IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 1, January 2023
Impact Factor: 6.252

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Gene Therapy

Miss. Vaishnavi S. Jadhav and Mr. Mahesh H. Mohite
Nootan College of Pharmacy, Kavathe Mahankal, Sangli, Maharashtra, India

Abstract: *The gene editing technology is now the hot topic for researchers the recent studies shows the future outcomes of this technology in reference to treat diseases and disorders that are not treatable till date. This technology could be a milestone in healthcare system this article projects the area of the gene editing technology especially the CRISPR gene editing technology . the CRISPR refers to the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). The technology is still being researched but the recent findings show the positive hopes for the scientists.*

Keywords: Gene Editing, CRISPR Technology, Cas, Gene Editing Technology, Limitations For CRISPR.

I. INTRODUCTION

The DNA sequence family known as CRISPR, which stands for clustered regularly interspaced short palindromic repeats, is present in the genomes of prokaryotic species like bacteria and archaea. The DNA fragments of bacteriophages that previously infected the prokaryote are where these sequences were derived. During successive infections, they are utilized to locate and eradicate DNA from bacteriophages that are identical to them. As a result, these sequences are essential to prokaryotes' antiviral (or anti-phage) defensive mechanism and offer a sort of acquired immunity. About 50% of sequenced bacterial genomes and about 90% of sequenced archaeal genomes include CRISPR.

CRISPRs were initially found in archaea (and later in bacteria) by Francisco Mojica, a biologist at the University of Alicante in Spain. He suggested that CRISPRs function as a component of the bacterial defense system that protects it from invading viruses. They are made up of genetic code repeats that are broken up by "spacer" sequences left behind from earlier invaders. The system functions as a genetic memory that aids the cell in recognising and eliminating re-entering invaders (also known as "bacteriophage") in the future. An investigative test of Mojica's idea was performed in 2007 by a group of researchers under the direction of Philippe Horvath.

The Zhang lab announced the first technique for creating CRISPR to modify the genome in mouse and human cells in January 2013.

With the use of CRISPR genome editing, scientists can swiftly produce cell and animal models that they may utilize to further the study of illnesses like cancer and mental illness. Furthermore, CRISPR is now being researched as a quick diagnosis. Through direct instruction and the distribution of more than 40,000 CRISPR components to university laboratories across the world, Feng Zhang and his colleagues have taught thousands of researchers in the use of CRISPR genome editing technology in an effort to support this kind of research globally.

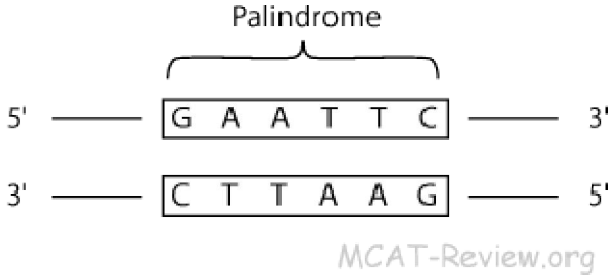
1.1 What Is CRISPR?

CRIPSR is a nuclease connected to CRISPRs, which stand for clustered regularly interspaced short palindromic repeats. The first report of these 29-nucleotide repeat sequences in bacteria was made in 1987, and they were separated by varied 32-nt spacer sequences. Bacteria have been shown to have repetitive DNA sequences known as CRISPR, together with "spacer" DNA sequences that precisely match virus sequences in between the repeats. It was later found that, in response to viral infection, bacteria convert these DNA components to RNA. To cut the viral DNA and offer defense against the virus, the RNA directs a nuclease protein to its location. "Cas" stands for "CRISPR-associated," and it refers to the nucleases. The protein Cas9 is an enzyme that acts like a pair of molecular scissors, capable of cutting strands of DNA. The standard Cas9 protein cuts the DNA at the target. When the cut is repaired, mutations are introduced that usually disable a gene. This is by far the most common use of CRISPR. It's called genome editing – or gene editing – but usually the results are not as precise as that term implies.(1)

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 1, January 2023
Impact Factor: 6.252

