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Gene Editing: A harmony between Development and Moral Issues in the CRISPR Era

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Abstract. CRISPR-Cas9, an acronym for CRISPR-associated protein 9 and Clustered Regularly Interspaced Short Palindromic Repeats, is a revolutionary molecular biology technology that has revolutionized the field of genome editing. Its remarkable accuracy, effectiveness, and adaptability attract interest in many scientific fields. This technique has huge potential for advancing future developments in a variety of industries, including biotechnology, agriculture, and medicine. This review article offers a comprehensive examination of the fundamentals, practical uses, difficulties, and potential future developments of CRISPR-Cas9 technology. The basic mechanisms of CRISPR-Cas9, which emerged from the adaptive immune systems of bacteria, have been explained, insisting on its unique capacity to accurately slice DNA and later rearrange genetic material. CRISPR-Cas9 has been aggressively adopted in a variety of research subject areas, from fundamental genetic study to therapeutic treatments, mainly to its simplicity, efficiency, and adaptability. The capacity of CRISPR-Cas9 to accurately modify disease-causing mutations is one of the technology's most significant uses. This technology has the potential to be used to treat genetic illnesses. The study discusses the progress made in correcting genetic defects in both somatic and germline cells, highlighting the promise of CRISPR-based treatment options for the treatment of inherited disorders. CRISPR-Cas9 has also made it easier to create genetically modified organisms (GMOs) with more desirable characteristics, which has transformed bioengineering and agricultural methods. The significance for responsible governance and public involvement are highlighted as moral and challenges with regulations associated with gene editing technology and genetically modified organisms (GMOs) are reviewed. In alongside genome editing, CRISPR-Cas9 has extended the scope of biological study through the development of revolutionary approaches such as live-cell imaging, epigenome editing, and gene control. The complicated nature of gene expression and cellular functions have been demonstrated by these advancements, providing up the possibilities to new methods of therapy and diagnostic technologies. But there are several obstacles to overcome before CRISPR-Cas9 is widely used. Immune responses, delivery strategies, and off-target effects seem to be major barriers to the practical use of CRISPR-based treatments. To address these issues, ongoing research programs have been highlighted that aim to improve delivery methods, mitigate immunogenicity, and enhance specificity. With plenty of potential for additional research and application, this analysis explores the most recent developments of the technology known as CRISPR, namely base editing, prime editing, and CRISPR-based diagnostics. A new age of precision medicine is also announced by an investigation of the potential of CRISPR-based medicines in the fight against neurological disorders, cancer, and infectious illnesses. At the end, CRISPR-Cas9 is an advanced way that has the potential to have a major impact on genetic research, industry, and medicine. Its accuracy and adaptability provide previously unthinkable opportunities for accurately modifying genomes. CRISPR-Cas9 has a wide range of uses, from looking into complicated genetic features to designing crops, repairing genetic abnormalities, and generating innovative treatments. With its debut, genetic manipulation enters a new age that promises revolutionary breakthroughs that will influence science and technology in the future. Even if there has been a lot of development, more collaboration between disciplines and ethical examination are necessary for optimal use of CRISPR technology while appropriately addressing its limitations.

Keywords –Gene editing, Genetic Engineering, Molecular biology, Biotechnology

INTRODUCTION

Utilizing targeted DNA sequence modifications in the genome, gene editing technology allows for the insertion, deletion, and other modifications of DNA fragments. In the beginning, gene editing was accomplished using homologous recombination, yet this method proved to be error-prone and inefficient. This led to the development of ZFNs, TALEN, and Meganuclease with the use of artificial nuclease modification for gene editing applications [1-3]. One non-specific DNA cleavage domain and one particular DNA binding domain make up these modified nucleases [4-5]. Double-strand breaks (DSBs) at certain genomic sites are a key obstacle that they addressed. That being said, the technique was arduous and difficult since each new target site required a redesign of the binding domain sequences [6]. CRISPR/Cas9 technology has displayed more construction efficiency than previous gene editing methods, which has sped up its adoption in both research and real-world applications [7]. The CRISPR/Cas9 system's ongoing developments have opened up a world of possibilities for genetic enhancement, gene therapy, and gene function studies, pushing the boundaries of life sciences research to previously unheard-of heights.

