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ADVANCES AND APPLICATIONS OF CRISPR/Cas9 TECHNOLOGY IN GENE EDITION

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SUMMARY
CRISPR/Cas9 technology has transformed the field of gene editing by providing a precise, efficient, and versatile tool to modify DNA in a wide variety of organisms. This system, based on the immune system of bacteria and archaea, consists of a guide RNA molecule and the Cas9 enzyme. The guide RNA is designed to recognize specific DNA sequences, and when it binds to its target, the Cas9 enzyme cuts the DNA at that site. From there, the natural cellular repair mechanisms come into play and can result in the insertion or deletion of bases or the introduction of a new DNA sequence. The technology has found numerous applications in various fields. In medicine, it is being used for research into genetic diseases, allowing the study of the molecular bases of various conditions and the development of gene therapies. The ability to edit genes in human cells and tissues opens up the possibility of treating inherited and acquired diseases. In addition, CRISPR/Cas9 has facilitated the creation of animal models to study diseases and evaluate new therapies. In the field of agriculture, it is being used to develop crops resistant to diseases, pests or adverse environmental conditions. Research is also being carried out to improve the nutritional quality of crops, increase agricultural productivity and reduce the use of agrochemicals. These applications can contribute to addressing global food and food security challenges. In biotechnology, the technology has great potential for the production of pharmaceuticals and enzymes. It allows the precise modification of microorganisms to increase the efficiency of the production of biochemical and pharmaceutical substances of interest. Likewise, CRISPR/Cas9 is used in the engineering of microorganisms for industrial applications, such as the production of bioplastics or biofuels. Recently, significant advances have been made in CRISPR/Cas9 technology to improve its efficiency and accuracy. Additional variants of CRISPR systems, such as Cas13 and Cas12, are being developed that extend the gene editing capabilities and address certain challenges and limitations present in the original Cas9 system. In addition, approaches are being investigated to reduce potential side effects and improve the specificity of gene editing. While this technology has fueled important advances, it also poses security and ethical challenges. The debate on gene editing in human embryos and the long-term implications of modifying the human genome continues. It is critical to ensure responsible and ethical use of technology, as well as address potential risks and legal considerations associated with gene editing. As this tool continues to be researched and refined, it is essential to carefully assess its safety and ethical implications, while promoting its responsible application for the benefit of society.

KEY WORDS: CRISPR-Cas9, gene editing, applications.

INTRODUCTION

The ability to precisely and efficiently edit genes has been a goal pursued by scientists for decades. CRISPR-Cas9 technology has emerged as a revolutionary tool in the field of gene editing, opening up new possibilities in DNA modification in a wide variety of organisms.[1-3]

The CRISPR-Cas9 system is inspired by the immune system of bacteria and archaea, which allows them to defend themselves against viruses and other foreign genetic elements. The acronym CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), which refers to highly repetitive DNA sequences present in bacterial genomes. These sequences are flanked by segments called spacers, which are genetic sequences derived from previous invading elements, such as viruses. On the other hand, Cas9 is an enzyme that acts like molecular scissors capable of cutting DNA at specific sites.[2,4,5]

Figure 1 shows the components of the CRISPR-Cas9 system.

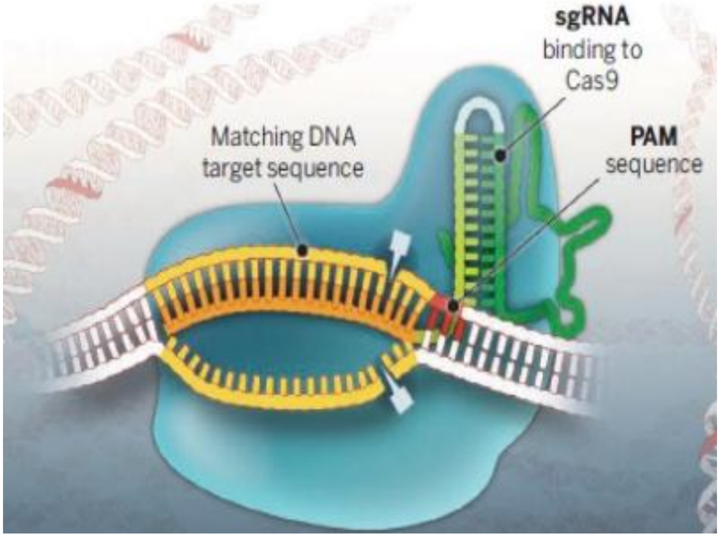

Figure 1. Main components of the CRISPR-Cas9 system. The cas9 protein recognizes the target DNA thanks to a **PAM sequence** (trinucleotide that varies depending on the cas9 used) located 3-4 nucleotides away from the cutting point and to an **sgRNA** that interacts with cas9 and guides it to the specific sequence by sequence complementarity.

