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CRISPR-CAS System: Current Applications and Future Prospect

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Abstract

The CRISPR-Cas system is a revolutionary technology that has transformed gene editing and has the potential to revolutionize many fields. It is a bacterial immune system that can be programmed to recognize and cleave specific DNA sequences using Cas proteins and guide RNAs. This technology has numerous biotechnological and bioengineering applications and is simpler to design with high targeting efficiency and multiplex editing capability compared to older gene editing technologies. This review aims to explore the general application, impact on molecular biology and medicine, limitations, and future potential of the CRISPR-Cas system. With its potential to revolutionize medicine, agriculture, and environmental science, the CRISPR-Cas system is a powerful gene editing tool that offers hope for a wide range of applications.

Keywords : CRISPR, gene, DNA

1. Introduction

The ability to precisely and strategically change DNA sequences, genome editing has recently transformed a number of academic disciplines. Traditionally, modified nucleases, or "molecular scissors," have been used to cause a double-strand break (DSB) in the DNA at a specified site within the genome, which is the first step in this procedure. It may take some time, though, to create new DNA specificities in these protein scaffolds.[1,2]

The science of genome editing has been revolutionized by the discovery of the CRISPR-Cas system. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) consists of a variety of short, repetitive DNA sequences separated by unique sequences called spacers.[3,4] Escherichia coli's genome featured the CRISPR-Cas system for the first time in 1987, but it wasn't until 2005 that it was found to be a component of an adaptive immune system in archaea or bacteria.[4] Since then, the CRISPR-Cas system has taken over as the go-to method for genome editing, providing a more flexible and effective means to alter DNA.[3] The easily programmable, multiplexable, and functionalizable nature of CRISPR has positioned it as a revolutionary component in genetic circuits that perform computation inside living cells.[5]

The capacity to quickly reconfigure the CRISPR-Cas system by switching out the RNA guide sequence makes it a highly adaptable set of molecular scissors, which is one of its main advantages.[3,4] With CRISPR-Cas systems now being widely used for genome engineering applications in molecular biology research, this has opened the door for a revolution in the life sciences.[3,6] They have made important advancements in organ xenotransplantation, cancer immunotherapy, and the correction of harmful mutations.[7]

Additionally, CRISPR-based diagnostics have become a potentially useful tool in medicine and other fields. They can be used for a variety of purposes, including as the genotype-specific detection of pathogens for individualized therapies, the detection of pathogens in plants, animals, or the environment, or the detection of pathogens in food to stop the spread of food-borne illnesses. For thousands of people with hereditary illnesses and few treatment options, CRISPR offers hope.[8]

The CRISPR-Cas system has revolutionized genome editing by providing a more effective and adaptable method of changing DNA. It is a very promising instrument for the future due to its broad variety of applications in molecular biology research, medicine, and other fields. That is why, the objective of this review is to learn more about CRISPR, its general application, its impact to molecular biology and medicine, its limitation and its potential for future study.

2. Discussion

2.1. Understanding CRISPR

CRISPR-Cas, or Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated, is a bacterial immune system that has been harnessed as a powerful tool for gene editing. The CRISPR-Cas system consists of Cas proteins, guide RNA, target DNA, and can be used to edit genes in a variety of organisms. There are several types of CRISPR-Cas systems found in bacteria, each with unique properties. The five main types of CRISPR-Cas systems are Type I, II, III, V, and VI, with Type II being the most commonly used for gene editing due to its simplicity and efficiency.[9]

The CRISPR-Cas system was first discovered in Escherichia coli in 1987 and was later found in various microbial species, including archaea and certain bacteria.[4,10,11] It was discovered to be an innate immune system in archaea and bacteria that fights against viruses. The system can be used for gene editing because it consists of Cas proteins and guide RNAs that recognize and cleave specific DNA sequences.[1,4,9,12]

The CRISPR-Cas system is highly specific and can be programmed to target any desired DNA sequence. CRISPR-Cas9 systems can modify element of DNA target of chromosomes, plasmids or phages in the cells.[6] The mechanism of CRISPR/Cas9 gene editing involves several steps. When foreign DNA enters a bacterial cell, it is recognized as non-self and triggers an immune response.[8] The bacterial cell detects the presence of foreign DNA using a protein called Cas1, which integrates fragments of the foreign DNA into the bacterial genome, creating a "memory" of the invader.[13] Once the foreign DNA has been integrated into the bacterial genome, another protein called Cas6 cleaves it into small RNA fragments called crRNAs (CRISPR RNAs).[8]

