Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 3
BLSME 2024

CRISPR/Cas9 Mechanism and Applications Progression

Shangyang Liu *

Department of Cell & Systems Biology, University of Toronto, 27 King's College Cir, Toronto, ON, CA M5S 1A1

* Corresponding author: 1811061109@mail.sit.edu.cn

Abstract. CRISPR (short for Clustered Regularly Interspaced Short Palindromic Repeats) and the accompanying endonuclease Cas9 are potent tools for gene editing in many applied disciplines. The CRISPR/Cas9 system primarily functions through two components, in which the single guide RNA (sgRNA) directs the Cas9 protein with endonuclease activity to carry out precise DNA cleavage. The cell's inherent repair mechanisms allow it to complete the subsequent reconnection of the broken genes. Gene knock-in or knock-out can therefore be achieved via this approach. This system was typically applied to prokaryotes in early studies. Its role as a component of their defense mechanism has been thoroughly studied. With rapid development over the past ten years, this system can now be effectively used in a multitude of eukaryotic cells, including yeast cells and mammalian cells. Because of its accuracy, effectiveness, and low cost, CRISPR/Cas9 has been widely used for human disease treatment and has shown great potential in gene therapies for hereditary diseases. However, since more late-stage clinical trials are required to validate its safety, its applications still face many challenges. This review investigates the mechanism of CRISPR/Cas9-mediated gene editing technology and highlights its recent applications in Alzheimer's disease, Sickle cell disease, and Duchenne muscular dystrophy, which are difficult to treat with current approaches. Some challenges the CRISPR/Cas9 system has encountered at this stage are described, and its prospects are also examined.

Keywords: CRISPR; Cas9; gene knock-out; Alzheimer's; Sickle cell disease.

1. Introduction

Genome editing is the term for a group of scientific techniques that allow the DNA of an organism to be modified [1]. CRISPR is a unique DNA sequence in which a little fragment of DNA keeps replicating with equally long spacers between each repetition [2]. It was first discovered in the E. coli genome and described by Japanese scientist Ishino and his team. However, they failed to determine its biological function at that time. Cas9 is an endonuclease that cleaves DNA at a place designated by gRNA. Later research revealed that CRISPR/Cas9 plays an essential role in the immune system that protects against plasmid transfer and phage invasion in bacteria and archaea [1]. When foreign plasmids or phages first infect bacteria or archaea, they develop immunity by inserting a short section of viral DNA into the CRISPR spacer region.

Previously acquired viral infections are stored in spacers as genetic memory [2]. Therefore, the CRISPR region will be transcribed for defense if the bacteria or archaea are reinfected with homologous DNA. It has been shown that by modifying short guide RNAs, CRISPR/Cas9 can accurately target practically any desired genomic site to repair pathogenic mutations [1]. Before it was successfully applied to eukaryotes, two frequently utilized gene editing technologies included zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [2-3]. Both ZFNs and TALENs are built to sever DNA strands at any desired position. However, the complexity of protein engineering, the high cost, and the time-consuming nature hinder the widespread application of these two gene editing technologies [2]. In contrast, the effectiveness, efficiency, and accuracy of CRISPR/Cas9 allow it to be applied in many research areas, including studying humans, plants, and microorganisms. One of the best gene editing technologies now available for all living cells is CRISPR/Cas9 [2].

Content from this work may be used under the terms of CC BY-NC 4.0 licence (https://creativecommons.org/licenses/by-nc/4.0/).
Published by Warwick Evans Publishing.

