The revolutionary role of CRISPR in disease treatment and prevention

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ABSTRACT

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a powerful genome editing technology that has revolutionized the field of genetic engineering. In recent years, CRISPR has emerged as a promising tool for the treatment and prevention of various genetic and non-genetic diseases. This review article provides an overview of the revolutionary role of CRISPR in disease treatment and prevention, covering various aspects of its application in diverse fields such as oncology, neurology, infectious diseases, and rare genetic disorders. We discuss the challenges and limitations of CRISPR, including off-target effects and delivery issues, as well as ethical considerations surrounding its use. Furthermore, we provide insights into the future of CRISPR-based therapies and highlight the potential of this technology for advancing precision medicine. In general, this review highlights the significant impact of CRISPR in disease treatment and prevention and emphasizes its potential to revolutionize the field of medicine in the coming years.

Keywords: CRISPR, sickle cell anemia, cancer, infectious diseases, HIV

INTRODUCTION

Clustered regularly interspaced short palindromic repeat (CRISPR)-Cas systems are currently in the spotlight of active research in biology. The first CRISPRs were detected more than three decades ago in *Escherichia coli* in the course of the analysis of the gene responsible for isozyme conversion of alkaline phosphatase (Ishino *et al*., 1987 In Ishino *et al*., 2018). However, it was not until the early 2000s that the potential for using CRISPR as a tool for gene editing was recognized (Jinek *et al.*, 2012). In 2012, Jennifer Doudna, Emmanuelle Charpentier, along with their colleagues identified the mechanism through which bacteria employ the CRISPR/cas 9 system to defend themselves against viruses. The researchers also proposed the idea of using the CRISPR/cas 9 system as a genome editing (Jinek *et al.*, 2012). This groundbreaking discovery opened the door for researchers to use CRISPR to make precise edits to the DNA of any organism, including humans.

Since then, CRISPR has been widely used in a variety of fields, including plant breeding, animal research, and human therapeutics (Liang *et al*., 2015). The potential applications of CRISPR are vast and include the ability to modify genes associated with diseases, increase crop yields, and even prevent organisms from going extinct (Liu *et al*., 2021). CRISPR allows for precise and efficient editing of the genome, enabling researchers to alter or repair specific genetic sequences associated with disease (Gaj *et al*., 2013). This revolutionary gene editing tool allows for precise modification of specific genomic sequences, enabling the correction of genetic mutations that lead to diseases (Zhang *et al.*, 2011).

The system utilizes a programmable enzyme called Cas9, which can be directed to specific genomic locations to cut and modify DNA sequences. This has led to the development of therapies for a range of genetic diseases, including sickle cell anemia (Asmamaw & Zawdie, 2021) beta-thalassemia (Papaemmanuil *et al*., 2016), and Duchenne muscular dystrophy (Mou *et al*., 2017), caused by mutations in specific genes. With CRISPR, it is now possible to identify and correct these mutations, offering the potential for effective treatments for diseases that were previously untreatable (Hsu *et al.*, 2013).

One major advantage of CRISPR-based therapies is their ability to specifically target and modify the genomic loci responsible for the underlying genetic defects. This allows for precise correction of the

genetic mutation, rather than the more general approach of traditional therapies such as enzyme replacement or gene supplementation. In the case of sickle cell anemia, CRISPR-based therapies have demonstrated the ability to restore normal hemoglobin production and improve clinical outcomes (Hailu *et al*., 2020).

CRISPR technology represents a revolutionary breakthrough in the field of disease treatment and prevention, offering unprecedented precision and efficiency in genome editing. While still in its early stages, CRISPR holds immense potential for addressing a wide range of genetic disorders and infectious diseases, as well as advancing our understanding of fundamental biological processes. This review aims to provide an overview of the current state of CRISPR-based therapeutics and highlight some of the most promising applications of this technology, while also discussing the key challenges that need to be overcome for its widespread adoption. Ultimately, the goal of this review is to offer insights into how CRISPR can transform the landscape of medicine and improve patient outcomes.