The CRISPR-Cas9 system is made up of two main components: a guide RNA molecule designed to recognize specific DNA sequences, and the Cas9 enzyme that acts like "molecular scissors" to cut DNA at the desired site. The guide RNA molecule is designed to be complementary to the target DNA sequence that it is intended to modify. When the guide RNA binds to the target DNA, Cas9 attaches to the guide RNA molecule and cuts the DNA at the specific site, creating a double-strand break.[6-9]

Figure 2 schematically shows the foundation of the CRISPR/Cas9 system.

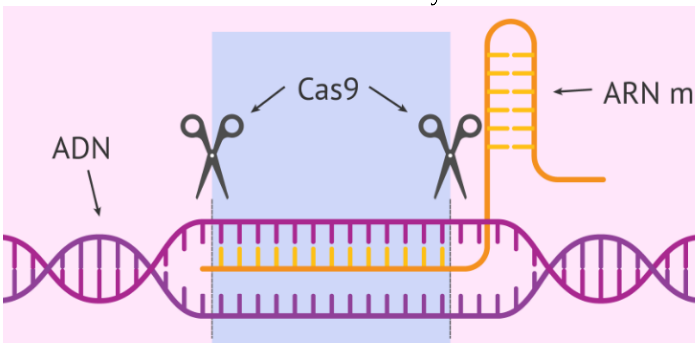

Figure 2. Fundamentals of the CRISPR/Cas9 system.

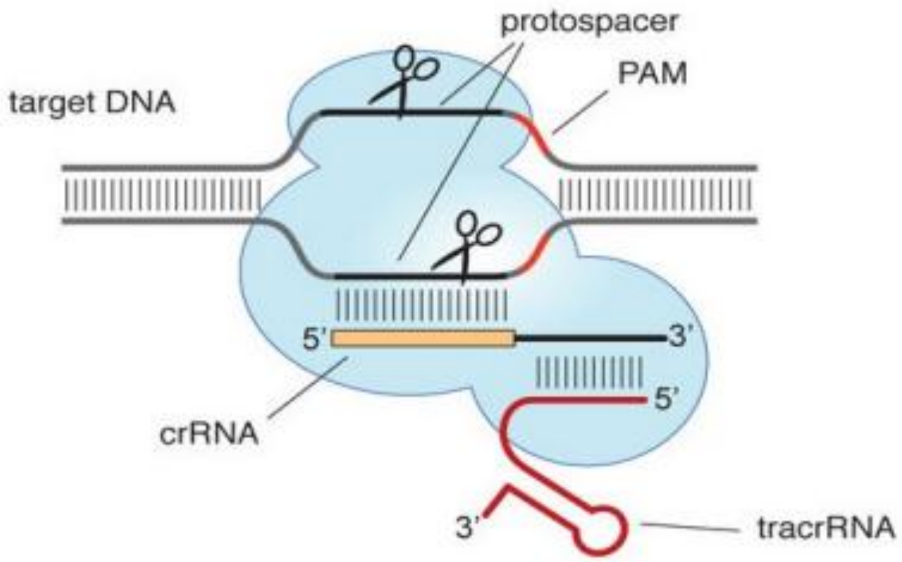

Figure 3. In blue, the mechanism of action of cas 9 with its guide RNA is observed. The catalytic domains cut the region complementary to RNA and close to PAM.

From this point, the cell's DNA repair mechanisms come into play. Two of the most common repair mechanisms are non-homologous end-joining (NHEJ) repair, which often results in base insertions or deletions, and homologous recombination (HR) repair, which can allow for the introduction of a sequence. of new DNA. These cellular repair mechanisms allow precise modifications in the DNA sequence, either to deactivate a specific gene, introduce new characteristics or correct genetic mutations (Figure 4).[10-12]