The CRISPR/Cas9 system was initially discovered in the year 1987 when repeating tandem arrays in Escherichia coli (E. Coli) were discovered [8]. These arrays are made up of unique sequences that are dispersed among conserved DNA repeats, that are found in many bacterial and archaeal genomes [8]. Later research has shown that 40% of bacterial genomes and nearly 90% of archaeal genomes include these unique sequences [6,9]. This class of repeating arrays is formally known as "clustered regularly interspaced short palindromic repeats" or CRISPR for short. This word first came to be utilized in 2002 [9]. Later, the CRISPR-associated protein (Cas) was identified, containing helicases or nucleases that are functionally connected with CRISPR [9].

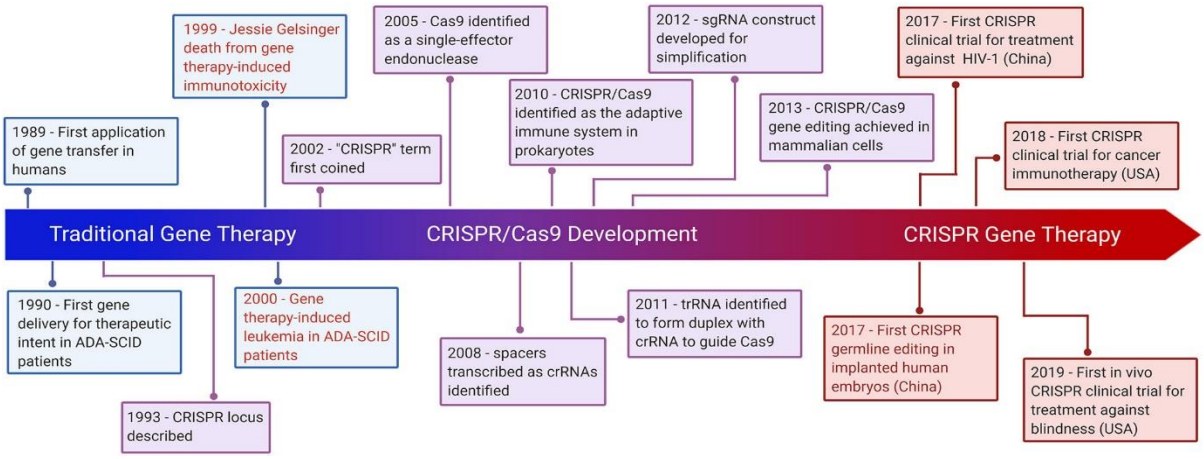

Figure 1. Timeline that covers the key moments in the evolution of CRISPR, standard gene therapy, and CRISPR gene therapy [10].

The degree of similarity between the CRISPR sequences and phage sequences is high—up to 100% [9]. This implies that phages may have been the source of CRISPR sequences. Research teams discovered a possible link between CRISPR and microbe immunity in 2005, which prompted a more thorough examination of CRISPR [12]. Researchers were able to speculate that CRISPR may be contributing to bacterial defense systems by employing antisense RNA to recognize and recall foreign nucleic acids that invade cells. The RNA interference (RNAi) process in eukaryotic self-immune function is similar to this defensive mechanism.

The CRISPR/Cas system's ability to support self-immune function was later shown in an experiment where Streptococcus thermophiles were infected with Lysozyme [12]. However, it is often considered that this system in some bacteria and archaea acts as an instance of "acquired immune system" evolved as a defense mechanism against genetic material that enters the cells from viruses (phages) or plasmids [12,13]. Because of this adaptive immune response, bacteria may identify and destroy certain foreign DNA sequences in a manner similar to the manner in which the human immune system identifies and destroys viruses [12, 13].

MECHANISM OF CRISPR-Cas9

The CRISPR-Cas9 system is an elegant and highly specific process, harnessing the innate defense mechanisms of bacteria to precisely modify the genetic material of organisms. It involves three critical components: the Cas9 nuclease enzyme, guide RNA (gRNA), and the CRISPR array.

The CRISPR array serves as the biological archive of past viral infections. Bacteria incorporate short fragments of viral DNA into this array during encounters with viruses, creating a genetic memory. This archive, marked by short palindromic repeats, provides the system with a database for future recognition of familiar threats.

