GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(REVIEW ARTICLE)

Check for updates

# Fundamentals of CRISPR-Cas9: Gene-editing technology and basic

America Fernanda Acosta-Soto [1], Diana Marisol López-Díaz [1], Jehosafat Esquivel-Ramírez [1,*], Joana Mora-Soriano [1] and Brissia Lazalde- Medina [2]

*[1] Faculty of Medicine and Nutrition, Universidad Juárez del Estado de Durango, Durango, Dgo., México.*
*[2] Department of Genetics, Faculty of Medicine and Nutrition, Universidad Juárez del Estado de Durango, Durango, Dgo, México.*

GSC Advanced Research and Reviews, 2024, 20(01), 042–049

Publication history: Received on 06 May 2024; revised on 23 June 2024; accepted on 26 June 2024

Article DOI: https://doi.org/10.30574/gscarr.2024.20.1.0223

## Abstract

The CRISPR/Cas9 system provides a robust and multiplexable genome editing tool, enabling researchers to precisely manipulate specific genomic elements and facilitating the elucidation of target gene function in biology and diseases. CRISPR/Cas9 consists of a nonspecific Cas9 nuclease and a set of programmable sequence-specific CRISPR RNA (crRNA), which can guide Cas9 to cleave DNA and generate double-strand breaks at target sites. Subsequent cellular DNA repair processes lead to desired insertions, deletions, or substitutions at target sites. The particularity of CRISPR/Cas9-mediated DNA cleavage requires target sequences matching crRNA and a protospacer adjacent motif located downstream of target sequences. Here, we review the molecular mechanism, applications, and challenges of CRISPR/Cas9-mediated genome editing and the clinical therapeutic potential of CRISPR/Cas9 in the future.

**Keywords:** CRISPR/Cas9; Genome editing; Therapeutic applications; History; Components

## 1. Introduction

Genetic perturbation enables scientists to probe gene function or correct mutations but is often intractable due to a technical challenge: site-specific nucleic acid-targeting. Targeted gene-editing has been achieved by induced double-stranded DNA (dsDNA) breaks in eukaryotic chromosomes but with challenging technologies based on engineering direct protein-DNA recognition. Transformative discoveries shaped the clustered regularly interspaced short palindromic repeat (CRISPR) CRISPR-associated (Cas) toolbox for genetic manipulation based on simpler RNA-guided DNA recognition. This toolbox now provides incredible scientific opportunities for curing genetic diseases, engineering desirable genetic traits, and new approaches to live-cell imaging, high-throughput functional genomic screens, and point-of-care diagnostics. This review summarizes the basic mechanisms of RNA-guided single-component CRISPR-Cas systems and their general applications[1].

Bacterial resistance process against foreign nucleic acid invasion:

- **Adaptation:** The bacteria recognizes foreign DNA and fragments it into smaller sequences which are then integrated into its CRISPR region.
- **Expression:** Upon encountering the same type of virus again, the bacteria transcribes the CRISPR sequences into pre-crRNA, which is processed into mature crRNA.
- **Interference:** The crRNA recognizes and binds to foreign DNA through complementary sequences and guides the Cas nuclease to cleave the invading DNA, thus destroying foreign nucleic acids, and achieving self-defense.

* Corresponding author: Esquivel- Ramírez Jehosafat

Genome editing using the CRISPR/Cas system:

The specific target sequence recognition of crRNA and DNA-cleavage activity of Cas nuclease, coupled with cellular DNA repair mechanisms, enable CRISPR/Cas-based genome editing.

Two repair pathways exist:

- **Non-homologous end joining (NHEJ):** Directly ligates broken DNA ends, which are prone to insertions or deletions (indels) that may create frameshift mutations resulting in gene knockout.
- **Homology-directed repair (HDR):** Integrates a homologous DNA fragment into the genome, achieving gene knock-in.

Genome editing employs engineered Cas9 and crRNA to target and cleave specific DNA sequences [2].

## 2. History of the CRISPR/Cas

CRISPRs, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, were first identified in the DNA of the bacterium Escherichia coli. Their discovery is credited to Ishino et al. in 1987, who were working at Osaka University, Japan [3].