When we discuss repeats in the genetic code, we are referring to the arrangement of the rungs on a DNA molecule's spiral ladder. Two chemical bases are linked together in each rung: Together, the bases adenine (A), thymine (T), and guanine (G) pair with the base cytosine (C). In a CRISPR region, these bases appear in the same order several times, and in these repeated segments, they form what's known as "palindromic" sequences, In a palindromic sequence, bases on one side of the DNA ladder mirror those on the opposing side when you read them in the other manner, comparable to how the word "racecar" reads the same forward as it does backward.(2)

Example -



With CRISPR/Cas, it's easy to disrupt a targeted gene, or, if a DNA template is added to the mix, insert a new sequence at the precise spot desired. The method has profoundly changed biomedical research, as it greatly reduces the time and expense of developing animal models with specific genomic changes.

Over the past ten years, a revolution in biomedical advancements has been sparked by the revolutionary discovery of a technology called CRISPR in 2012. It allows researchers to specifically target, edit, change, and regulate genes as well as insert any enzyme or protein they desire at any point in the genome. They can now comprehend how certain genes affect cells in a way that was previously unthinkable and discover novel therapy targets as a result. For the cell to utilize as a template while repairing the DNA break, scientists can now introduce new DNA fragments to the genome of living things. In this approach, the genome can be altered or a disease-causing mutation can be swapped out for a healthy version.(.3,4)

Recent findings that use prokaryotes' adaptive immune systems to undertake targeted genome editing are revolutionizing the biological sciences. The identification of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) proteins has broadened the applications of genetic research in tens of thousands of labs worldwide and is changing how we think about gene therapy. Traditional gene therapy has generated considerable controversy since it relies on viral vector delivery of therapeutic transgenes, which can result in immunogenic toxicity and insertional oncogenesis. While viral vectors continue to be a crucial delivery method, CRISPR technology offers a comparatively easy-to-use option for site-specific gene editing, allaying certain worries associated with conventional gene therapy. (5)

1.2 How does CRISPR work?

This somewhat brute-force technique to gene transfer is used in traditional gene therapy. A healthy copy of a gene is transported into cells by a benign virus or another type of "vector" to make up for a disease-causing faulty gene. However, by removing the incorrect DNA and replacing it with the proper sequence, CRISPR may directly restore the damaged gene. That should, in theory, function far better than introducing a new gene as it removes the possibility that a foreign gene may accidentally end up in the wrong location in a cell's genome and activate a cancer gene. Additionally, a CRISPR-repaired gene will be controlled by its native promoter, preventing the cell from producing too much or too little of its products.(6)

A Cas9 nuclease plus a short non-coding gRNA make up the CRISPR-Cas9 system (Figure 1). A target-specific CRISPR RNA (crRNA) and an additional trans-activating crRNA are the two molecules that make up the gRNA (tracer RNA). The Cas9 protein is directed to a specific genomic location by the gRNA unit, where the Cas9 nuclease causes a double strand break at the designated genomic target region.(Fig.2)

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 1, January 2023
Impact Factor: 6.252

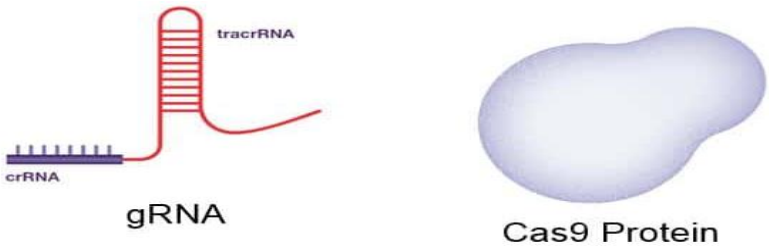


Figure 1: The CRISPR gRNA and Cas9 protein.

