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Advantages and Challenges of CRISPR-Cas9 Applications in Animal Modeling: A Concise

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Abstract

CRISPR is an extraordinarily powerful technique regulating any target gene across the genome with promising therapy intentions. CRISPR-Cas9 is a convenient tool for gene manipulation. Notwithstanding this, the broad consequence of human gene editing, particularly germinal genes, cannot be predicted. Firstly, once edited, the genes would be part of the human population for successive generations and may be impossible to remove from humanity; secondly, success is not guaranteed; thirdly, the fidelity of editing, as it could affect unrelated genes or unspecified segments of DNA; and last but not least, its influence on gene interaction, network, and signaling pathways could be difficult to be predicted. CRISPR-Cas9 mostly includes precise genome editing, rapidity and cost-effectiveness, creation of disease models, study of gene function, applications in gene therapy and translation research and wide diversity for species. The technique also ignited the moral and ethical concerns of scientific community. Ethics and safety approval for gene modification in human cells is required by the National Institutes of Health (NIH). The NIH does not currently fund studies of CRISPR in human embryos and opposes the CRISPR utilization in germline cells because these alterations would be permanent and heritable. The technology has promised with the most profound implications for cancer therapy. Recent advances in CRISPR-based technology is redefining how cancer is studied and potentially improves anti-cancer therapies. One way to improve the technology is to use machine-learning approaches to comprehending CRISPR errors and predicting more specific edits and repairing outcomes.

Keywords: CRISPR-Cas9, genome editing, animal modeling, advantages, disadvantages

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Introduction

Animal modeling, also known as animal experimentation or animal testing, refers to the use of animals in scientific research and experimentation. It involves studying animals in order to gain insights into various biological processes, diseases, and potential treatments. Animal models are utilized in a wide range of scientific disciplines, including medicine, pharmacology, toxicology, genetics, and behavioral research (1). They play a crucial role in advancing our understanding of human biology and disease mechanisms, as well as in the development and testing of new drugs, therapies, and medical procedures. Commonly used animal models include

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mice, rats, rabbits, dogs, and non-human primates. These animals are chosen based on their genetic similarity to humans, physiological similarities, and practical considerations such as size, availability, and ease of handling. Animal modeling involves various types of experiments, such as basic research, disease modeling, drug development and safety testing and surgical and medical procedures (2, 3). Animals are utilized to study fundamental biological processes, genetics, and behavior. This research helps scientists understand normal physiology and how it can go wrong in disease states. Animals are genetically modified or exposed to specific conditions to mimic human diseases. This allows researchers to study disease progression, test potential treatments, and investigate underlying mechanisms. Animals are utilized to evaluate the efficacy and safety of new drugs before they are tested in humans. This includes assessing drug absorption, distribution, metabolism, and toxicity (4, 5).

CRISPR-Cas 9 acts by several stages with benefits including exact precision, versatility, efficiency, cost-effectiveness, potential for therapeutic applications, disease modeling and ethical considerations. CRISPR-Cas9 allows scientists to target specific genes with high precision, enabling precise modifications or edits to the DNA sequence (5, 6). It can be utilized to add, delete, or modify specific sections of the genome. It can be utilized in a wide range of organisms, including plants, animals, and even humans. This versatility makes it a valuable tool for studying gene function and developing potential therapies for genetic diseases. Compared to previous gene-editing techniques, CRISPR-Cas9 is relatively easy to use and highly efficient. It enables researchers to make targeted edits in a shorter time frame and with fewer resources. The simplicity and efficiency of CRISPR-Cas9 have significantly reduced the cost of gene editing experiments (7). This accessibility has democratized genetic research and allowed more scientists to explore its potential applications. CRISPR-Cas9 holds promise for treating genetic disorders by correcting disease-causing mutations. It offers the potential to develop personalized gene therapies tailored to an individual's genetic makeup. CRISPR-Cas9 can be utilized to improve crop yields, enhance nutritional content, and increase resistance to pests, diseases, and environmental stressors. This technology has the potential to contribute to sustainable agriculture and food security (8, 9). CRISPR-Cas9 enables researchers to create disease models by introducing specific genetic mutations into cells or organisms. These models can help understand disease mechanisms and facilitate the development of new drugs and treatments. While not a direct benefit of CRISPR-Cas9, its development has sparked important discussions around the ethical implications of gene editing. These conversations are crucial for establishing guidelines and regulations to ensure responsible use of this technology (10). CRISPR-Cas9 offers tremendous potential, while there are still challenges and limitations to overcome, such as off-target effects and delivery methods. Ongoing research and advancements in gene-editing technologies aim to address these concerns and further enhance the benefits of CRISPR-Cas9 (10, 11). The objective of this review study was to assess CRISPR-Cas 9 in animal modeling.