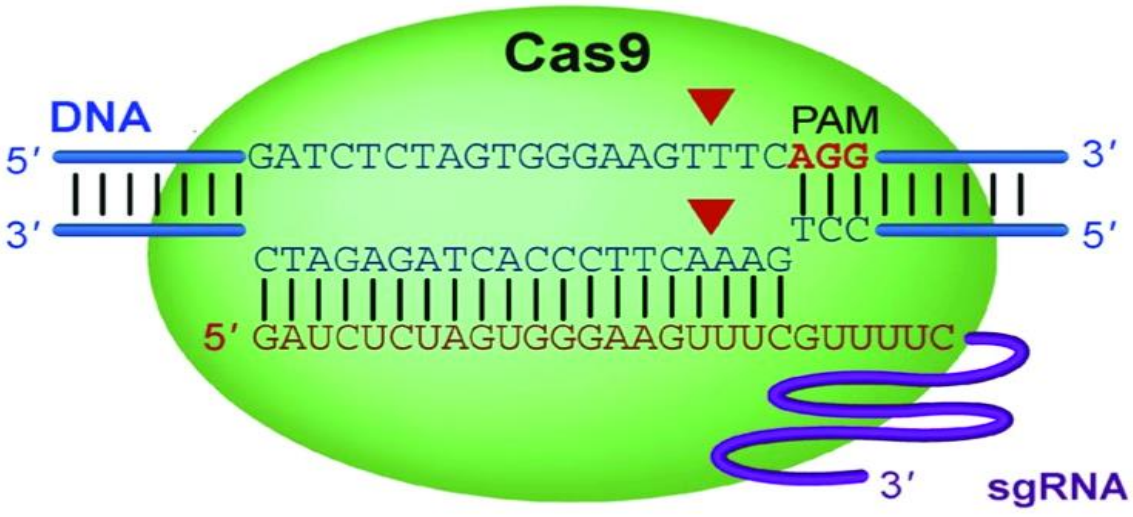

Figure 2: The CRISPR-Cas9 system. The purple sgRNA directs the Cas9 protein to locations on the genome that include sequences that are complementary to the sgRNA's 5' end [14]. A PAM must be used to follow the target DNA sequence [14].

At the heart of this system lies the Cas9 nuclease enzyme, often referred to as the molecular scissors. In particular, Cas9 is essential to the CRISPR/Cas9 system because it splits the target DNA at particular sites in response to guide RNA (gRNA) instructions. This enzymatic activity is a crucial aspect of the precision offered by CRISPR-Cas9, as it allows for site-specific modifications without causing widespread genomic damage [12,15].

The gRNA acts as the molecular guide directing Cas9 to the precise location on the DNA sequence that requires modification. The Cas9 enzyme is properly guided to the right position inside the target DNA sequence by the guide RNA (gRNA) [16]. The gRNA enables exact positioning of Cas9, enabling the cleavage of DNA at the intended location, by matching the target DNA sequence. The exact targeting of certain genes by researchers is made possible by the unique specificity of the CRISPR-Cas9 system, which is largely dependent on the design and manufacture of the gRNA [16,17].

For the start of editing a genome with CRISPR-Cas9, a guide RNA that specifically matches with the intended target DNA sequence must be designed and synthesized. The careful development of this gRNA allows it to identify and attach to the targeted genomic locus for editing. To achieve the required accuracy in the editing process, it is vital to ensure that the gRNA corresponds to the target DNA sequence. After the gRNA is produced and synthesized, it joins with the enzyme, which is, Cas9 enzyme to constitute a complex that leads the enzyme to the intended spot in the genome and starts the correct modification process. When the guide RNA finds the target sequence, it directs the Cas9 enzyme to attach to the DNA at that specific spot, causing Cas9 to cause a double-strand break and providing a chance for modifications [18].

Cellular repair mechanisms come into play to mend the broken DNA. Two main pathways are involved: non-homologous end joining (NHEJ) which leads to insertions/deletions (indels), and homology-directed repair (HDR) for targeted insertions using a repair template [15-17]. The choice between these pathways dictates the nature of the genetic modifications [14,18].

The adaptable nature of the CRISPR-Cas9 system grows from its capacity to produce customized guide RNAs (gRNAs) which specifically target particular DNA regions, making it suitable for a wide range of usages in various animals [18].

