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REVIEW

Benefits of Using the CRISPR/Cas9 System for the Correction of Genetic Mutations

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Abstract—The discovery of the CRISPR/Cas9 gene scissors technology was a breakthrough in the development of gene therapy methods and attracted the attention of the entire scientific community. This technology provides prospects for the treatment of many neurodegenerative diseases and cancers that were previously considered almost incurable. To date, the mechanisms of the development and progression of these groups of nosologies are becoming increasingly studied due to discoveries in the field of molecular biology and genetics. However, the application of the CRISPR/Cas9 technology has some limitations and difficulties that should be considered. The development of works on the creation and selection of specific gene carriers, a thorough safety assessment of modern genetic manipulations, and the integrated participation of specialists from different fields of science will be important to solve the problems associated with the use of the CRISPR/Cas9 technology and achieve the desired therapeutic effect.

Keywords: human diseases, CRISPR/Cas9, genome editing, therapy, drug development

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INTRODUCTION

History of Discovery

The rapid development of molecular biological technologies in recent years has opened up the possibility of their application in the treatment of previously incurable diseases. One of them is the genetic scissors technology known as CRISPR/Cas9. This technology was developed after bacterial CRISPR/Cas systems were discovered and then carefully studied, and the protective function of these repeating palindromic structures was proven [1, 2].

In 1987, a group of Japanese scientists managed to identify unique CRISPR loci formed by palindromic repeats in the genome of the bacterium *Escherichia coli*. This discovery became the starting point in the development of this area of genetics. Scientists continued research in this area, identifying the structural and functional features of CRISPR. In 1993, Francisco Mojica identified short palindromic repeats specific to CRISPR, and the results of their study determined the name of this complex structure (clustered regularly interspaced short palindromic repeats, CRISPR) [1–3].

Later, in 2002, genes encoding CRISPR-associated proteins (Cas) were identified within CRISPR loci. CRISPR/Cas systems differ in the composition of *cas* genes, which allowed one to develop the currently used classification of these genetic structures. In 2013, the possibility of CRISPR functioning not only in bacterial, but also in eukaryotic, cells was demonstrated. In 2020, Jennifer Doudna and Emmanuelle Charpentier were awarded the Nobel Prize in Chemistry for pioneering gene editing techniques using CRISPR/Cas9. A rich history of discoveries, increased interest from the global scientific community, and numerous studies demonstrate the relevance of the CRISPR/Cas9 technology today [3–5].

CRISPR STRUCTURE AND MECHANISM

The immune function of CRISPR/Cas is to recognize specific foreign nucleotide sequences and store a memory of them in order to subsequently degrade them under reentering the cell. In the CRISPR/Cas structure, the initial element of the chain is a functional region of the genetic sequence encoding Cas proteins that determine the type of the CRISPR/Cas

system. Further along the bacterial deoxyribonucleic acid (DNA) chain there is a leader sequence, including a promoter and regularly located short palindromic repeats separated by spacers. When foreign genetic material reenters, the CRISPR/Cas system uses spacers as a genetic memory to recognize and destroy the infection [1, 6–8].

The mechanism of CRISPR/Cas immunological defense includes three sequential stages: adaptation, expression and interference. When a foreign agent penetrates the host cell, the adaptation stage begins, during which fragments (short sections of foreign sequences) called spacers are formed with the participation of Cas proteins, supplementing CRISPR systems with the information about this agent. Adaptation is carried out using Cas1–Cas2 proteins and the protospacer adjacent motif (PAM). The protein complex affects double-stranded DNA (dsDNA) of phages and plasmids, causing a double-strand break (DSB) and the release of a foreign genome fragment [2, 3, 9–11]. The reaction of foreign DNA degradation is supported by the multiprotein complex RecBCD, which acts as a helicase and nuclease. Preference in the selection of mobile genetic elements (MGEs) in the chain of these reactions plays an important role in differentiation of foreign and own genetic structures, allowing one to avoid autoaggression [11–14].

The process of spacer integration is carried out by the integration host factor (IHF). The IHF folds host DNA into a U-shaped configuration, after which the spacer is integrated into the host genome between the leader sequence and the 5'-phosphate of the proximal row of spacer repeats [5, 13–15].