CRISPR-Cas9 in animal modeling

Chemical and physical methods have long been utilized to induce random mutations in the DNA sequence. Although these methods have a number of advantages, they suffer from certain limitations. For instance, deletion or single mutations such as missense and nonsense usually have functional consequences (12). The birth of transgenic genetically modified animal models is tied to the birth of recombinant DNA. The field of genome editing was revolutionized by discovering certain nucleases that could create double-stranded breaks at specific locations. Some salient examples of such programmable

nucleases are Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR) RNA guided nucleases (13, 14). Non-homologous end-joining repair at double-strands breaks (DSBs) can result in insertion or deletion mutations. Precise alteration can be created in the presence of a designed homologous donor DNA template (Figure 1).

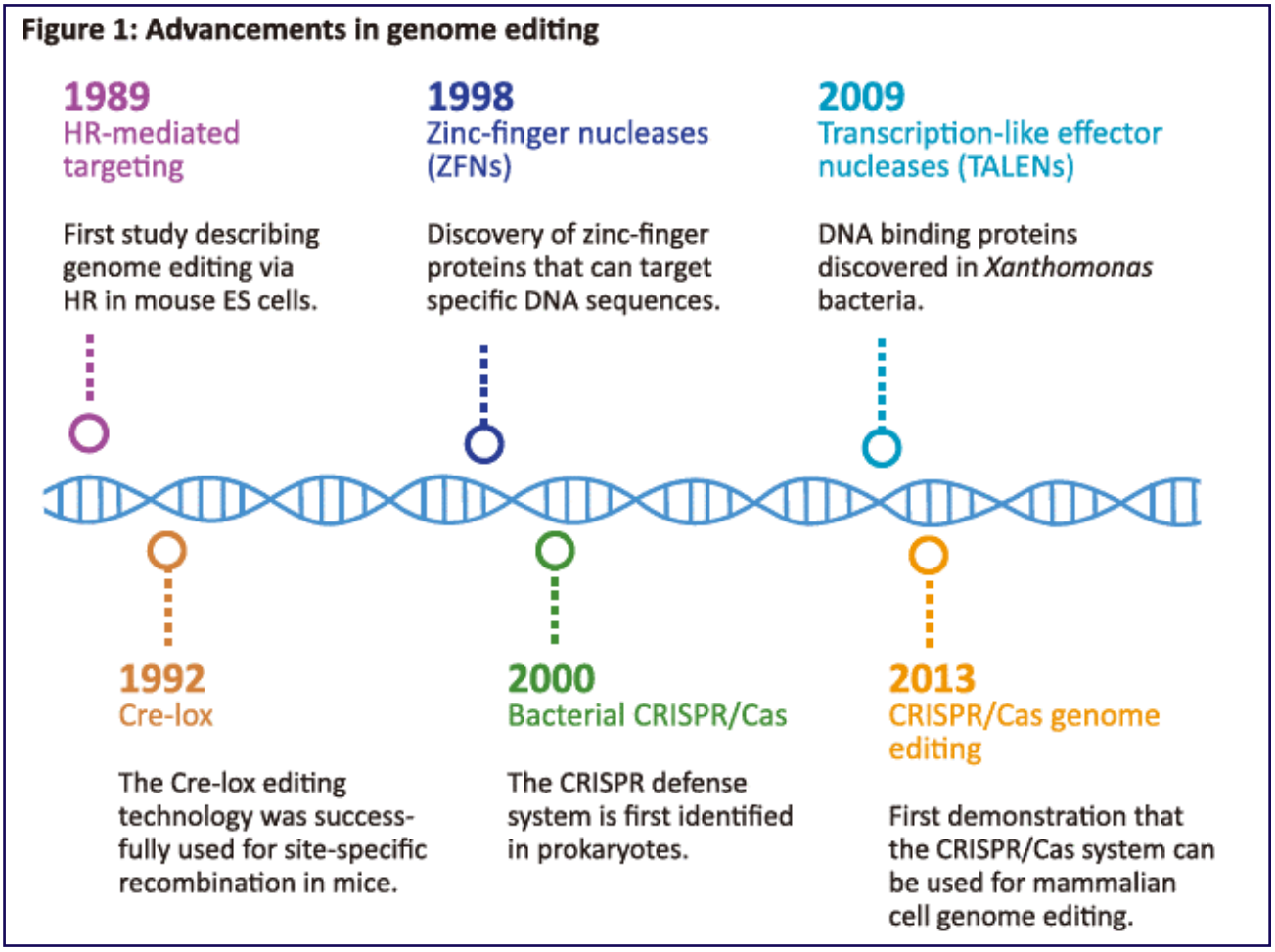

Figure 1. Timeline of key events in gene editing (15)

CRISPR-Cas9 is a powerful gene-editing tool that has revolutionized the field of genetic research. It allows scientists to make precise changes to an organism's DNA, including animal models, by targeting specific genes and introducing modifications.

Animal modeling using CRISPR-Cas9 includes several steps:

1. Designing the guide RNA (gRNA): The gRNA is a short RNA sequence that guides the Cas9 enzyme to the target gene. Scientists design the gRNA to specifically bind to the desired gene sequence (16).

2. Delivery of CRISPR components: The Cas9 enzyme and the gRNA are delivered into the cells of the animal model. This can be done through various methods such as microinjection into embryos, viral vectors, or electroporation (17).

