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REVIEW

Genetic Editing with CRISPR Cas9: recent Biomedical and Biotechnological Applications

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Abstract

The use of a novel and powerful technology that allows for the precise editing of the genetic material of various organisms is becoming widespread. This technology derives from bacterial and archaeal defense machinery and is called CRISPR Cas9. Unlike other gene editing tools that exclusively rely on proteins, CRISPR Cas9 utilizes interactions between the target DNA and an RNA sequence that guides the Cas9 enzyme to alter the structure of a target gene. Various genome locations can be edited thanks to the ease of programming different guide RNA sequences, facilitating its use and implementation. Furthermore, the non-active version of the Cas9 protein, guided by its corresponding RNA, can be utilized for visualization processes of genetic material or, more recently, for the regulation of the transcription process. Considering the recent advances and possibilities in biomedical and biotechnological research, we must understand that the exploration of this technology is just beginning, and its eventual applications will influence the world around us on multiple levels. In this review, we describe the biological foundations of the functioning of the Cas9 nuclease, together with selected applications of its use in editing and regulating specific sections of the genetic material of various organisms. We also discuss some bioethical issues surrounding this subject.

Keywords: CRISPR Cas9; gene editing; monogenic disease; cancer biology; antiviral therapy; bioethics

1. Introduction

The central dogma of molecular biology first expressed in 1957 and regularly reformulated since 1970, describes the flow of genetic information from DNA to RNA and RNA to proteins within cells [1]. While there are known exceptions or extensions to this assertion, understanding how genes directly determine the characteristics of living organisms holds significant importance and utility for researchers worldwide [2]. Furthermore, this conceptual tenant of biology implies the possibility of altering the fundamental characteristics of living beings through the edition of their genetic information [3].

Since the beginning of this century, gene editing has been explored with increasing success, utilizing zinc finger nucleases (ZFN) [4] and transcription activator-like effector nucleases (TALEN) [5]. Consequently, the ability to edit and regulate genomes has become more attainable. A cell's genetic material precise manipulation entails a two-step process involving DNA-protein interactions. Firstly, proteins recognize and join a specific DNA segment, and second, this machinery cuts the DNA double helix or regulates transcription around the target genetic material segment [6]. In the case of the above genetic editing strategy, a protein transcription factor recognizes its

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target DNA segment and dimerizes with the restriction enzyme FokI to generate a double helix break and introduce modifications to the target DNA [7]. However, since these techniques rely on the direct interaction of proteins with DNA, targeting new locations in the genome requires the continuous generation of new proteins, which poses significant technical challenges and limits the use of these technologies in some cases [8, 9]. See **Figure 1** and **Figure 2**.

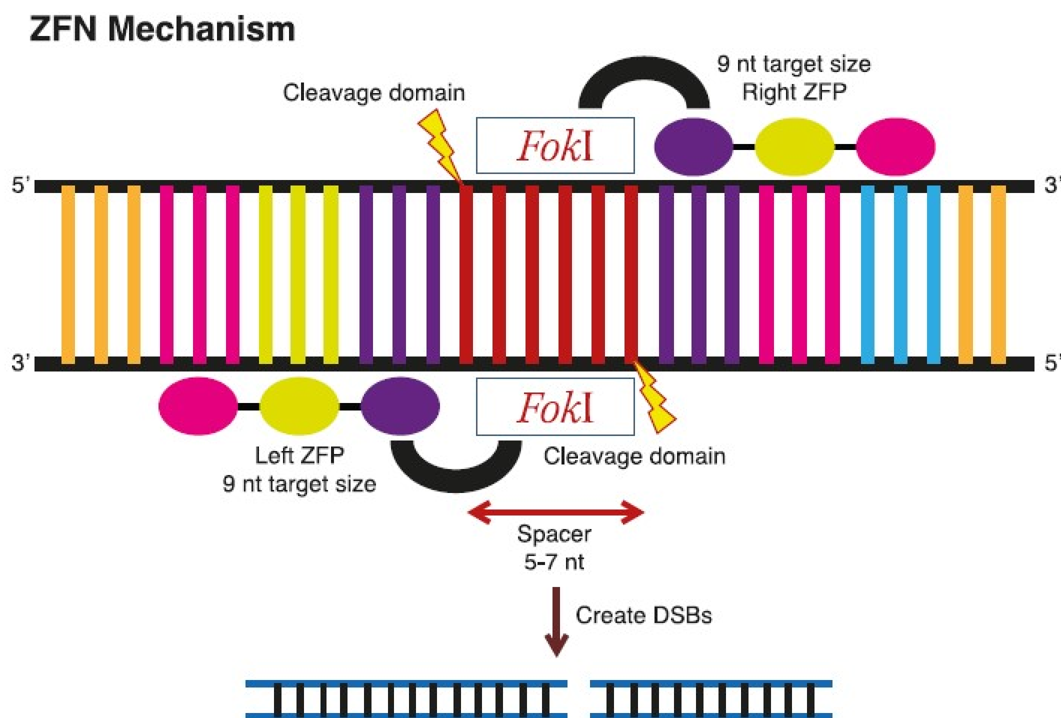

Figure 1. Sketch of a zinc finger nuclease (ZFN) dimer linked to its DNA target. Each ZFN contains the FokI cleavage domain linked to a series of three to six zinc fingers (three are shown here) that have been specifically designed to recognize sequences, flanking the cleavage domain. A small number of bases (usually five or six) separate ZFN targets.

Alternatively, for just over ten years, the use of the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats) and its associated protein Cas9 (CRISPR associated) was proposed. CRISPR Cas9 is a technological development enabling genomic editing and regulation through a simpler, more efficient, and cost-effective mechanism. It has facilitated the creation and implementation of revolutionary tools with ample application in biological and medical sciences [10, 11].

In stark contrast to the previously mentioned use of DNA-editing proteins, the CRISPR system and its associated protein Cas9, responsible for breaking the DNA double helix, rely on the recognition and interaction with target DNA sequences mediated by the coupling with a short RNA sequence known as single guide RNA (sgRNA). This sgRNA sequence is complementary to a specific DNA region, thus directing the Cas9 protein to that specific genome location. By modifying the sgRNA sequence any desired genome region can be targeted. This feature has positioned CRISPR Cas9 as the leading gene-editing strategy today [12, 13, 14], enabling its implementation in multiple organisms [15, 16, 17]. **Table 1** compares some relevant characteristics of the most common genome editing technologies.

Table 1. Comparison between some relevant characteristics of the most common genome editing technologies.

Parameter	ZFN's	TALENs	CRISPR Cas9
Design simplicity	Complex, it needs customized protein for every DNA sequence.	Complex, identical repeats are multiple, technical issues of engineering are common.	Simpler, available sgRNA versions can be designed in a relatively easy manner.
Engineering feasibility	Low	Low	High
Specificity: Low/High	Low	High	Low (it is possible to be increased due to Nickases)
Cost	Low	Medium	Low
Efficiency	Normal	Normal	High
Mutagenic throughput	Low	Moderate	High

In this review we describe the molecular foundations of gene editing using CRISPR Cas9, as well as some of its recent applications in the biomedical and biotechnological fields. Additionally, we discuss some ethical aspects related to its application.

