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DNA EDITING AND THE PROSPECTS FOR TREATING DISEASES WITH CRISPR-CAS9

Paola Moraes de Freitas

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Abstract: The discovery of the CRISPR-Cas9 technique represented a major breakthrough in the history of biotechnology and medicine, bringing major advances to genetic research and the treatment of diseases, including genetic disorders. The method stands out for its low cost and precision in genome editing, however, it still raises ethical questions and concerns about possible errors. In this study, a narrative review was carried out using the *SciELO, PubMed, ScienceDirect* and Google Scholar databases, with articles from 2014 to 2023, surveying the exploration of CRISPR-Cas9, its therapeutic applications in complex diseases, such as the manipulation of cancer-related genes and the existing challenges regarding the efficient delivery of the technique, as well as ethical issues, which have implications for gene editing in the germ line, with implications for future generations. In general, it is described as "molecular scissors", which allow two strands of the DNA double helix to be cut using the Cas-9 enzyme, making it possible to insert a new strand, replace elements in the DNA chain or correct genetic errors. The case of scientist He Jiakui, who created genetically modified babies, highlighted the need for strict ethical regulations. Considering the analysis presented, it is a technique with great potential, which promises great efficiency in its results, but brings with it a series of ethical concerns.
Keywords: *CRISPR-Cas9, gene therapy,* CRISPR-Cas9 cancer, genetic mutation and gene therapy.

INTRODUCTION

The discovery of the CRISPR-Cas9 technique was a milestone in the history of biotechnology and medicine, because for a long time the world lived with fears and hopes about the possibilities of genetic interventions in human beings (SGANZERLA et al., 2020). The method has proven to be truly revolutionary, exhibiting broad potential for genetic research and medicine, providing hope for the treatment of numerous diseases, such as rare genetic disorders, as well as complex diseases such as cancer. Its precision in reading and editing the genome, combined with a low cost in view of gene editing technology, can solve long-standing genetic problems by preventing them. Thus, this technique opens up countless possibilities for solving challenges that were previously considered irreversible. However, given the numerous risks, the possibility of errors is alarming, especially in terms of ethics (HUPFFER et al., 2020).

It is known as molecular scissors because it is able to cut two strands of the DNA double helix using the Cas-9 enzyme, making it possible to insert a new strand. It has been widely accepted due to its ease of use, specificity and manipulation *in vitro* and *in vivo* (SGANZERLA et al., 2020).

It was in the 1980s that researcher Ishino and his collaborators observed a specific sequence of the *iap* gene present in the bacterium *Escherichia coli*, where they found an unusual structure. They then identified similar repeats by randomly sequencing the entire genome in numerous other bacteria. These repetition sequences, grouped together and regularly *interspaced* by unique interacting sequences of constant length, were named *''Short Regular Spaced Repeats (SRSRs)"* (LINS et al., 2018).

Thus, the name CRISPR refers to a section of DNA in bacteria and archaea, and also to the technique that uses genes from this sec-

tion as a tool in genome editing. It works like a defense system: when a bacteriophage (virus that infects bacteria) infects a cell, it inserts the viral DNA that is introduced into the bacterial DNA, precisely in the place called CRISPR. Through this mechanism, the bacteriophage's attacks are recorded over time, creating an infection file, so that the organism gains the ability to recognize the same aggressor in possible future reinfections, enabling a rapid immune response (MARTINEZ-OLIVIA, 2020).

In the middle of the 21st century, researchers Emmanuelle Charpentier and Jennifer Doudna began research to understand how bacteria attack viral infections. During this study, they discovered that bacteria have an adaptive immune system, called CRISPR, which allows them to detect viral DNA and destroy it. In the course of their research, they identified a protein found in *Streptococcus Pyogenes*, called Cas9, which is capable of seeking out, cleaving and degrading the virus's DNA. And by studying this protein, Cas9, they realized that they could use it as a tool for editing genomes (LIBERALESSO et al., 2021). With this, they won the 2020 Nobel Prize in Chemistry.

In short, this technique makes it possible to replace elements in the DNA chain, allowing genetic errors to be corrected or beneficial characters to be inserted, inhibiting or activating certain codons as desired (BERNARDES et al., 2021).