2. Mechanisms

CRISPR/Cas can be classified into two classes, both for breaking down nucleic acids. Class I systems employ a group of several Cas proteins, whereas Class II systems only one is needed. Due to the efficiency of CRISPR/Cas9, it has undergone sufficient research and evolved into the most powerful gene-editing technology [4]. SgRNA, one of the primary components, is made by combining crRNA and tracrRNA. The other component, the Cas9 protein, comprises six domains, REC1, REC2, Bridge Helix (BH), PAM Interacting (PI), and two nuclease domains, HNH and RuvC. A Cas9/sgRNA complex is created when the Cas9 protein surrounds a single guide RNA and complementarily binds to it via the REC1 and REC2 domains. Without interacting with sgRNA, the Cas9 nuclease stays inactive [2, 5]. The complex uses the Cas9 PI domain to scan the whole genome for a PAM sequence, a 2–5 base pair conserved DNA sequence downstream from the cleavage site. The Cas9/sgRNA complex begins unwinding the double-stranded DNA once an appropriate PAM sequence is recognized, starting with the nucleotide closest to the PAM and advancing one nucleotide at a time toward the end away from the PAM [5]. As the DNA duplex unwinds, the spacer sequence of the sgRNA displaces the non-complementary DNA strand to form a complementary base pairing with the target sequence. A stable Cas9-sgRNA-DNA complex is thus produced. The Cas9 protein is then activated for cleavage. The complementary DNA strands are cleaved by the HNH domain, while non-complementary DNA strands are cleaved by the RuvC domain. As a result, blunt-ended double-stranded breaks (DSBs) are generated [5]. Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are two pathways that the cell naturally uses for repairing DSBs [2]. NHEJ facilitates DSB repairs by directly joining the break sequences without providing exogenous homologous DNA. However, NHEJ is prone to errors. For example, it could result in frameshift mutation or premature stop codon caused by insertions or deletions (indels) at the cleavage site [2, 4]. HDR is relatively accurate since the donor DNA template is used. It allows for precise insertion of the correct DNA at the cleavage site. Therefore, a significant number of donor DNA templates are needed for HDR to precisely modify the genome of a target cell in CRISPR/Cas9 gene editing. The sequence of interest for insertion or modification is contained in this donor template, which is flanked by DNA fragments homologous to the blunt-ended DSBs. With the help of exogenous DNA templates, the cell's natural DNA repair pathways can complete the insertion of new genes or the knockout of existing ones, thereby making genome modifications [2, 4].

3. Applications

3.1. Alzheimer’s disease (AD)

AD is a chronic neurodegenerative disorder that impacts the health of millions of individuals around the world. Memory loss, apathy, depression, and irritability are some primary symptoms [6]. Patients gradually lose cognitive ability and their ability to form memories because of permanent neuronal loss. Significant features of AD include high levels of amyloid-beta 42 (Aβ42), hyperactivation of glial cells, excess phosphorylated Tau, damaged synapses, and deficient neurotrophin signaling [7]. Numerous issues regarding the pathophysiology and mechanisms of AD remain unclear, even though a multitude of research has been conducted. Treatment for AD patients is made more challenging by the complexity of the pathogenesis and the wide variation in clinical presentations. Additionally, the discovery of more potent therapeutic strategies for AD faces significant obstacles due to the irreversible nature of the disease process.

The formation and aggregation of Aβ caused by secretases in the brain are explained by the amyloid hypothesis. β-secretase 1 (BACE1) cleaves amyloid precursor protein (APP) to create Aβ monomers, which then assemble into oligomers, resulting in the generation and deposition of Aβ plaques. γ-secretase is responsible for cleaving the C99 fragment produced by BACE1-cleaved APP to yield Aβ monomers Aβ40 and Aβ42. An increase in the level of Aβ42 induces the organization of Aβ plaques [6-7]. According to the Tau hypothesis, the formation of Neurofibrillary tangles (NFTs) is related to the microtubule-associated protein Tau. 3R and 4R, two Tau isoforms prominent in adult human

axons, are targets for several phosphates and kinases. 3R and 4R may accumulate and become hyperphosphorylated in neural tissue, leading to NFT formation [6-7]. These two hypotheses are generally accepted to describe the critical factors that trigger AD progression. Referring to the amyloid beta hypothesis, therapeutic drugs for AD are developed to inhibit the synthesis and aggregation of Aβ and facilitate the removal of harmful peptides from the brain. However, most clinical trials have failed since the drugs have only been shown to temporarily relieve AD symptoms [7]. Due to the failure of currently available disease-modifying approaches, it is necessary to consider other alternative treatments. The gene-editing technology CRISPR/Cas9 has promise for treating AD.