ADVANCEMENTS IN CRISPR-Cas9

Scientists have been growing and enhancing the utilization of CRISPR/Cas9 in order to accomplish numerous genetic alterations. By using appropriate DNA repair processes, this includes targeted disruptions of certain genes, knock-ins, deletions, and precise editing. Since the non-homologous end joining (NHEJ) route is active throughout the cell cycle, especially during the G1/M phase transition, it is often selected for CRISPR/Cas9 activities [19]. NHEJ is very efficient, although it can make mistakes that result in insertions or deletions at DNA double-strand breaks (DSBs) [20]. Gene function is disrupted by these changes, which result in frame-shift mutations. As such, NHEJ is frequently used to create gene knockouts [21]. It is also possible to achieve complete gene deletions by using two guide RNAs (sgRNAs), one of which targets the gene's start and the other its end, resulting in the creation of two DSBs. In the meantime, accurate gene edits and knock-ins are the main uses of the homology-directed repair (HDR) process [21]. When compared to NHEJ, HDR is a more effective repair method. For the repair of double-strand breaks (DSBs), homology arms on both sides of the knock-in sequence are used as a donor template in homology-directed repair (HDR). Sequences in these homology arms correspond to the areas around the DSBs. Though it is less error-prone, HDR is noticeably less efficient than NHEJ [21]. HDR operates merely during the S and G2/M stages of the cell cycle, in comparison with NHEJ [22]. Since stem cells are usually quiescent, there is limited utilization for HDR-mediated gene editing in actively dividing cells [23]. This restriction makes it less useful in clinical situations [23].

Gene knockin production has been revolutionized by the unveiling of homology-independent targeted integration (HITI), which allows the process in both dividing and nondividing cells via the non-homologous end joining (NHEJ) pathway [24]. In HITI, a donor vector with the Cas9/gRNA target sequence surrounds a targeted transgene. The NHEJ repair mechanism begins to operate when Cas9 breaks down both the donor vector and the genome at the same time, producing double-strand breaks (DSBs) in both. The transgene is incorporated into the genome by this process. Gene knockins are more effectively produced by HITI than by HDR because NHEJ is active over the whole cell cycle [19]. However, before HITI is used for medicinal purposes, there are issues that need to be resolved. As of present, HITI's knockin efficiency in nondividing cells is usually less than 5% [24], and Cas9's possible off-target effects might result in transgene integration in undesired regions. On the other hand, having a high-fidelity Cas9 nuclease and carefully choosing the Cas9/gRNA target sequences can help reduce these off-target effects [25,26].

CRISPR/Cas9 gene editing uses an additional path called microhomology-mediated end joining (MMEJ) [27]. When there are small (5–25 base pair) microhomology areas on each of the sides of double-strand breaks (DSBs), it becomes active. The intervening sequence may be deleted as an effect of the annealing of these microhomology sequences. Targeted knockin of transgenes can be done through a technique termed precise integration into target chromosome (PITCh), which was recently published by Nakade and collaborators and depends on MMEJ [27]. Cas9 cleaves the genome and the PITCh donor vector in PITCh, displaying their microhomology sequences. This starts the process by which transgenes are incorporated into the genome at the DSB sites via MMEJ [27]. Notably, MMEJ functions when HDR is dormant, namely in the M and early S stages. Crucially, MMEJ shows between two and three times the effectiveness than HDR at achieving transgene focused knockin [27].

Regardless of the DNA repair path used, the formation of double-strand breaks (DSBs) after Cas9 cleavage raises concerns about genotoxicity [28]. As a result, base editing—a method that modifies point mutations in genes without requiring DNA cleavage—was created [29,30]. Through the use of a cytidine deaminase and a catalytically inactive Cas9 (dCas9) enzyme, the genome's C or G nucleotides can be repaired, allowing C > T or G > A mutations to be converted [28,29]. There was a

constraint at first since adenine nor thymine deaminases were accessible. But thereafter, an adenosine deaminase was created by building a tRNA deaminase and using protein evolution [30]. The conversion of T > C or A > G mutations in the genome is facilitated by this adenosine deaminase [30]. Base editing therefore provides a potentially secure and effective way to edit point mutations. Base editing is carried out by combining dCas9 with adenosine or cytidine deaminase.

Every one of the four transition mutations can be converted by means of base editing, but transversion mutations cannot be dealt with in this way. But Anzalone and team's latest work has developed a flexible prime-editing method that does not require a donor template to accomplish targeted insertions, deletions, and conversions of all 12 combinations of point mutations [31]. A Cas9 nickase and a prime editing guide RNA (pegRNA) are two necessary elements of prime editing [31]. PegRNA is an elongated version of sgRNA that can hybridize with the 3' end of the nicked genomic DNA because it has an RNA template with the desired edit and a primer binding site [31]. The single-strand nick that the catalytically impaired Cas9 nickase makes in the genomic DNA facilitates contact with the 3' end of the pegRNA [31].