At that time, sequencing these challenging DNA fragments took several months, yet neither their origin nor their significance in the bacterial cell were fully understood by their discoverers. Although the biological function of the CRISPR system had not yet been elucidated in the early research in this field, scientists had already proposed a way to utilize the information encoded in CRISPR loci in medical research, specifically for genotyping various bacterial strains: initially in Mycobacterium tuberculosis [4].

Mojica et al. were the first to realize that all these bacterial and archaeal sequences were functionally related . The term CRISPR was proposed by Jansen et al. in 2002 and became generally accepted by the community working with these sequences, which avoided further confusion caused by many different names for related repeat sequences. Comparative genomics studies revealed common features of CRISPRs, namely (i) they are located in intergenic regions, (ii) they contain multiple short direct repeats with very little sequence variation, (iii) the repeats are interspersed with nonconserved sequences, and (iv) a common leader sequence of several hundred base pairs is located on one side of the cluster of repeats [5].

Then, the CRISPR-associated protein (Cas), which functions as a nuclease or helicase and is functionally related to CRISPR, was discovered. The CRISPR sequences are homologous to phage sequences, with homology levels reaching up to 100%. This suggests that the CRISPR sequences may originate from phages. In 2005, three research groups independently found evidence suggesting that CRISPR may be linked to the immunity of microorganisms, prompting scientists to scrutinize CRISPR more closely. Researchers speculated that CRISPR might participate in the defense mechanism of bacteria, hypothesizing that it could utilize antisense RNA to store information and recognize exogenous nucleic acids invading cells. This defense mechanism resembles the RNAi mechanism involved in eukaryotic self-immune function. The self-immune function mediated by the CRISPR/Cas system was soon demonstrated in an experiment where Lysozyme was used to infect Streptococcus thermophiles. CRISPR/Cas is considered to be the "acquired immune system" evolved by bacteria or archaea to resist foreign DNA from plasmids or phages [2].

In 2012, two independent research laboratories reported that the CRISPR/Cas system could be reconstructed in vitro, and the reconstituted CRISPR/Cas systems exhibited biological functions and were capable of cutting an individual DNA sequence. This provides a foundation for using CRISPR/Cas as a genetic editing tool. Their studies and others also show that, to make the CRISPR/Cas system work in genome editing, three component parts are needed: the Cas enzyme, crRNA, and tracRNA. Jinek and colleagues (2012) demonstrated that CRISPR RNA (crRNA) and trans-activating crRNA (tracRNA) can be constructed together to form a single chimeric synthetic RNA molecule, called single guide RNA (sgRNA), which functions effectively in genome editing activities. This research is significant for simplifying DNA construction to make the CRISPR/Cas system a biotechnological tool for genome editing, which is always much simpler compared to previously used genome editing tools such as ZFNs and TALENs [6].

CRISPR-Cas systems provide microbes with RNA-guided adaptive immunity to foreign genetic elements by directing nucleases to bind and cut specific nucleic acid sequences. Through a process termed adaptation, microbes capture snippets of foreign genetic elements and incorporate them into their genomic CRISPR-array. Transcription of CRISPR

arrays creates CRISPR-RNAs (crRNA) that bind to Cas nucleases and provide specificity by base-pairing with target nucleic acids [1]. For genome editing applications, the necessity of protospacer-adjacent motif (PAM) recognition by Cas9 and Cas12a proteins constrains targeting and affects editing efficiency and flexibility [7].

## 3. Basic components of CRISPR-Cas9

The CRISPR-Cas system is a groundbreaking genetic engineering technique that allows for the exact alteration of DNA sequences. Constructed up of vital elements, it allows for remarkably accurate genetic modification.

The CRISPR array is formed of small foreign DNA pieces known as spacers inserted between repeating DNA sequences. These spacers are acquired from prior plasmid or viral infections [8].

Gene editing Cas nucleases and their variations designed to improve specificity, increase efficiency, and increase the potential target range (Koonin, 2019). The Cas proteins involved in interference compose up the CRISPR effector complexes, which in certain CRISPR-Cas systems also aid in the maturation of crRNAs [9].

Cas proteins appear in a variety of forms, including effector and adaptive proteins. Two adaptive Cas proteins called Cas1 and Cas2 are engaged in creating new spacers from foreign DNA. Effector Cas proteins, such as Cas9, Cas12, and Cas13, recognize and cleave targets [10].

