduce the expression of Aβ, CRISPR/Cas9 can eliminate target genes. The conversion of APOE 4 to APOE 3 was demonstrated to significantly improve the pathological features associated with AD. The conversion from APOE4 to APOE3 can also be reduced based on this mechanism the hyperphosphorylation of tau protein at a certain limit. At present, the proteins and genes related to AD are the main treatment directions of AD, namely APP and Aβ. The dysregulation of the gene subnetwork of nerve cell was the main cause of LOAD, Identified ATP6V1 A as a key regulator. ATP6V1A gene is targeted, and its expression is reduced, and nerve cell activity is suppressed. This discovery opens a new direction to treat AD. However, this technology still has shortcomings such as off-target effects, specificity of sgRNA mechanisms, and human immunity to Cas9. The underlying ethical issues still need to be considered by humans. Since CRISPR technology has been widely used in the treatment of neurodegenerative diseases, this research will specifically analyze its application performance in PD and AD.

2. Analysis of PD and AD based on CRISPRS technology

2.1. PD

Genetic screening was conducted using CRISPRS technology to identify PARKIN abundance regulators, which helped us understand the determinants of mitochondrial autophagy [1]. The ubiquitin chain synthesized by PARKIN (an E3 ligase) is a target for PINK1 kinase to generate phosphoS65 ubiquitin (pUb) prompts mitochondria to engulf themselves and envelop them into vesicles. Mitochondrial dysfunction is associated with Parkinson's disease. PARKIN can integrate the operational modes between quality control factors, providing an environment for mitochondrial protease degradation and inhibit mitochondrial antigen presentation. Inducible Neurogen in 2 (iNGN2) neurons derived from human induced pluripotent stem cells (iPSCs) (THAP11 targeted by CRISPR/Cas9) exhibit de inhibition by enhancing pUb accumulation of PARK2 transcription. The regulation of PARKIN level regulates mitochondrial autophagy in related cells. The disadvantage is that artificially controlling mitochondrial autophagy through PARKIN level regulation may lead to other diseases, and the safety of clinical trials cannot be guaranteed.

Research has shown that CRISPR/sgRNA directed co activators (SAM) can serve as a clinical treatment for PD astrocyte proliferation was observation of a large gray matter mass and the main components of the basal ganglia between the soles of the foot and the tegmental layer of the midbrain in animals used by scientists to study human diseases and physiological processes, as well as death reports of PD [2], indicating the immune response of astrocytes in Parkinson's symptoms. The endogenous tyrosine hydroxylase gene (Th) is an enzyme that promotes the production of DA in astrocytes. Using SAM to awaken Th in astrocytes can manufacturing dopamine in the main component of the basal ganglia in rats with 6-OHDA injury, implantation of cells into the striatum of 6-OHDA biased PD rats resulted in improved motor behavior compared to normal diseased rats. Under normal circumstances, The SAM system does not undergo DNA double-strand structural breaks that can lead to mutations and alter gene expression as is the case with CRISPR, providing the possibility of curing PD. The advantage of this study is that DSB will not occur, providing assurance for safety.

Guangxi Bama miniature pigs cultivated with CRISPR/Cas9 carry PD-causing mutations. Scientists use large animals to study human diseases and physiological processes to validate the treatment process of PD [3]. Editing target genes and their transcripts using CRISPR/Cas9 integrate with SCNT technology, making three missense mutations can lead to PD in Guangxi Bama miniature

pigs in SCNA were successfully cultivated. The transgenic expression of synaptic nuclear protein is all in mice, but the mouse model does not sufficient demonstrate the main pathologies of Parkinson's in humans, and the formation time of PD may be longer than the lifespan of mice. To gain a more comprehensive understanding of PD in the human body, experiments should be conducted on long-lived animal models. The reason why miniature pigs are used to simulate brain diseases is because they have a neurology analogous to that of humans. Additionally, miniature pigs have a long lifespan, relatively short gestation periods, and produce more offspring. But SN dopamine neurons were not lost was observed in 3-month-old gene edited miniature pigs (PD specific pathological brain changes). Continuous testing is needed to determine whether these genetically modified animals exhibit pathological features similar to PD, in order to validate Guangxi Bama miniature pig as a large-scale animal model capable of accurately demonstrating human Parkinson's pathology and to solve the problem of inaccurate replication of the main pathological features of human PD in mouse animal models.

2.2. AD

In order to selective discard APP SW or APP WT in human fibroblasts, three different lengths were generated and tested of gRNA and a non-mutated version of the gRNA targeting APPwt, and confirmed by ELISA analysis that Aβ levels in human cells could be effectively reduced by two of them, while in the primary cortical neurons and hippocampus of transgenic mice expressing the APP SW mutation [4], the CRISPR/Cas9 strategy can target disruption of mutant APP alleles, leading to the formation of indels, resulting in DNA frameshift sequences. However, the effectiveness of this therapy is not significant and there is still a long way to go.