Each crRNA is specific to a particular sequence in the invading virus or plasmid. The crRNA must be combined with another RNA molecule called tracrRNA (trans-activating RNA) to form a functional guide RNA. The guide RNA directs an enzyme called Cas9 to its target sequence in the invading DNA. When Cas9 binds to its target sequence, it becomes activated and can cut both strands of the DNA.[4,9]

The CRISPR-Cas system has numerous biotechnological and bioengineering applications.[1,14] It is simpler to design and has high targeting efficiency and multiplex editing capability compared to older gene editing technologies.[14] The technology is rapidly evolving and has the potential to revolutionize medicine, agriculture, and environmental science.[15]

CRISPR-Cas system is a powerful gene editing tool that has been harnessed from a bacterial immune system. Its mechanism of action involves Cas proteins and guide RNAs that recognize and cleave specific DNA sequences. The CRISPR-Cas system has numerous biotechnological and bioengineering applications and has the potential to revolutionize many fields.[16]

2.2. Applications of CRISPR

There are diverse applications of CRISPR gene editing technology in different fields such as cancer research, genetic diseases, virology, and even food safety. Some key results from these studies include the successful use of CRISPR gene therapy for cancer treatment in multiple myeloma and sickle cell disease.[8] CRISPR/Cas9 genome editing system has been used to explore cancer-related genes and establish tumor-bearing animal models, contributing to our understanding of cancer genomics.[3,17] CRISPR/Cas9 has also been utilized in genetic engineering of animal models to understand disease mechanisms and improve therapeutic outcomes.[18]

Furthermore, CRISPR-Cas technology has been repurposed as a tool for genome editing in virology and vaccinology, with applications in understanding virus-host interactions and developing recombinant viral vaccines.[19] The use of CRISPR-Cas9 base editors has been suggested as a safer approach for gene correction and disruption, with potential clinical applications in cancer immunotherapy and gene therapy.[20] CRISPR/Cas9 has also shown promise in the treatment of various genetic and non-genetic diseases, including cardiovascular diseases, neurodegenerative diseases, and some X-linked diseases.[11]

CRISPR technology has been applied in a wide range of fields, from genetic circuits engineering to stem-cell reprogramming, vaccine development, and antibody production.[11] Finally, understanding the natural mechanisms used by CRISPR-Cas in prokaryotes can aid in developing strategies for controlling food pathogens by editing genes associated with virulence modulation and reversal of antimicrobial resistance.[21] While these studies show the immense potential of CRISPR gene editing technology, ethical considerations and potential unintended consequences, such as off-target effects and unintended mutations, must also be carefully considered.[20]

2.3. CRISPR Impact to Molecular Biology and Medicine

CRISPR-Cas9 technology has the potential to impact the field of molecular biology in multiple ways, including treating genetic disorders, developing new cancer treatments, improving drug discovery, creating disease-resistant crops, improving animal breeding, studying gene function, creating gene therapies, and providing personalized medicine based on an individual's genetic makeup.[7,12,15] The technology has been used to correct mutations in DNA that cause genetic disorders such as sickle cell anemia or cystic fibrosis.[12] It has also been used to develop new cancer treatments by targeting specific genes involved in tumor growth, such as PD-1, which is involved in suppressing the immune system's response to cancer cells.[12] CRISPR-Cas9 technology can be used to create disease-resistant crops by editing the genes responsible for susceptibility to certain diseases, improving crop yields and reducing reliance on pesticides. The technology can also be used to improve animal breeding by creating animals with desirable traits such as increased muscle mass or resistance to disease.[12]

In the field of medicine, CRISPR-Cas9 technology has been used to correct the E4 allele of the apolipoprotein E gene (APOE) to the E3 isoform in stem-cell-derived neurons to decrease the phosphorylated tau production, which reduces the risk of Alzheimer’s disease significantly.[11] The technology has also been used to disrupt HPV oncogenes E6 and E7 in vitro, causing infected cell cycle arrest and apoptosis, as well as for more than 30 clinical trials to treat cancers, some of which have achieved partial success.[11] Moreover, CRISPR-based diagnostics have multiple applications in medicine and beyond, including determining pathogen genotypes, detecting human, animal, plant, or environmental pathogens, and enabling tailored treatments.[3]

Despite the promise of CRISPR technology, this technology could be exploited biologically weapons to fight society. Also, with treatment the efficacy of CRISPR is considered an important drug to fight for serious malignancies such as cancer. However, major barriers to clinical use are potential issues with safe delivery it is addressed by designing a versatile carrier.[5] Also ethical considerations surrounding the use of CRISPR gene therapy in humans persist, with questions remaining about the long-term safety and efficacy of these treatments, as well as issues related to informed consent and equitable access to treatment. Moreover, unintended mutations, are a potential concern.[8]

