Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.1S,
January-June 2024: P.947-956

Print version ISSN 0970-0765
Online version ISSN 2320-3188

Original Research Article
Content Available online at: www.bpasjournals.com

OVERVIEW ON THE ROLE OF CRISPR TECHNOLOGY IN CLINICAL APPLICATIONS

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Abstract
One of the most significant scientific advances in DNA editing is CRISPR-Cas technology, which is based on repetitive sequences derived from bacterial DNA and has been shown to have immunological properties. Through genetic editing, scientists have altered these sequences to target the genes in order to treat diseases by adding or removing specific sequences from the target genes' DNA. As a result, this technology is regarded as a revolution in the medical field since it treats many diseases more quickly, accurately, and efficiently. In this review, we went over the various forms of CRISPR technology and discussed the most significant medical conditions for which this technology can be used to treat patients' illnesses and problems, additionally, the most significant difficulties this technology faces were noted.

Key Words: CRISPR Cas, CRISPR Types, Gene editing, Diseases

Introduction

Recent advances in genetics and biology have paved the way for future scientific discoveries by manipulating DNA and examining its helical structure, which has allowed scientists to learn more about the makeup of the genome [1]. The field of genetic engineering has witnessed significant advancements in various domains, including the identification of genetic variation and differences, genome modification, and manipulation of living organisms' genomes and cells. Among these technologies, CRISPR/Cas has revolutionized biomedical research, because of the numerous laboratory applications of this technology, it is now possible to correct errors in the genome [2]. Because of its low cost, high efficiency, and straightforward design, this technology has become one of the most common in recent years. It has demonstrated great potential in the study of genes and genomic functions in humans, animals, plants, and microorganisms [3]. Because it is quick and easy to do, by adding, removing, and changing nucleotides in the genes of bacterial and eukaryotic cells [4]. Because of this technology's importance and role in treating diseases and health problems, this review aims to identify this technology, identify its different types, and highlight the most important disease conditions that it works on. It also highlights the challenges that arise when applying this technology, which is one of the most important scientific achievements that scientists and researchers seek to use to solve many health problems.

Discover of CRISPR/Cas System

This system of regularly interspaced short palindromic repeats was first discovered when a

group of Japanese scientists studied the bacterium *Escherichia coli* in 1987 and later discovered it in other bacteria, until 2005, when scientists found similarities in DNA sequences, this led to the hypothesis that the sequences are derived from the bacterial adaptive immune system [5]. The active CRISPR RNA, tracrRNA, crRNA, and several CRISPR-Cas genes that encode for Cas proteins with endonuclease activation are all transcribed from these repetitive sequences [6]. This system, called the prokaryotic adaptive immune system, is present in bacteria and archaea. It was discovered that this system serves as an adaptive immune response intended to target and sever the DNA of the virus when it attacks a host. Scientists were able to make genetic modifications by re-engineering this immunity to the specific parts of the genetic material to be targeted, extremely particular to the kind of cells that need to be changed [7]. These sequence repeats have a role in attacking and identifying pathogen infection because it was discovered that they are identical to the DNA sequences of foreign viruses and plasmids [8]. Cas proteins split and chop the genetic material into tiny pieces when foreign organisms like viruses attack prokaryotic cells. These fragments then overlap and combine with the CRISPR DNA. The crRNA identifies and recognizes the pathogen once it attacks the prokaryotic cells, at which point it binds to it. It activates Cas proteins, which cleave the targeted region of the pathogen's DNA [6].

When prokaryotic cells are attacked by foreign organisms such as viruses, Cas proteins fragment and cut the genetic material into small pieces, which then overlap and merge with the CRISPR DNA. Once the same pathogen attacks the prokaryotic cells, it is identified and recognized by the crRNA and then binds to it, and stimulates Cas proteins that cut the target portion of the genetic material of the pathogen [6].It was discovered that this system operates in three steps, the adaptation stage during this stage, the DNA of the virus is combined with the CRISPR sequences of the bacteria. At this point, the bacteria acquire fresh DNA sequences from the pathogen, the expression process is a representation of the second stage, which involves copying, processing and conversion of the CRISPR RNA (crRNA) into a single crRNA with repeated regions and spacers. The last stage known as interference, involves trying to shield the bacteria from infections by external pathogens like viruses by dividing the pathogen's DNA through Cas proteins [9].