3. Gene editing: Once inside the cells, the Cas9 enzyme binds to the target gene sequence guided by the gRNA. It then cuts the DNA at the targeted location. At this point, researchers can introduce specific modifications, such as inserting, deleting, or replacing DNA segments (18).

4. Repair mechanism: After the DNA is cut, the cell's natural repair mechanisms come into

repair at double-strands breaks (DSBs) can result in insertion or deletion mutations. Precise alteration can be created in the presence of a designed homologous donor DNA template (Figure 1) play. There are two main repair pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ often leads to small insertions or deletions, resulting in gene disruption or loss of function. HDR can be used to introduce specific changes by providing a DNA template for repair (19).

5. Screening and selection: After the gene editing process, researchers screen the edited cells or embryos to identify those with the desired modifications. This can involve techniques such as PCR, DNA sequencing, or phenotypic analysis (20).

6. Generation of animal models: Once the desired modifications are confirmed, the edited cells or embryos are used to generate animal models. This can be done by implanting edited embryos into surrogate mothers by culturing edited cells and generating chimeric animals. Animal models created using CRISPR-Cas9 technology have been instrumental in studying the function of specific genes, understanding disease mechanisms, and developing potential therapies. They have contributed to advancements in various fields, including biomedical research, agriculture, and conservation biology (21, 22).