2.4. CRISPR Limitation and Challenge

The use of CRISPR technology in genome editing faces several limitations and challenges. One of the main limitations is its inability to efficiently edit certain types of cells, such as neurons or muscle cells.[7,8,16,22] Off-target effects can lead to unintended consequences such as mutations or chromosomal rearrangements.[8,11,18] Strategies to overcome these challenges include using nickases to reduce off-target effects or pre-made ribonucleoprotein complexes to improve efficiency.[8]

Another challenge for the widespread clinical use of CRISPR/Cas9 in human therapy is the development of safe and effective in vivo delivery.[11,17] The CRISPR-Cas9 system can make significant changes in the cells genome by knocking out and knocking in of genome. For this purpose, the gRNA must specifically bind to its intended target, in other words, it must not be off-target. Thus, one of the key challenges of the CRISPR/Cas9 system is the accurate prediction of off- targets.[5] Current strategies, such as electroporation, ultrasound-propelled nanomotors, adeno-associated virus (AAV), and liposomes have limitations that need to be addressed.[11] As mentioned, AAV is one of the CRISPR/Cas9 delivery systems used frequently for in vivo study. However, one of the obstacles with this virus is its small size capacity, while the plasmid size of the CRISPR system is large (>9 kb). Thus, to solve this problem, the delivery system must be designed with high loading capacity or use another efficient Cas9 variants that have a smaller size.[5] A transient delivery system is preferred for editing the genome for therapeutic purposes to avoid mutagenesis and immunogenicity.[6]

Despite these challenges, advancements in CRISPR technology include new delivery methods such as nanoparticles or viral vectors, which can improve specificity and efficiency. The development of prime editing, which allows for more precise editing with fewer off-target effects than traditional base editors, is also promising.[8] That is why, while CRISPR technology has great potential in biodefense and cancer therapy, its limitations and challenges such as off-target effects, low efficiency, packaging difficulties, and delivery issues need to be addressed for its widespread clinical use.

2.5. Future Study of CRISPR

The development and application of CRISPR-Cas systems have provided vast opportunities for future research in multiple fields. One area for future research is improving the specificity and efficiency of gene editing using the CRISPR-Cas system, as mentioned by Nidhi, et. al.[12] Xu, et. al. also convey the same issue/[7] This can be achieved by exploring the use of new Cas enzymes with different properties, such as base editors, prime editors, and high-fidelity Cas enzymes. Another area for future research is developing new delivery methods for the CRISPR-Cas system to improve its effectiveness, as mentioned in.[12]

However, as highlighted by Mohanty[15] and Ghosh[18], there are challenges that need to be addressed in the use of CRISPR-Cas systems, such as ethical concerns, potential infectious diseases caused by Cas9 from S. aureus and S. pyogenes, and the development of immunity against Cas9 proteins. Therefore, future research should also focus on addressing these challenges to ensure the safe and effective use of CRISPR-Cas systems in biomedical applications.[15,18]

Moreover, Guo, et. al., highlights the potential of CRISPR-Cas technologies in treating gene-related diseases, such as monogenetic diseases and X-linked diseases.[11] Clinical trials have been underway to test the effectiveness of Cas9-mediated gene editing in treating these diseases. In cancer therapy, CRISPR-Cas systems have been applied in drug target screening and immune therapy, as mentioned by Ghosh, et. al.[18] Future research can further explore the use of CRISPR-Cas systems in these areas to improve cancer treatment outcomes.

Lastly, as mentioned by Knott[23] and Aman[3], CRISPR-Cas systems have immense potential in biological research and biotechnological applications. Future research can explore the use of CRISPR-Cas systems in developing diagnostic devices that can rapidly and specifically detect pathogens in human, animal, food, and environmental samples. Overall, the development and application of CRISPR-Cas systems have opened up vast possibilities for future research in multiple fields, and addressing the challenges associated with its use can further unlock its potential in biomedical and environmental applications.[3,23]

3. Conclusion

The CRISPR-Cas system is a powerful and highly specific gene-editing tool that has revolutionized the field of molecular biology. With its ability to precisely and strategically change DNA sequences, it has broad applications in various fields, including medicine, agriculture, and environmental science. The CRISPR-Cas system is simpler to design and has high targeting efficiency and multiplex editing capability compared to older gene editing technologies. It has already made important advancements in organ xenotransplantation, cancer immunotherapy, and the correction of harmful mutations. CRISPR-based diagnostics have become a potentially useful tool in medicine and other fields. While the technology is rapidly evolving, there are still limitations and challenges to overcome, such as off-target effects and ethical concerns. Nevertheless, the CRISPR-Cas system has tremendous potential for future study and can lead to significant advancements in various areas of research.