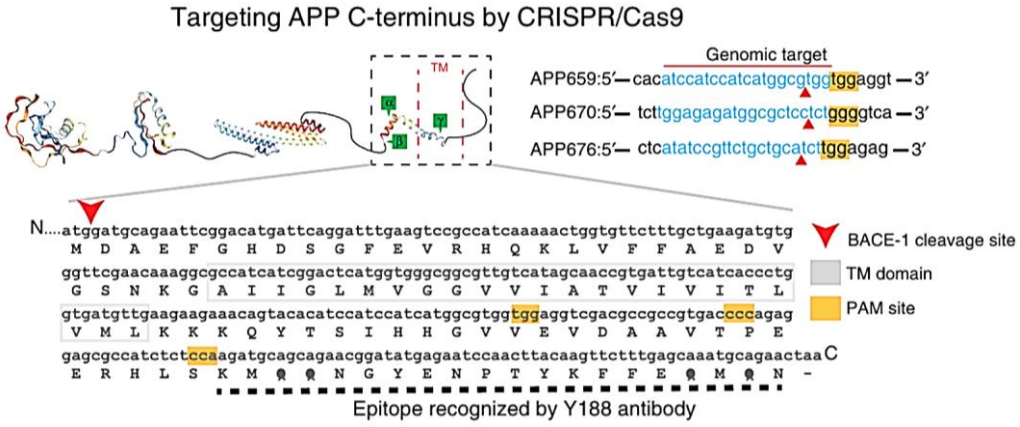


Figure 1. Schematic and C-terminal sequence of mouse APP [5]

Sun et al. identified three human and mouse conserved genes at APP about the C-terminus and synthesized sgRNAs targeting these regions [5], as shown in Fig. 1. They designed stable H4 glioma cell lines expressing APP:VN and BACE-1:VC single copies Comparison of editing efficiency of sgRNAs, and although the TGG PAM of APP-659 was conserved in human APP, upstream sgRNA target sequences differ only for two nucleotides, mo-APP-sgRNA still could not edit human APP. However, sgRNAs targeting human APP targeting sequences stably editing APP in HEK293 and human embryonic stem cells. Application CRISPR edit does not change the holonomic level of the Holo-APP. In addition, they examined the role of APP in the ad like environment of the heterozygous knockout iPSC lines with sgRNA of the most common familial AD APP. Immunoblotting using antibodies confirmed stable and C-terminal selective APP editing in WT and APP-London iPSC lines. Further experimental purposes the APP-C99 construct ruled out the effect of sgRNA on APP-γ cleavage, suggesting that this editing strategy has a selective effect on APP-β cleavage. This gene editing strategy has been further shown to better regulate the amyloid pathway.

Through CRISPR/Cas9 technology, the phagocytosis of microglia was enhanced by the disappearance of mCD33 gene, which increased the Aβ clearance rate and weakened the pathological phenotype of AD. Bhattacharjee et al. demonstrated hCD33m is a function acquisition type [6]. This

function acquisition effect can be clarified by the phagocytosis and the increase of the transfer bias in convey microglia hCD33m, and given the rs201074739 CD33 deletion allele and lack of AD protection, these results support a typical example in which the gain-of-function role of hCD33m is important in reducing rs12459419T CD33 allele-mediated susceptibility to AD.

3. Conclusion

Currently, CRISPR is a new direction in treating neurological disorders. It has played an important role in many neurodegenerative diseases and is still being developed. However, the technology still has many problems, such as off-target mismatching and specific tissue targeting. At the same time, low efficiency and insufficient effectiveness are also problems that still need to be solved. More efficient delivery systems are the goal of a great deal of research at present. The advent of CRISPR/Cas9 has improved the situation. CRISPR/Cas9 is favored by more people because of its low off-target rate and high efficiency. Due to the development of lipid nanoparticle (LNP)-based CRISPR/Cas ribonucleoprotein (RNP) technology, CRISPR/Cas9 technology has created conditions for the research and clinical treatment of neurodegenerative diseases. In summary, CRISPR/Cas9 is the most successful invention in gene editing technology. In clinical gene therapy, gene scissors play a key role. While other gene editing technologies are still difficult to make progress due to the shortcomings of long cycle time, high cytotoxicity, high price, and difficult delivery. The crispr-cas9 system is expected to be widely applied in clinical treatment about neurodegenerative diseases.

Authors Contribution

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

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