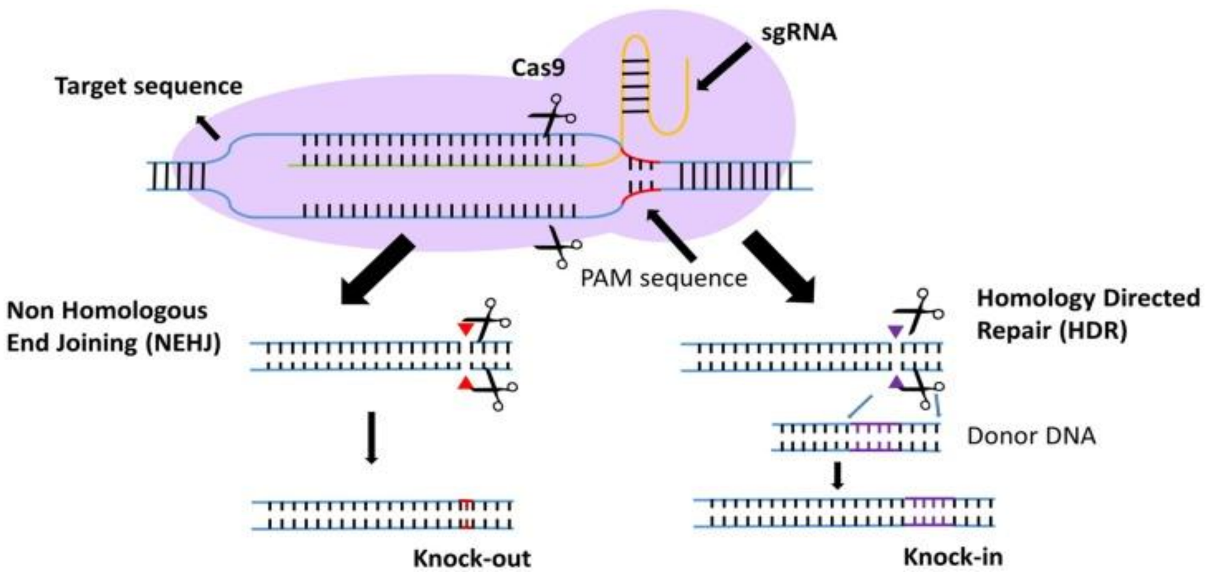

Figure 4. Gene editing using CRISPR cas9.

CRISPR/Cas9 technology has proven to be a highly efficient and low-cost gene editing tool compared to previous technologies. Its wide application in biological research has led to important advances in the understanding of the genetic mechanisms of various diseases, as well as in the development of gene therapies and the improvement of agricultural crops.[5,9,11]

FUNDAMENTALS OF CRISPR/CAS9 TECHNOLOGY

The CRISPR/Cas9 system is based on a natural defense mechanism that bacteria and archaea use to protect themselves from viruses. The acronym CRISPR refers to clustered regularly interspaced short palindromic repeats, which are repetitive DNA sequences present in bacterial genomes. Spacers, on the other hand, are genetic sequences derived from viruses or other invading elements that have previously attacked the bacterium.

The process begins when a bacterium is infected by a virus. In response to infection, the bacterium integrates fragments of the viral genome into its CRISPR repeats, forming a kind of "genetic memory" of the attack. The bacterium transcribes and processes these CRISPR sequences into small RNA molecules, known as guide RNAs.[2-7]

Guide RNA is the key component in CRISPR/Cas9 technology. It is an RNA sequence synthesized in the laboratory to be complementary to the DNA sequence to be modified. This guide RNA sequence directs the Cas9 enzyme to the specific place in the DNA where the cut is desired.[12-15]

The Cas9 enzyme is an endonuclease, that is, an enzyme that can cut DNA. Once the guide RNA has bound to the target DNA through complementary sequence recognition, the Cas9 enzyme binds to the guide RNA and forms a hairpin-shaped structure. This allows Cas9 to cut the two DNA strands at the specific location determined by the guide RNA.[16-20]

Once the DNA is cut, the cell attempts to repair the break using its cellular repair mechanisms. The two main mechanisms are non-homologous end-joining repair (NHEJ) and homologous recombination repair (HR).

Non-homology end junction repair (NHEJ) is the most common mechanism and occurs when broken ends of DNA are rejoined. However, this process often introduces base insertions or deletions at the cleavage site, which can result in inactivation of the gene or alteration of its function.

Homologous recombination repair (HR), in contrast, uses a similar DNA sequence present in another copy of the gene or in a donor DNA molecule to repair the break. This allows for the introduction of a new DNA sequence at the cleavage site, providing the opportunity for precise and targeted genetic modification.

CRISPR/Cas9 technology has been noted for its efficiency and versatility compared to previous gene editing techniques such as zinc finger nucleases and TALEN proteins. The ease of design and synthesis of guide RNA molecules, as well as Cas9's ability to target specific DNA sequences, have made CRISPR/Cas9 technology widely adopted by the scientific community.[10,14,17]

Therefore, CRISPR/Cas9 technology is based on a bacterial defense system and uses a combination of guide RNA and the Cas9 enzyme to precisely edit DNA. By harnessing natural cellular repair mechanisms, targeted genetic modifications can be made, opening up a world of possibilities in biological research and practical application in medicine, agriculture, and biotechnology.