Once a virus or plasmid invades a host's genome, its crRNA—which usually stays attached to the processing complex—is used as a guide to identify the protospacer or a closely related region. The Cas nuclease subsequently cleaves and inactivates the target sequence [11].

Trans-activating crRNA (tracrRNA), which is usually located between the cas genes and the CRISPR array region, is necessary for the formation of the functional ribonucleoprotein complex and the processing of crRNA. TrasrRNA permits it to pair with the repeat fragment of the pre-crRNA and perform DNA breakage with the assistance of the Cas nuclease [12].

In order to make genome editing applications easier to utilize, tracrRNA and crRNA can be chemically combined to form a single-guide RNA (sgRNA). This single-guide RNA (sgRNA), makes targeting easier [13].

PAM refers to "Protospacer Adjacent Motif," a short sequence of DNA which is located close to the target location in the genome. It is necessary for the Cas protein to connect to the target DNA and recognize it [9].

Type II CRISPR/Cas9 is the most frequently used CRISPR/Cas system. The Watson–Crick complementary nucleotides between the foreign DNA with a short PAM and the Cas9 protein's guide RNA are the main means by which the protein identifies and attaches to foreign nucleic acids. By altering the guide RNA sequence, the CRISPR/Cas9 system can modify any target DNA site which includes a PAM pattern [14].

## 4. Mechanism of action

The CRISPR/Cas9 system contains three main components: RNA-guided Cas9 endonuclease, CRISPR RNA (crRNA) and CRISPR transactivation RNA (tracrRNA).

Structural studies of cas9 from studies Streptococcus Pyogenes (spcas9) have revealed a bilobed architecture composed oficial the recognition lobe (REC) and the nuclease lobe (NUC). The REC lobe consists of two domains: REC and the bridging helix, while the NUC lobe has three domains: Protospacer adjacent motif interacting, HNH and RUVC [15].

The REC lobe is responsible for brinding to SgRNA, and the BH domain intiates the cleavage activity after binding to DNA, the PI domain is responsible for binding to the motif adjacent to the protospacer (PAM), contributing to the local separation of the strands of the target DNA duplex and the formation the SgRNA-DNA hybrid. The HNH domain cuts the ADN strand complementary to the guide RNA via a sing-metal mechanism, while the RUVC domain cuts the non-complementary strand via a two-metal mechanism, resulting in double strand breaks in the DNA.

Once the DNA is cut, one of several DNA repair systems performs the correction, which can lead to the desired genetic modification [16]. NHEJ repair is imprecise and can cause base insertions and deletions, changing the open reading frame. HR repair is accurate and can achieve gene-specific repair when homologous templates are introduced [17].

Interestingly, Cas9 endonucleases from different species can identify different PAMs despite their structural similarities. For example, Cas9 endonuclease Cas9 from *Francisella novicida* (FnCas9) recognises the sequence 5′-YG-3' (Y-pyrimidines), whereas Cas9 from Staphylococcus aureus (SaCas 9) identifies 5′-NNNNGATT [16].

In the case of SpCas9, the generation of a double-strand break (DSB) depends on a 20-nucleotide-long spacer and the 5'-NGG PAM. Cas9 systems are dual RNA-guided: a crRNA is responsible for DNA targeting and hybridises with the tracrRNA, forming a complex with Cas9. The crRNA and tracrRNA functions can be combined into a single designed guide RNA (gRNA) [18]. This RNA-DNA scaffold guides the Cas9 enzyme to bind and cleave DNA, resulting in a double-strand break that is repaired by two pathways: non-homologous end joining, which is error-prone, and homology-directed repair (HR), which is more accurate.

The NHEJ repair pathway is the more active mechanism and often causes small nucleotide insertions or deletions (indels) at the site of the double-strand break. Because NHEJ is a non-specific repair system, it has important practical implications, such as the generation of a diverse set of mutations [19].

CRISPR-associated Cas9 proteins rely on RNA guidance for targeting specificity. In engineered CRISPR-Cas9 systems, Cas9 interacts with the guide RNA (gRNA) backbone. Complementary pairing of the spacer portion of the gRNA to a DNA target sequence together with a PAM results in the generation of a double-strand break in the DNA by Cas9's nuclease domains, RuvC and HNH [20].