2. Origin of the CRISPR Cas9 system

Many bacteria, and nearly 95 % of the archaea described to date, possess an immune system that counters the attack of bacteriophage viruses by cutting the invading viral genetic material. However, bacteria go beyond simply degrading invading viruses or plasmids into pieces. These

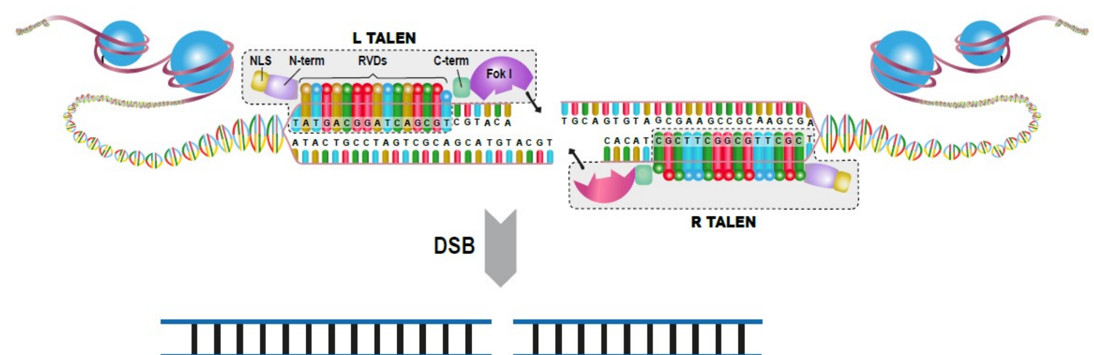

Figure 2. Sketch of a TALEN: Similar to ZFNs, TALENs comprise a non-specific DNA cleavage domain (FokI) fused to a customizable sequence-specific DNA binding domain (RVDs) to generate DSBs. This DNA binding domain consists of a highly conserved repeat sequence of the transcription activator-like effector (TALE), which is a protein originally discovered in the phytopathogenic bacterium *Xanthomonas* that naturally alters gene transcription in host plant cells.

microorganisms can integrate invading DNA fragments into their genetic material, thus creating the CRISPR Cas9 system in conjunction with the Cas9 protein nuclease, which degrades DNA sections [18, 19].

To illustrate the functioning of the bacterial defense system against a viral infection, one considers two phases. In the first phase, a bacterium gets infected by a bacteriophage virus. Thanks to its constitutive nucleases, a bacterium partially degrades the invading genetic material, integrating these foreign DNA fragments into its genetic material. In the second phase, when the same virus infects the bacterium again, the CRISPR locus transcribes two RNA molecules (crRNA and tracrRNA) from DNA segments of the same invader previously integrated into the bacterial genome [20]. These RNA molecules guide the Cas9 nuclease with greater precision, resulting in cuts in specific segments of the exogenous DNA efficiently, thereby deactivating it and halting the infection [21]. For biotechnological applications, the complex of crRNA and tracrRNA characteristics of bacteria was successfully simplified by a single guide RNA (sgRNA) [22]. See **Figure 3**.

The Cas9 protein exerts its nuclease activity on the genome region to be degraded, called the Protospacer Adjacent Motif (PAM). This PAM region is recognized jointly by the Cas9 protein and sgRNA. This PAM region is specific to each Cas nuclease and depends on the type of bacteria that produces it [23].

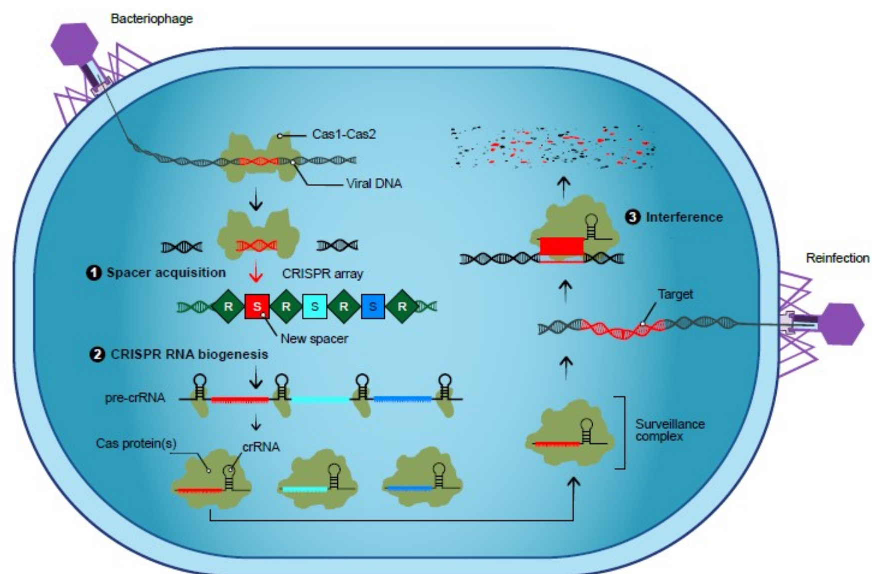

Figure 3. The CRISPR/Cas9 system is the bacterial defense mechanism. In the first step, selected sequences of the invading DNA, so-called protospacers, are integrated into the CRISPR locus. After transcription from the CRISPR locus, the resulting pre-crRNAs are processed into mature crRNAs, each consisting of a single spacer and a repeat sequence. These crRNAs are bound by the Cas endonuclease (step 2), forming the active effector surveillance complex, which recognizes the foreign nucleic acid by base cleavage during subsequent reinfection and degrades it, leading to interference (step 3).

3. Molecular mechanism of the CRISPR/Cas9 system

The bacterium *Streptococcus pyogene*'s Cas9 enzyme is a multi-domain endonuclease consisting of 1368 amino acids. Through the action of its two nuclease domains, HNH and RuvC, which catalyze the breakage of the complementary and non-complementary strands of the DNA, respectively, the enzyme cuts the double helix of the target DNA three base pairs upstream of the PAM sequence [24]. Recognition of the target DNA depends strictly on the presence of a short protospacer adjacent motif (PAM) immediately downstream of the region of the DNA that pairs its bases with the sgRNA [25].

A "seed" region close to the PAM sequence, consisting of eight to twelve nucleotides in the target DNA-sgRNA junction section, is essential for Cas9 binding. While interactions in the seed region are sufficient for target binding, cleavage of the target section of DNA requires more extensive target DNA-sgRNA interactions. However, Cas9 tolerates mismatches within the guide-target region; this fact is the likely leading cause of gene editing effects outside the target region. The analysis of crystal structures and molecular dynamics studies of Cas9 in its free and nucleic acid-bound states revealed a rearrangement resulting from the binding of Cas9 to DNA, driven precisely by the sgRNA [26].

Achieving site-specific DNA recognition and cleavage requires Cas9 to assemble with a sgRNA molecule to form an active complex. The 20-nucleotide spacer sequence of the sgRNA confers specificity to the target DNA and plays a crucial role in Cas9 recruitment.

In CRISPR systems, mismatches in the seed region can severely impair or even abrogate nuclease action and cleavage of the target DNA, while close homology in the seed region often leads to off-target binding events [27].

Once Cas9 binds to its guide RNA, the complex is ready to search for a complementary target DNA. Target search and recognition require a complementary base pairing between the 20-nucleotide spacer sequence of the sgRNA and a segment in the target DNA, along with a conserved PAM sequence adjacent to the target site. Consequently, single mutations in the PAM sequence can inactivate the cleavage activity of Cas9. The native PAM sequence for the commonly used *S. pyogene*'s Cas9 nuclease is 5-NGG-3, where N can be any of the four DNA bases. Various experiments have shown that Cas9 initiates the target DNA search process by seeking a suitable PAM sequence before determining possible guide RNA complementarity [28].