AD can be split into familial Alzheimer's disease (FAD) and sporadic Alzheimer's disease (SAD). CRISPR/Cas9 targets gene mutations for FAD treatment. The presenilin 1 (PSEN1) and presenilin 2 (PSEN2) genes are mutated in most cases of FAD. These mutations interfere with amyloid metabolism, resulting in increased Aβ42 levels. Recent research demonstrates that CRISPR/Cas9 can balance the Aβ42/40 ratio by fixing these autosomal dominant mutations [7]. FAD is also related to APP mutations, which lead to increased β-secretase cleavage of APP and consequently increased Aβ protein levels. It has been reported that CRISPR/Cas9 can delete APP alleles to reduce the expression of Aβ protein [6]. As a result, FAD patients who have APP mutations may benefit from this gene therapy. CRISPR/Cas9 edits the essential enzyme of Aβ protein and APOE genotype for SAD treatment. Sun et al. used a CRISPR/Cas9-based technique to selectively alter endogenous APP at the extreme C-terminus, preventing further interaction with BACE1. The generation of Aβ eventually declines [8]. Therefore, gene therapy delivered by the CRISPR/Cas9 system applies to SAD patients. The APOE4 isoform is one of the greatest risk factors for SAD. APOE2 and APOE3 are the other two isoforms of APOE. The only distinction among the isoforms is the substitution of a single amino acid. In an APOE4 study by Wadhwani et al., E3 neurons had less Tau phosphorylation when the E4 allele was corrected by the CRISPR/Cas9 method in iPSCs from two AD patients to the E3/E3 genotype [9]. Additionally, isogenic conversion of APOE4 to APOE3 has been identified to reduce various AD-associated pathologies [6]. Therefore, altering the isoforms of APOE with CRISPR/Cas9 could successfully mitigate the risk of AD brought on by the APOE4 allele.

3.2. Sickle cell disease (SCD)

SCD is an inherited blood disorder in which the capacity of hemoglobin to carry oxygen throughout the body is damaged. A single nucleotide changes in exon 1 of the β-globin gene (HBB) is the cause of SCD. The resultant hemoglobin S (HbS) polymerizes and aggregates in a deoxygenated state, leading to sickling of red blood cells. SCD patients usually suffer from severe pain, organ failure, and even early death due to chronic hemolysis and hemolytic anemia generated by damaged RBCs [10]. Over the past few decades, there has been no improvement in the average life expectancy of SCD patients. The approved drugs can only reduce acute complications. There have always been limitations on how SCD may be treated. Until recently, bone marrow transplantation was the sole viable treatment for SCD patients, but it comes with many risks and side effects. For example, when employing matched but unrelated donors, the mortality rate from the transplantation rises considerably. Only a few SCD patients have access to a matched-related donor. It has been unable to be widely adopted due to the safety concerns.

Ex vivo engineering of autologous hematopoietic stem cells (HSCs) followed by transplantation of transgenic cells shows the possibility for permanent treatment of SCD [10]. This autologous gene therapy is currently expected to be achieved in two ways, one is to directly correct the pathogenic point mutation through CRISPR/Cas9 to express normal adult hemoglobin, and the other is to edit the HbF regulatory element or knock down the HbF repressor by CRISPR/Cas9 to induce the expression of fetal hemoglobin (HbF). High levels of HbF are beneficial for mitigating SCD. Gene-corrected HSCs are preferable to SCD HSCs in vivo, which is essential for the success of autologous gene therapy for SCD. Current SCD gene editing trials attempt to induce HbF expression since this is easier than directly correcting the point mutation. For example, NHEJ is needed for the induction of HbF expression, which is relatively simple to accomplish in HSCs.