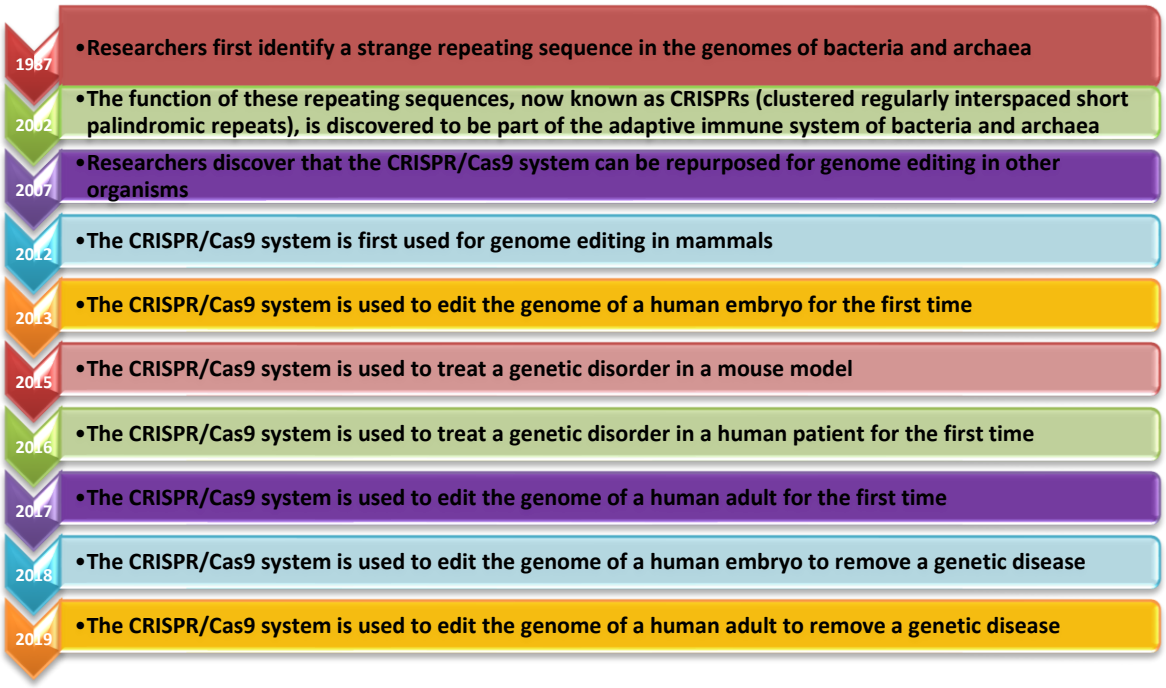

Figure 1: Timeline of key events in the development and advancement of the CRISPR/Cas9 system for genome editing.

CRISPR-Cas biology and principle

CRISPR is a natural defense mechanism found in bacteria and other microorganisms that helps to protect against infection by viruses and other foreign elements. CRISPR is made up of short DNA sequences that are separated by longer "spacer" sequences, and it is associated with a group of proteins known as CRISPR-associated (Cas) proteins (Rath *et al*., 2015). The CRISPR-Cas system has been adapted for use as a powerful tool for genome engineering, and it has revolutionized the field of molecular biology. The most well-known CRISPR-Cas system is CRISPR-Cas9. CRISPR-Cas9 works by recognizing and cutting specific sequences of DNA, and it has been adapted for use in genome editing applications (Doudna & Charpentier, 2014).

To use CRISPR-Cas9 for genome engineering, researchers design a small RNA molecule that recognizes and binds to a specific target sequence in the genome. The RNA molecule is then paired with the Cas9 enzyme, which uses the RNA as a guide to cut the DNA at the target site. This creates a break in the DNA molecule, which can then be repaired by the cell's natural DNA repair mechanisms.

Depending on the desired outcome, the repair process can result in the insertion, deletion, or substitution of a specific sequence in the genome (Asmamaw *et al*., 2021).