Recognition occurs through three-dimensional collisions, in which Cas9 rapidly dissociates from DNA that does not contain the appropriate PAM sequence, with a dwell time depending on the complementarity between the guide RNA and adjacent DNA when an appropriate PAM is present. Once Cas9 has found a target site with the appropriate PAM, it triggers local DNA fusion at the nucleation site adjacent to the PAM, followed by the invasion of the RNA strand to form an RNA-DNA hybrid and a displaced DNA strand. Adequate complementarity between the seed region of the sgRNA and the target DNA is necessary for Cas9-mediated DNA cleavage.

The PAM sequence's first base, designated N, remains paired with its counterpart but does not interact with Cas9. The conserved PAM GG dinucleotides read directly into the major groove through specific hydrogen bond interactions with two arginine residues (R1333 and R1335) located in a hairpin of the terminal domain of Cas9. Upon PAM recognition and subsequent formation of the RNA-DNA duplex, the Cas9 enzyme is activated for DNA cleavage. Cas9 uses two nuclease domains: a well-conserved RuvC domain consisting of three split RuvC motifs

and an HNH domain residing in the middle of the protein. Each domain cleaves one strand of the target dsDNA at a specific 3-bp site of the NGG PAM sequence to produce a predominantly blunt-ended double-strand break (DSB) [28, 29].

4. Fundamentals of gene editing with CRISPR Cas9

The widely accepted model for the functioning of the Cas9 protein postulates that, in the absence of a sgRNA, it is self-inhibited by a conformation in which its different sections for DNA recognition and nucleotide removal block one another. Once the Cas9 nuclease is activated, through its interaction with the sgRNA, this protein changes its initial conformation, initiating a three-dimensional diffusion process locating the target region of the DNA and performing a local separation of the DNA double helix. Then, the protein carries out its biological action, removing one or more nucleotides from both strands of the DNA double helix, which are adjacent to the PAM segment [30, 31]. The loss of the integrity in the DNA double helix triggers an automatic response within the cell nucleus, where enzymes repair the genetic material. Depending on factors such as the cell cycle stage, a cell can initiate two alternative strategies to repair its DNA [32].

Firstly, the cell can use non-homologous end joining (NHEJ), which involves correcting DNA integrity via random insertions of nucleotides to join the affected DNA strands. In the vast majority of cases, this leads to mutations that inactivate genes, causing shifts in the genetic reading frames or affecting the primary structure and, consequently, the functioning of the encoded protein [33]. Secondly, the cell can utilize a specific segment of DNA to repair the affected genetic material. This strategy is known as homology-directed repair (HDR). The HDR pathway entails related sub-pathways that utilize DNA strand invasion and template-directed DNA repair synthesis to achieve a high-fidelity repair [34]. Thanks to this process, genetic material corrections have been made in multiple experiments using CRISPR Cas9, functionalizing a Cas9 protein with a particular DNA segment that the cell uses as a template to insert specific indicated nucleotides, thus avoiding random repair and making precise changes or corrections in the sequence [35, 36, 37], see **Figure 4**.

In the case of the Cas system derived from *S. pyogenes*, the PAM section (NGG, where N refers to any nucleotide, followed by two guanines) is present on average every eight base pairs inside the DNA, implying that almost any gene can be edited [38]. Cas systems from other bacterial species have different target PAMs, further expanding the range of sequences amenable to editing [39].

The Cas9 variant isolated from *S. pyogenes* is the best-known nuclease in CRISPR experiments but has certain limitations. First, its complementarity specifications are not very strict and can recognize other PAM sequences, such as 5'-NAG-3' and 5'-NGA-3'. Thus, it can sometimes cut non-specific DNA sequences [40]. These off-target effects could cause detrimental effects to a cell and are especially concerning for the application of this method in humans. Second, its relatively large size challenges its delivery into cells with common viral vectors such as adeno-associated viruses (AAV). Finally, the strict PAM sequence requirement limits the target DNA that can be manipulated by this method [41].

Cas9 can also be isolated from several bacterial species. Bacterial Cas9 nucleases differ mainly in the PAM sequence they require for cleavage. However, some of them stand out for their particular features. *Staphylococcus aureus* Cas9 is an increasingly popular Cas nuclease; of only 1053 amino acids in length, it is approximately 1 kb smaller than the traditional *S. pyogenes* Cas9. This

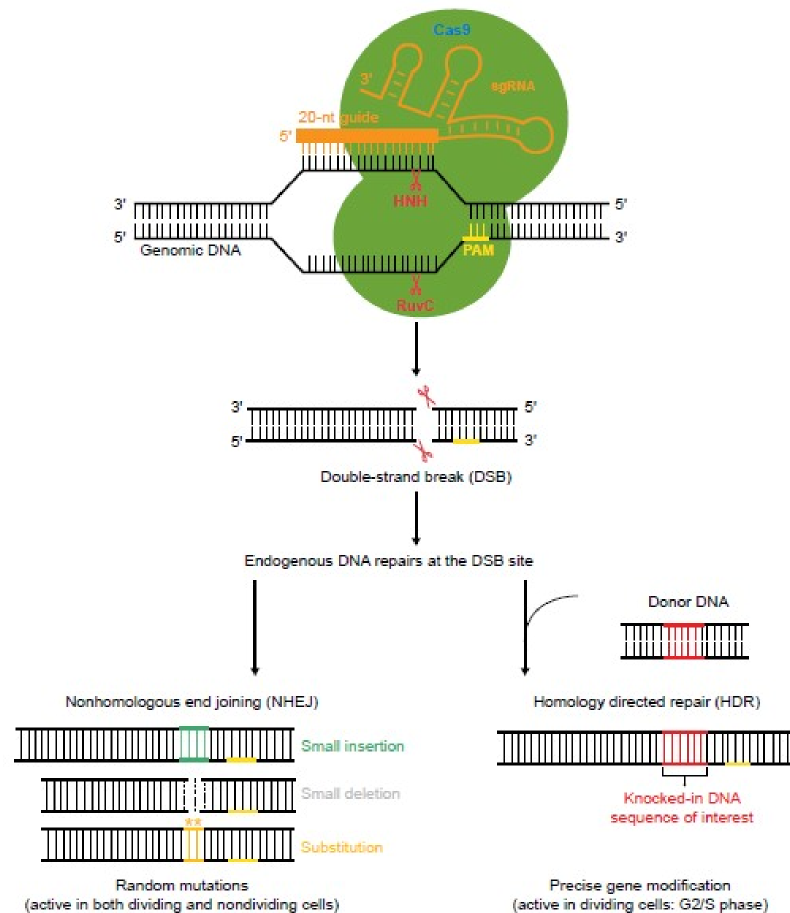

Figure 4. CRISPR Cas9: RNA-guided endonuclease. Cas9 (green) binds its target DNA via a protospacer-sgRNA (yellow) duplex. The two catalytic subunits (the HNH and RuvC-like domains) introduce a blunt-ended double-strand break (DSB) three nucleotides upstream of the protospacer-adjacent motif (PAM). DSBs can be repaired by the non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms. NHEJ introduces smaller insertions and deletions, resulting in an alteration of the gene sequence. In contrast, entire genetic sequences can be replaced or introduced with HDR, where the provided donor DNA serves as a template for DNA repair.

size difference allows for *S. aureus* Cas9 to be straightforwardly packaged into viral vectors, such as FDA-approved AAVs, making it ideal for clinical applications. Furthermore, *S. aureus* Cas9 recognizes a 5'-NNG-3' PAM sequence and generates blunt-ended DSBs [42].