Types of CRISPR/Cas System

There are two variants of the CRISPR-Cas system: Class I and Class II. According to [10], these two classes are further subdivided into six types: types I, III, and IV are part of the first class, and types II, V, and VI are part of the second. The first, second, and fifth types of CRISPR systems have been shown to recognize and bind to DNA; the third type modifies DNA and RNA; the fourth type has an ambiguous effect on DNA and RNA; and the sixth type is involved in RNA editing [11]. Cas endoribonuclease cleaves pre-crRNA in the first type of CRISPR-Cas system to produce mature crRNA, which then interacts with Cas proteins [12].

The first type is made up of a set of influencing factors like Cas3 and a set of proteins that are divided into different subtypes I-A, I-B, I-C, I-U, I-D, I-E, and I-F based on how many of them there are and how often they appear in the chain. According to [13], the proteins that comprise the first type are Cas4, Cas5, Cas6, Cas7, Cas8 (Cse1), and Cas11 (Cse2). The cascade is a group of Cas-crRNA proteins that are involved in the recognition of foreign genetic sites. Upon binding to foreign genetic material, the Cas nuclease Cas3 is stimulated, resulting in the

degradation of the targeted genetic material [12]. It has been discovered that several single subunits belonging to the second class of CRISPR systems are involved in the process of genetic engineering and modification in eukaryotic cells, CRISPR-Cas12a and CRISPR Cas9 systems are classified as type five and type two respectively, and the CRISPR-Cas13 system modifies RNA, it is categorized as type VI [14][15]. While the CRISPR-associated transposase CAST systems, CRISPR-Cas12f (Cas14), and CRISPR-Cas12J are of the fifth type, which is categorized as Class 2, because they are among the contemporary technologies that are involved in genetic modification [16][17].

CRISPR technology and clinical applications

Because CRISPR-Cas technology can cut, delete, insert, or replace genes, it has been applied in numerous gene therapy applications, treating a variety of pathological conditions. The following are some examples of these uses of CRISPR technology:

Antibiotics Resistance of Bacteria

Antibiotics are primarily used to treat infections and infections caused by bacterial pathogens. However, because of improper and careless antibiotic use, multiple antibiotic resistance has emerged, and it is possible that resistance will spread from one type to another, which has contributed to the development of bacterial resistance to antibiotics. Nevertheless, by utilizing cutting-edge technologies like CRISPR/Cas9, which specifically targets the genome of resistant bacteria, it is now possible to overcome these obstacles [18]. Research has demonstrated that CRISPR technology can be used to make resistant bacteria more susceptible to antibiotics. It was discovered that the use of CRISPR/Cas9 technology assisted in the eradication of coli bacteria, which are resistant to multiple antibiotics because they produce the beta-lactamase ESBL and have plasmids that aid in the transmission of the resistance trait. It was observed that the sequences genes SHV and TEM of beta-lactamase gene that can be altered to increase their susceptibility to antibiotics, Since the CRISPR system has proven effective at editing the genes of *Staphylococcus. aureus* bacteria resistant to clindamycin and methicillin, scientists have also turned to it in recent years to restore the sensitivity of bacteria carrying resistance genes to antibiotics carried on plasmids [4]. The gyrA gene mutations that cause *Escherichia. coli* to become resistant to quinolone antibiotics have also been found using CRISPR-Cas9 technology, his task was to pinpoint the two nucleotides (248 and 259) in the gene that alter amino acids 83 and 87, Which causes *Escherichia. coli* to become resistant to these antibiotics [19].

Coronavirus Infection

The coronavirus that caused the COVID-19 pandemic to spread globally is called SARS-CoV-2, SARS-CoV-2 resembles the receptors for angiotensin converting enzyme 2 (ACE-2) found in the lungs in epithelial cells, because it contains glycoproteins that help it enter the cells. It was discovered that it starts editing RNA as soon as it gets inside cells. It then starts to replicate its DNA in the cytoplasm to make new copies in order to infect other healthy cells [20]. Thus, one of the most effective therapeutic approaches is to target the genes of the virus that influence replication, preventing the virus from completing its replication cycle. The advent of CRISPR technology and its application in gene editing has made it possible to work on genetic

