Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/60/20241431

The utilization of CRISPR-Cas9 with human pluripotent stem cell for Parkinson's disease modeling

Yiping He[1], Xiaowen Xing[1,2]

[1]College of Life Science & Technology, Huazhong University of Science and Technology, Wuhan, Hubei, China

[2]u202113029@hust.edu.cn

Abstract. Parkinson's disease (PD), also often referred to as "parkinsonism," is a neurodegenerative disorder. Those afflicted with this condition are frequently elderly, which results in a considerable decline in their quality of life and overall well-being. Although there are major breakthroughs in PD research, there remains an incomplete grasp of the disease's pathogenesis and the formulation of innovative treatment approaches. This has led to an urgent need for a technology that can recapitulate all the features of human PD. The emergence of induced pluripotent stem cells (iPSC) coupled with the rising appeal of Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 (CRISPR-Cas9) technology, the construction of PD models through the combination of iPSC and CRISPR-Cas9 has emerged as a crucial area of research in the field of PD. Employing patients' somatic cells, transformed into iPSC and integrated with CRISPR-Cas9 for genetic modification, has facilitated the duplication of the genetic makeup of neural cells associated with PD, thereby facilitating the investigation of the influence of diverse genotypes on disease progression and treatment response. This review outlines the application and future development of iPSC and CRISPR-Cas9 technologies for the study of Parkinson's disease pathogenesis. The technology enables scientists to gain a deeper understanding of PD and paves the way for the development of novel therapeutic approaches in the future.

Keywords: Parkinson disease, models, CRISPR-Cas9, iPSC.

1. Introduction

Parkinson's disease (PD) manifests as an advancing neurodegenerative disorder, marked by shaking, stiffness, and unstable posture. This common neurological disorder is a global health concern which is estimated to affect 4 million people worldwide [1].

The prevailing belief is that the primary motor manifestations of PD, notably bradykinesia and rigidity, stem from the demise of dopaminergic neurons in the substantia nigra pars compacta (SNc) [2]. However, the pathogenesis of PD is not determined by a single gene. Despite the investigation of PD-related genes, including LRRK2, PINK1, and SNCA [3], the underlying molecular mechanisms remain incompletely understood. Conversely, the pathogenesis of PD is complex and involves a convergence of genetic, environmental, and other yet unidentified factors. Moreover, the study of the interaction between these multiple factors represents a significant challenge. Scientists need to create superior PD

DOI: 10.54254/2753-8818/60/20241431

models to tackle these challenges and explore PD's development and possible treatments in a simplified manner.

Before iPSC technology, acquiring cells with altered genes required directly sampling from patients, a challenging procedure for the delicate brain. iPSC technology allows constructing PD models from fibroblasts of PD patients. Introducing transcription factors into somatic cells reprograms them into ESC-like pluripotent stem cells. These iPSCs differentiate into dopaminergic neurons or other PD-associated cells. This enables patient-specific disease models to study pathology and drug responses at the cellular level. CRISPR-Cas9 technology allows precise gene editing in iPSCs, correcting disease-causing genes and assessing the impact on neuronal function. Integrating CRISPR-Cas9 with iPSCs facilitates accurate gene modification and tailored disease studies, advancing PD research and therapy development.

2. The current state of research and application of CRISPR-Cas9 in PD

2.1. Principle of CRISPR-Cas9 Technology

The CRISPR-Cas system is comprised of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas), which confer adaptive immunity to exogenous elements in bacteria and archaea. Classification of the CRISPR-Cas system encompasses three distinct categories (I, II, and III) along with 12 varied subtypes, each differing in terms of genes, quantity, and dimensions [4]. The categorization of the CRISPR-Cas system mainly hinges on the Cas gene and the specific protein it produces. During the targeting stage, freshly immunized CRISPR arrays undergo transcription into extended precursor CRISPR RNA (pre-crRNA), which is subsequently converted into mature CRISPR RNA (crRNA). Mature crRNA binds to Cas, recognizing and destroying foreign nucleic acids. In the initial phase of acquiring primer spacers, novel substrates for spacer acquisition are created concurrently with the destruction of targets and then assimilated into the CRISPR array.