So far, researchers have utilized Cas9 variants from *Streptococcus thermophilus* [43], *Neisseria meningitidis* [44], and *Campylobacter jejuni* [45], among others, in CRISPR experiments [46]. Another variant of Cas9 was isolated from the bacteria *Streptococcus canis*. Its sequence has a striking similarity (89.2% sequence homology) to that of *S. pyogenes* Cas9 but differs in one salient aspect. It requires a less stringent PAM sequence (5'-NNG-3 instead of 5'-NGG-3') for its activity, greatly expanding the genomic target for CRISPR editing [47]. More recently, a Cas9 variant was identified in *Staphylococcus auricularis*, which recognizes a 5'-NGG-3' PAM sequence, has high editing activity, and is sufficiently small for delivery in AAV [48].

The fidelity of the CRISPR system is directly related to its tolerance to DNA mismatches. The possibility that Cas9 cuts DNA at off-target sites is a crucial safety concern, especially for future clinical applications of CRISPR. To address this issue, researchers developed variants by mutating

the catalytic domains of Cas9 while retaining specificity. As a result, these variants cut a single strand of DNA at the desired locus rather than creating a double-strand break [49]. Since a break in one of the DNA strands is repaired with higher fidelity than a double-strand break (DSB), two nickases targeting opposite DNA strands, each with a different guide RNA, could be used to create a DSB with high fidelity. Using this approach, Cho *et al*. reported that it generated a relatively long, 1-kb deletion in human cells with off-target effects reduced below the limit of detection [50].

This technology's implementation has positively impacted the efficiency of transgenic organism generation, from fungi and plants to a wide array of animals. Furthermore, this technology greatly facilitates the generation of disease models, particularly for genetic disorders and diseases such as cancer, enhancing our understanding of the molecular dynamics of these pathological processes [51].

CRISPR Cas9 was used to edit multiple locations within the genetic material by simultaneously introducing several sgRNAs. This approach has potential applications in generating large-scale chromosome rearrangements within cells. For instance, the induction of a pair of double helix breaks in nearby regions within the same chromosome can lead to specific deletions or inversions of the intermediate DNA segment. Meanwhile, generating simultaneous double helix breaks in different chromosomes leads to specific translocations. These targeted genetic material rearrangements may be useful in creating disease models by producing, in a controlled manner, the chromosomal changes that characterize human diseases such as cancer and some inherited genetic disorders [52, 53].

Below, we describe examples of recent gene editing applications achieved with CRISPR Cas9 technology. These examples, which include gene silencing, gene activation, gene insertion, targeted mutagenesis, gene correction applications, etc., illustrate some of the most promising applications of this technology in biomedical and biotechnological fields.

5. Biomedical and Biotechnological Applications of CRISPR Cas9

5.1. CRISPR Cas9 based technology for Cell Biology

Gene editing mediated by the CRISPR Cas9 system has found wide application in reverse genetics studies, aiming to understand the role of specific genes. Moreover, this technique has been implemented in disease modeling, impacting positively the development of novel pharmacological treatments for genetic and infectious diseases. This is primarily possible due to the relative ease of redirecting Cas to a new site in the DNA by creating a new sgRNA that pairs with the desired DNA targeting site close to the PAM.

Thanks to gene editing tools, the fundamental aspects of the functioning of various cell types and tissues have been explored. CRISPR Cas9 has assisted the expansion of knowledge on the cellular and molecular biology of different cell types. Romanelli *et al.*, [54] developed a transgenic mouse line producing the Cas9 proteín within brown adipocytes. The authors implemented gene editing in brown mouse adipocytes to analyze the function of various active genes in such cells. They found that the local administration of adenoviruses containing CRISPR Cas9 and various sgRNAs in the brown adipose tissue of adult mice allowed gene editing of those cells and reduced by at least 90% the expression of proteins such as adiponectin, adipose triglyceride lipase, fatty acid synthase, perilipin 1, and stearoyl-CoA desaturase 1. Also, the administration of multiple sgRNAs led to simultaneous knockout of up to three genes without substantial accumulation of mutations

outside the target gene segment. Additionally, the implementation of CRISPR Cas9 allowed the creation of a mouse model with a deactivated inducible uncoupling protein (Ucp1), providing access to the evaluation of the effects of Ucp1 loss in adaptive thermogenesis processes. These biotechnological advances made it possible to demonstrate increases in heat production through the acceleration of peroxisomal lipid degradation processes.

Similarly, the communication processes between cells underlie the proper functioning of organs and tissues and maintain balance in the body. In this context, β-catenin, a central protein for cell adhesion and with a fundamental role in cell signaling processes related to cell proliferation and survival, emerged as a valid study target for gene editing. To study the impact of deletion of the CTNNB1 gene, which codes for the β-catenin protein, Guan *et al.*, [55] used CRISPR Cas9 to silence this gene and thus analyze its influence on cell behavior. The results showed that the deletion of the target gene resulted in the collapse of the cadherin-β catenin- α catenin complex, which in turn reduced the adhesion and proliferation capacities of the cells under study while lowering the expression of various genes related to cell cycle progression.

To advance the understanding of the mechanisms involved in lipid metabolism, conditions derived from mutations in the MTTP and SAR1B genes, such as abetalipoproteinemia and chylomicron retention disease, were targets for CRISPR Cas9 gene editing. Bordat and his group [56] explored potential therapeutic options, developing knockout cell models using the CRISPR Cas9 technique. The analysis of the results confirmed the introduction of mutations with subsequent inactivation of the corresponding proteins, demonstrating altered formation of lipid and triglyceride droplets and cholesterol secretion in the genetically edited cells. The silencing of the MTTP gene led to a more severe phenotype than the silencing of the SAR1B gene, confirming clinical observations. The cellular models generated provided an effective tool for experimenting with therapeutic strategies and enabled advancing the understanding of the mechanisms involved in lipid metabolism.

As mentioned earlier, the intervention in a cell by the CRISPR Cas9 system results in breaks in specific segments of its genome. Typically, the cellular machinery mends these breaks through non-homologous repair, characterized by being an imprecise mechanism and highly prone to generating insertions or deletions of genetic material. This process involves the simple reconnection of the cut segments.

In contrast, the homologous repair pathway is much more precise but lacks efficiency. This repair requires a DNA template, which, under cellular conditions, is available only in the S and G2 phases of the cell cycle or conveniently can be an artificial exogenous DNA source. Consequently, this genome editing technology allows for the insertion of exogenous sequences at the cutting site [57].

Thus, DNA repair templates can be designed for genetic modifications, such as nucleotide substitutions or insertions of gene segments. However, these modifications are not without risks, as insertions and deletions can occur with the intended insertion [58].

In this context, Meca-Cortés and his team accomplished the insertion of therapeutic genes into specific genomic regions of human mesenchymal stem cells. They utilized electroporation as a transfection method and Crispr Cas9 as a strategy for inserting a 3kb DNA segment encoding the synthesis of cytotoxic proteins against glioblastoma. Consequently, the transfected mesenchymal cells exhibited a notable therapeutic capacity against tumor cells in vivo. This determination indicated that the therapeutic efficacy stems directly from the expression of the inserted genes [59].