ZINC-Finger nucleases

Combining a C_2H_2 zinc-finger DNA binding domain and a FokI nuclease domain creates artificial proteins known as ZFNs (Figure 2). C_2H_2 zinc-finger domain is composed of 30 amino acids. Its secondary structure has two anti-parallel B-sheets by a zinc ion coordinated by two cysteines and an α-helix with two histidine residues (23). The α-helix, also known as the recognition helix, is responsible for identifying three nucleotides, thereby arrays of the 3-6 ZF modules should assemble to recognize9-18 nucleotides. These parts then fuse to a FolK expression vector, and the final chimeric protein is created. Since each FokI nuclease makes a nick in one strand, for having double-strand breaks at a target site, two molecules of ZFNs are needed. Naturally, the cell tries to repair these breaks with a non-homologous end joining (NHEJ) pathway; however, in the presence of a DNA sequence complementary to the break sites, the homology-directed repair pathway (HDR) inserts a new sequence at the break sites (24-26).

Transcription Activator-like Effector Nucleases

TALENs are chimeric proteins containing two domains: DNA binding domain (Transcription Activator-Like Effector or TALEs) that recognizes a target sequence and a nuclease domain from FokI nucleases. One TALE domain recognizes one nucleotide; therefore, to identify a target sequence, 12-20 TALE domains should be assembled. DNA-binding domain is futilized with FokI expression cassettes, and finally, TALEN proteins are expressed. TALEN molecules are required to create a DSB. Like ZFNs, specific TALEN tools should be designed and built for each target, rendering the process laborious, time-consuming, and expensive (27-29).

Cre-lox system

The demand for an efficient genetic engineering tool arose with the growth of animal disease models. Cre-lox technology has been utilized since the early 1990s and played a pivotal role in developing transgenic mouse models. The Cre-lox

system allowed researchers to control gene expression. Site-specific recombinase DNA enzyme called Cre, identifies the locations of 3 pairs of genes called loxp. The Cre enzyme mediates recombination, resulting in knocking out of the target genes between loxP sites (30, 31).

In spite of their effectiveness, these methods require extensive expertise in protein engineering, which is a bottleneck for the majority of research laboratories. The progress in genome editing technology has identified essential prerequisites for a widely utilized method in genetic engineering. Such technology should be highly efficient, cost-effective, and convenient (32).

CRISPR-Cas9

CRISPR can be traced back to its origin in the late 1980s. In 2002, these sequences were given a name: Clustered Regularly Interspaced Short Palindromic Repeats, or CRISPR. Cas genes have always been linked to the CRISPR site and exhibit helical and endonucleases characteristics. The presence of multiple CRISPR chromosomal sites and the endonucleases associated with them suggest that CRISPRs are motile agents and Cas proteins play a role in their genetic integration. The length of CRISPR arrays varies, but they all share a specific feature in their sequence, which is the presence of repeat-spacer sequences (17).

Until a decade ago, the acquired immune system was thought to exist solely in eukaryotes. The discovery of CRISPR and Cas proteins led to the hypothesis that most bacteria and archaea have a complex developed immune system. In 2005, three independent research groups announced that CRISPR spacers were derived from viral and non-chromosomal sources. Overall, thesefindings encourage researchers to use CRISPR as an acquired bacterial immune system (18). Since then, five types of CRISPR-Cas systems have been identified in bacteria. In 2013 (Figure 3), researchers utilized the type II system in the bacterium *Streptococcus pyogenes* (SpCas9) to edit the genome of mammalian cells.

CRISPR-Cas9 system can generate DSBs in genes to knock out mutations through NHEJ and Knock in mutations through HDR (if donor DNA is present).