The composition of CRISPR-Cas9 is a type Ⅱ CRISPR complex that utilizes a single protein, Cas9. In addition to the Cas9 protein and the guide crRNA sequence, CRISPR-Cas9 employs a second RNA molecule, trans-activated crRNA (tracrRNA), which forms a double-stranded stem with the 39 end of the crRNA and assists in recruiting the Cas9 protein to the crRNA. To more effectively identify and neutralize foreign DNA, the CRISPR-Cas9 system must possess a DNA sequence that aligns with the spacer, in addition to the Cas9 protein's capacity to recognize a protospacer adjacent motif (PAM) situated in close proximity to the target sequence [5].

The current approach is to heterologously express Cas9 and two RNA sequences by viral transfection, plasmid transfection and microinjection, or directly into guide RNA.Cas9 targets the target sequences and makes double-strand breaks, and DNA repair is then initiated via non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms to achieve precise knockout and knock-in [5].

2.2. Establishing PD models via CRISPR-Cas9

Despite years of research by numerous molecular biology and biochemistry researchers, the pathogenesis of PD remains unclear, thereby demonstrating the complexity of PD. To elucidate the pathogenesis of PD, it is imperative to develop efficacious and precise models that can be employed to study the disease and observe its progression. As an emerging technology in recent years, CRISPR-Cas9 has built effective disease models in different fields through its gene editing ability. For neurodegenerative diseases related to gene mutations such as PD, it is essential and advanced to use CRISPR-Cas9 technology to build models that used for revealing pathophysiological mechanisms of disease or drug screening and individual specificity.

2.2.1. Modelling PD with animals via CRISPR-Cas9

Animal models are needed to establish disease models for PD and to develop therapies. Presently, CRISPR-Cas9 stands as an exceedingly effective genomic reprogramming system in various animal species, including pigs, rats, mice, zebrafish, bacteria, and more.

DOI: 10.54254/2753-8818/60/20241431

The MCI-Park model, a diminutive mouse model, was created using CRISPR-Cas9 in mice, generating dopamine neurons devoid of NDUSF2, responsible for encoding mitochondrial complex I. These mice, without NDUSF2, display neurodegenerative alterations [6]. Dopamine signaling in the striatum is dependent on the negative regulation of cell cycle protein-dependent kinase 5 (*CDK5*). Recently, a study by Zhou et al. [7] found the manifestation of defective locomotor activity and a disruption in the activity/rest behavior in mice by knockdown of *CDK5* using CRISPR-Cas9. Importantly, the study revealed a decrease in dendritic length and a lower count of active synapses in the mouse brain, suggesting a crucial role of CDK5 in controlling the progression of neurodegenerative conditions like PD.

CRISPR-Cas9 in large mammals offers insights into PD beyond what small rodent models provide, serving as effective models for PD research. Studies involving CRISPR-Cas9 and SCNT in pigs targeting genes like *Snca*, *Park2*, *Pink1*, and *Park7* reveal varied results: while some show PD-like phenotypes, others do not exhibit significant neurodegeneration or clinical PD signs.

Future improvements may focus on reducing vector size, off-target effects, and optimizing delivery systems via CRISPR-Cas9 in animal models.

2.2.2. Cellular models of PD by CRISPR-Cas9

Numerous research efforts have utilized iPSCs obtained from PD patients exhibiting a distinct gene mutation responsible for the disease to develop models. The iPSCs have evolved into neural stem cells, known to display a phenotype linked to neuronal cells in the brains of individuals with PD. The construction of disease models at the cellular level has enabled the identification of a few key gene mutations that induce the development of PD. These include the *LRRK2*, *SNCA* and *PARKIN* genes [3]. Additionally, studies show that iPSC cells' neural stem cells in people with the LRRK2 p.G2019S mutation are more susceptible to proteasomal stress and exhibit genetic anomalies in nuclear envelope structure, clonal growth, and neuronal variety [8]. Subsequently, researchers utilizing CRISPR-Cas9 technology to revert point mutations to wild type were observed to reverse the phenotypes, resulting in the suggestion that changes in nuclear structure could be a clinical indicator of PD.

The use of iPSCs to construct cellular-level models of PD has facilitated the study of disease-causing gene mutations, which has made it easier and more efficient to mimic the phenotypes associated with PD patient signs, such as neuronal cells in the brain. Cellular disease models offer a distinct avenue for investigating the pathogenesis of PD, offering a complementary perspective to animal disease models, that is, a human-derived disease model.