Similarly, Tung and collaborators introduced a system based on CRISPR Cas9 to activate and repair genes in mouse hematopoietic stem cells. CRISPR/Cas9 ribonucleoproteins, in conjunction with donor DNA templates, crafted as recombinant adeno-associated viruses, led to the activation of genes at the Lmnb1 and Actb loci of mouse hematopoietic cells in vitro. Subsequently, these cells successfully reconstituted immune cell lineages in recipient mice. Through this approach, it became feasible to restore B and T cell development in vivo, confirming their functionality and presenting an effective strategy for studying gene function in the hematopoietic system and modeling hematological disorders [60].

5.2. Treatment for Monogenic Disorders

Genetic disorders and congenital abnormalities occur in approximately 2 % - 5 % of all live births, accounting for up to 30 % of pediatric hospital admissions and contributing to nearly 50 % of childhood deaths in industrialized countries [61].

To date, approximately 8000 different types of these pathologies have been described. Most monogenic diseases are caused by individual nucleotide changes within a particular gene, and disorders of medical relevance, such as sickle cell anemia, Becker and Duchenne muscular dystrophies, and Huntington's disease, among many others, belong to this category [62].

Although significant advances have recently been made in understanding the molecular and pathophysiological processes underlying these disorders, we are still far from providing definitive treatments to affected patients. In this scenario, the possibilities offered by gene editing technologies such as CRISPR Cas9 represent a possibility of developing effective therapies.

Huntington's disease, a neurodegenerative disorder characterized by a progressive decline in cognitive, motor, and psychiatric function caused by a repeated expansion of a specific section of the Huntington (HTT) gene, could be a target for gene therapy through the implementation of CRISPR Cas9. The development of the disease results in the production of a mutant protein that forms inclusions, destroys neurons, and affects the general functioning of the brain. Using CRISPR Cas9 in a mouse model, Ekman and his group [63] developed a gene-editing protocol, disrupting the expression of the mutant HTT gene, demonstrating a 50 % decrease in neuronal inclusions and establishing a significant increase in mice motor functions and lifespan. In the case of Huntington's disease, gene editing mediated by CRISPR Cas9 could confer protection to neurons against degeneration of the striatum.

Analogously, Duchenne muscular dystrophy involves mutations that alter the structure of the DMD gene, which codes for the protein dystrophin, impairing the integrity of the muscle fibers of affected individuals and gradually deteriorating their muscle cells. Deleting one or more specific segments of the DMD gene can result in a truncated but still functional protein. Tabebordbar and colleagues [64] developed and tested a CRISPR Cas9 gene editing approach in a mouse to induce the deletion of an exon of the DMD gene, thereby generating the expression of an alternate version of dystrophin. Consequently, the target DNA was edited, and the reading frame of the DMD gene was restored in myofibers, cardiomyocytes, and muscle stem cells after local or systemic administration. The treatment with CRISPR Cas9 recovered, in part, muscle functional deficiencies and generated a group of endogenously corrected myogenic precursors in mouse myocytes.

Similarly, one of the leading molecular causes of Alzheimer's disease, the most common cause of dementia, is altered levels of the *Tau* protein in brain tissue. The MATP gene encodes the *Tau* protein. To date, six isoforms of this protein have been found in the central nervous system, illustrating

its biological importance. The *Tau* protein maintains the stability and binding of cytoskeleton components. Under pathological conditions, *Tau* separates from microtubules and aggregates, forming neurofibrillary tangles, especially in cholinergic cells, leading to a deficit in acetylcholine production and impaired neuronal synapses [65].

Through a large-scale analysis of the use of CRISPR Cas9 DNA editing technology, Sanchez and his group [66] identified 481 genes whose modification directly affects brain *Tau* levels. Specifically, the alteration of 215 of the identified genes increases the levels of the pathologic protein, and the inactivation of 266 genes reduces the levels of the *Tau* protein. These findings allow researchers to make significant advances in our understanding of the biology of the disease and establish new strategies to modify the levels of the pathogenic protein. In addition, tuberous sclerosis protein 1 (TSC1), a critical component of the mTOR signaling pathway, was found to regulate negatively *Tau* levels in neurons. Thus, this finding provides another specific approach to potentially control Alzheimer's disease progression.

CRISPR Cas9 can also help activate specific genes. For example, through a CRISPR Cas9 variant called CRISPR On, the SCN1A gene responsible for encoding the synthesis of Nav1.1 proteins, which are voltage-gated sodium channels of great relevance in the functioning of GABAergic neurons, can be activated. Mutations of this gene occur in approximately 80% of patients with Dravet syndrome, who develop epileptic seizures (tonic-clonic or clonic), often after febrile episodes that occur between 4 and 10 months after birth. This syndrome frequently produces intellectual disability, autism, and sudden death. After the design of the sgRNA sequences and their entry into cells of a murine model through an adeno-associated virus, Yamagata *et al.* [67] showed that the CRISPR On strategy increases the expression of the SCN1A gene in the brains of SCN1A haplodeficient mice. This improvement ameliorated epileptic phenotypes, increased the temperature threshold of febrile seizures, and favored the behavioral manifestations of the mice in the study.

Worldwide, approximately 300 000 people are born with sickle cell anemia annually, making it the most prevalent monogenic blood disorder globally [68]. Sickle cell anemia develops from a single nucleotide change in the HBB gene, leading to the substitution of a hydrophilic glutamic acid molecule with a hydrophobic valine molecule at position 6 of the corresponding protein. This alteration causes the resultant hemoglobin to polymerize under acidic or hypoxic conditions, distorting erythrocytes and reducing their functionality and longevity. This, in turn, results in chronic hemolysis, accompanied by episodes of severe pain, organ damage, and premature mortality [69]. Presently, standard treatments primarily focus on providing symptomatic relief without fundamentally addressing the root cause of the disorder. The most impactful therapeutic measures involve periodic blood transfusions, administrating hydroxyurea or other small molecules, and controlling pain as needed [70]. Allogeneic hematopoietic stem cell transplantation is the ultimate therapy for sickle cell anemia. Nevertheless, the availability of immunologically compatible donors is limited, and there is a risk of host rejection [71].

In this context, it is worth noting that despite the development of various gene therapy clinical trials utilizing lentiviral vectors for sickle cell anemia management, challenges persist regarding the safety and efficiency of transduction. An alternative approach involves the correction of the HBB gene in hematopoietic stem cells using CRISPR/Cas9 and a recombinant adeno-associated virus, specifically rAAV6, as the donor DNA template. Based on previous results, [72, 73, 74] The first human clinical trial, approved by the FDA for the correction of gene damage, was opened. To gain a better understanding of the molecular mechanism of genome editing using CRISPR Cas9 and rAAV6 and to ascertain the safety of genome-edited pharmaceuticals, Xu and his group [75]

developed assays to detect changes in the reagents involved and cellular responses during and after genome editing. The researchers consistently observed high frequencies of genetic correction, along with stability and preservation of the activity of the rAAV6 donor template virus, even if not stored at -80 °C. In conclusion, the methodology demonstrated high efficiency, reproducibility, and safety, characterized by low off-target activity, minimal translocations, absence of tumorigenesis in mice, and low transcriptional perturbation.