In addition, this programmable endonuclease technology allows researchers to examine the function of multiple genes at the same time, simultaneously targeting multiple loci in a single experiment, which significantly accelerates the understanding of pathological processes that involve large sets of genes or mutations, such as tumor development. Using single guide RNA (sgRNA) libraries, CRISPR--based genomic screenings can be harnessed to identify a biological or disease resistance target, such as novel tumor suppressors or oncogenes, and to rapidly evaluate drug targets. As such, CRISPR-Cas9-mediated genome engineering holds immense promise for treating or even curing genetic diseases, including many forms of cancer and neurodegeneration, as well as sickle cell anemia, cystic fibrosis, Duchenne muscular dystrophy, viral infections, immune disorders and cardiovascular diseases (JIANG et al., 2017).

A central concern is the ethical challenges, because in addition to the ability to edit genes for the treatment of genetic diseases, there are profound moral questions, such as the fact that human nature has been altered, bringing with it the limits of human intervention in biological evolution. Another point is the concern about gene editing in human embryos, raising questions about the possibility of permanent interventions in the bloodline.

The aim of this study was to conduct a literature review on CRISPR-Cas9 technology, covering its principles of operation, applications in medicine, science and its ethical limits.

METHODOLOGY

This study is a literature review, using scientific articles from the SciELO (*Scientific Electronic Library Online),* PubMed (*National Institutes of Health), ScienceDirect* and Google Scholar databases as a source of research. A search was carried out for articles in Portuguese, Spanish and English, between 2014 and 2023, using terms such as *''CRISPR-Cas9''*, *''gene therapy''*, ''CRISPR-Cas9 cancer", ''genetic mutation" and ''gene therapy" as keywords.

DEVELOPMENT

THE CRISPR-CAS9 TECHNIQUE

CRISPR consists of single spacer sequences delineated by short, repetitive, palindromic sequences that encode Cas proteins (KOLLI et al., 2017). This system has been suggested to play roles in DNA repair and regulation, as well as protection against foreign agents, such as viruses, in prokaryotes (LINS et al., 2018). It was first described as an adaptive immune system in bacteria and archaea, and has now been engineered as an RNA-guided endonucleases for genome editing (MA et al., 2014).

To edit the genome, scientists have joined together a single chimeric molecule containing crRNA and tracrRNA, known as sgRNA or gRNA. Thus, sgRNA has two basic characteristics: a sequence of 20-25 nucleotides at the 5' position - which joins the specific sequence of the target DNA, and a complementary repeated sequence in crRNA and tracRNA, which pair up to form a double stranded clamp, made up of 42 conserved nucleotides, essential for recognition by the Cas9 enzyme. In addition, the genome editing system based on *S. pyogenes* contains another transcription terminator sequence, in a hairpin, made up of 40 nucleotides. This construction is sufficient to generate the association between crRNA, tracRNA and the Cas9 enzyme, forming a complex directed at the specific site of the target DNA, developing the cleavage of the DNA double strand (VASCONCELOS et al., 2015).

The crRNA:tracRNA:Cas9 complex slides along the strand of invading DNA until it finds the appropriate PAM sequence, which is located just below the target sequence in the complementary (non-target) strand of genomic DNA. After this, Cas9 promotes the opening of the DNA double strand in the position immediately above the PAM, enabling the pairing of sgRNA with the DNA strand complementary to the crRNA *spacer* (ANDERS et al., 2014; STERNBERG et al., 2014).

The HNH and RuvC domains, with nuclease activity in Cas9, precisely cleave the DNA at the third nucleotide adjacent to the PAM, inducing a double-strand break (DSB) in the invading DNA molecule. DSBs initiated by CRISPR-Cas9 can be corrected by the DNA repair mechanisms NHEJ or HDR. Commonly, NHEJ generates random mutations (*indels*), which can be deletions or insertions of different sizes, causing changes in the reading window and leading to gene *knockout* or the interruption of regulatory elements in *cis* in promoters or *enhancer* sequences (VASCONCELOS et al., 2015).

Since its first appearance in 2012, the sgRNA-guided Cas9 system has been applied to modify endogenous genes and a wide range of cells and organisms, including bacteria, yeast, plants, roundworms, silkworms, fruit flies, zebrafish, frogs, rabbits, mice, pigs, monkeys and different human cells (MA et al., 2014).

THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS9 SYSTEM

CRISPR-Cas9 technology has emerged as a revolutionary system capable of transforming the therapeutic landscape. The applications are vast and complex, allowing for the correction of specific genetic mutations, as well as the modulation of complex cellular pathways in common diseases.

Editing takes place through the cleavage of both target DNA chains, followed by the triggering of one of two DNA repair mechanisms: homology-directed repair (HDR), which addresses errors in the insertion or deletion of a specific DNA sequence and can modify the expression of proteins, or the non-homologous end joining (NHEJ) mechanism, which includes homologous recombination with donor DNA sequences in the target DNA (BRAGA et al, 2023).

The growing number of bibliographic reviews and research in the field on the use of CRISPR-Cas9 to inhibit, activate or modify genes in order to treat or strengthen the body's defense system is widely evident in academia (BERNARDES, 2021).

Its use to treat genetic diseases caused by mutations in a single gene is one of the important applications, such as cystic fibrosis (CF), Duchenne muscular dystrophy (DMD) and hemoglobinopathies.

The CRISPR-Cas9 study is also being used on viruses with a DNA genome or which reveal a DNA phase in their life cycle. In this way, it is possible to research successful editing studies in the genomes of some viruses, such as hepatitis B, papillomavirus, herpesvirus, human immunodeficiency virus type 1 (CASTRIGNANO, 2017).

The technique can be applied directly to the molecular basis of the HIV virus due to its high specificity and effectiveness. It has come to be used in tests for gene therapy, as existing treatments do not promote a cure (OLIVEIRA et al., 2018).

There are two cellular types of gene therapy: germline, where genetic alterations are inherited, and somatic lineage, limiting a patient's modifications without impacting on future generations. This approach has great potential in the fight against genetic diseases. The rapid advance of CRISPR-Cas9 has enabled translational tests to be carried out on human somatic cells, the first applications with an emphasis on the therapeutic sphere, describing optimization steps for the safety and efficacy of the system (GONÇALVES et al., 2017).

According to Silva et al. (2020), in the vast majority of cases of genetic diseases for which replacement treatment or feeding methods are not applicable, the most effective alternative is the genetic modification of ex vivo cells in culture, which will then be transplanted. These genetically modified cells can stimulate the immune system and even synthesize therapeutic molecules to improve the patient's clinical condition.

CRISPR-CAS9 IN CANCER THERAPY

Cancer is one of the leading causes of disease-related mortality, with an increasing incidence worldwide. At the same time, progress has been made in the prevention and treatment of many malignant tumors, leading to prolonged survival or even a cure (ZHAN et al., 2019). Technological advances, such as CRISPR-Cas9-mediated genome editing technology, make it possible to precisely manipulate almost any genomic sequence, enabling the functional elucidation of genes involved in carcinogenesis and the correction of cancer-causing mutations. However, despite their advantages and potential, how CRISPR-Cas9 editing tools are efficiently delivered to target cells *in vivo* and how to avoid or reduce unintended off-target effects remain major challenges, which are crucial for their clinical applications (CHEN et al., 2019).

Considering that cancer is a genetic disease resulting from accumulative genetic and epigenetic abnormalities, it is rational to suggest that correcting oncogenic genome/epigenome abnormalities through CRISPR-Cas9 could be a promising therapeutic strategy against cancer (CHEN et al., 2019).

Different concepts of CRISPR/Cas9-mediated cancer therapy, including manipulation of tumor-related genes, tumor immunotherapy, tumor research modeling and overcoming resistance to anticancer drugs, are established in various types of cancer. To achieve effective and precise cancer treatment, the CRISPR/Cas9 components must go directly to the target cells, passing through different physical barriers. In addition, the gene editing process requires the transportation of the functional Cas9 protein and sgRNA into the nucleus at the same time (XU et al., 2021).

Drug discovery and development is a long and complex process of identifying new drugs and bringing them to market. Generally, this process begins with the hypothesis that disrupting a specific biological target will result in a beneficial effect that will change the course of a disease. In the field of oncology, drug discovery attempts to identify molecules against genetic anomalies in oncogenes and tumor suppressor genes that lead to the development of tumors (MARTINEZ-LAGE., et al 2018).