APPLICATIONS OF CRISPR-Cas9

CRISPR-Cas9 has become an outstanding method with tremendous possibilities in many fields, including medicine and gene therapy. By directly targeting the mutations causing genetic illnesses, this precise gene-editing method holds the prospect of healing many ailments. The promise of CRISPR-based therapeutics is demonstrated by conditions including Duchenne muscular dystrophy, sickle cell disease, and cystic fibrosis [32,33]. CRISPR-Cas9 may be able to fix the hemoglobin gene mutation that causes sickle cell disease in hematopoietic stem cells, potentially providing a treatment. Similarly, CRISPR-Cas9 may be utilized for targeting mutations within the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which is linked to cystic fibrosis, a potentially fatal disease that affects the lungs and digestive system [34]. Moreover, CRISPR-Cas9 may be able to mitigate the symptoms of Duchenne muscular dystrophy, a severe disorder that results in muscle atrophy brought on by mutations in the dystrophin gene, by boosting the production of functional dystrophin protein [32,33].

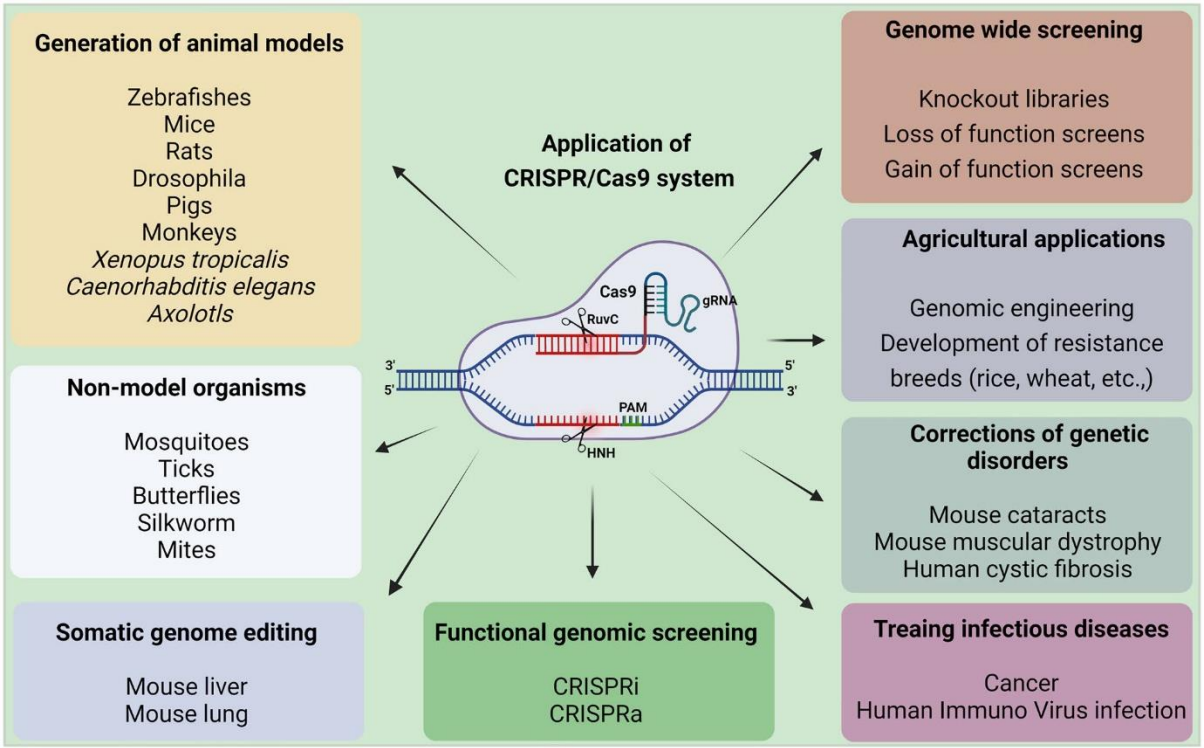

Figure 3. Areas of applications of the CRISPR System. The Genome engineering has drastically changed with the advent of CRISPR/Cas9. Its unmatched swiftness, precision, and affordability have created countless opportunities in a wide range of sectors. After it was discovered, scientists have the ability to treat diseases, develop new medicines, and advance the field of precision medicine by utilizing CRISPR/Cas9. Its applications do not stop at improving human health. Furthermore, CRISPR/Cas9 is being utilized to manufacture biofuel, enhance agricultural quality, study the biology of various insects, and do somatic genome editing [18].