This system is relatively easy to exploit and requires only two components, the Cas9 and a copy of the crRNA-tracrRNA, known as the guide RNA (gRNA) (19). Cas9 protein is a golden tool for genetic engineering and genetically modified organisms. Owing to its programmability, Cas9 has been proposed as a useful molecular tool in genomic editing in a variety of organisms and cells, including human cells, mice, zebrafish, Drosophila (20, 21),*Caenorhabditis elegans*, rats (22), pigs (23), monkeys (24), and plant cells. Both in *vitro* and *in vivo* genome manipulation modes are easily possible using guide RNAs, artificially designed and synthesized gRNAs. Therefore, Cas9 is a fascinating alternative to the usual protein-based methods such as ZFNs and TALEN. In eukaryotic cells, the double incisions of DSB created by Cas9 are repaired by NHEJ or HDR, which can be utilized for insertion, deletion, and substitution in the incision (25).

Genome editing in cells by CRISPR-Cas9

CRISPR-Cas9 is a revolutionary gene-editing tool that allows researchers to makeprecise alterations to an organism DNA. Regarding an overview of how CRISPR-Cas9 works, there are several stages including guide RNA (gRNA) design, Cas9

protein binding, the guide RNA binding to the Cas9 protein, target recognition and DNA cleavage or DNA repair (8).

After the DNA is cut, the cell's natural repair mechanisms come into play. There are two primary repair pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR) (32).

- NHEJ: This repair pathway rejoins the loose ends of the DNA strands, often resulting in small insertions or deletions at the cut site. These mutations can disrupt the function of the gene.

- HDR: If a repair template with a desired DNA sequence is provided along with the CRISPR components, the cell can utilize this template to make precise alterations at the target site.

By introducing CRISPR-Cas9 components into cells, scientists can edit the DNA at specific locations. This technology has significant implications for various fields, including basic research, biotechnology, and potentially treating genetic diseases. Most researchers prefer CRISPR-Cas9 to other methods, such as ZFNs and TALENs, owing to its preciseness, ease of use, and the fact that it can modify eukaryotic cells. Since making CRISPR-Cas9 vectors and reagents is a convenient task, the genome-editing field has been revolutionized faster in recent years, resulting in creation of custom engineered cells and animal models (26). One of the most critical challenges in this field is the requirement for embryonic stem cells (ES) to create genetically modified animal models. Hence, generating DSBs efficiently by direct injection of CRISPR reagents in the fertilized zygotes or embryos would simplify the process (27).

CRISPR-Cas9 and lower organisms

Various genetic models, such as zebrafish, *Caenorhabditis elegans*, and *Drosophila*, also benefits from CRISPR's technology and genome modification. It is worthwhile to mention that different studies have reported hereditary germinal modification, the introduction of high efficiency of specific mutations, and transgenic, tissue-specific editing in flies (33-38), fish (39-45), and worms (46-56).

Zebrafish is known as a classic model because it possesses inimitable features such as external fertilization, transparent embryos, high fertility, and rapid growth. In 2013, the synthesized Cas9 mRNA and gRNA targeting *fumarate hydratase (fh)* were confirmed to work in vivo to induce targeted genetic modifications in zebrafish with high efficiency (42). After one month, it was found that Cas9 / gRNA successfully induced biallelic conversion of *etsrp* and *gata5* in zebrafish somatic cells, leading to abnormal intersegment vessels and cardia bifida (57). Thus, *fh* was the first engineered gene by CRISPER in zebrafish, which cautilized generation of zebrafish-codon-optimized Cas9 and enhanced genome editing efficiency (58). A CRISPR/Cas9 vector system was employed for tissue-specific gene disruption. The *urod* gene, encoding uroporphyrinogen decarboxylase, was disrupted under the control of different tissue-specific promoters, simulating human hepatic cutaneous porphyria in zebrafish (39).

In *Drosophila,* effective mutagenesis of the yellow gene was cautilized by injecting Drosophila embryos with single-guide RNA (sgRNA) targeting the second exon of the gene, resulting in stable germline mutations in animals (33). The knock-out *Drosophila* models has been also made using the CRISPR/Cas9 method of gene editing (59).