During the expression stage, genetic information is transcribed to form a long pre-crRNA transcript and it is processed with the formation of a short mature crRNA (guide RNA) containing a spacer sequence and a part of the adjacent repeat. This stage includes synthesis of protein complexes that will interact with foreign agents at subsequent stages.

Finally, interference is the final step of CRISPR/Cas immune defense. At this stage, crRNA serves as a guide for Cas proteins and recognizes a specific target sequence in foreign DNA or RNA. When the target is recognized, a cascade of reactions is activated, which leads to the degradation of foreign nucleic acid structures and protection of the host cell. It is worth noting the fact of the strategic benefit of the palindromic structure of CRISPR repeats, which provides a high rate of transcription of certain spacers, which allows a quick and effective response to retrospectively relevant invasive elements [16–19].

LIMITATIONS OF CRISPR/CAS9 APPLICATION

The benefits of using the CRISPR/Cas9 system open up broad prospects for its clinical application. However, before introducing this technology into practice, it is necessary to overcome a number of problems associated with it.

The most urgent problem is the method of delivering CRISPR/Cas9 to the target cell. Many options are currently being explored to implement efficient transportation of the system components. One promising means is represented by mRNA, which is a compact and convenient structure with high activity in genome editing and control of the volume of targeted delivery of these structures into the cell. Another option is plasmid DNA encoding the Cas9 protein. This technology is more bulky and limited in its target effect, but it has an advantage over mRNA, being more stable and convenient at the design stage [3, 12].

Viral and nonviral delivery methods such as electroporation, microinjection and lipid nanoparticles are worth mentioning. However, the therapeutic application of such technologies is limited due to the relatively low delivery efficiency compared with, for example, viral vectors such as adeno-associated virus (AAV), adenovirus, and lentivirus. The advantages of viral methods are high specificity and relatively low immunogenicity; however, the limitation of 4.7 kbps capacity for these structures is the main problem for efficient AAV-mediated CRISPR/Cas9 transport [3, 10, 12].

Also, one of the main problems associated with the use of the CRISPR/Cas9 system is the off-target effect of genetic scissors, which can lead to random pathological mutations. Efforts have been made to increase the specificity of the system, including placing the Cas9 gene under the control of the minimal HIV-1 promoter mediated by a transactivator of transcription (Tat), thereby avoiding unwanted expression of Cas9. It was also demonstrated that the Cas9/gRNA ribonucleoprotein (Cas9 RNP) can be degraded post factum, allowing the active protein structure to be inactivated after editing the target DNA, providing maximum on-target and minimal off-target activity. However, there is evidence that the use of RNP in some cell types can induce innate immunopathological reactions [9, 10, 13].

BENEFITS OF APPLYING CRISPR/CAS9 TECHNOLOGY

The prospects for using the CRISPR/Cas9 system in medicine are huge for the treatment of many human diseases. This technique can be used to combat viral infections and correct genetic mutations that lead to the development of hereditary and acquired diseases that were previously considered incurable. Strategies for correcting genetic material in patients with cancer, neurological and allergic diseases, cardiovascular diseases, hereditary blood diseases and metabolic disorders are currently being studied [10, 16, 19, 20].

HEREDITARY DISEASES

Duchenne Muscular Dystrophy

Progressive Duchenne muscular dystrophy is a neuromuscular disorder inherited in an X-linked recessive pattern. It predominantly affects males in childhood and is caused by a mutation in the *DMD* gene, which encodes the dystrophin protein. This mutation leads to a qualitative or quantitative impairment of dystrophin function.

Past studies demonstrated the effectiveness of using CRISPR/Cas9 technology to treat Duchenne muscular dystrophy in a mouse model. In the experiment, the *DMD* gene was artificially mutated using CRISPR/Cas9 technology and placed into a zygote. As a result of the experiment in the germline mouse model of Duchenne muscular dystrophy, the correction of the *DMD* gene mutation was achieved and the phenotype was fixed in newborn mice with an efficiency of 2–100% [20, 21].

These experimental models with induced targeted mutations or edited mutations in the target gene can serve as an important source of information for uncovering the mechanisms underlying disease development and progression. However, additional studies are needed to evaluate the safety and effectiveness of this approach in human beings, as well as obtaining all necessary approvals for the use of this technology in clinical practice [20–22].