## 5. Biomedical applications

The CRISPR/Cas9 technology holds vast potential in the realm of biomedicine. This powerful technology enables precise modifications of the genome, offering essential tools for studying disease mechanisms and developing therapeutic approaches. Establishing disease models enables detailed investigations into disease progression and the testing of potential treatment methods. Moreover, CRISPR/Cas9 is invaluable for large-scale gene functional studies and genome-wide screenings. The versatility of the CRISPR/Cas9 system extends beyond basic research, as it holds tremendous potential in various areas, including gene therapy, infectious disease diagnostics and bioengineering [21].

### 5.1. Disease treatment

Cancer represents a major menace to human well-being and stands as the second highest contributor to mortality Due to their complex origins and the variations observed among individual patients, achieving a complete cure for cancer remains challenging. However, with the advent of genomic exploration and the advancements in molecular biology techniques, novel avenues for cancer treatment are emerging. In addition to conventional therapeutic approaches like surgery, radiotherapy, chemotherapy and immunotherapy, harnessing the power of CRISPR/Cas9 gene editing for tumor cells and immune cells holds great promise in pinpointing the specific genetic mutations underlying cancer and elucidating their mechanisms. This genetic precision provided by CRISPR/Cas9 offers a fresh perspective and a potential new strategy for the effective treatment of tumors

### 5.2. The potential of CRISPR/Cas9 in tissue engineering

Getting a firm grip on the behavior of the cells utilized in the regeneration process is one of the main difficulties in tissue engineering. Scientists can accurately change DNA sequences in cells using the potent genome editing technology CRISPR/Cas9). In tissue engineering cells, certain genes may be altered using CRISPR/Cas9 to improve their capacity for regeneration, encourage differentiation into cell types, and optimize their behavior in response to signals. For instance, CRISPR/Cas9 might be used to change the genes that regulate stem cells' differentiation and proliferation, which are frequently employed in tissue engineering applications .Scientists might influence the development of stem cells into cell types, such as bone or cartilage cells, that may be utilized to rebuild damaged tissues by manipulating the expression of these genes. Moreover, CRISPR/Cas9 has the potential to alter immune response-related genes, which could enhance the compatibility of transplanted tissues with the recipient's immune system. This could lower the possibility of rejection and increase the effectiveness of tissue engineering treatments over time [22].

### 5.3. Infectious diseases

The CRISPR–Cas9 system has the potential to treat infectious diseases by directly targeting viral genomes. Research has demonstrated successful use in inhibiting viruses such as HIV[25] and hepatitis B. It can also be used to target and disrupt viral genomes, potentially inhibiting viral replication and reducing viral load. Researchers have employed CRISPR-based strategies to develop diagnostic assays for virus detection, enabling rapid and sensitive identification of viral pathogens. CRISPR–Cas9 has been explored for its ability to target and disrupt antibiotic resistance genes,

potentially restoring the sensitivity of bacteria to antibiotics, and researchers have harnessed CRISPR to identify and remove specific virulence factors in bacteria, potentially attenuating their pathogenicity.Researchers have also employed CRISPR–Cas9 to edit the malaria parasite genome, potentially disrupting its lifecycle and reducing its ability to infect humans. CRISPR–Cas systems, including Cas13, have been repurposed for developing sensitive and specific diagnostic tools for detecting nucleic acids in infectious agents. This approach has potential applications in point-of-care diagnostics [23].

## 6. Challenges and limitations

Although CRISPR/Cas9 is a well-established gene editing tool and has been widely used in research and treatment of various diseases, its clinical application is still in its early stages. There are several challenges that need to be addressed, including off-target effects, delivery methods, efficiency and safety.

Off-target effects are a major limitation of CRISPR/Cas9 technology and are considered a significant risk in in vivo gene therapy [17]. These effects have been observed with a frequency of ≥50%. To address this concern, several strategies have been developed that include Cas9 variants designed to reduce these effects and optimise guide designs.

One effective strategy uses Cas9 nickase (Cas9n), a variant that induces single-strand breaks (SSB), in combination with an sgRNA pair that targets both DNA strands at the desired location to produce the double-strand break (DSB). In addition, Cas9 variants have been developed specifically designed to reduce off-target effects while maintaining editing efficiency.

SpCas9-HF1 is one such high-fidelity variant that exploits the "excess energy" model, which proposes that excessive affinity between Cas9 and target DNA could allow off-target effects. By introducing mutations at four residues involved in direct hydrogen bonding between Cas9 and the phosphate backbone of the target DNA, SpCas9-HF1 has been shown to have no detectable off-target activity compared to wild-type SpCas9 [24].