Single-guide RNA (sgRNA) and Plasmids expressing Cas9 under separate promoters are able to be microinjected

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into the gonad in order to generate gene-edited *C. elegans models* (60). In addition, the CRISPR-Cas9 Inheritance Genome Edition has been performed in *C. elegans*. Expression of Cas9 protein, with specific sgRNAs targeting the coding sequences of the unc-119 and *dpy-13* genes, generates indels in these two genes, and the resultant animals showed previously identified phenotypes, such as uncoordinated (*Unc*) and Dumpy (*Dpy*) (49).

CRISPR-Cas9 and Rodents

One of the most common organisms for generating genetic models is the laboratory mouse due to providing the ES cells (61). However, the ES cells retrieved from rats were not strong enough to maintain pluripotency. Given the fact that the CRISPR tool can be utilized directly on zygotes (without the need for ES cells), this technology can be straightforwardly implemented in rats (62).

In general, for use in both early cancer studies to describe the function of cancer genes and screening for anti-cancer drugs, a laboratory mouse has been reconstructed to mimic human cancers (63). Rapid lung tumors were developed by rearranging *Eml4-ALK* in adult mice to test the drug response using crizotinib, an *ALK* inhibitor, for lung cancer targeted therapy (64). Other remarkable examples of new models include CRISPR-based liver, pancreas, brain, and hematopoietic tumors (65-71).

Genome-editing tools, in particular CRISPR technology, hold promise to be utilized as a truly revolutionary technique in mammalian models. These include mice, where CRISPR alters the landscape of genetic analysis, rats, pigs, and non-human primates. Traditional genetic tools developed in mouse have been challenging to adapt. Targeted genomic manipulation in the rat was transformed by the advent of ZFN and TALEN technology, which provided a means to create mutant strains without the need for ES cell cultures. In addition, although ZFNs and TALENs seem to be adequate, it has been difficult to adopt the newest CRISPR tools to produce a range of germline mutant strains, as well as knock-in fluorescent reporters in addition to Cre and loxP conditional mutants that have been proven effective in mouse model (26, 72). As is already evident in cell culture models, the simplicity of CRISPR will likely become the dominant genome-engineering tool in the rat in near future, and this will translate to an expanded array of rat disease models.

Genetically engineered mouse models utilized in biomedical research fall into one of four major categories: (1) simple knock-outs; (2) point mutation (a slight change in the gene sequence) knock-ins; (3) large cassette insertions (e.g., reporter or recombinase knock-ins such as GFP or Cre); and (4) genomic segments replacements (e.g., conditional knock-out models having two loxP sites flanking a target region, also known as floxed alleles). The first two categories (simple knock-outs and point mutation knock-ins) are the most widely utilized models; the second two categories (reporter/Cre knock-ins and floxed alleles) are demanding models. Several thousands of floxed and reporter/Cre knock-in models are created annually throughout the world. The Easi-CRISPR method was developed recently and is shown to be robust in creating floxed and reporter/recombinase knock-in models at the efficiencies of 30% to 60%, occasionally reaching 100% (73, 74).

CRISPR-Cas9 and Higher Organisms

In addition to site-specific modifications in the genomes, TALENs and CRISPR/Cas also have potential applications in various disease models and gene correction techniques. For example, human stem cell-based disease models were generated using TALENs, and different disease-related genes were analyzed, including *APOB* for human hepatitis C virus (HCV) replication, *SORT1* (encoding sortilin) for *ApoB* secretion in hepatocytes, insulin resistance in adipocytes and motor

neuron death, and *PLIN1* for lipolysis in adipocytes. More importantly, heritable disease models have been easily generated. For instance, lentivirus-delivered sgRNA:Cas9 genome editing was successfully exploited to generate mouse models of myeloid malignancy by modifying five genes in mouse hematopoietic stem cell (HSC) (75, 76).

