



REVIEW ARTICLE

CRISPR TECHNOLOGY: FROM MEDICINE TO AGRICULTURE

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ABSTRACT

Gene editing is the process of altering DNA at specific locations in various cell types and organisms. It involves inserting, removing or replacing DNA, which can shut down certain genes, introduce new genetic traits, or fix harmful gene mutations. Because of its simple design, low cost, high efficiency, good repeatability, and quick results, CRISPR-Cas systems have become the most popular tool in molecular biology labs around the world. In this review, I will cover an overview of CRISPR-Cas systems, including their innovations, uses in human diseases research and gene therapy, as well as the challenges and opportunities they face in real-world applications.

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INTRODUCTION

Genome editing allows for precise changes to DNA at specific places in different cell types and organisms. These changes can turn off certain genes, add new traits, or correct harmful mutations. With fast progress in life sciences, this technology has become a top tool for studying gene functions, understanding hereditary diseases, finding gene therapy targets, and creating improved crop varieties. Among the main genome editing tools- zinc finger nucleases (ZFNs), transcription activator- like effector nucleases (TALENs), and RNA-guided CRISPR-Cas nucleases-CRISPR-Cas systems stand out because they are simple, low cost, highly efficient, consistently repeatable and can be quickly deployed. These advantages have made CRISPR-Cas leading genome- editing technology in labs worldwide. (1-2)

INNOVATIONS IN CRISPR-Cas SYSTEM: CRISPR- Cas systems have become the most important tool for genome editing in molecular biology since their ability to edit DNA was proven in 2012. They have led to major advances in fixing harmful mutations, identifying essential genes for cancer immunotherapy, and addressing issues in xenotransplantation. However, there are still limitations such as off- target effects, PAM sequence requirements that limit where they can be used, and less than ideal efficiency and accuracy. Because of this, many efforts have focused on improving these systems.

Dead Cas9 (dCas9) Systems: Introducing certain mutations- H840A and D10A – into the HNH and RuvC domains of Cas9 makes it non- functional, yielding dCas9. Although dCas9 can still bind to DNA, it cannot cut it. When dCas9 is linked to activators or repressors, it can be used for CRISPRi, allowing regulation of specific genes. Adding fluorescent proteins can help with genome imaging, and including epigenetic modifiers can allow for targeted changes in the genome. This system is easy to use and can handle multiple gene changes at once. (1,2,11)

Base Editing Systems: To improve precision in making genetic changes, base editors combine dCas9 with enzymes that change specific bases, such as cytosine deaminases (CBEs) or adenine deaminases (ABEs). These systems can change C-G to T-A or A-T to G-C without breaking the DNA strands. By avoiding double- strand breaks, these changes are more predictable. However, the limited area they can edit makes them less versatile. For example, editing C- rich regions can lead to off- target changes. Ongoing improvements are addressing these issues. Base editors have been used efficiently across many types of cells, including human embryos, bacteria, plants, and animals. These tools are finding use in research, biotechnology, and therapy. In theory, they could fix over 3,956 known genetic variants through C to T or A to G changes. (1,2,11)

Cas variants for Expanded PAM Recognition: The standard Cas9 enzyme, SpCas9, needs a specific sequence called a PAM at the target site's end. There are alternative PAMs like NGA and NAG, but these are less effective, covering only about 1/16th of the human genome. Scientists have created modified versions of Cas9 that work with a wider range of PAMs. For example, xCas9, developed using phage- assisted evolution, can use NG, GAA and GAT PAMs. Other versions can work with NRRH, NRCH, NRTN, NGN(SpG), or nearly PAM- free sites (SpRY). These versions can correct genetic variants that were previously hard to edit, although their efficiency and activity are still limited and require further refinement. (1,2,11)