BCL11A has been demonstrated to be the major HbF repressor protein that can inhibit HbF expression by interfering with the γ-globin gene (HBG) promoter. Although knockdown of the BCL11A gene can generate strong HbF induction, BCL11A is still necessary for expression in some non-erythroid lineages. Individuals with BCL11A deletions and haploinsufficiency have increased HbF expression but often suffer from intellectual disability and developmental delay [11]. The erythroid-specific enhancer, a certain area in intron 2 of the BCL11A gene, has been later discovered to be a more clinically useful and practical target for HbF induction than the BCL11A gene. A series of guide RNAs are used to edit this enhancer in erythroid precursors, which leads to a massive induction of HbF without impacting erythropoiesis while preserving BCL11A expression and function in a non-erythroid environment [10-11]. According to research by Wu et al., cleavage of the GATA1 binding site at the +58 BCL11A erythroid enhancer mediated by CRISPR/Cas9 in hematopoietic stem and progenitor cells (HSPCs) could result in the reduction of BCL11A expression in an erythroid environment and the induction of fetal γ-globin. This can produce erythroid progeny with the highest levels of HbF induction [12]. Significant induction of HbF can also be accomplished by disrupting the BCL11A binding site in the HBG promoters. According to research by Wang et al., a single nucleotide change mediated by CRISPR/Cas9-associated base editing at the BCL11A binding site in HSPCs could generate the disruption of BCL11A binding and an increase in HBG expression. Base editing is a newly developed technology that does not create DSBs, which may cause damage to the genome, thus it may be a safer therapeutic strategy [13]. Overall, CRISPR/Cas9 is seen as a possible method for treatment, but extra research is needed to completely comprehend the mechanisms that control globin expression and its related factors to increase the likelihood of success in clinical settings.

3.3. Duchenne muscular dystrophy (DMD)

DMD is an X-linked recessive genetic disorder in which the production of dystrophin, a protein necessary for preserving the strength of the muscle fibers and the stability of their membranes, is affected. DMD is brought on by spontaneous or inherited mutations in the DMD gene that codes for the protein dystrophin. Muscle fibers are unable to contract normally in DMD patients. They experience progressive muscle degeneration and generally die of cardiac or respiratory failure in early adulthood [14]. The DMD gene contains 79 exons that encode four main functional domains of the dystrophin protein: ABD1, central rod domain, cysteine-rich domain, and carboxyl terminus. This gene experiences frequent mutations in exons, such as deletions, insertions, duplications, and point mutations, resulting in out-of-frame transcripts [14-15]. Open reading frames (ORFs) of the DMD gene are disrupted by these out-of-frame mutations, which prevents the dystrophin protein from being expressed. Currently, available treatments for DMD, such as corticosteroid drug therapy, can only relieve and improve the symptoms of the disease. Although other treatments are being researched, they are proceeding slowly. Nevertheless, a novel and promising gene therapy mediated by CRISPR/Cas9 has been introduced to induce DNA excision or deletion to restore ORF to effectively treat DMD patients suffering disruptive ORF generated by deletion and duplication mutations [15].

Deletion of several exons destroys ORF, whereas deletion of the DMD gene fragments or specific exons maintains ORF. Using a pair of gRNAs mediated by CRISPR/Cas9 to target both sides of the desired region can generate DSBs and delete internal genomic sequences. This enables the excision of necessary exons to restore ORF and produce dystrophin protein that is internally truncated but partially functional. Numerous research has been conducted over the past decade to demonstrate the feasibility of this approach. To date, exons 2-10 and exons 45-55 serve as the most often deleted exons to effectively restore ORF. Research suggests that targeting exons 3-9 for deletion using CRISPR/Cas9 may assist in correcting ABD1 mutations for the treatment of DMD. The excision of exon 51 can lead to effective ORF restoration and thus treat nearly 13% of DMD patients. Additionally, patients who have an in-frame deletion of exons 45-55 may only experience mild DMD symptoms. For example, their exercise tolerance may be enhanced, and their average life span may be extended. This deletion has shown significant advantages, providing treatments for up to 60% of DMD patients [15].