Pigs are a highly relevant model for human disease because they closely reflect many aspects of human physiology. For instance, while existing mouse models of monogenic disorders such as cystic fibrosis (CF) and familial adenomatous polyposis (FAP) do not precisely reflect clinical symptoms, counterpart pig mutants demonstrate the classic disease progression observed in human beings, implying they are superior models for investigating particular diseases. Generally, pigs have not been a popular research model due to their size, high expense, and limited ability to create specific mutants; however, the deployment of easy, one-step CRISPR-mediated genetic engineering should pave the way for more common use of pig models in preclinical research. In fact, it would be interesting to see whether those who already use pig models for preclinical evaluation of wound-healing therapies as well as cardiovascular and metabolic treatments would adopt the novel tools to more closely model genetic-based disease (77, 78).

Advantages of CRISPR-Cas in Animal Modeling

CRISPR-Cas9 has revolutionized genetic engineering and offers several advantages in animal modeling. These mostly include precise genome editing, rapidity and cost-effectiveness, creation of disease models, study of gene function, applications in gene therapy and translation research and wide diversity for species (6, 79).

CRISPR allows researchers to make precise alterations in the DNA sequence of animals, including the addition, deletion, or modification of specific genes. It enables targeted modifications at a higher efficiency compared to traditional genetic engineering techniques. CRISPR technology is relatively rapid and cost-effective compared to older methods, such as gene targeting by homologous recombination. This allows for efficient generation of genetically modified animal models, reducing the time and resources required for research. CRISPR enables the development of animal models that closely mimic human diseases by introducing disease-associated mutations (9, 11). These models provide valuable insights into disease mechanisms, drug testing, and potential therapeutic interventions. CRISPR facilitates the investigation of gene function by creating knockout or knock-in animal models. Knockout models involve disabling a specific gene, allowing researchers to study the impact of its absence on various biological processes. Knock-in models involve inserting specific DNA sequences, such as reporter genes or disease-causing mutations, to study their effects (2, 3, 15). CRISPR has shown great promise in the field of gene therapy. Animal models can be utilized to test and optimize CRISPR-based therapies for various genetic disorders. The technology allows targeted correction of disease-causing mutations, providing potential treatment avenues. Animal models generated using CRISPR technology can bridge the gap between basic research and clinical applications (7). By accurately recapitulating human diseases, these models enable the evaluation of novel therapeutic approaches before advancing to human trials. CRISPR can be applied to a wide range of animal species, including mice, rats, pigs, and non-human primates. This versatility allows researchers to study various aspects of human biology and diseases in different animal models. While CRISPR technology offers significant advantages, ethical considerations and careful

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regulation should be taken into account when using it in animal research to ensure the well-being of the animals involved (72).

Challenges and obstacles

CRISPR is a potent technology for controlling any target gene across the genome with the goal of treating or preventing certain deficiencies/diseases. Nevertheless, the broad consequence of human gene editing, particularly germinal genes, cannot be forecasted. Before we begin editing, we should be certain of the outcomes of this strategy. Firstly, once edited, the germline gene will be present in the human population for generations and will appear to be intractable to remove; secondly, there is no guarantee of success; thirdly, the accuracy of editing as it may affect other unspecified genes or DNA segments; and, finally, the impact on gene interaction network, and pathways will be difficult to detect or predict (80, 81). The off-target effect is somewhat inherent to the CRISPR system: CRISPR-Cas9 is a bacterial adaptive immune system, and viruses constantly mutate, especially under selective pressure from the immune system; Cas9 is in turn programmed to attack slightly mutated (different) viral sequences. Off-targeting would be a major issue, particularly if the Cas9 cleaved at other sites, resulting in the loss of function of an essential gene.