PARKINSON'S DISEASE

Parkinson's disease, also known as shaking palsy, is a neurodegenerative disease that progresses as a result of a neuroinflammatory response. This reaction is caused by exogenous neurotoxins and hyperreactivity of the neuroglial system, leading to an autoimmune process. The most intense reaction occurs in the striatopallidal system and the substantia nigra of the reticular formation. As a result of a neuroinflammatory response, neuroglial cells acquire an amoeba-like shape and begin to produce excessive amounts of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), 1-methyl-4-phenyl-1,2, 3,6-tetrahydropyridine (MPTP), a number of interleukins (IL-12, IL-6 IL-1β, and IL-1), and glial fibrillary acidic protein (GFAP). As a result of this process, neurons die and are replaced by neuroglial cells, which leads to the development of reactive gliosis. These changes are aggravated by the activation of the transcription factor NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) and protein kinase C delta (PKCδ), which accelerates death of neurons [23].

The results of research by a group of scientists from the University of California, Berkeley, and the California Institute of Technology (United States) uncovered approaches to editing nuclear and mitochondrial genomes using CRISPR/Cas9. Using expressed Cas9 gRNAs targeting cyclooxygenase 1 and 3 (Cox1 and Cox3 are genes encoding cyclooxygenase enzymes 1 and 3, respectively), targeted initiation of mitochondrial DNA cleavage at specific loci occurs. The experiment using this technique found that knockout of adult peroxisome proliferator receptor γ (PPAR γ), protein coactivator-1α (PGC-1α), and the master regulator of mitochondrial disease biogenesis leads to death of dopaminergic neurons [23].

These studies also highlight the importance of the prokinetin 2 (PK2) family of proteins in mitochondrial biogenesis and the protection of mitochondria from neurodegenerative processes [23, 25]. Increased levels of PK2 in postmortem brains in Parkinson's disease are a consequence of intravital protective effects in response to neurodegeneration, and increased levels of PGC-1α and BCL2 achieved through PK2 expression contribute to the preservation of mitochondrial bioactivity in response to neurotoxic stress. In addition, it has been demonstrated in an experiment on glial cells that AAV-mediated delivery of PK2 can reduce biogenic processes of reactive astrocytes and increase gene expression of A2 astrocytes with an alternative neuroprotective phenotype, which counters neuro-inflammation caused by reactive A1 astrocytes. This opens up new prospects in studying the relationship between mitochondrial dysfunction and autoimmune neuro-inflammation, as well as allowing us to more precisely determine the role of PK2 in these processes. It is also important to note that these studies demonstrate the effectiveness of CRISPR/Cas9 in investigating complex mechanisms related to cell biology and diseases [24, 25].

It is worth paying attention to the protein synuclein α (SNCA), which plays an important role in the occurrence of sporadic Parkinson's disease. There is evidence that by changing the gene associated with this protein, it is possible to prevent the development of the disease. However, the use of the CRISPR/Cas9 method in the combination with human induced pluripotent stem cells (hiPSCs) can lead to the restoration of the A53T mutation in hiPSCs without harming the differentiation process of dopaminergic neurons responsible for the production of the hormone dopamine [23].

Another important protein is leucine-reach repeat kinase 2 (LRRK2), which is associated with the occurrence of Parkinson's disease. Mutations G2019S and R1441C in this gene can lead to mitochondrial biosynthesis disorders. An experiment was conducted using the CRISPR/Cas9 method to replace the entire repeat region in hiPSCs with representative alleles Rep1 257, Rep1 259, Rep1 261, and Rep1 263 to inactivate the G2019S and R1441C mutations in the *LRRK2* gene. These alleles also contributed to increased expression of the SNCA protein, which gives a hope for the success in further research in this area [23–25].

Alzheimer's disease (AD), a neurodegenerative disease, is manifested by the gradual onset of disorders of memory and higher brain functions in presenile or old age, leading to dementia, and is characterized by neuropathological, neuro-imaging, and biochemical signs. AD pathogenesis is associated with the accumulation of the β-amyloid (Aβ) protein, abnormal neurofibrillary tangles. and damage to the mitochondria of neurons, which leads to their death [20, 26]. AD is the best-known neurodegenerative disease for which there is no satisfactory therapy that is safe and effective for patients. Current medications approved by the Food and Drug Administration (FDA) provide only temporary relief of some symptoms [27].