modification in viruses, which is relevant to studies on the potential therapeutic applications of viruses [21]. Cas13, which functions as an enzyme, and guide RNA (gRNA), a sequence of RNA that targets the viral RNA, make up CRISPR technology in mammalian cells, one type of CRISPR that functions in bacteria by preventing RNA synthesis is called Cas13. When it is present inside mammalian cells, it has been discovered to play a part in the process of preventing and inhibiting viral RNA and ending reproduction [22]. And it has The SHERLOCKTM CRISPR SARS-CoV-2 test kit was authorized by federal authorities to be used in laboratory diagnostic procedures, so this technology was also utilized when the coronavirus spread as a tool for virus diagnosis [23]. This test uses gRNA, which complements a portion of the virus's genetic sequence, to guide the CRISPR system in examining the genome of SARS-CoV-2 in order to detect Covid. After determining the desired sequence in the sample, the CRISPR system is then turned on and generates a signal in the form of a fluorescent marker that can be detected [24]. Additionally, the DETECTR test, which was authorized by Mammoth Biosciences, identified the virus using the CRISPR-Cas12 system in certain samples obtained from nasal or oral swabs of patients [25].

Infection with the Acquired Human Immunodeficiency Virus (HIV)

In recent years, researchers have discovered that it is possible to use CRISPR/Cas9 technology for the purpose of eliminating HIV infection by working to integrate Cas9 with human induced pluripotent stem cells, primary CD4+ T cells, and infected CD4+ T cells, where the gRNA is targeted, which works to prevent the infection from occurring. The new HIV-1 virus [26]. This technology was used to modify the recipient cells' genes, represented by CXCR4 and CCR5, in order to make these cells resistant to infection, even though viruses with high resistance to Cas9/sgRNA have emerged and are effective in the reproduction process, In order to completely eradicate the virus and its infection, this technology has also been used in animal models, such as mice, where work has been done to remove the virus's genome from infected cells [27].

Cystic Fibrosis

The development of gene editing techniques, such as CRISPR Cas9 technology, has aided in the treatment of numerous genetic diseases and the creation of gene and cell therapies to address these conditions [28]. As is the case with CF Transmembrane-conductance Regulator gene (CFTR) genetic mutations leading to genetic diseases such as cystic fibrosis [29]. Neon channel protein, a CFTR protein, is found on the upper surface of epithelial cells, including those in the intestine, reproductive system, lung, and pancreas. It is controlled by protein kinase-A [30]. Thus, a mutation in this gene results in the structural instability of this regulatory protein and the loss of its function, ultimately leading to the development of cystic fibrosis [31]. Research has revealed that there are over 100,000 cases of cystic fibrosis patients globally [32]. While the application of CRISPR/Cas-9 technology to genetic disease gene editing is still in its infancy, it has emerged as a promising and cutting-edge tool [33]. Following the transplantation of intestinal stem cells from patients suffering from cystic fibrosis, the researchers were able to correct the mutation in the regulatory gene CFTR, resulting in the proper expression of the gene necessary for the protein's function. Consequently, cystic fibrosis patients could be treated with Cas-9 [34]. Cystic fibrosis genes can be modified through the

use of CRISPR technology because of its great efficacy, efficiency, and straightforward design, utilizing Cas9 endonuclease to create new cases of cystic fibrosis by transplanting the diseased patients' cells into living creatures [35]. Thus, genetically modified cystic fibrosis cell lines have unique properties through cell culture, which aids in research testing and the selection of suitable therapies and drugs [36].

Sickle Cell Disease

A genetic mutation in the hemoglobin gene's B subunit causes sickle cell anemia, a disease that runs in families (HBB), which are regarded as point mutations because they function at the sixth amino acid site by substituting the phenyl amino acid for the glutamic amino acid, polymerizing the hemoglobin gene. This reduces the patient's average lifespan due to hemolysis, red blood cell deformation, anemia, damage to peripheral organs, and blood vessel blockage [38]. The utilization of CRISPR/Cas9 technology, which repairs genetic mutations in hematopoietic stem cells derived from patients or stimulates hemoglobin gene expression to produce genetically modified and pluripotent stem cells, has become one of the therapeutic options for treating cases of sickle cell anemia in patients with it, thanks in part to the tremendous advancements made in the field of gene editing using CRISPR technology [39].

According to[40], it was discovered that the CRISPR-Cas9 system has unique properties exemplified by its straightforward design and operation in addition to its high efficiency. This indicates that the system plays a significant role in genetic modification, because it breaks double-stranded DNA and creates double-strand breaks (DSBs), this system primarily functions on double-stranded DNA, which facilitates genetic modification. According to [41], the Cas9 system plays an immune role that allows it to specifically target the target gene and modify or add bases to the DNA sequence. Cas9 was discovered to be composed of two sections: the nuclease lobe (NUC), which contains the elements that cut DNA and the sections that bind to the target DNA, and the recognition region (REC), which functions to bind gRNA [42]. It binds to the target site using Cas9 endonuclease and makes use of single guide RNA sequences (gRNA) of RNA [43].