Although good guides (with no or minimum off-target recognition sites) can diminish the potential for off-target mutations, the potential for the introduction of undesired modification is still open to scientific debate. It requires further work to make the tool safer. A careful evaluation or practical experimentation with possible off-target effects is crucial, mainly if the tool is utilized for any therapeutic purpose. Current off-target prediction algorithms are based on sequence alignment and discard targets with user-defined potential mismatches, but this does not eliminate all the gRNAs that are potential off-targets (82). Although improvements are being made to reduce off-target effects, there is still another issue: "on-target" damage. During the repair process, unwanted deletions, rearrangements, and insertion of DNA sequences may occur. This collateral damage can cause some genes to be turned on or off, which could have serious consequences for the therapeutic use of the CRISPR-Cas9 tool. Addressing these issues will allow the scientific community to make better use of this tool in the future studies.

To avoid unanticipated bioethical issues, several challenges, such as undesired side effects and individual choice, should be addressed before future clinical applications. Profound moral and ethical questions will also arise. It is mandatory to get ethics and safety approval for gene alteration in human cells by the National Institutes of Health (NIH). NIH currently does not fund CRISPR studies on human embryos and opposes CRISPR use on germline cells (in an egg, sperm, or embryo) because any such alterations would be permanent and heritable (83).

CRISPR opens up a new world of treatments, curing diseases and even eradicating them so that future generations are not affected. CRISPR/Cas9 genome editing has promised rapid breakthroughs in scientific, agricultural, and medical sciences, with cancer research having the most profound influence. The advancement and deployment of CRISPR-based technology is altering the way how cancer is studied and, ultimately, how it may improve therapeutics (83). Machine learning algorithms to help identify CRISPR errors and predict more specific edits and repair outcomes are one way to improve the technology. This would help select the best sgRNA and aid in screening for any unwanted effects (84). CRISPR opens up a new world of treatments, curing diseases and even eradicating them so that future generations are not affected. CRISPR/Cas9 genome editing has promised rapid breakthroughs in scientific, agricultural, and medical sciences, with cancer research having the most profound influence. The advancement and deployment of CRISPR-based technology is altering the way

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how cancer is studied and, ultimately, how it may improve therapeutics (83). Machine learning algorithms to help identify CRISPR errors and predict more specific edits and repair outcomes are one way to improve the technology. This would help select the best sgRNA and aid in screening for any unwanted effects (84).

Conclusion

The advantages of CRISPR Cas outweigh its disadvantage if designed properly in genome editing for various purposes. CRISPR is an extraordinarily powerful technique in regulating any target gene across the genome with promising therapy intentions or potentially preventing a myriad of deficiencies/diseases. CRISPR-Cas9 is a convenient tool for gene manipulation. Notwithstanding this, the broad consequence of human gene editing, particularly germinal genes, cannot be predicted. Before editing, we need to predict the outcomes and results associated with this approach. Firstly, once edited, the genes would be part of the human population for successive generations and may be impossible to remove from humanity; secondly, success not guaranteed; thirdly, the fidelity of editing, as it could affect unrelated genes or unspecified segments of DNA; and last but not least, its influence on gene interaction, network, and signaling pathways could be difficult to predict. Various challenges such as unexpected side effects and individual choices ought to be addressed prior to future clinical applications to avoid possible bioethical problems. The technique also ignited the moral and ethical concerns of scientific community. Ethics and safety approval for gene modification in human cells is required by the National Institutes of Health (NIH). The NIH does not currently fund studies of CRISPR in human embryos and opposes the CRISPR utilization in germline cells because these alterations would be permanent and heritable. The technology has promised the potential for rapid change in agricultural and medical sciences, with the most profound implications for cancer therapy. Recent advances in CRISPR-based technology is redefining how cancer is studied and potentially improves anti-cancer therapies. One way to improve the technology is to use machine learning approaches to comprehending CRISPR errors and predicting more specific edits and repair outcomes.

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Conflict of Interest

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Ethical Considerations

Not applicable.

Compliance with Ethical Standards

This article does not contain any studies with animals performed by any of the authors.

Code of Ethics

Not applicable.

Author's Contributions

A.G. and M.M. designed the study and wrote the draft. E.N. G.N. M.K. M.G. A.S. visualized, edited and approved the study.

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