References

1. Paul B, Montoya G. CRISPR-Cas12a: Functional overview and applications. Biomed J. 2020 Feb 1;43(1):8–17.
2. Manghwar H, Lindsey K, Zhang X, Jin S. CRISPR/Cas System: Recent Advances and Future Prospects for Genome Editing. Trends Plant Sci. 2019 Dec 1;24(12):1102–25.
3. Aman R, Mahas A, Mahfouz M. Nucleic Acid Detection Using CRISPR/Cas Biosensing Technologies. ACS Synth Biol. 2020 Jun 19;9(6):1226–33.
4. Kim S, Ji S, Koh HR. Crispr as a diagnostic tool. Biomolecules. 2021 Aug 1;11(8).
5. Shivram H, Cress BF, Knott GJ, Doudna JA. Controlling and enhancing CRISPR systems. Nat Chem Biol. 2021 Jan 1;17(1):10–9.

6. Ghaemi A, Bagheri E, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M. CRISPR-cas9 genome editing delivery systems for targeted cancer therapy. Life Sci. 2021 Feb 15;267.
7. Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J. 2020 Jan 1;18:2401–15.
8. Uddin F, Rudin CM, Sen T. CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future. Front Oncol. 2020 Aug 7;10(1387).
9. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering crispr: A review of the challenges and approaches. Drug Deliv. 2018;25(1):1234–57.
10. Chira S, Nutu A, Isacescu E, Bica C, Pop L, Ciocan C, et al. Genome Editing Approaches with CRISPR/Cas9 for Cancer Treatment: Critical Appraisal of Preclinical and Clinical Utility, Challenges, and Future Research. Cells. 2022 Sep 1;11(18).
11. Guo N, Liu J Bin, Li W, Ma YS, Fu D. The power and the promise of CRISPR/Cas9 genome editing for clinical application with gene therapy. J Adv Res. 2022 Sep 1;40:135–52.
12. Nidhi S, Anand U, Oleksak P, Tripathi P, Lal JA, Thomas G, et al. Novel crispr–cas systems: An updated review of the current achievements, applications, and future research perspectives. Int J Mol Sci. 2021 Apr 1;22(7).
13. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther. 2020 Dec 1;5(1).
14. Lau CH. Applications of CRISPR-Cas in Bioengineering, Biotechnology, and Translational Research. CRISPR J. 2018 Dec;1(6):379–404.
15. Mohanty S, Dash A, Pradhan CK. CRISPR-Cas9 Technology: A magical tool for DNA editing. International Journal of Biosciences and Bioengineering. 2019;1(1).
16. Arroyo-Olarte RD, Bravo Rodríguez R, Morales-Ríos E. Genome editing in bacteria: Crispr-cas and beyond. Microorganisms. 2021 Apr 1;9(4).
17. Wang SW, Gao C, Zheng YM, Yi L, Lu JC, Huang XY, et al. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Mol Cancer. 2022 Dec 1;21(1).
18. Ghosh D, Venkataramani P, Nandi S, Bhattacharjee S. CRISPR-Cas9 a boon or bane: The bumpy road ahead to cancer therapeutics 06 Biological Sciences 0604 Genetics. Cancer Cell Int. 2019 Jan 8;19(1).
19. Tang N, Zhang Y, Shen Z, Yao Y, Nair V. Application of CRISPR-Cas9 Editing for Virus Engineering and the Development of Recombinant Viral Vaccines. CRISPR Journal. 2021 Aug 1;4(4):477–90.
20. Lahr WS, Sipe CJ, Skeate JG, Webber BR, Moriarity BS. CRISPR-Cas9 base editors and their current role in human therapeutics. Cytotherapy. 2023 Mar 1;25(3):270–6.
21. Rodrigues RC, Tagliaferri TL, Mendes TA de O. Potential of the endogenous and artificially inserted CRISPR-Cas system for controlling virulence and antimicrobial resistance of food pathogens. Food Chemistry Advances. 2023 Oct 1;2.
22. Chavez M, Chen X, Finn PB, Qi LS. Advances in CRISPR therapeutics. Nat Rev Nephrol. 2023 Jan 1;19(1):9–22.
23. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science 361. 2018;866–9.