The potential of CRISPR-Cas9 extends beyond treating genetic disorders. Researchers are also exploring its use in fighting HIV infection by removing or disrupting the virus's genetic material. In cell culture models, scientists have demonstrated the ability to edit HIV-1 proviral DNA using CRISPR-Cas9, suggesting the possibility of developing novel therapies for HIV and other viral infections. Furthermore, CRISPR-Cas9 has shown promise in the field of cancer immunotherapy [35]. By editing the genes of immune cells, such as T cells or natural killer (NK) cells, researchers can enhance their ability to recognize and target cancer cells more effectively [16,35]. This approach could lead to personalized cancer treatments tailored to individual patients' specific genetic profiles [16,36]. However, it is crucial to consider the ethical implications surrounding gene therapy and human germline editing as we explore the immense potential of CRISPR-Cas9 in medicine [35,36]. Regulatory bodies and ethical guidelines must be established to ensure the responsible and safe use of this technology [36].

CRISPR-Cas9 also has the potential to revolutionize the agricultural sector by enabling the precise modification of plant genomes. This technology can be leveraged to improve crop yields, enhance nutritional value, increase disease resistance, and develop crops that are more tolerant to environmental stresses [34-36]. By precisely targeting and modifying genes associated with traits such as photosynthetic efficiency, nutrient uptake, and growth regulation, CRISPR-Cas9 can be used to develop high-yielding crop varieties. This could help address the global challenge of food security and meet the increasing demand for food production. Plant pathogens pose a significant threat to crop production worldwide. CRISPR-Cas9 can be used to edit plant genes responsible for disease resistance, enabling the development of crops that are more resilient to various pathogens [32]. This approach could reduce the need for chemical pesticides and promote sustainable agriculture practices. Additionally, CRISPR-Cas9 can be utilized to enhance the nutritional content of crops by modifying genes involved in the biosynthesis of essential vitamins, minerals, and other beneficial compounds [35,36]. This could help address malnutrition and improve the overall nutritional quality of food crops [34,35]. Environmental stresses, such as drought, salinity, and extreme temperatures, can significantly impact crop yields and productivity [34-36]. By editing genes related to stress response pathways, CRISPR-Cas9 can be used to develop crop varieties that are more tolerant to these adverse conditions, ensuring food security and resilience in the face of climate change [34,35]. While the potential benefits of CRISPR-Cas9 in agriculture are significant, it is essential to address concerns regarding the environmental impact, potential risks, and regulatory frameworks surrounding the use of genetically modified organisms (GMOs) in food production [36].

The applications of CRISPR-Cas9 extend far beyond medicine and agriculture. This versatile technology has numerous applications in various fields, including biofuel production, environmental remediation, and basic research. CRISPR-Cas9 can be used to modify microorganisms, such as algae or bacteria, to enhance their ability to produce biofuels more efficiently [34]. By editing genes involved in metabolic pathways or stress tolerance, researchers can engineer organisms optimized for biofuel production, contributing to the development of sustainable energy sources. CRISPR-Cas9 also has potential applications in environmental remediation by engineering microorganisms or plants capable of breaking down pollutants or sequestering greenhouse gases. For example, bacteria could be modified to degrade specific environmental contaminants, or plants could be engineered to absorb and store excess carbon dioxide from the atmosphere [35]. Eventually, CRISPR-Cas9 has developed into a vital tool for fundamental research, allowing researchers to study the complexity of many biological processes, look into the mechanisms behind illness, and analyze the role of genes. With accurate gene editing and following phenotypic observations, scientists may learn more about the complex mechanisms of living organisms and improve the understanding of basic biological concepts [33-36].

CHALLENGES AND FUTURE DIRECTIONS

CRISPR-Cas9, despite its revolutionary potential, faces several challenges that require attention for its safe and responsible implementation [37]. One major concern is off-target effects, where the Cas9 enzyme mistakenly cuts unintended DNA sequences. These unintended cuts can lead to harmful mutations and disrupt gene function. Researchers are actively developing strategies to improve the specificity of CRISPR-Cas9, including optimizing guide RNA design, utilizing engineered Cas9 variants, and employing bioinformatics tools for off-target prediction and minimization [38].