The epithelial-mesenchymal transition alters the expression of adhesion molecules on the cell surface, enabling malignant tumor cells originating from epithelial cells to acquire the capacity for migration and invasion, achieving metastasis in distant locations. It is an effective method for identifying the signals that regulate the transition from epithelial cells to mesenchymal cells, helping to understand the genetic basis of biological processes during development and in diseases such as cancer (WANG et al., 2022).

As an example, triple negative breast cancer (TNBC), characterized by its high malignancy and heterogeneity, there are no targeted therapies. *BET bromodomain* inhibitors (BBDIs) are a potential drug to treat TNBC, but the inherent and acquired resistance of tumors to BBDIs limits their clinical application (WANG et al., 2022).

Studies conducted by Shu et al. (2020), identified a synthetic lethal interaction with BBDIs and genes that confer resistance to BBDIs when deleted. The results showed that CDK4/6 inhibitors and paclitaxel present a strong synergy between BBDIs, while the absence of components of the SFN/SWI complex results in resistance to BBDIs. As a result, single-cell RNA sequencing in BBDI-sensitive and -resistant cell lines showed a high degree of heterogeneity between the samples, indicating that resistance to BBDIs can be both pre-existing and acquired.

ETHICAL CHALLENGES

During the 20th century, humanity witnessed a period of fear and hope regarding genetic interventions in human beings. Each new technological advance in the field of genetics gave rise to a new wave of concern and hope for the future. Towards the end of the 20th century, the world was surprised by the birth of Dolly the sheep, the first clone of an adult animal, the result of years of research led by geneticists Ian Wilmut and Keith Campbell in Edinburgh, Scotland. It raised ethical questions about human cloning, the use of embryonic stem cells and the production of embryos for research (SGANZERLA et al., 2020).

The possibility of editing the human germ line is one of the most controversial, i.e. being able to modify the genome of gametes and embryos. The risks of this modification cannot be predicted, especially since the changes will be passed down from generation to generation (GÓMEZ-TATAY et al., 2019.).

When it comes to ‘’somatic” and ‘’germline” gene therapies, a distinction needs to be made: the first category involves genetic alterations that are not passed on to children, such as numerous gene therapies used in the last decade to treat diseases of the immune system, while the second category involves genetic alterations that are passed on through germ cells (sperm and egg). The germ line represents an “extraordinary line” to begin with, and it’s easy to see the difference between therapy in children or adults and intentional modifications to the germ line. Science historian Nathaniel Comfort wrote “they are not used to treat disease in one individual, but to prevent it (or decrease the risk) in future individuals”, but this depends to some extent on the type of germline modification (FOHT, 2016).

On November 26, 2018, Chinese scientist He Jiankui shocked the world’s scientific community by announcing the creation of the world’s first genetically modified babies,

implanted in a woman who gave birth to twin girls called Lulu and Nana, so that they would become resistant to the HIV virus, preventing the development of HIV and eliminating the disease. Following the announcement, the world's leading science and medicine institutions reacted strongly, condemning this "scientific" execution and highlighting the need to create ethical and legal protections so that experiments like these are not repeated (SGANZERLA et al., 2020).

It is known that in one of the embryos, both CCR5 copies were inactivated (Nana), while in the second only one was modified (Lulu). As such, only Nana has a chance of being protected from HIV infection in the future, at least from the main variants of the virus that enter cells by binding to the CCR5 receptor. Kiran Musunuru, a cardiologist and professor of medicine at the University of Pennsylvania, said that the first babies of the "CRISPR-Cas9" generation were unfortunately not born as a result of a "historic scientific achievement, but rather a historic ethical fiasco". The international scandal put He under house arrest, and then a three-year prison sentence, from which he has already been released (GOSTIMSKAIA, 2022).

FINAL CONSIDERATIONS

The revolutionary CRISPR-Cas9 technique offers a series of possibilities for genetic research and medicine, bringing with it new perspectives for the treatment of diseases, including cancer. In addition to its low cost, it promises efficiency in genome editing, providing solutions to challenges that were previously considered irreversible, but despite its commitment, it brings with it a series of ethical concerns about possible errors. The case of the modified twins in China served as a wake-up call for the scientific community.

In general, it is a method with great therapeutic potential, but it is necessary to balance its benefits with careful ethical reflection and strict regulation.

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