Other variants developed include evoCas9 and HiFiCas9, both with altered amino acid residues in the Rec3 domain, which is involved in nucleotide recognition. Desensitising the Rec3 domain increases the dependence on the specificity of the DNA:RNA heteroduplex to induce DSB, thus reducing off-target effects while maintaining editing efficiency.

One of the most recent variants is Cas9_R63A/Q768A. In this variant, the R63A mutation destabilises R-loop formation in the presence of mismatches, and the Q768A mutation increases sensitivity to distal MAP mismatches.

Despite the different strategies, the main goal of generating so many Cas9 variants with reduced off-target effects has ultimately been to decrease the overall interactions between Cas9 and DNA, and to give a stronger role to the DNA:RNA heteroduplex in facilitating edits [25].

## 7. Ethical considerations

However, despite the apparent advantages of the CRISPR-Cas9 system, numerous ethical and technical difficulties stand in the way of researchers dreaming of curing life-threatening diseases, especially if the genetic changes resulting from these manipulations can be inherited.

Discussions about the safety and, more importantly, ethical issues arising from the possibility of CRISPR-Cas9 gene therapy began shortly after the first publications showing the use of this system in human cells. A significant step in initiating these formal discussions was taken by Doudna, who organised a conference on scientific, medical, legal and ethical issues related to genomic modification. This conference was held in Napa Valley, California, in January 2015 [26].

The Nuremberg Code, the Declaration of Helsinki and the Belmont Report are based on fundamental ethical principles of biomedical research, such as respect for the individual, informed patient consent, understanding of risks and benefits, voluntary participation, fairness in the conduct of experiments, and the utmost professionalism of researchers. These principles are particularly relevant to the events of November 2018, when Chinese scientist Jiankui He announced the birth of the first babies undergoing genetic modification using the CRISPR-Cas9 system [27].

## 8. Recent advances and future directions

A particularly useful technique in several domains, including biotechnology, transcriptomics, functional genomics, and gene therapy, is CRISPR/Cas9. Genome editing has been transformed by programmable nucleases, especially CRISPR/Cas9, which allow for cell line changes and validations of knockouts. They have contributed significantly to the creation of animal models and enhanced plant varieties in addition to cell screening [28].

The application of plant biotechnology has proven indispensable in mitigating food insecurity resulting from population expansion and climate change. It has been utilized to improve characteristics in crops such as oilseed rape, rice, and soybean [29].

Despite its promise, there are still issues, most notably with effective CRISPR/Cas9 delivery to target cells in therapeutic settings. Nonetheless, attempts are being made to create more effective delivery systems without sacrificing cell viability. In addition to improving our knowledge of biological systems, CRISPR/Cas9 presents opportunities for the development of innovative therapeutics and the treatment of hereditary illnesses, such as universal CAR-T cells, an immuno-oncology strategy [30].

CRISPR/Cas9 has had a substantial impact on a number of study areas and has the potential to advance scientific understanding and solve societal issues in the future.

## 9. Conclusions

CRISPR/Cas9 has gained rapid development in recent years, providing an adaptable and accessible tool for genome manipulation and visualization. CRISPR/Cas9 holds tremendous potential as a therapeutic for diverse diseases related to genetic disorders and cancers. The CRISPR/Cas9-based therapies have been evaluated in clinical trials. To date, most of these trials are focused on adoptive cell therapies, in which the target cells ought to be readily accessible. The delivery of CRISPR/Cas9 system in clinical trials mainly depends on physical approaches and viral vectors, which are seriously hampered by cell injury and safety concerns. The development of non-viral nanocarriers would contribute to extending the medical applications of CRISPR/Cas9 system.