2.3. The utilization of CRISPR-Cas9 in PD research

For a considerable period, scientists have been engaged in the search for a robust and adaptable instrument for the modulation of genetic information, both in vitro and in vivo. The advent and accessibility of the CRISPR-Cas system have had a profound and far-reaching impact on the entire scientific community. The discovery and utilization of the CRISPR-Cas system has had a profound and enduring impact on the advancement of biological knowledge. It is challenging to identify any technological innovation that has had a more profound and sustained impact on biological progress than the CRISPR-Cas method. This chapter will examine one of the CRISPR isoforms, the CRISPR-Cas9 system, in the context of PD.

The underlying causes of PD remain largely unclear, and current treatments remain symptomatic. To address this, scientists have developed various disease models to study PD pathogenesis more comprehensively. Experimental models are primarily classified into two categories: model organisms and in vitro cultured cells. These models can analyze PD diseases at different levels. Two principal approaches to constructing PD models using CRISPR-Cas9 are:

2.3.1. Knockdown of Disease-Related Genes

DOI: 10.54254/2753-8818/60/20241431

This allows for the exploration of specific gene effects on the disease. For instance, the generation of tango14 null mutants in Drosophila by Xue et al., which resulted in reduced lifespan, locomotor defects, and cholesterol accumulation, correlating with PD pathogenesis [9].

2.3.2. Targeted Editing of GWAS-Identified Risk Variants

This approach clarifies the potential pathogenesis of specific risk variants. For example, in zebrafish, targeting the exotic region of the Park7-/- gene generated a DJ-1-deficient model, providing a novel background for studying DJ-1 function [10].

CRISPR-Cas9 has also been used to deliver vectors targeting PINK1 and DJ-1 genes in the monkey brain, resulting in significant clinical signs and pathological features of PD [11]. Additionally, knockout models of PARK2 and PINK1 in porcine fetal fibroblasts achieved using CRISPR-Cas9 and SCNT did not show neurodegenerative changes or PD phenotypes, indicating variability in gene-targeting results [12].

Precision editing properties of CRISPR-Cas9 have enabled the introduction of genes like LRRK2-G2019S into hiPSC lines, allowing assessment of potential risk loci's contribution to the PD cell phenotype [13]. This technology can also generate genetically controlled isogenic pluripotent stem cells to identify the cis-acting effects of PD-associated risk SNP genotypes on gene expression. CRISPR-Cas9 offers crucial understanding of the disease's development and possible treatment objectives. Continued advancements in this technology will enhance the accuracy and efficiency of these models, ultimately contributing to more effective treatments for PD.

3. The utilization of iPSC technology and CRISPR-Cas9 in PD

3.1. Induced pluripotent stem cell (iPSC) technology

The advent of iPSC technology has transformed the field of PD research, facilitating the transformation of differentiated somatic cells into a pluripotent state akin to embryonic stem cells (ESCs). This process, driven by the Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc), entails significant changes in epigenetics, including alterations in DNA methylation, histones, and chromatin restructuring. Pluripotent cells have the capability to transform into various cell types originating from the three germ layers, facilitating diverse applications in PD research and therapy. iPSCs are generated by introducing transcription factors into somatic cells using viral or non-viral methods, forming colonies validated for pluripotency. These cells can differentiate into specific cell types using growth factors, cytokines, and gene editing techniques like CRISPR-Cas9, crucial for modeling PD. iPSCs capture patient-specific mutations, enabling the examination of disease processes, drug screening, and the development of personalized cell therapies. Differentiation protocols mimic embryological development, producing dopaminergic neurons (DAns) that provide accurate PD models. Despite challenges like tumorigenicity and genetic stability, advances in gene editing and scalable methods hold promise for clinical applications. iPSC-derived models with *SNCA*, *LRRK2*, *PINK1*, and *GBA* mutations offer insights into PD pathogenesis and potential treatments, including cell therapy and patient-specific transplantation.

3.2. CRISPR-Cas9 combining iPSC technology in PD

The precise control of gene expression is a fundamental aspect of functional genomics, and a range of approaches, including knockout, knock-in, knock-down and overexpression, are employed in gene editing. The construction of iPSC cell lines and the ability to obtain a variety of target cell types enhances the ability to model human diseases and develop therapies. Currently, the most prevalent approaches are RNAi (shRNA/siRNA)-based and CRISPR-Cas9 gene editing systems. The CRISPR-Cas9 system is capable of directly modifying the genome, enabling precise gene knockdown, in contrast to the more limited approach of merely reducing the level of gene expression. Furthermore, the CRISPR-Cas9 system allows for genome-wide knockdown and can simultaneously target multiple genes. CRISPR has been demonstrated to have a higher knockdown efficiency than RNAi, and is more flexible in targeting genes throughout the genome.