In gene therapy of AD, three genes that are associated with the disease progression can be targeted: *APP*, *MAPT*, and *APOE*. However, additional studies are needed to evaluate the effectiveness and safety of AD gene therapy. Gene therapy of AD using the CRISPR/Cas9 technology is aimed at regulating A@[beta] protein expression and can be achieved in several ways. Researchers have showed that viral insertion-deletion (InDel) mutations of APP alleles using the CRISPR/Cas9 technology can reduce Aβ protein expression. Another target of gene therapy is the regulation of the γ-secretase protease, a large intramembrane protein complex that is regulated by γ-secretase activating protein (GSAP). A reduction of GSAP expression significantly reduces Aβ levels. The functions of γ-secretase are regulated by expression of key components of the complex: GSAP and PSEN2. Changes in amyloid metabolism cause an increase in the Aβ42/40 ratio and Aβ42 levels and/or a decrease in the Aβ40 synthesis [28–30].

ONCOLOGY

Recent studies aimed at investigating the nature of tumor growth demonstrate a correlation between the development of the oncological process and mutations in genes that regulate signaling pathways. Traditional methods of drug therapy used today have only a compensatory ability, while genetic engineering methods have the potential to correct genetic mutations that lead to oncogenesis. Another antitumor aspect in medicine is counteracting the immune mechanisms of the tumor process. The emergence of immune checkpoint blockade (ICB) therapy, such as a blocker of programmed cell death 1 (PD-1), its ligand 1 (PD-L1), or cytotoxic T-lymphocyte antigen-4 (CTLA-4), led to a revolution in the treatment of many types of tumors. To date, drugs such as ipilimumab (anti-CTLA-4), pembrolizumab, nivolumab (anti-PD-1), and atezolizumab (anti-PD-L1) have been approved for clinical use by the FDA [31].

Despite significant advances in cancer immunotherapy, there are still many challenges in this field, such as low response rates and tumor drug resistance, which necessitate further efforts to elucidate the mechanisms underlying sensitivity or resistance to the antitumor immune response, and the development of more effective immunotherapeutic strategies. Recently, the CRISPR/Cas9 technology has been successfully applied to eliminate potential factors regulating tumor anti-immune mechanisms, thereby providing a new paradigm for target discovery.

In a report on studies carried out last year, scientists reported how they aimed to destroy the Epstein-Barr viruses (EBVs) in patient's cells that were derived from Burkitt lymphoma with EBV infection. Tumor cells showed decreased proliferation as a result of CRISPR targeting EBV. In addition, CRISPR can specifically destroy the oncogenes E6 or E7 of human papillomavirus, which are integrated into the genome of cervical carcinoma cells. These oncogenes cause degradation of the P53 tumor suppressor gene and destabilization of the retinoblastoma protein, leading to the development of various types of cancer. E6 and E7 knockout by CRISPR was associated with increased protein levels of P53 and Rb, as well as increased cancer cell death [20, 31, 32].