In the same way, around 300 gene mutations in the adult β-globin gene give rise to hereditary diseases known as β-thalassemias. These conditions portray a low or absent adult hemoglobin (HbA) production [76]. Together with sickle cell anemia, thalassemia syndromes constitute the genetic diseases with the highest impact in developing countries. The lack of genetic counseling and prenatal diagnosis in these regions has contributed to maintaining a very high frequency of these conditions in the population. The management of patients with β-thalassemia consists primarily of blood transfusions, chelation therapy, and, alternatively, bone marrow transplantation [77]. In a recent communication, Cosenza and his group [78] presented the correction of the β039-thalassemia mutation, one of the most frequent mutations observed in various regions worldwide, through the implementation of CRISPR Cas9. This nonsense mutation induces the introduction of a stop codon, leading to a truncated, non-functional β-globin and the β0-globin phenotype. The results obtained post-genetic intervention demonstrated the presence of normal β-globin genes after correcting the β039-thalassemia mutation in erythroid precursor cells from homozygous patients with β039-thalassemia. Following the intervention, the authors observed an accumulation of corrected β-globin mRNA and a significant and efficient "de novo" production of β-globin and adult hemoglobin (HbA).

Upon analyzing the potential genomic toxicity of the editing procedure, researchers have found low levels of insertions or deletions, evidencing the absence of effects outside of the target gene. Thus, the developed protocol could be a starting point for creating efficient and safe processes for editing erythroid cells derived from patients with thalassemia.

Furthermore, the advancement of our understanding of human genome editing processes has significantly influenced medical endeavors to treat a broad spectrum of human disorders, ranging from infections to cancer. The various possibilities provided by the CRISPR Cas9 technology in generating specific gene knockout processes have raised valid questions about its capability to perform effective genetic editing in primary T-cells. This prospect emerges as a highly relevant alternative with the potential to revolutionize the field of immunotherapy [79].

CAR T-cell therapy entails the genetic modification of autologous T-cells from patients to express a CAR (chimeric antigen receptor). CARs are synthetic receptors that typically comprise an extracellular antibody-derived target-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling moiety capable of activating cells [80].

CAR-programmed T-cells can specifically recognize and destroy cells expressing particular antigens without the restriction of the major histocompatibility complex (MHC). Clinical data have demonstrated that CAR T-cell therapy can induce complete and durable remissions in patients with various hematologic and solid cancers, achieving striking response rates of 80 % – 100 % [81].

Despite the promising efficacy of CAR T-cell therapy, several challenges await solutions, including insufficient quantity and poor quality of autologous T-cells, exhaustion of CAR T-cells, potential self-destruction, and uncontrollable proliferation. The scientific community is continually working on optimizing the designs of CAR-programmed T-cells to overcome these limitations.

In this context, incorporating additional functional elements into the next generation of cells, such as chemokine receptor genes to enhance T-cell trafficking, interleukin genes to increase potency, or on-off switches or suicide genes to improve security, represents valuable strategies in the present day [81, 82].

In this scenario, implementing the CRISPR Cas9 technology will further expand the landscape of T-cell engineering. In addition to activating functional genes, such as those related to interleukins and suicide processes, other strategies, including the elimination of endogenous genes to develop universal cells available on the market or the alteration of inhibitory receptors to improve the efficiency and safety of cells, among others, validate the inference that CRISPR Cas9 technology is ushering in a new era for CAR T-cell therapy [83].

5.3. Applications in Cancer Biology

Despite multiple advances in fields such as radiotherapy, chemotherapy, and surgery, which constitute the main tools to treat cancer, individualized treatments for this disease are still in their early stages of development. Additionally, the high toxicity of the drugs and radiation used in conventional therapies justifies the need for new therapeutic approaches against cancer. Among the recent advances in molecular biology, which make genetic material editing a viable process and allow direct observation of responses derived from genetic modulation, CRISPR-Cas9 technology naturally stands out. This tool provides researchers with opportunities to evaluate, for instance, how tumors respond directly to drug therapies. Moreover, CRISPR-Cas9 emerged as a tool to generate oncolytic viruses [84] or enhance immune cell responses [85].

In this context, we highlight Waldt and collaborators' work [86], who, using CRISPR-Cas9, generated an animal model for studying potential drugs to treat the most frequent primary intracranial tumor: meningioma. Meningioma is a cancer that arises in the meningeal membranes surrounding the brain and spinal cord. Apart from surgery and radiotherapy, treatment options for meningioma are usually limited. More than 50 percent of meningiomas originate from mutations in the neurofibromatosis type 2 (NF2) gene, which encodes the synthesis of the tumor suppressor protein called Merlin. Merlin is responsible for controlling cell cycle progression and the expression of genes involved in cell growth arrest and regulation of cell apoptosis.

To identify drugs that can specifically target patients with meningiomas driven by mutations in the NF2 gene, the researchers selected a group of cells in which the NF2 gene was silenced with CRISPR-Cas9 and implanted into the skulls of mice. Thus, the authors also generated an animal model to study potential drugs to treat meningioma. With this advancement, it became possible to develop the pharmacological inhibition of the focal adhesion protein kinases (FAKs), thereby potentially developing an effective therapy for meningiomas caused by NF2 gene mutations. Similarly, it was demonstrated that mutations in the gene that codes for the epidermal growth factor receptor protein (EGFR) play a central role in the progression of about 15% of non-small cell lung cancer cases.

To treat cancer caused by mutations in the EGFR gene, Koo *et al.* [87] used CRISPR Cas9 and the corresponding sgRNA to eliminate the mutant allele in cancer cells. By altering a single nucleotide within the target gene, they achieved a precise break in the target gene sequence with high specificity, leading to the destruction of cancer cells and tumor reduction in a mouse model of human lung cancer.

Similarly, osteosarcoma, the most common type of primary malignant bone tumor with an average survival period of less than one year after metastasis, was targeted for CRISPR Cas9 research. Feng and his group [88] focused on protein kinases (CDKs), which regulate cellular functions and are essential for tumor generation and evolution. The authors used the CRISPR Cas9 system in human osteosarcoma cell lines to evaluate the potential impact of deleting the CDK11 and its corresponding protein on the migratory activity of osteosarcoma cells. The knockdown of CDK11 by CRISPR Cas9 significantly decreased cell viability and induced cell death in osteosarcoma cell lines, reaffirming the therapeutic value of using CRISPR Cas9 to treat this type of cancer.

However, the development of tumors is a complex process, and not all mutations contribute equally to this process. Consequently, distinguishing those essential mutations for tumor growth and survival is pivotal. Nevertheless, the complexity of this task is evident.

To investigate some of the genetic vulnerabilities of cancer cells, Sayed and colleagues [89] utilized a colorectal carcinoma cell line. They engineered 100 different sgRNAs, which, along with a Cas9 nuclease, generated mutations in key genes within malignant cells. Analysis of the genetic material of cancer cells revealed that mutations in the UTP14A gene directly affect cell growth and tumor evolution. This finding allowed the researchers to recognize the UTP14A tumor gene as a protoagonist in cancer proliferation and survival.

Similarly, tumor cell metastasis in pancreatic cancers is enhanced by the hypoxic microenvironment of tumors. In this type of cells, high levels of the HIF-1α protein, responsible for regulating various survival and proliferation processes in hypoxic environments, are of great relevance. Taking these insights into account, Li and his group [90] implemented CRISPR Cas9 to suppress HIF-1α protein levels in tumors. To achieve controlled delivery of the gene editing system, the researchers used liposomes functionalized with peptides related to proteins of the tumor cells as a vehicle. This enabled the effective delivery of the Cas9 protein and the corresponding sgRNA. Consequently, deep penetration of gene editing in tumor spheroids was achieved, resulting in significant antimetastatic effects and increasing the survival time of the studied animals without inducing toxicity in normal pancreatic cells.