RNA- Targeting CRISPR- Cas Systems: In addition to DNA, CRISPR- Cas Systems can also edit RNA. Type VI systems, like the Cas13 family (Vas13a/C2c2, Cas13b, Cas13c, Cas13d), target and cut RNA strands. These can be used for knocking down RNA, imaging transcripts, adjusting splicing and detecting viruses. When dead Cas13(dCas13) is paired with enzymes like ADAR2, it can create systems like REPAIR (changing A to I /G) and RESCUE (changing C to U). RNA editing offers better efficiency, specificity, reversibility, and fewer ethical concerns compared to DNA-based methods. It supports research and early- stage disease studies. (1,2,11)

Prime Editing Systems: Prime editing, developed by Anzalone and his team, allows for inserting, deleting and all 12 types of base changes without requiring double- strand breaks or donor DNA. It uses a modified Cas9 (nCAs9) linked to a reverse transcriptase and guided RNAs(pegRNAs) that specify the target and the sequence to insert. After the DNA is nicked, reverse transcription adds the new DNA directly. This method expands the range and accuracy of genetic changes and could potentially correct 89% of known harmful mutations. Further improvements are needed for more widespread use. (1,2,11)

APPLICATIONS OF CRISPR IN MEDICINE

PERSONALISED GENE EDITING IN INFANTS

Personalised genome editing has moved quickly from research testing to real world use, marking a big change in modern medicine. In last ten years, progress has led to treatments that are tailored to individuals' patients and can be done inside the body. In 2025, this was shown with the case of "Baby KJ", who received a custom gene editing treatment for a major milestone, proving that personalized gene editing is both safe and effective for serious genetic diseases that can be life-threatening. CPS1 deficiency is a severe disorder of the urea cycle, which leads to high ammonia levels in the blood, brain damage, and high risk of death without a liver transplant. The usual treatments, like limiting protein intake, using ammonia-scavenging drugs, and transplanting a liver, are only temporary and risky, not a cure. In Baby KJ's case, a quick whole-genome test found a harmful genetic change in the CPS1 gene that could be fixed with base editing. A team of doctors, geneticists, and regulatory experts developed a custom therapy: a special mRNA that carries the base editing tool and a patient- specific guide RNA, all packed in nanoparticles that target the liver. This approach aims to fix the genetic change in liver cells, restore normal CPS1 activity, and prevent dangerous ammonia buildup. (3,7)

Creating this treatment involved testing it in animals that were genetically matches to the patient, checking for harmful side effects using whole- genome sequencing and computer models, and making the therapy under strict manufacturing rules with emergency approval. The treatment was given through repeated injections during a time when Baby KJ's condition was getting worse, and the team kept a close eye on ammonia levels, liver function, the child development, how well the gene change was fixed, and if there was any unexpected changes in other parts of the genome. The results were promising Baby KJ's metabolism stabilized and they were less dependent on other treatments. Their development also improved more than it would have under regular care. Long- term monitoring is still needed to see how permanent the gene change is and to check for any hidden risks.(3)

INDIGENOUS CRISPR- BASED GENE THERAPY FOR SICKLE CELL DISEASE

Sickle cell disease is caused by a single change in the gene that makes haemoglobin, the protein in red blood cells that carries oxygen. This change makes the red blood cells sickle – shaped, which can block blood flow, damage organs, and shorten life. Traditional treatments like hydroxyurea can help manage symptoms but need to be taken every day. Bone marrow transplants can cause the disease, but they aren't always possible because of a lack of matching donors and the risks involved. Gene therapy aims to fix the genetic problem in two ways: adding a healthy copy of the gene or using CRISPR-Cas9 to correct the mutation. Some therapies approved by FDA have been very successful. For example, Lyfgenia reduced severe sickle cell crises in patients and kept their haemoglobin levels high. Casgevy helped nearly 94% of patients avoid sickle cell crises and increased the production of fetal haemoglobin (1,4,5).