CONCLUSIONS

Gene manipulations, which once seemed like science fiction, have now become a reality. Hard-to-treat neurodegenerative diseases can be cured with gene therapy thanks to advances in vector systems. Gene manipulations combined with modern technologies for gene delivery into cells of the central nervous system promises to change approaches to the clinical treatment of both hereditary and sporadic neurodegenerative diseases and also provide new opportunities for the treatment of a number of rare and genetic diseases. However, unresolved problems remain, including the development of more efficient vector systems, a thorough safety assessment of current gene manipulation tools, and transparency and collaboration among members of the scientific community. Improving methods for using genetic scissors and finding effective carriers for gene delivery are bringing us closer to a new era in medicine.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Bhaya, D., Davison, M., and Barrangou, R., CRISPR–Cas systems in bacteria and archaea: Versatile small RNAs for adaptive defense and regulation, *Annu. Rev. Genet.,* 2011, vol. 45, pp. 273–297. https://doi.org/10.1146/annurev-genet-110410-132430
2. Charpentier, E. and Marraffini, L.A., Harnessing, CRISPR–Cas9 immunity for genetic engineering, *Curr. Opin. Microbiol.,* 2014, vol. 19, pp. 114–119. https://doi.org/10.1016/j.mib.2014.07.001
3. Heidenreich, M. and Zhang, F., Applications of CRISPR–Cas systems in neuroscience, *Nat. Rev. Neurosci.,* 2016, vol. 17, no. 1, pp. 36–44. https://doi.org/10.1038/nrn.2015.2
4. Ishino, Y., Krupovic, M., and Forterre, P., History of CRISPR–Cas from encounter with a mysterious repeated sequence to genome editing technology, *J. Bacteriol.,* 2018, vol. 200, no. 7, p. e00580-17. https://doi.org/10.1128/JB.00580-17
5. Maxwell, K.L., Phages fight back: Inactivation of the CRISPR–Cas bacterial immune system by anti-CRISPR proteins, *PLoS Pathog.,* 2016, vol. 12, no. 1, p. e1005282. https://doi.org/10.1371/journal.ppat.1005282
6. Amitai, G. and Sorek, R., CRISPR–Cas adaptation: Insights into the mechanism of action, *Nat. Rev. Microbiol.,* 2016, vol. 14, no. 2, pp. 67–76. https://doi.org/10.1038/nrmicro.2015.14
7. Burstein, D., Harrington, L.B., Strutt, S.C., Probst, A.J., Anantharaman, K., Thomas, B.C., et al., New CRISPR–Cas systems from uncultivated microbes, *Nature,* 2017, vol. 542, no. 7640, pp. 237–241. https://doi.org/10.1038/nature21059
8. Burstein, D., Sun, C.L., Brown, C.T., Sharon, I., Anantharaman, K., Probst, A.J., et al., Major bacterial lineages are essentially devoid of CRISPR–Cas viral defence systems, *Nat. Commun.,* 2016, vol. 7, p. 10613. https://doi.org/10.1038/ncomms10613
9. Huang, C.H., Lee, K.C., and Doudna, J.A., Applications of CRISPR–Cas enzymes in cancer therapeutics and detection, *Trends Cancer,* 2018, vol. 4, no. 7, pp. 499–512. https://doi.org/10.1016/j.trecan.2018.05.006
10. Komor, A.C., Badran, A.H., and Liu, D.R., CRISPR-based technologies for the manipulation of eukaryotic genomes, *Cell,* 2017, vol. 168, nos. 1–2, pp. 20–36. https://doi.org/10.1016/j.cell.2016.10.044
11. Koonin, E.V., Makarova, K.S., and Zhang, F., Diversity, classification and evolution of CRISPR–Cas systems, *Curr. Opin. Microbiol.,* 2017, vol. 37, pp. 67–78. https://doi.org/10.1016/j.mib.2017.05.008
12. Knott, G.J. and Doudna, J.A., CRISPR–Cas guides the future of genetic engineering, *Science,* 2018, vol. 361, no. 6405, pp. 866–869. https://doi.org/10.1126/science.aat5011
13. Ma, H., Marti-Gutierrez, N., Park, S.W., Wu, J., Lee, Y., Suzuki, K., et al., Correction of a pathogenic gene mutation in human embryos, *Nature,* 2017, vol. 548, no. 7668, pp. 413–419. https://doi.org/10.1038/nature23305
14. Marraffini, L.A., CRISPR–Cas immunity in prokaryotes, *Nature,* 2015, vol. 526, no. 7571, pp. 55–61. https://doi.org/10.1038/nature15386
15. Mohanraju, P., Makarova, K.S., Zetsche, B., Zhang, F., Koonin, E.V., and van der Oost, J., Diverse evolutionary roots and mechanistic variations of the CRISPR–Cas systems, *Science,* 2016, vol. 353, no. 6299, p. aad5147. https://doi.org/10.1126/science.aad5147
16. Moreno, A.M. and Mali, P., Therapeutic genome engineering via CRISPR–Cas systems, *Wiley Interdiscip. Rev.: Syst. Biol. Med.,* 2017, vol. 9, no. 4. https://doi.org/10.1002/wsbm.1380
17. Pawluk, A., Davidson, A.R., and Maxwell, K.L., Anti-CRISPR: Discovery, mechanism and function, *Nat. Rev. Microbiol.,* 2018, vol. 16, no. 1, pp. 12–17. https://doi.org/10.1038/nrmicro.2017.120
18. Rath, D., Amlinger, L., Rath, A., and Lundgren, M., The CRISPR–Cas immune system: biology, mechanisms and applications, *Biochimie,* 2015, vol. 117, pp. 119–128. https://doi.org/10.1016/j.biochi.2015.03.025
19. Sander, J.D. and Joung, J.K., CRISPR–Cas systems for editing, regulating and targeting genomes, *Nat. Biotechnol.,* 2014, vol. 32, no. 4, pp. 347–355. https://doi.org/10.1038/nbt.2842
20. Sharma, G., Sharma, A.R., Bhattacharya, M., Lee, S.S., and Chakraborty, C., CRISPR–Cas9: A preclinical and clinical perspective for the treatment of human diseases, *Mol. Ther.,* 2021, vol. 29, no. 2, pp. 571–586. https://doi.org/10.1016/j.ymthe.2020.09.028
21. 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 Dis.,* 2020, vol. 8, no. 2, pp. 146–156. https://doi.org/10.1016/j.gendis.2019.12.007
22. Pickar-Oliver, A., Gough, V., Bohning, J.D., Liu, S., Robinson-Hamm, J.N., Daniels, H., et al., Full-length dystrophin restoration via targeted exon integration by AAV-CRISPR in a humanized mouse model of Duchenne muscular dystrophy, *Mol. Ther.,* 2021, vol. 29, no. 11, pp. 3243–3257. https://doi.org/10.1016/j.ymthe.2021.09.003
23. Rahman, M.U., Bilal, M., Shah, J.A., Kaushik, A., Teissedre, P.L., and Kujawska, M., CRISPR–Cas9-based technology and its relevance to gene editing in Parkinson's disease, *Pharmaceutics,* 2022, vol. 14, no. 6, p. 1252. https://doi.org/10.3390/pharmaceutics14061252
24. Luo, J., Padhi, P., Jin, H., Anantharam, V., Zenitsky, G., Wang, Q., et al., Utilization of the CRISPR–Cas9 gene editing system to dissect neuroinflammatory and neuropharmacological mechanisms in Parkinson's disease, *J. Neuroimmune Pharmacol.,* 2019, vol. 14, no. 4, pp. 595–607. https://doi.org/10.1007/s11481-019-09844-3
25. Vermilyea, S.C., Babinski, A., Tran, N., To, S., Guthrie, S., Kluss, J.H., et al., In vitro CRISPR/Cas9-di-