The use of CRISPR in disease treatment

Sickle Cell Anaemia

Sickle cell anemia is a genetic disorder that is caused by a mutation in the Hemoglobin Subunit Beta **(**HBB) gene, which codes for the production of a protein called beta-globin. This mutation leads to the production of abnormal hemoglobin, which causes red blood cells to become sickle-shaped. These abnormal red blood cells have a shortened lifespan and are prone to clogging blood vessels. This can cause a variety of symptoms, including anemia, severe pain, and organ damage (Inusa *et al*., 2019). One of the most promising applications of CRISPR is in the treatment of inherited genetic diseases. These diseases are caused by mutations in specific genes that are passed down from a person's parents, and are often difficult to treat or cure. CRISPR has the ability to precisely target and edit these mutant genes, potentially providing a cure for these diseases (Hsu *et al*., 2013). For example, CRISPR has been used to correct the genetic mutation that causes sickle cell anemia (Frangoul *et al*., 2021). This could be done by cutting the DNA at the site of the mutation and repairing it with a healthy copy of the gene. Using CRISPR, researchers were able to correct this mutation in human hematopoietic stem cells, resulting in the production of normal red blood cells (Frangoul *et al*., 2021). This demonstrates the potential for CRISPR to provide a cure for inherited diseases.

Another approach being explored is using CRISPR to stimulate the production of fetal hemoglobin, which can compensate for the abnormal adult hemoglobin in people with sickle cell anemia. This approach has also shown promise in animal studies and is currently being tested in clinical trials (Demirci *et al*., 2021).

Cancer

In addition to its use in genetic disorders, CRISPR has also been investigated as a potential tool for treating cancer. Cancer is caused by mutations in genes that regulate cell growth and division, leading to uncontrolled proliferation and the formation of tumors (Hanahan and Weinberg, 2011). By using CRISPR to target and remove these mutations, researchers have used CRISPR to delete or modify genes that drive the growth and proliferation of cancer cells, leading to significant tumor regression in animal models (Gaj *et al.*, 2013). Moreover, CRISPR has been used to enhance the effectiveness of other cancer therapies, such as chemotherapy and radiation, by increasing the sensitivity of cancer cells to these treatments (Martinez-Lage *et al*., 2018). Cyranoski (2016) reported the first CRISPR human trial to treat patients with metastatic non-small cell lung cancer who had failed to respond to chemotherapy, radiation, and other therapies with CRISPR-edited T cells (knockout PD-1 gene). CRISPR has also been used in the development of new drugs. For example, CRISPR has been used to modify enzymes in order to create more effective drugs for the treatment of cancer (Zhang *et al*., 2017). These findings suggest that CRISPR could be used to develop targeted therapies for cancer, potentially leading to more effective and less toxic treatments for this devastating disease.

Cystic fibrosis

CRISPR has been used to correct the genetic mutation that causes Cystic fibrosis (Fan *et al*., 2018). Cystic fibrosis (CF) is an autosomal recessive disorder that affects the respiratory, digestive, and reproductive systems. It is caused by a mutation in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This protein plays a critical role in regulating the flow of electrolytes, such as sodium and chloride, across cell membranes. When the CFTR protein is not functioning properly, it can lead to the buildup of thick, sticky mucus in the lungs and other organs, leading to serious respiratory and digestive problems (Maule *et al.*, 2020). The genetic mutation that causes CF can be corrected using CRISPR gene editing technology, which allows for the precise modification of specific parts of the genome. Fan *et al*., (2018) described the generation of a sheep model for CF using CRISPR/Cas9 genome editing and somatic cell nuclear transfer (SCNT) techniques.

The cells were generated with *CFTR* gene disruption and used them for production of $CFTR^{-/-}$ and $CFTR^{+/-}$ lambs. The newborn $CFTR^{-/-}$ sheep developed severe disease consistent with CF pathology in humans. In human cells and animal models, CRISPR has shown promise as a potential treatment for CF.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a genetic disorder that affects muscle strength and function. It is caused by a mutation in the gene encoding the dystrophin protein, which is essential for maintaining the structural integrity of muscle fibers (Duan *et al.*, 2021). The absence of dystrophin leads to progressive muscle weakness and degeneration, and it can also affect other organs, such as the heart and lungs. CRISPR gene editing technology has the potential to treat DMD by correcting the genetic mutation that causes the disease. In human cells and animal models, CRISPR has been shown to be a powerful tool for modifying specific parts of the genome with great precision. Long *et al*. (2014) performed the first proof-of-concept for in vivo CRISPR-mediated gene editing in DMD. They injected zygotes with SpCas9, sgRNA, and an exogenous DNA template for homology-directed repair (HDR). The resultant mice were mosaic for corrected dystrophin, varying from 2 to 100% correction.