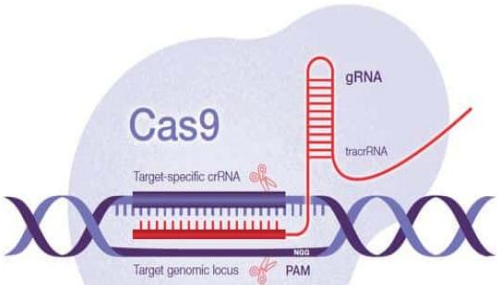


Figure 2: The CRISPR-Cas9 system

In bacteria, CRISPR loci are made up of repeats that are separated by spacers, which are segments of foreign DNA that are around 30 base pairs long. Long precursor transcripts of the repeat-spacer array are processed inside repeat sequences to produce short crRNAs that designate the target sequences (also known as protospacers) that the Cas9 nuclease will cleave. Then, exogenous genetic components are identified and silenced at the DNA level using CRISPR spacers.

Importantly, for cleavage to take place, the protospacer adjacent motif (PAM) sequence, a particular three-nucleotide sequence, must be present directly downstream of the 3' end of the target region. The target DNA has this PAM sequence (NGG for Cas9), but the gRNA that targets it does not.

After CRISPR-Cas9 has cut the DNA, the double-stranded break can be repaired by one of two components of the cell's natural repair system (Figure 3).

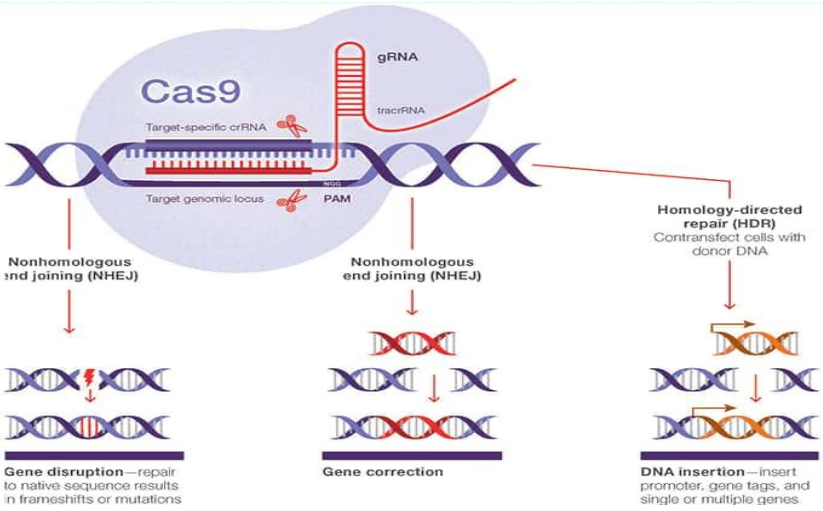


Figure 3: Double-stranded break and repair pathways using CRISPR-Cas9. Three base pairs upstream of the NGG in the PAM sequence at the 3' end of the target sequence, cleavage takes place on both strands.

- Non homologous end joining (NHEJ) produces a heterogeneous population of cells with various insertions or deletions (indels) around the gRNA-defined break in the absence of a repair template. A functional knockout can be created by using this approach to create cell lines with random deletions around a particular sequence.(7)

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 1, January 2023
Impact Factor: 6.252

- As an alternative, the error-free homology-directed repair (HDR) method can introduce a user-defined sequence alteration at a particular location within the genome if a repair template is made available. This procedure can be used to tag endogenous genes with reportable moieties, over express a new gene, or develop disease-relevant cell models.(7)

1.3 Role of CRISPR Technology in Health Science

The CRISPR technology being a novel technology with widespread of applications in health science. This technology is developing rapidly and the new outposts of this technology are being revealed now with its novel approaches which are as follows,

A. Gene Editing

The method is tremendously crucial in biotechnology and medicine because it makes it possible to accurately, affordably, and simply modify genomes in vivo. It may be applied to the development of novel pharmaceuticals, food items, and genetically modified organisms as well as the management of infections and pests. It may potentially be used to treat genetic disorders that run in families as well as somatic mutation-related illnesses like cancer. Its application to human germ line genetic manipulation, however, is very debatable.

B. Creating Cell and Animal Models

Research to find a treatment for lethal diseases, such as cancer and mental illness, has advanced quickly because to CRISPR genome editing. It enables fast cell and animal model construction and genome-wide gene mapping to identify key genes in biological processes.