RECENT ADVANCES IN CRISPR-CAS9

In recent years, CRISPR/Cas9 technology has undergone significant advances that have improved its efficiency, precision, and versatility. These advances have further expanded the application possibilities of gene editing in various fields. Some of the most outstanding advances are[3,7,9,12,13,15]:

-Gene editing in complex tissues and organisms: One of the initial challenges of CRISPR/Cas9 technology was its applicability in complex tissues and organisms. However, researchers have developed new strategies to overcome this limitation. More efficient delivery systems, such as modified viral vectors and nanoparticles, have been designed that allow delivery of CRISPR/Cas9 to specific tissues. In addition, Cas9 variants have been developed that are more efficient in gene editing in multicellular organisms, opening the door to applications in regenerative medicine and gene therapy.

-Precise genome editing: The precision of gene editing has been a matter of concern. Researchers have made efforts to improve the specificity of CRISPR/Cas9 and reduce potential unwanted modifications to the genome. Cas9 variants, such as high-fidelity Cas9 (Cas9-HF) and high-specificity Cas9 (eSpCas9), have been developed that have a lower propensity to generate unwanted DNA changes. In addition, molecular engineering strategies, such as the use of bifunctional guide RNAs and high-specificity guide RNA design, have been implemented to increase the precision of gene editing.

-Individual Base Editing: In addition to the ability to perform base insertions or deletions, precise editing of individual bases in the genome has been achieved using CRISPR-Cas9. This approach, known as base editing, allows correction of point mutations without requiring a double-stranded DNA break. Variants of Cas9 have been developed, such as Cas9 deaminase (dCas9) fused to base-editing enzymes, which can change one specific base to another with high precision. This advance has promising applications in the correction of genetic diseases caused by point mutations.

-Expansion of the range of objectives: In addition to gene editing, CRISPR/Cas9 technology has been used for a variety of applications beyond DNA modification. Researchers have developed Cas9 variants, such as Cas13 and Cas12, that have the ability to target and manipulate RNA rather than DNA. This has enabled the development of techniques such as viral RNA detection, gene regulation at the transcriptional level, and non-coding RNA editing. These applications open up new opportunities in basic and applied research.

- Addressing complex diseases: CRISPR/Cas9 technology has demonstrated its potential to address complex and genetically heterogeneous diseases. Significant advances have been made in gene therapy using CRISPR/Cas9 to correct gene mutations responsible for inherited diseases such as muscular dystrophy and sickle cell disease. Furthermore, approaches are being developed to modulate gene expression in complex diseases, such as cancer, by regulating key genes involved in tumor progression.

These and other advances in CRISPR/Cas9 technology are driving research in biology and medicine, opening up new prospects in gene therapy, agricultural crop breeding, food production, and biotechnology in general. As gene editing techniques continue to be refined and optimized, it is expected to continue to revolutionize our approach to modifying and understanding the genomes of different organisms. However, it is important to be aware of the ethical and safety challenges associated with this technology and ensure responsible use to maximize its benefits and minimize potential risks.[17,19,20]

CONCLUSIONS

CRISPR/Cas9 technology has proven to be a revolutionary tool in the field of gene editing. Its efficiency, precision, and versatility have made it a powerful tool for researchers and scientists in a wide range of disciplines. Over the years, significant advances have been made in optimizing and applying this technology, further expanding its potential and making it possible to address complex scientific and medical challenges.

In the field of medicine, CRISPR/Cas9 has shown great potential in gene therapy, offering the possibility of correcting genetic mutations responsible for hereditary diseases. Preclinical and clinical studies in animals and in human cells have provided promising results, and it is hoped that effective and personalized treatments for various genetic diseases will be possible in the future.

In agriculture, technology has opened up new opportunities to improve agricultural crops, increase resistance to disease, improve food quality and yield, and reduce reliance on pesticides. This could have a significant impact on food security and the sustainability of agriculture.

However, despite the exciting advances, it is important to address the ethical and security challenges associated with CRISPR/Cas9 technology. Gene editing raises questions about the manipulation of the human germ line, the responsible use of this technology, and potential unforeseen impacts on ecosystems and biodiversity. It is crucial that the scientific community, regulators and society at large participate in ethical discussions and in the development of strong regulatory frameworks to ensure responsible and ethical use of CRISPR/Cas9 technology.

In short, the technology has revolutionized gene editing and has the potential to have a significant impact in fields as diverse as medicine, agriculture, and biotechnology. If used responsibly and ethically, this technology can offer innovative solutions to scientific and medical challenges, and improve people's quality of life. However, it is essential to continue research and carefully evaluate the ethical, legal and safety aspects to ensure that benefits are maximized and potential risks are minimized.

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