Another approach to treating DMD relies on NHEJ, a natural DNA repair pathway used to cause small insertions and deletions (indels) in out-of-frame mutated exons or sequences. This can remodel the DMD gene to enable dystrophin expression. This approach is carried out by gRNA that can insert or subtract a few nucleotides in the exon upstream of the mutation. As a result, a targeted frameshift is created to restore ORF downstream of the small indel [14-15]. According to research conducted by Min et al., exon 45 was reframed after utilizing a pair of sgRNAs to insert a single adenine nucleotide at its 5′ boundary through NHEJ, which boosted dystrophin expression [16]. Moreover, Amoasii et al. created a gRNA mediated by the CRISPR/Cas9 system, targeting a specific sequence on exon 51. This is done to induce indels that can reframe exon 51 or interrupt the intron 50 splice acceptor site for exon 51 skipping. Dystrophin expression restoration can be achieved by either reframing exon 51 or skipping exon 51, ranging from 3 to 90% [17]. Currently, these approaches have been applied to large animal models and made considerable progress. Overall, CRISPR/Cas9 has a large potential to alleviate the suffering of DMD patients and prolong their lifespans through direct exon skipping and indel-derived reframing.

4. Conclusion

The CRISPR/Cas9 technology has significantly impacted the fields of molecular biology and gene therapy due to its simplicity, adaptability, and effectiveness. It has been widely used all around the world. The study of CRISPR/Cas9 gene editing to cure human diseases progresses rapidly. As discussed in this review article, CRISPR/Cas9 holds considerable promise for the treatment of Alzheimer's disease, Sickle cell disease, and Duchenne muscular dystrophy to replace existing ineffective drug therapies. CRISPR/Cas9 is a relatively new technology, though, and now is typically only used on seriously ill patients who are at risk of dying. The safety and efficacy of CRISPR/Cas9 technology need to be proven by additional clinical trials, particularly late ones, as most clinical trials are now in phase I or phase II. For some complicated diseases, more reliable long-term research using large animal models is needed before translation to the clinic. In addition, CRISPR/Cas9 technology may face ethical charges because it is applied to early-stage embryonic genomes for gene editing to treat certain genetic diseases. Therefore, laws and regulations related to CRISPR/Cas9 gene editing need to be formulated and improved. Another drawback of CRISPR/Cas9 is off-target cleavage at unexpected genome sites. This may destroy normally functional genes and could become an extremely serious risk. Fortunately, CRISPR/Cas12a over Cas9 may offer advantages in this regard. For example, it only needs one RNA molecule, the crRNA, and promotes HDR instead of NHEJ since it generates staggered DSBs. However, further study is necessary to confirm its safety and effectiveness. Overall, CRISPR/Cas9, as a powerful gene editing technology, brings great hope to researchers in treating various human diseases and provides potential gene therapies for patients.

References

[1] Li, T., Yang, Y., Qi, H., Cui, W., Zhang, L., Fu, X., He, X., Liu, M., Li, P., et al., “CRISPR/Cas9 Therapeutics: Progress and Prospects,” Signal Transduction and Targeted Therapy, 8 (1), (2023).

[2] Mengstie, M.A., and Wondimu, B.Z., “Mechanism and applications of CRISPR/cas-9-mediated genome editing,” Biologics: Targets and Therapy, Volume 15, 353 – 361 (2021).

[3] Zhang, Y.., Yin, H., “Genome editing with mrna encoding ZFN, Talen, and cas9,” Molecular Therapy, 27 (4), 735 – 746 (2019).