Huntington's disease

Huntington's disease (HD) is a genetic disorder that affects the brain and nervous system. It is caused by a mutation in the gene that encodes the huntingtin protein, which plays a critical role in brain development and function. The mutated huntingtin protein can lead to the death of brain cells, resulting in progressive physical and mental decline. Researchers have used CRISPR to correct the genetic mutation that causes HD in human cells and animal models. Ekman *et al*., (2019) reported that the CRISPR-Cas9-mediated disruption of the mutant HTT gene resulted in a ∼50% decline in neuronal inclusions and significantly improved lifespan and certain motor deficits.

Infectious diseases

CRISPR also has the potential to revolutionize the treatment of infectious diseases. The CRISPR-Cas9 system has been used to knock out genes in bacterial and viral pathogens, potentially leading to the development of new antimicrobial therapies (Bikard *et al*., 2014).

Human Immunodeficiency Virus (HIV)

CRISPR has been shown to be effective in the treatment of infectious diseases, such as HIV and influenza (Gaj *et al.*, 2013). In order to decrease HIV-1 infection and eradicate the provirus, CRISPR has been used to target cellular co-factors or the HIV-1 genome. It has also been used to trigger transcriptional activation of latent virus in latent viral reservoirs for elimination. In both human cells and animal models, this adaptable gene editing technology has been used to effectively prevent and reduce HIV-1/AIDS (Xiao *et al.*, 2019).

Zika Virus

The study by Hoffmann *et al*. (2020) used the CRISPR-Cas9 system to perform genetic screens in order to identify genes that may play a role in the host response to Zika virus infection. Specifically, the researchers used CRISPR to knock out or disrupt specific genes in human cells and then exposed the cells to Zika virus. By analyzing the cells that were resistant to the virus, the researchers were able to identify genes that may be important for the host response to Zika virus infection. The authors of the study suggest that these findings could be useful for the development of new therapies for Zika virus infection. These findings suggest that CRISPR could be used to develop new, more effective treatments for infectious diseases.

Hereditary tyrosinemia

CRISPR also has been used to successfully treat a pig model of Hereditary tyrosinemia type I (HT1), a rare metabolic disorder caused by a deficiency in the enzyme fumarylacetoacetate hydrolase (FAH) (Gu *et al*., 2018). Using CRISPR-mediated gene editing, Gu *et al*., (2021) reported an improved single-step method to establish a biallelic ($FAH^{-/-}$) mutant porcine model using CRISPR-Cas9 and cytoplasmic microinjection. The feasibility of rescuing HT1 pigs through inactivating the 4-hydroxyphenylpyruvic acid dioxygenase (*HPD*) gene, which functions upstream of the pathogenic pathway, rather than by directly correcting the disease-causing gene as occurs with traditional gene therapy was also tested. Direct intracytoplasmic delivery of CRISPR-Cas9 targeting *HPD* before intrauterine death reprogrammed the tyrosine metabolism pathway and protected pigs against *FAH* deficiency-induced LLI.

Eye conditions

CRISPR/Cas9 has been explored as an efficient therapeutic tool for the treatment of different genetic eye defects. It has been widely used in ophthalmology research by using mouse models to correct pathogenic mutations in the eye stem cells. CRISPR technology has shown promising results in the treatment of eye conditions such as age-related macular degeneration (AMD) (Kim *et al*., 2017) and retinitis pigmentosa (RP) (Bakondi *et al*., 2016).