5.4. Antiviral applications

Although humanity has overcome the most significant impact of the COVID-19 pandemic, some infectious diseases, such as those caused by the human immunodeficiency virus (HIV), the human papillomavirus (HPV), or Hepatitis B virus (HBV), among others, persist as potential causes for pandemics. The CRISPR Cas9 gene editing technology can provide humanity with new therapeutic alternatives to fight viruses. This technology confers novel antiviral capabilities to animal hosts by modifying their genomes or altering processes related to viral replication. Even though this technology offers notable opportunities to develop new treatments for human viral infectious diseases, numerous difficulties and limitations still need to be overcome.

In this context, Abbott *et al.* [91] developed a strategy based on CRISPR Cas9 for SARS-COV2 virus infection in human lung epithelial cells. The researchers designed specific RNA sequences to guide the Cas13 nuclease to selected regions of the virus genome. This intervention significantly reduced viral loads in the studied cells. A parallel bioinformatics analysis conducted alongside the viral gene editing showed that targeting only six regions of the genome of known coronaviruses could inhibit by 90% the infection processes by these pathogens.

As previously mentioned, viral gene editing, using CRISPR Cas9, can be implemented to prevent the production of various viral infection proteins, for instance, those directing the integration of viral genetic material into the host cell genome, the synthesis of new copies of the virus, or the synthesis of envelope proteins. Herskovitz and his group [92] identified various regions of the genetic material of the HIV-1 virus, including the TAT gene and the env gene, and, when treating HIV-1-infected human leukocytes, they observed significantly lower levels of virus replication. Ribonucleoprotein and sgRNA delivery via electroporation into latently infected cells resulted in up to 100 % viral excision.

This study demonstrated the validity of using this genomic editing strategy to eliminate the HIV-1 virus. However, challenges remain. For example, the genetic diversity of viruses, arising during their replication processes inside infected cells, results in viral resistance to multiple direct-acting antiviral molecules. In this scenario, recent CRISPR-based antiviral strategies aim at degrading viral nucleic acids in eukaryotic cells. For instance, the Cas13 enzyme, a nuclease related to the CRISPR system, can selectively target and effectively degrade viral RNA.

In this context, the versatility of the CRISPR Cas toolbox is one of its main advantages. Until now, researchers have primarily focused on the well-characterized Cas9 protein. However, recent approaches have demonstrated the antiviral potential of other Cas nucleases, such as Cas13. While Cas9 serves for DNA manipulation, the Cas13 family enables the targeting of RNA sequences. This feature is crucial in antiviral therapy since viruses can possess RNA or DNA as genetic material. Cas9 is effective against DNA and RNA viruses with dsDNA intermediates in cells. In contrast, Cas13 can directly attack RNA viruses, offering a safer alternative for in vivo applications as it cannot induce permanent genetic alterations in host cells.

Thus, Ashraf *et al.*, used the CRISPR Cas13 system to inhibit the proliferation of the hepatitis C virus (HCV) in mammalian cells [93]. Through computational analysis, the team identified various target sites within highly conserved regions of the pathogen's genetic material, revealing significant HCV replication inhibition and translation reduction in infected cells, with minimal effects on cell viability. Thus, the CRISPR Cas13 system prevents in vitro HCV infection, indicating its potential as a programmable therapeutic antiviral strategy.

Currently, the infection caused by the Human papillomavirus (HPV) does not have a complete cure, mainly due to the ability of the virus to modulate its activity in host cells. This feature allows the virus to evade host immune defenses and hinders its genetic material removal from latently infected cells. Recent studies have shown that HPV-induced tumor formation processes are related to viral genes E6 and E7 activity. Consequently, these genes are considered two leading therapeutic targets in gene therapy; precisely. Using a model system based on cervical cancer driven by the ongoing expression of the HPV E6 and E7 proteins, Jubair *et al.*, [94] demonstrated that CRISPR Cas9, delivered systemically in vivo through the use of liposomes, resulted in tumor elimination and complete survival in treated animals. The findings presented in this study provide compelling evidence for the efficacy of in vivo CRISPR Cas9 editing as a therapeutic intervention for preexisting tumors, even in the presence of substantial genetic payloads.

6. Bioethical aspects

The characteristics of CRISPR Cas9 gene editing technology, including its simplicity, effectiveness, and relatively low cost have led to a rapid increase in investigations exploring its potential therapeutic applications in various diseases [13, 95, 96]. As previously mentioned, this technology holds promise to develop experimental animal models, advancing our understanding of the

pathophysiology of multiple diseases and paving the way to cure genetic diseases and increase resistance against infections and other pathologies. Furthermore, gene editing can be performed in all organisms, potentially impacting the entire human environment.

Ensuring the safe and ethical applications of CRISPR Cas9 gene editing is a complex process with multiple regulatory challenges. The discussion surrounding the responsible use of gene editing has intensified, particularly following revelations regarding its application in human embryos edition [97, 98]. Thus, it is fundamental to discuss the case of He Jiankui, a scientist and biophysicist at Shenzhen University of Science and Technology in China and a Biotech entrepreneur, who drew the international scientific community's attention in November 2018 as he announced that, for the first time, he had genetically modified the genetic material of human babies. Jiankui raised the babies who were born by a surrogate mother. According to the researcher's statements, the twins, Lulu and Nana, were born that same month, and the genetic intervention procedure, developed with CRISPR-Cas9, was carried out safely. He claimed the twins would "look exactly like any other baby" [99].

In a subsequent interview, Jiankui stated that his research was driven by a duty to scientific advancement. He emphasized that after confirming the feasibility of the challenge, society must decide whether to ban or allow this type of technology. He expressed, "I understand that my work is controversial, but I believe families need this technology" [100].

During the international gene-editing conference held in November 2018 in Hong Kong, Jiankui explained that his objective with the controversial study was not to cure or prevent hereditary diseases but to attempt to generate a characteristic that few people possess: the ability to resist HIV infection. In this sense, it is crucial to note that the study, which culminated in the generation of babies with a "personalized" genome using CRISPR Cas9, does not constitute an official publication critically reviewed by other scientists, nor does it serve as an affirmation of the reliability and veracity of modern science [101].

As soon as Jiankui's statements became public, the medical and scientific community categorically condemned his work and drew attention to the urgent need to create a legal framework to prevent the repetition of this type of experiment.

At first glance, the negative impact caused by Jiankui's work could rely on the possibility of babies developing unexpected and even heritable health conditions; however, this study may open the door to attempts to produce genetically altered babies with given physical, intellectual, or athletic characteristics. It is also valid to mention that scientists fear negative reactions from society to research with genetic editing that does not necessarily involve embryos and that represents, as has been established throughout this document, great potential for treatment or disease prevention [102].

Numerous personalities, including Jennifer Doudna, a biochemist at the University of California, Berkeley, and one of the developers of the CRISPR Cas9 technique, were horrified by the study. They considered it illegal and once again demanded that similar studies be banned. The Chinese government itself stated that this study violated the country's regulations in the area of genetics.