The effective and safe delivery strategy remains a primary challenge for clinical applications of CRISPR/Cas9 system. Typically, three modes of CRISPR/Cas9 system are available for delivery: DNA plasmids, mRNA/sgRNA, and Cas9 RNP. The vectors should be rationally designed according to the action mechanism and physicochemical properties of each CRISPR/Cas9 mode for maximized genome editing efficiency, and minimized off-target risks. To date, various delivery systems for different CRISPR/Cas9 forms were developed including lipids, polymers, gold nanoparticles, DNA nanoclew, and cell penetrating peptides. Thereinto, the cationic lipid-based CRISPR/Cas9 plasmid delivery is the most commonly used strategy due to the mature technology of lipid nanoparticles and the structural simplicity, natural biostability, sequence editability, and compressibility of the plasmid. Nevertheless, the unavoidable drawbacks of delayed onset and integration risk drives the development of alternative approaches. The ideal strategy is supposed to be the direct delivery of Cas9 RNP into the nucleus, which could circumvent the expression of Cas9/sgRNA components for transient function, high genome-editing efficiency, and minimum off-target effect. The direct Cas9 RNP delivery strategy requires the smart vectors with multiple functions of Cas9 RNP encapsulation, internalization, endosomal escape, nuclear trafficking, and payload liberation. This represents a fertile research direction that can advance the CRISPR/Cas9 technology.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

## References

[1] Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science [Internet]. 2019 [cited 2024 Apr 29];361(6405):866–9.

[2] Zhu Y. Advances in CRISPR/Cas9. Biomed Res Int. 2022 Sep 23;2022:9978571. doi: 10.1155/2022/9978571. PMID: 36193328; PMCID: PMC9525763.

[3] Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol. 1987 Dec;169(12):5429-33. doi: 10.1128/jb.169.12.5429-5433.1987. PMID: 3316184; PMCID: PMC213968.

[4] Groenen PM, Bunschoten AE, van Soolingen D, van Embden JD. Nature of DNA polymorphism in the direct repeat cluster of Mycobacterium tuberculosis; application for strain differentiation by a novel typing method. Mol Microbiol. 1993 Dec;10(5):1057-65. doi: 10.1111/j.1365-2958.1993.tb00976.x. PMID: 7934856.

[5] Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. J Bacteriol. 2018 Mar 12;200(7):e00580-17. doi: 10.1128/JB.00580-17. PMID: 29358495; PMCID: PMC5847661.

[6] Zhang D, Zhang Z, Unver T, Zhang B. CRISPR/Cas: A powerful tool for gene function study and crop improvement. J Adv Res. 2020 Oct 21;29:207-221. doi: 10.1016/j.jare.2020.10.003. PMID: 33842017; PMCID: PMC8020163

[7] Walton RT, Christie KA, Whittaker MN, Kleinstiver BP. Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science [Internet]. 2020 [cited 2024 Apr 29];368(6488):290–6.

[8] Rahman S, Ikram AR, Azeem F, Tahir ul Qamar M, Shaheen T, Mehboob-ur-Rahman. Precision genome editing with CRISPR-Cas9. In: Methods in Molecular Biology. New York, NY: Springer US; 2024. p. 355–72.

[9] Shmakov SA, Barth ZK, Makarova KS, Wolf YI, Brover V, Peters JE, Koonin EV. Widespread CRISPR-derived RNA regulatory elements in CRISPR-Cas systems. Nucleic Acids Res. 2023 Aug 25;51(15):8150-8168. doi: 10.1093/nar/gkad495. PMID: 37283088; PMCID: PMC10450183.

[10] Gostimskaya I. CRISPR–Cas9: A history of its discovery and ethical considerations of its use in genome editing. Biochemistry (Mosc) [Internet]. 2022 [cited 2024 Apr 30];87(8):777–88.

[11] Koonin EV, Makarova KS. Mobile Genetic Elements and Evolution of CRISPR-Cas Systems: All the Way There and Back. Genome Biol Evol. 2017 Oct 1;9(10):2812-2825. doi: 10.1093/gbe/evx192. PMID: 28985291; PMCID: PMC5737515.

[12] McDonald ND, Regmi A, Morreale DP, Borowski JD, Boyd EF. CRISPR-Cas systems are present predominantly on mobile genetic elements in Vibrio species. BMC Genomics. 2019 Feb 4;20(1):105. doi: 10.1186/s12864-019-5439-1. PMID: 30717668; PMCID: PMC6360697.

[13] Le Rhun A, Escalera-Maurer A, Bratovič M, Charpentier E. CRISPR-Cas in Streptococcus pyogenes. RNA Biol. 2019 Apr;16(4):380-389. doi: 10.1080/15476286.2019.1582974. Epub 2019 Mar 11. PMID: 30856357; PMCID: PMC6546361.