Age-related macular degeneration

AMD is a leading cause of vision loss in people over the age of 60 and is characterized by the degeneration of the macula, a small area of the retina responsible for central vision. There is no cure for AMD, but treatments such as anti-vascular endothelial growth factor (VEGF) therapies and dietary supplements have been shown to slow its progression (Kim *et al*., 2017). However, CRISPR has been used to successfully edit the genetic mutations that cause AMD in animal models, by targeting and repairing the mutated genes that cause the disease (Kim *et al*., 2017). In a study published in the journal Genome Research, Kim *et al*., (2017) used CRISPR to edit the genes of mice with a form of AMD and found that it improved their vision and slowed the progression of the disease.

Retinitis pigmentosa

Researchers have also investigated the use of CRISPR to treat RP. RP is a rare genetic disorder that causes progressive vision loss due to the degeneration of the retina. There is currently no cure for RP, but treatments such as vitamin A supplements and low vision aids can help manage the condition (Bakondi *et al*., 2016). CRISPR/Cas9 technology has been applied in some in vitro and in vivo experiments to treat RP, such as removing a mutation in the rhodopsin gene in a rat model of adRP. (RhoS334). In a study Bakondi *et al*., (2016), the exon 1 closely upstream of a PAM exclusive toward the RhoS334 locus was the target of an intravitreal delivery of sgRNA/Cas9 plasmid in S334ter-3 rats. Genome analysis of transfected retinal cells from two separate rats showed cleavage efficacy of 33 and 36%. Through immunohistological analysis, subsequent injection of the sgRNA/Cas9 plasmid has demonstrated improved eye perception and extensive retinal protection. In another study by Gumerson *et al*., (2022), they reported the use of CRISPR to edit the genes of mice with RP and found that it improved their vision. These results suggest that CRISPR could potentially be used to treat these vision-threatening conditions in the future.

Table 1: Application of CRISPR in the treatment of genetic diseases

Disease	Application of CRISPR	Reference
Sickle cell disease	Correction of genetic mutation	Frangoul *et al*., (2021)
Cystic fibrosis	Correction of genetic mutation	Fan *et al*., (2018)
Duchenne muscular dystrophy	Correction of genetic mutation	Long *et al*., (2014)
Huntington's disease	Correction of genetic mutation	Ekman *et al*., (2019)
Tay-Sachs disease	Correction of genetic mutation	Ou *et al*., (2020)
Retinal degeneration	Correction of genetic mutation	Bakondi *et al*., (2016)
Breast cancer	Edit genome to make cells more sensitive to chemotherapy	Parashar *et al*., (2019)
HIV	Edit genome to eliminate virus	Xu *et al*., (2017); Xiao *et al*., (2019)
Muscular dystrophy	Edit genome to improve muscle function	Long *et al*., (2014)
Pancreatic cancer	Knock-out genes implicated in disease progression	Watanabe *et al*., (2018)
Alzheimer's disease	Introduce a short deletion in the transcriptional start codon in exon 1 of the Mapt gene	Tan *et al*., (2018)
Genetic deafness	Edit genome of inner ear cells to restore hearing	Gao *et al*., (2018)
Blood disorders	Edit genome of hematopoietic stem cells	Vuelta *et al*., (2020)
Genetic eye disorders	Edit genome of eye cells	Kim *et al*., (2017)
Genetic skin disorders	Edit genome of skin cells	Wu *et al*., (2017)

The use of CRISPR in disease prevention

In addition to its potential for treating inherited diseases, CRISPR has also been explored as a tool for preventing the onset of diseases. Many infectious diseases are caused by pathogens that have evolved to evade the immune system, making them difficult to treat (Doerflinger *et al.* 2017). By using CRISPR to modify the genomes of these pathogens, researchers have been able to make them more susceptible to the immune system and thus more easily preventable (Bikard *et al*., 2014). For example, researchers have used CRISPR to delete the CCR5 gene, which is a key receptor for the HIV virus (Xu *et al*., 2017). By deleting this gene, researchers were able to protect human cells from HIV infection (Xu *et al*., 2017). CRISPR has also been used to modify the flu virus to make it less virulent, potentially paving the way for the development of more effective flu vaccines (Bikard *et al*., 2014).

CRISPR can be used to modify the genome of mosquito populations, making them resistant to the malaria parasite thereby reducing their ability to transmit malaria, dengue fever (Hammond *et al*., 2016), and Zika fever (Hoffmann *et al*., 2021). This demonstrates the potential for CRISPR to be used as a preventative measure against infectious diseases and could have a significant impact on global health, as these diseases continue to be major public health challenges in many developing countries.