DOI: 10.54254/2753-8818/60/20241431

The iPSC model has been employed to construct PD-related models using the CRISPR-Cas9 system. Creating an iPSC line using a PD patient carrying a PARK7 mutation and a CRISPR-modified Gibco human episomal iPSC line to replicate the PARK7 mutation [14]. These models provided a condition for the study of the DJ - 1, thus finding the defensive role in nerve cells. CRISPR-Cas9 editing of iPSC lineage PD cells can also be utilized to construct homozygous PD astrocytes lacking the *LRRK2 G2019S* mutation, with the objective of determining gene pathogenicity by functional comparison with homozygous mutants [8]. This study of nerve cells to block the pathogenic crosstalk injury provides a new thought of treatment, designed to save the nerve damage in the early days, prevent the mass diffusion.

Integrating CRISPR-Cas9 with iPSC technologies facilitates mutation creation at genes responsible for diseases, while maintaining identical genetic profiles in the modified cells. This facilitates the evaluation of variations in phenotypes and subsequent impacts. Additionally, comprehensive CRISPR screenings across the genome enable rapid identification of genetic alterations in neuronal types, aiding in uncovering novel pathways and pinpointing new treatment targets.

4. Challenges and issues of CRISPR-Cas9 in PD research

It is irrefutable that the advent of CRISPR-Cas technology has precipitated a plethora of scientific advancements. Nevertheless, there are still numerous limitations and unavoidable problems in the practical application of CRISPR-Cas9 technology. The Cas9 proteins may act on non-targeted genomic sites, leading to undesirable results of cleavage and the occurrence of serious off-target phenomena, which greatly reduce the efficiency and accuracy of gene editing by CRISPR-Cas9. A quartet of primary tactics has been formulated to minimize unintended consequences. These include the improvement of Cas9 protein through genetic engineering, which involves increasing the fidelity of the Cas9 protein [15]. Another approach is the synthesis of high-fidelity sgRNA, which is achieved by adjusting the length of sgRNA and chemical modification [16]. Additionally, there is the establishment of a DSB-independent CRISPR-Cas9 system, as well as the improvement of delivery platforms.

A major hurdle in the use of CRISPR-based treatments as antimicrobial agents lies in pinpointing appropriate carriers for introducing external DNA into the bacteria targeted. Currently, two dominant delivery modalities exist: viral vectors, including AAV, lentivirus, retrovirus, and emerging phages; and non-viral delivery, mainly nanoparticles, electroporation, microinjection, and conjugate delivery. A number of in vitro and in vivo studies have been published on phage release of the antimicrobial CRISPR-Cas system [14] indicating phage-based vector as CRISPR-Cas delivery platform is feasible to some extent.

5. Conclusion

The CRISPR-Cas9 technology serves as an effective instrument for altering genomes, comprising the Cas9 protein and a guide RNA (gRNA) that directs Cas9 to a specific DNA sequence, where it introduces a double-strand break. This break is repaired by the cell's mechanisms, allowing for targeted gene modifications. CRISPR-Cas9 has revolutionized PD research by enabling precise genetic modifications in model organisms and iPSC-derived cellular models. CRISPR-Cas9 has been employed in developing models for Parkinson's Disease in mice, rats, zebrafish, and non-human primates, facilitating the investigation of disease processes and evaluation of possible treatments. In Drosophila, targeting the *tango14* gene revealed its role in lipid metabolism and PD pathogenesis. In zebrafish, targeting genes like *Park7* and *CDK5* elucidated their roles in neurodegeneration. Human iPSCs reprogrammed to dopaminergic neurons provide a precise PD model, preserving endogenous cellular machinery and transcriptional feedback, essential for understanding the disease. These models have demonstrated the efficacy of potential treatments and revealed genetic susceptibility factors. Advances in CRISPR-Cas9 aim to reduce off-target effects and improve delivery systems, making it a vital tool in PD research and potential clinical applications. Despite challenges like tumorigenicity and genetic stability, the integration of CRISPR-Cas9 and iPSC technology holds considerable potential for the creation of tailored medical treatments for PD.