From the academic perspective, the university to which Jiankui was affiliated stated that the researcher falsified ethics approval documents, used gene editing methods that were neither safe nor effective, and deliberately avoided supervision. Consequently, the university terminated his contract and initiated an investigation. The institution expressed that the editing of human embryos by the researcher alarmingly violated academic ethics and codes of conduct.

The Chinese scientific community also expressed its fierce opposition to the experiments in question, stating that any attempt to make changes to implanted human embryos was “madness” and that giving birth to a baby under such conditions is highly risky. In several countries, the study was deemed monstrous, highly irresponsible, unethical, and dangerous [103].

Ongoing discussions among the scientific and medical communities, bioethicists, and governments focus on the responsible use of gene editing while not impeding scientific research. Specifically, when it comes to editing the genome of human germ cells, the possibility of inherited changes must be carefully considered, as such changes could affect future generations. Regarding gene editing in somatic human cells, a thorough analysis of the circumstances justifying the alteration of the genetic material is necessary.

Generally, clinical trials on somatic cell genome editing cannot begin without due regulatory approval of an investigation dossier. This clinical protocol typically requires an institutional review board and continuous revision, sometimes encouraging public debate. Of course, country regulations vary in the research stage at which a treatment can be marketed and the terms for its withdrawal.

Gene edition-based treatment approval for clinical use depends chiefly on identifying whether expected benefits outweigh therapeutical risks, both labeled and intended. Clinical trial data are reviewed thoroughly within a structured framework that identifies needs, alternatives, areas of uncertainty, and avenues for risk management.

Product review teams must weigh scientific and clinical evidence and consider stakeholders’ conflicting perspectives on the value of benefits and tolerability of risks. They should consider the existence and effectiveness of alternative treatments, the severity of the disease, the risk tolerance of affected patients, and the possibility of obtaining additional information from post-marketing data. Such decisions require finding the right balance between high-quality evidence and early access, between benefit and risk, and between protecting the public and fostering innovation, which can improve health outcomes [104].

In global terms, the social, bioethical, and legal consequences of genome editing in human germline cells dominate academic and scientific debates. Researchers generally agree on allowing CRISPR Cas9 to generate human disease models and understanding their progression at the molecular level. However, they insist on prohibiting its use for enhancement and eugenics [105].

Through the analysis of safety concerns, difficulties of *in vivo* application, and ethical issues, it is possible to foresee that genetic editing of human embryos will not be viable in the immediate future. However, it is possible to assume that if solutions to these problems arise, CRISPR Cas9 could eventually be applied in germline cells [106]. While research into therapeutic uses of CRISPR Cas9 in the medical field and for clinical purposes will continue, legal frameworks that can eliminate violations of germline genome editing will need to be reevaluated, as currently, the risk of untargeted hereditary genetic mutation outweighs the potential benefits of treatment [107].

In addition to its potential to edit the human genome, CRISPR Cas9 also offers the opportunity to impact ecosystems at various scales through changing animal and plant genomes. For instance, by editing the genomes of strategic crops and livestock, their yields can be significantly increased. CRISPR Cas9 is also a likely viable tool for controlling populations of disease-carrying animals, such as malaria-carrying mosquitoes [108]. Within this context, primary concerns include our ability to predict the ecological impact of such genetically modified organisms and our capability to contain or control them once released into the wild.

The responsible development of Cas9 technology relies on our ability as a scientific community and as a society to establish and enact comprehensive policies governing genome editing technology. As the biotechnological and medical fields progress, it is crucial to create suitable conditions to ensure the safe and responsible utilization of gene editing technologies like CRISPR Cas9.

7. Conclusions

The impact of gene editing with CRISPR Cas9 has revolutionized multiple disciplines, including genomics, genetics, medicine, and cell physiology, among others. However, its complete potential remains unrealized, and breakthroughs are expected. Numerous research groups currently work on developing therapeutic alternatives for various oncological, neurological, muscular, and infectious diseases using CRISPR-Cas9. While there are high expectations for the potential benefits of precise gene editing, it is crucial to ponder the bioethical implications associated with this tool.

In the case of gene editing in humans, it is essential to restrict its application to somatic tissues to avoid potential modifications to future generations. Thus, the responsible use of CRISPR Cas9 represents a valuable tool for advancing scientific and technological knowledge.

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9. Conflict of interest

The authors declare having no conflicts of interest.

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Edición genética con CRISPR Cas9: aplicaciones recientes en biomedicina y biotecnología

Resumen: El uso de una tecnología novedosa y potente que permite la edición precisa del material genético de diversos organismos se está generalizando. Esta tecnología deriva de la maquinaria de defensa bacteriana y arqueobacteriana y se denomina CRISPR Cas9. A diferencia de otras herramientas de edición genética que dependen exclusivamente de proteínas, CRISPR Cas9 se basa en interacciones entre el ADN diana y una secuencia de ARN que guía la acción de la enzima Cas9 para alterar la estructura de un gen diana. Se pueden editar varias ubicaciones del genoma gracias a la facilidad de programación de diferentes secuencias de ARN guía, lo que facilita su uso e implementación. Además, versiones no activas de la proteína Cas9, guiadas por sus ARN correspondientes, pueden ser utilizadas en procesos de visualización de material genético o, más recientemente, para regular el proceso de transcripción. Teniendo en cuenta los recientes avances y posibilidades en la investigación biomédica y biotecnológica, debemos entender que la exploración de esta tecnología apenas comienza, y que sus eventuales aplicaciones influirán en el mundo que nos rodea en múltiples niveles. En esta revisión, describimos los fundamentos biológicos del funcionamiento de la nucleasa Cas9, así como aplicaciones seleccionadas de su uso en la edición y regulación de secciones específicas del material genético de diversos organismos. También discutimos algunas cuestiones bioéticas en torno a este tema.

Palabras Clave: crispr cas9; edición genética; enfermedad monogénica; biología del cáncer; terapia antiviral; bioética.

Edição gênica com CRISPR Cas9: aplicações recentes em biomedicina e biotecnologia

Resumo: O uso de uma nova e poderosa tecnologia que permite a edição precisa do material genético de vários organismos está se disseminando amplamente. Essa tecnologia deriva da maquinaria de defesa bacteriana e arqueobacteriana e é chamada de CRISPR Cas9. Ao contrário de outras ferramentas de edição genética que dependem exclusivamente de proteínas, CRISPR Cas9 depende de interações entre o DNA-alvo e uma sequência de RNA que guia a ação da enzima Cas9 para alterar a estrutura de um gene alvo. Vários locais no genoma podem ser editados graças à facilidade de programação de diferentes sequências de RNA guia, facilitando o uso e a implementação da tecnologia. Além disso, versões não ativas da proteína Cas9, guiadas por seus RNAs correspondentes, podem ser utilizadas em processos de visualização de material genético ou, mais recentemente, para regular o processo de transcrição. Considerando os recentes avanços e possibilidades na pesquisa biomédica e biotecnológica, devemos entender que a exploração dessa tecnologia está apenas começando, e que suas eventuais aplicações influenciarão o mundo ao nosso redor em múltiplos níveis. Nesta revisão, descrevemos os fundamentos biológicos do funcionamento da nuclease Cas9, bem como aplicações selecionadas de seu uso na edição e regulação de seções específicas do material genético de vários organismos. Também discutimos algumas questões bioéticas em torno desse tema.

Palavras-chave: CRISPR Cas9; edição gênica; doença monogênica; biologia do câncer; terapia antiviral; bioética

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