Furthermore, CRISPR has the potential to prevent the transmission of genetic diseases from parent to child. By editing the germline cells, the DNA of which is passed down to future generations, CRISPR could potentially eliminate the risk of inherited genetic disorders (Hsu *et al*., 2013). For example, preimplantation genetic diagnosis (PGD) is a commonly used method for screening embryos for inherited genetic diseases before implantation. However, this method is limited to a small number of genetic conditions that can be identified through genetic testing. CRISPR-based therapies have the potential to be used in conjunction with PGD to edit out genetic mutations before the embryo is implanted, suggesting that this approach has the potential to prevent the transmission of inherited genetic diseases (Ranisch, 2020). This could enable the prevention of a wide range of inherited diseases, including many that are currently untreatable.

Challenges

Despite the promising potential of CRISPR, there are still many challenges that need to be addressed before it can be widely used in the clinic. One major challenge is the risk of off-target effects, which occur when CRISPR targets and modifies unintended sections of the genome (Liu *et al*., 2021). Another concern is the potential for CRISPR to introduce new mutations or disrupt essential genes, leading to unintended consequences such as the development of new diseases or the worsening of existing conditions (Hsu *et al*., 2013). To minimize the risk of off-target effects, researchers have developed a number of strategies, such as using modified versions of CRISPR/Cas9 that have improved specificity, or using computational tools to predict and avoid potential off-target sites. However, off-target effects remain a concern, and further research is needed to better understand and mitigate this risk.

Additionally, there are ethical concerns surrounding the use of CRISPR in humans, particularly in the context of germline editing (Schleidgen *et al*., 2020), including the potential for it to be used for non-therapeutic purposes, such as enhancing certain traits or abilities (Gyngell *et al.*, 2017). Also, the application of CRISPR raises significant ethical concerns, as it could potentially lead to the creation of "designer babies" and the further widening of socio-economic disparities (Doudna and Charpentier, 2014). To address these concerns, it is important to have robust ethical guidelines in place to ensure that CRISPR is used responsibly and in accordance with the principles of beneficence and non-maleficence. It is also important to engage in public dialogue and to consider the potential long-term consequences of using CRISPR for non-therapeutic purposes (Naeem *et al*., 2020).

Regardless of these challenges, the potential for CRISPR to revolutionize disease treatment and prevention is undeniable. Its ability to precisely modify specific sections of the genome has opened up new possibilities for the treatment of genetic disorders, cancer, and infectious diseases. As researchers continue to refine and optimize the use of CRISPR, it is likely that we will see significant progress in the treatment and prevention of a wide range of diseases.

CONCLUSION

CRISPR technology has already shown tremendous promise in treating a variety of genetic diseases such as sickle cell anemia, cancer, HIV, cystic fibrosis, Huntington's disease, etc. These are diseases that are caused by mutations in specific genes, and by using CRISPR to correct these mutations, scientists can provide permanent cures for these debilitating conditions. Additionally, CRISPR can be used to prevent inherited diseases by editing the genes of embryos, a technique known as germline editing. CRISPR also has significant implications for developing new antimicrobial therapies. With the rise of antibiotic-resistant bacteria, there is an urgent need for new treatments to combat infectious diseases. CRISPR-based therapies offer a promising alternative to traditional antibiotics, as they can target specific pathogens and prevent the development of resistance.

Despite the enormous potential of CRISPR, there are still many challenges that need to be addressed before it can be widely adopted. One major concern is off-target effects, where CRISPR makes unintended edits to the genome. Additionally, ethical concerns surrounding the use of CRISPR for germline editing and the potential for unintended consequences in the environment must also be carefully considered.

In the future, research in CRISPR technology will focus on addressing these challenges, improving its efficiency and accuracy, and expanding its applications beyond genetic diseases and antimicrobial therapies. With continued investment in research and development, CRISPR holds the potential to transform the landscape of medicine, agriculture, and food security, and revolutionize our approach to disease treatment and prevention.

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