[4] Xu, X., Liu, C., Wang, Y., Koivisto, O., Zhou, J., Shu, Y., and Zhang, H., “Nanotechnology-based delivery of CRISPR/Cas9 for cancer treatment,” Advanced Drug Delivery Reviews, 176, 113891 (2021).

[5] Feng, Y., Liu, S., Chen, R., and Xie, A., “Target binding and residence: A new determinant of DNA double-strand break repair pathway choice in CRISPR/cas9 genome editing,” Journal of Zhejiang University-SCIENCE B, 22 (1), 73 – 86 (2021).

[6] Lu, L., Yu, X., Cai, Y., Sun, M., and Yang, H., “Application of CRISPR/Cas9 in alzheimer’s disease,” Frontiers in Neuroscience, 15, (2021).

[7] Bhardwaj, S., Kesari, K.K., Rachamalla, M., Mani, S., Ashraf, G.Md., Jha, S.K., Kumar, P., Ambasta, R.K., Dureja, H., et al., "CRISPR/Cas9 gene editing: New hope for alzheimer's disease therapeutics," Journal of Advanced Research, 40, 207 – 221 (2022).

[8] Sun, J., Carlson-Stevermer, J., Das, U., Shen, M., Delenclos, M., Snead, A.M., Koo, S.Y., Wang, L., Qiao, D., et al., "CRISPR/Cas9 editing of App C-terminus attenuates β-cleavage and promotes α-cleavage," Nature Communications, 10 (1), (2019).

[9] Wadhwani, A.R., Affaneh, A., Van Gulden, S., and Kessler, J.A., "Neuronal apolipoprotein E4 increases cell death and phosphorylated tau release in alzheimer disease," Annals of Neurology, 85 (5), 726 – 739 (2019).

[10] Park, S.H., and Bao, G., "CRISPR/Cas9 gene editing for curing sickle cell disease," Transfusion and Apheresis Science, 60 (1), 103060 (2021).

[11] Demirci, S., Leonard, A., Essawi, K., and Tisdale, J.F., "CRISPR-Cas9 to induce fetal hemoglobin for the treatment of sickle cell disease," Molecular Therapy - Methods & Clinical Development, 23, 276 – 285 (2021).

[12] Wu, Y., Zeng, J., Roscoe, B.P., Liu, P., Yao, Q., Lazzarotto, C.R., Clement, K., Cole, M.A., Luk, K., et al., "Highly efficient therapeutic gene editing of human hematopoietic stem cells," Nature Medicine, 25 (5), 776 – 783 (2019).

[13] Wang, L., Li, L., Ma, Y., Hu, H., Li, Q., Yang, Y., Liu, W., Yin, S., Li, W., et al., "Reactivation of γ-globin expression through cas9 or base editor to treat β-hemoglobinopathies," Cell Research, 30 (3), 276 – 278 (2020).

[14] Happi Mbakam, C., Lamothe, G., Tremblay, G., and Tremblay, J.P., "CRISPR-Cas9 gene therapy for Duchenne muscular dystrophy," Neurotherapeutics, 19 (3), 931 – 941 (2022).

[15] Mollanoori, H., Rahmati, Y., Hassani, B., Havasi Mehr, M., and Teimourian, S., "Promising therapeutic approaches using CRISPR/cas9 genome editing technology in the treatment of Duchenne muscular dystrophy," Genes & Diseases, 8 (2), 146 – 156 (2021).

[16] Min, Y.-L., Li, H., Rodriguez-Caycedo, C., Mireault, A.A., Huang, J., Shelton, J.M., McAnally, J.R., Amoasii, L., Mammen, P.P., et al., "CRISPR-Cas9 corrects duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells," Science Advances, 5 (3), (2019).

[17] Amoasii, L., Hildyard, J.C., Li, H., Sanchez-Ortiz, E., Mireault, A., Caballero, D., Harron, R., Stathopoulou, T.-R., Massey, C., et al., "Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy," Science, 362 (6410), 86 – 91 (2018).