Cancer

One of the more complicated genetic diseases, cancer is caused by mutations that alter DNA. These mutations inhibit the ability of genes that suppress tumor growth and activate oncogenes, which alter DNA gene expression. It affects different organs in living things and thwarts the body's defense and immune systems, infecting healthy cells and interfering with the normal operation of different tissues [44]. In order to treat cancerous conditions, CRISPR-Cas9 technology has been used in gene editing [45]. This technology is unique in that it aims to treat cancerous tumors with exceptional results by precisely modifying genes. This modality specifically targets gene mutations that lead to the growth and spread of the tumor [46]. This is accomplished by the interaction between Cas9 and tumor suppressor and cancer-promoting genes, which results in genetic alterations in these genes [47]. It was discovered that the mutations produced by this method could either cause the genes to regain their normal function or cause the genes that promote cancer to stop functioning, It plays a part in determining the genes responsible for various cancer conditions [48]. Furthermore, CRISPR technology is employed to detect interactions that take place in critical genes, by focusing on a subset of

genes within a single cell, the most significant genetic interactions that are taking place can be found and understanding the effects of particular medications additionally, it is possible to ascertain whether members of the immediate family are affected by these interactions that cause cancer, CRISPR technology is also used to find interactions that take place in critical genes. By focusing on a subset of genes in a single cell, it is possible to determine which genetic interactions are most significant and the effects of particular medications, furthermore, it can be ascertained if these reactions that cause cancer also impact on first degree family members. [49][50]. CRISPR technology is currently being used to treat a variety of cancers, including blood cancer and lymphomas. Furthermore, CRISPR-Cas has been used to treat lung cancer in China in recent years [51]. In America, CRISPR-Cas clinical trials for cancer immunotherapies have also recently concluded [52]. It has been possible to identify certain uncommon mutations using CRISPR-Cas technology, such as those found in non-small cell lung cancer patients, Inactivated Cas9 (dCas9) was used in conjunction with magnetic beads, CRISPR technology, and the qPCR allele to identify EGFR mutations in patient DNA samples. These mutations included deletion of exon 9, T790M, and L858R [53]. The genes Trp53, Nf1, and Ptch1, which cause myeloma, were also eliminated using CRISPR-Cas9 technology, bBesides its function in removing genes that result in brain tumors [54].

Challenges Facing CRISPR Technology

The use of CRISPR technology to modify genes and interfere with gene therapy has sparked a lot of ethical questions and concerns because it may result in DNA mutations and unwanted changes to genes that are passed on to future generations [55]. Due to sequence similarity within the genome, using CRISPR technology for genetic modification in organisms with large genomes may result in errors in the binding of RNA to the target site in the gene, which could lead to the occurrence of genetic mutations in other non-target sequences [56]. In order to prevent this, scientists have worked on particular tactics whose goal is to remove mistakes that arise after binding to the target site. These tactics include the use of nickase Cas9 and anti-Cas9 proteins after it binds to the target site, as well as the development of bioinformation technology with the goal of designing a carrier nuclic acid that is more accurate [57].

One of the challenges with using this technique to modify genes is that it may result in unwanted genetic modifications. Recently, it has been noted that there have been large deletions of nitrogenous bases and other base rearrangements in the DNA, in addition to its effect on certain alleles and consequent loss of chromosomes [58]. Furthermore, it was discovered that the improper application of this technology and a lack of thorough understanding of it could result in issues for both people and the environment because genetic modifications could lead to diseases that are uncontrollably severe and severe due to the emergence of new generations of pathogenic organisms, it is important to highlight that these cutting-edge technologies are not applied in a way that impact on different living things [59]. Although this technology has made great strides in the area of genetic modification of DNA, it is not without its share of obstacles and difficulties that restrict its application to within the bounds of scientific and ethical guidelines and principles in order to accomplish its intended goals.

Conclusions

Based on this review, conclude this technology, which modifies genes by removing, replacing, or adding new sequences, has made it easier for scientists and researchers to treat diseases genetically. Thus, altering genes with the aid of this technology helped regulate their capacity for self-expression thus controlling the chances of contracting diseases. It has been applied to numerous medical issues, including treat bacteria that are resistant to antibiotics and viral infections, In addition to treating some genetic illnesses like sickle cell disease and cystic fibrosis it also treats cancer. Therefor this promising technology, in spite of its obstacles, holds great promise for treating various health issues, including hereditary diseases.

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