DOI: 10.54254/2753-8818/60/20241431

Authors Contribution

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

References

[1] Hayes MT. (2019). Parkinson's Disease and Parkinsonism. Am J Med, 132(7), 802-807. https://pubmed.ncbi.nlm.nih.gov/30890425/

[2] Surmeier DJ. (2018). Determinants of dopaminergic neuron loss in Parkinson's disease. FEBS J, 285(19), 3657-3668. https://pubmed.ncbi.nlm.nih.gov/30028088/

[3] Chia SJ, Tan EK, Chao YX. (2020). Historical Perspective: Models of Parkinson's Disease. Int J Mol Sci, 21(7), 2464. https://pubmed.ncbi.nlm.nih.gov/32252301/

[4] Makarova KS, Aravind L, Wolf YI, et al. (2011). Unification of Cas protein families and a simple scenario for the origin and evolution of CRISPR-Cas systems. Biol Direct, 6, 38. https://pubmed.ncbi.nlm.nih.gov/21756346/

[5] Jinek M, Chylinski K, Fonfara I, et al. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science, 337(6096), 816-21. https://pubmed.ncbi.nlm.nih.gov/22745249/

[6] González-Rodríguez P, Zampese E, Stout KA, et al. (2021). Disruption of mitochondrial complex I induces progressive parkinsonism. Nature, 599(7886), 650-656. https://pubmed.ncbi.nlm.nih.gov/34732887/

[7] Zhou H, Zhang J, Shi H, et al. (2022). Downregulation of CDK5 signaling in the dorsal striatum alters striatal microcircuits implicating the association of pathologies with circadian behavior in mice. Mol Brain, 15(1), 53. https://pubmed.ncbi.nlm.nih.gov/34818055/8

[8] Liu GH, Qu J, Suzuki K, et al. (2012). Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. Nature, 491(7425), 603-7. https://pubmed.ncbi.nlm.nih.gov/23075850/

[9] Xue J, Zhu Y, Wei L, et al. (2022). Loss of Drosophila NUS1 results in cholesterol accumulation and Parkinson's disease-related neurodegeneration. FASEB J., 36(7), e22411. https://pubmed.ncbi.nlm.nih.gov/35695805/

[10] Edson AJ, Hushagen HA, Frøyset AK, et al. (2019). Dysregulation in the Brain Protein Profile of Zebrafish Lacking the Parkinson's Disease-Related Protein DJ-1. Mol Neurobiol, 56(12), 8306-8322. https://pubmed.ncbi.nlm.nih.gov/31218647/

[11] Li H, Wu S, Ma X, et al. (2021). Co-editing PINK1 and DJ-1 Genes Via Adeno-Associated Virus-Delivered CRISPR/Cas9 System in Adult Monkey Brain Elicits Classical Parkinsonian Phenotype. Neurosci Bull, 37(9), 1271-1288. https://pubmed.ncbi.nlm.nih.gov/34165772/

[12] Zhou X, Xin J, Fan N, et al. (2015). Generation of CRISPR/Cas9-mediated gene-targeted pigs via somatic cell nuclear transfer. Cell Mol Life Sci, 72(6), 1175-84. https://pubmed.ncbi.nlm.nih.gov/25274063/

[13] Qing X, Walter J, Jarazo J, et al. (2017). CRISPR/Cas9 and piggyBac-mediated footprint-free LRRK2-G2019S knock-in reveals neuronal complexity phenotypes and α-Synuclein modulation in dopaminergic neurons. Stem Cell Res, 24, 44-50. https://pubmed.ncbi.nlm.nih.gov/28826027/

[14] Mazza MC, Beilina A, Roosen DA, et al. (2021). Generation of iPSC line from a Parkinson patient with PARK7 mutation and CRISPR-edited Gibco human episomal iPSC line to mimic PARK7 mutation. Stem Cell Res, 55, 102506. https://pubmed.ncbi.nlm.nih.gov/34419745/

[15] Frock RL, Hu J, Meyers RM, et al. (2015). Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. Nat Biotechnol, 33(2), 179-86. https://pubmed.ncbi.nlm.nih.gov/25503383/

[16] Cho SW, Kim S, Kim Y, et al. (2014). Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res, 24(1), 132-41. https://pubmed.ncbi.nlm.nih.gov/24253446/