Delivering the CRISPR-Cas9 machinery (Cas9 enzyme, guide RNA, and repair template if needed) effectively into target cells or tissues, particularly for applications within living organisms, presents another significant challenge. Various delivery methods like viral vectors, nanoparticles, and electroporation are being explored, each with its own advantages and limitations. Overcoming these delivery hurdles is crucial for the successful use of CRISPR-Cas9 in therapeutics and agriculture [37-39].

The immense power of CRISPR-Cas9 has ignited ethical discussions and concerns surrounding its responsible use [38]. Issues like germline editing, potential for human enhancement, and unintended consequences raise the need for strict ethical guidelines and regulatory frameworks. It needs constant communication and cooperation between scientists, decision-makers, and the general public to guarantee the morally and responsibly developed and utilized CRISPR-Cas9 [40].

Public acceptance and perception, particularly regarding gene therapy and genetically modified organisms, can significantly impact the adoption and implementation of CRISPR-Cas9 [41]. Addressing public anxieties, fostering open communication, and educating the public about both the potential benefits and risks of this technology are crucial steps towards gaining widespread acceptance and support [42].

The field of CRISPR-Cas9 is constantly evolving as experts look into new possibilities for expanding its range of applications and solve present limits. Future directions include developing novel CRISPR tools like base editors and prime editors, which enable precise genome modifications without double-stranded breaks. Furthermore, the development of Cas9 versions with improved selectivity and lowered off-target activity, along with advanced computational techniques for off-target prediction and guide RNA design, offer potential for improved accuracy. Enhancing delivery methods through novel strategies like lipid nanoparticles or engineered viral vectors is another area of active research [43]. Furthermore, researchers are looking into the use of CRISPR-Cas9 in new fields like disease modeling and synthetic biology. The potential to combine CRISPR-Cas9 with other emerging technologies like gene drives or gene circuits opens doors to addressing complex challenges in areas like population control or biosensing [44].

The first gene-edited babies have come into existence and the world is now concentrating on them since they may be immune to AIDS [12]. It is critical to keep a close eye on the quick development of CRISPR/Cas9 technology in order to reduce dangers and maximize advantages. Genetic inequality might result from parents who are motivated to modify their children's DNA to suit their preferences. This discovery emphasizes how people may change genetic coding to satisfy a range of needs and

wants. Safety concerns have been clarified by recent findings, especially with regard to DNA repair processes. Although gene editing aims to improve immune response and fight leukemia [46], there is a chance that DSBs will damage stem cells [47,48]. The possibility of genetic disparity after birth is highlighted by the birth of these gene-edited children. The absence of an in-depth investigation on CRISPR/Cas9 raises safety concerns since it increases the risk of unintended results from off-target impacts.

It is critical to preserve a balance between responsible usage and scientific advancement as CRISPR-Cas9 technology advances. This includes dealing with ethical problems while making sure this effective tool is utilized safely and effectively.

CONCLUSION

CRISPR-Cas9 is an emerging method for genome editing that presents an extensive range of possibilities in many different areas. This is being utilized to deal with hereditary medical conditions, personalize cancer therapies, reform agriculture, as well as explore renewable energy sources. The basic mechanism is a highly precise Cas9 enzyme that targets and modifies DNA regions under the instructions provided by specific RNA. But there are difficulties that must be overcome in order guarantee the ethical and responsible use of this technology, including the likelihood of unintentional modifications, complex distribution, and legal problems. In an attempt to cross over these obstacles, researchers are actively attempting to improve CRISPR-Cas9 delivery techniques and accuracy. Moreover, there is a lot of hope for the future in the ongoing creation of fresh CRISPR applications and instruments in various fields. Establishing robust ethical guidelines and regulatory frameworks is crucial to navigate the complex societal implications of this powerful technology. Moving forward, interdisciplinary collaboration among scientists, policymakers, and the public will be essential. This collaborative approach will unlock the full potential of CRISPR-Cas9 while addressing societal concerns and promoting responsible innovation. The future of CRISPR-Cas9 is bright, with the potential to reshape our understanding of biology, revolutionize medical treatments, enhance food security, and propel us towards new frontiers in biotechnology.

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