In India, BIRSA-101 is the first local CRISPR- based gene therapy for sickle cell disease. It was developed by the CSIR-Institute of Genomics and Integrative Biology (IGIB) using a special version of the CRISPR- Cas9 system from a bacteria called *Francisella novicida*. This system is precise and targets the specific genetic change in a patient's blood stem cell, which are taken from the bone marrow and genetically corrected in the lab before being returned to the body (1,5). The treatment uses a modified version of the CRISPR-Cas9 system that is efficient, has fewer off- target effects, and works well with a wider range of DNA sequences. It uses these tools to correct the faulty gene in blood stem cells, which then grow into healthy red blood cells that produce normal haemoglobin. This one- time treatment aims to fix the problem permanently, reducing the risk of sickle cell crises, chronic red blood cell breakdown and organ damage. BIRSA-101 was launched in 2025 and is part of India's efforts to tackle sickle cell disease, especially in communities where the disease is common. The therapy is being tested in a Phase 1 trial at AIIMS in 2026 and if successful; will move to larger trials. It is expected to be more affordable than similar therapies that are available globally. Early tests in lab models showed that this CRISPR system is more accurate than other versions, which is a big plus for developing safe and efficient treatments. (1,4,5)

APPLICATION OF CRISPR IN AGRICULTURE: Genome- Edited Rice varieties for higher yield

CRISPR- Cas9 allows scientists to cut specific parts of the DNA. These cuts are then repaired by the cell's natural process, which can lead to small changes that can either turn off a gene or make it work differently. This method helps to quickly improve important traits that affect crop growth, such as how much rice can be produced, how well it handles stress, and the quality of the grain. This is faster than traditional breeding methods, which can take 6 to 7 years. In rice, scientists target genes like OsCKX2, which affects how many grains grow on each plant, and other that help the plant survive in harsh weather conditions like drought and salty soil.

DRR Dhan 100 (Kamala): This rice variety is a modified version of the Samba Mahsuri strain. It uses CRISPR to change the OsCKX2 gene, which leads to more grains on each plant, increasing the yield by about 130 days and is strong against lodging, which is when plants fall over. It can handle moderate's drought, has strong roots, and is good for areas with less inputs, like the southern states of Andhra Pradesh and Telangana. This varieties uses less water and fertilizer and reduce greenhouse gas emissions by about 20%.

Pusa DST Rice 1: This rice variety was created by using CRISPR to turn off a gene that helps the plant use water that could promote drought and salt resistance. This leads to fewer pores on the leaves, which helps the plant keep more water. It also improves how many plants grow from one seed and greatly increases the yield in salty and dry soil (up to 30% increase) and overall, by up to 20%. This makes it ideal for areas in Eastern India, like Bihar and Jharkhand, where weather is unpredictable. Field studies have confirmed that this varieties performs better under harsh environmental conditions. (10,12)

CHALLENGES AND ETHICAL CONSIDERATIONS: Even though CRISPR has the potential to change medicine and farming, there are still many challenges to overcome. One major concern is off-target changes, which happen when CRISPR accidentally edits parts of the DNA that it wasn't meant to. These changes can lead to unexpected results or even cancer. Additionally, the long- term effects of changing genetic material in human cells or plants are not yet fully understood, so ongoing research and monitoring are necessary. There are also ethical issues, especially when it comes to making genetic changes in human embryos. These changes could affect future generations, raising questions about fairness and the right to choose for future people. Also, the high cost of these therapies could make them only available to rich people, widening the gap in healthy outcomes between different groups of people. Because of these concerns, strong rules and guidelines are needed. These should include international cooperation, strict testing before use, and fair ways to involve everyone in decision- making. As CRISPR moves from lab tests to real world use, it's important to handle these issues carefully to ensure that its benefits are used responsibly and fairly. (1,8,9)

CONCLUSION

Since CRISPR- Cas technologies have made it easier and more flexible to change, control, and study genes, they are considered a major breakthrough in molecular biology in century. So far, CRISPR-Cas systems have been widely used in studying gene functions, developing treatments for human

diseases, creating targeted drugs, building animal models, and improving livestock breeding. These applications show how much potential they have for future growth. However, there are still some challenges that need to be addresses before they can be fully used in real-world situations. A lot of work still needs to be done to check their long- term safety and effectiveness.

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