rected Gene Editing to Model LRRK2 G2019S Parkinson's disease in common marmosets, *Sci. Rep.,* 2020, vol. 10, no. 1, p. 3447. https://doi.org/10.1038/s41598-020-60273-2

26. Lu, L., Yu, X., Cai, Y., Sun, M., and Yang, H., Application of CRISPR/Cas9 in Alzheimer's disease, *Front. Neurosci.,* 2021, vol. 15, p. 803894. https://doi.org/10.3389/fnins.2021.803894
27. Huang, L.K., Chao, S.P., and Hu, C.J., Clinical trials of new drugs for Alzheimer disease, *J. Biomed. Sci.,* 2020, vol. 27, no. 1, p. 18. https://doi.org/10.1186/s12929-019-0609-7
28. Hanafy, A.S., Schoch, S., and Lamprecht, A., CRISPR/Cas9 delivery potentials in Alzheimer's Disease management: A mini review, *Pharmaceutics,* 2020, vol. 12, no. 9, p. 801. https://doi.org/10.3390/pharmaceutics12090801
29. Bhardwaj, S., Kesari, K.K., Rachamalla, M., Mani, S., Ashraf, G.M., Jha, S.K., et al., CRISPR/Cas9 gene editing: New hope for Alzheimer's disease therapeutics, *J. Adv. Res.,* 2022, vol. 40, pp. 207–221. https://doi.org/10.1016/j.jare.2021.07.001
30. Jeong, W., Lee, H., Cho, S., and Seo, J., ApoE4-induced cholesterol dysregulation and its brain cell type-specific implications in the pathogenesis of Alzheimer's disease, *Mol. Cell,* 2019, vol. 42, no. 11, pp. 739–746. https://doi.org/10.14348/molcells.2019.0200
31. Sarkar, E. and Khan, A., Erratic journey of CRISPR/Cas9 in oncology from benchwork to successful-clinical therapy, *Cancer Treat. Rev. Commun.,* 2021, vol. 27, p. 100289. https://doi.org/10.1016/j.ctarc.2020.100289
32. Zhan, T., Rindtorff, N., Betge, J., Ebert, M.P., and Boutros, M., CRISPR/Cas9 for cancer research and therapy, *Semin. Cancer Biol.,* 2019, vol. 55, pp. 106–119. https://doi.org/10.1016/j.semcancer.2018.04.001

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