[14] Tripathi S, Khatri P, Fatima Z, Pandey RP, Hameed S. A landscape of CRISPR/Cas technique for emerging viral disease diagnostics and therapeutics: Progress and prospects. Pathogens [Internet]. 2022 [cited 2024 Apr 29];12(1):56.

[15] Sánchez Artigas, Rolando, Díaz Armas, María Teresa, Rodríguez Duque, Raisa, Miguel Soca, Pedro Enrique. Principles and medical applications of gene editing using CRISPR/Cas. Medisur [Internet]. 2021 Dec [cited 2024 May 16] ; 19( 6 ): 1005-1014. Epub 30-Dec-2021.

[16] Leonova EI, Gainetdinov RR. CRISPR/Cas9 Technology in Translational Biomedicine. Cell Physiol Biochem [Internet]. 2020 [cited 2024 Apr 29];54(3):354–70.

[17] Zhang M-L, Li H-B, Jin Y. Application and perspective of CRISPR/Cas9 genome editing technology in human diseases modeling and gene therapy. Front Genet [Internet]. 2024 [cited April 29, 2024];15.

[18] Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J [Internet]. 2020;18:2401–15.

[19] Khoshandam M, Soltaninejad H, Hamidieh AA, Hosseinkhani S. CRISPR, CAR-T, and NK: Current applications and future perspectives. Genes Dis [Internet]. 2024 [cited 2024 Apr 29];11(4):101121.

[20] Pickar-Oliver A, Gersbach CA. The next generation of CRISPR–Cas technologies and applications. Nat Rev Mol Cell Biol [Internet]. 2019 [citado el 29 de abril de 2024];20(8):490–507.

[21] Du Y, Liu Y, Hu J, Peng X, Liu Z. CRISPR/Cas9 systems: Delivery technologies and biomedical applications. Asian J Pharm Sci [Internet]. 2023 [cited 2024 Apr 29];18(6):100854.

[22] Dubey AK, Mostafavi E. Biomaterials-mediated CRISPR/Cas9 delivery: recent challenges and opportunities in gene therapy. Front Chem [Internet]. 2023 [cited April, 29, 2024];11.

[23] Kolanu ND. CRISPR–Cas9 gene editing: Curing genetic diseases by inherited epigenetic modifications. Glob Med Genet [Internet]. 2024 [citado el 29 de abril de 2024];11(01):113–22.

[24] Ahmad S, Wei X, Sheng Z, Hu P, Tang S. CRISPR/Cas9 for development of disease resistance in plants: recent progress, limitations and future prospects. Brief Funct Genomics [Internet]. 2020 [cited 2024 May 31];19(1):26–39.

[25] Uddin F, Rudin CM, Sen T. CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future. Front Oncol. 2020 Aug 7;10:1387. doi: 10.3389/fonc.2020.01387. PMID: 32850447; PMCID: PMC7427626.

[26] Memi F, Ntokou A, Papangeli I. CRISPR/Cas9 gene-editing: Research technologies, clinical applications and ethical considerations. Seminars In Perinatology [Internet]. 1 December 2018;42(8):487-500.

[27] Gostimskaya I. CRISPR–Cas9: A history of its discovery and ethical considerations of its use in genome editing. Biochemistry (Mosc) [Internet]. 2022 [cited 2024 Apr 29];87(8):777–88.

[28] Tyumentseva M, Tyumentsev A, Akimkin V. CRISPR/Cas9 landscape: Current state and future perspectives. Int J Mol Sci [Internet]. 2023 [cited 2024 Apr 29];24(22):16077. Uddin F, Rudin CM, Sen T. CRISPR gene therapy: Applications, limitations, and implications for the future. Front Oncol [Internet]. 2020 [cited April 29, 2024];10.

[29] Cong Gan W, P.K. Ling A. CRISPR/Cas9 in plant biotechnology: applications and challenges. BioTechnologia [Internet]. 2022 [cited 2024 Apr 29];103(1):81–93.

[30] Wang SW, Gao C, Zheng YM, Yi L, Lu JC, Huang XY, Cai JB, Zhang PF, Cui YH, Ke AW. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Mol Cancer. 2022 Feb 21;21(1):57. doi: 10.1186/s12943-022-01518-8. PMID: 35189910; PMCID: PMC8862238.