C. Multiplex Genome Editing

While still in its infancy, the use of CRISPR in conjunction with other components, like as non-pathogenic viruses, has demonstrated promising outcomes in the treatment of neurodegenerative illnesses. Multiple genes will be able to be edited in a single step by combining CRISPR-Cas9 with several guide RNAs.

D. Xenotransplantation

There have been several ethical and moral problems with the procedure. Despite this, individuals waiting on an ever-increasing organ transplant list may be saved via Xenotransplantation, which involves growing human organs in animals. The development of genetically modified pigs with human organs demonstrates the potential of CRISPR/Cas9 gene editing technology to assist in the synthesis of organs with necessary immunological and regulatory characteristics.

E. Modulating Antibiotics

Due to its programmable sequence-specific character and effectiveness as a powerful antibacterial tool, CRISPR technology has emerged during the past 10 years. This can preserve the microbiota, a property that antibiotics lack, in addition to eliminating certain bacteria or virulence features from the population. When delivered to the chromosome via various biological carriers, including as phages and plasmids, CRISPR antimicrobials have shown to be fatal to microorganisms. These can also get rid of plasmids that contain genes for antibiotic resistance and make bacteria more sensitive to antibiotics.

F. Diagnostics

Different CRISPR systems exist. Six kinds and 22 subtypes have so far been identified and studied. Types II, V, and VI of these are used in diagnostics. CRISPR enzymes can be used to identify serious diseases like cancer as well as viral or bacterial illnesses. The SARS-CoV-2 virus may already be found utilizing diagnostic tests created by researchers employing the Cas12 and Cas13 enzymes. Additionally, because CRISPR diagnostic tests may be carried out using inexpensive chemicals and paper-based lateral flow assays, they can lower lab and patient expenses.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 1, January 2023
Impact Factor: 6.252

Scientists have developed a new way to make sophisticated antibiotics using gene editing. The method can be applied to treat neglected diseases, stop pandemics, and fight antibiotic resistance. New non-ribosomal peptide synthetase (NRPS) enzymes that provide therapeutically significant antibiotics are being developed using CRISPR-cas9 gene editing. Complex enzymes have proved challenging to modify in the past in order to create novel antibiotics. They generate large quantities of natural antibiotics, including penicillin .(8)

1.4 Limitations of CRISPR Gene Therapy

A. Off-Target Effects

The use of Cas proteins may be restricted or have negative effects if genome editing methods break DNA not only at the intended target site but also in unintended locations, known as off-target effects. 120 Chromosome rearrangements brought on by off-target effects could unintentionally affect some inadequately matched genomic locations and restrict the use of CRISPR-Cas editing systems for therapeutic purposes. 121 Additionally, off-target effects can disrupt the functioning of crucial human genes and vital function loss, which causes various physiological or signaling abnormalities, off-target effects can also disrupt the function of essential human genes and vital function loss, which causes various physiological or signaling abnormalities. Off-target effects can also disrupt the functioning of essential human genes and cause vital function loss, which causes 122 Studies showed that compared to some of the other common gene-editing techniques, CRISPR-Cas tools may be more susceptible to off-target(9,10) effects.

B. Unavailability of PAM

The absence of PAM in the intended gene loci is the primary problem with genome editing methods. But a variety of Cas-nuclease variations, like SpCas9 and Cas12a, are now readily available and are reducing PAM limitation. These types of developments will give genome editing flexibility for the required precise targets. In other aspects, artificial intelligence is crucial and has been included into experimental design to accurately forecast target sequences.(10)

C. Drug Delivery Approach

The biggest barriers to the development of CRISPR/Cas9 technologies as cancer therapies are the low editing efficiency in tumors' and probable toxicity of the currently used delivery vehicles. When genome editing tools need to be used successfully in the targeted organisms or cells, having a suitable and efficient alternative delivery approach is essential for CRISPR/Cas9 distribution. The Cas9 system's in vivo delivery has so far proven difficult. The Cas9-based gene editing platform has been delivered using both physical methods and viral vectors. For in vitro delivery, physical techniques are more practical.(10)

D. DNA-Damage Toxicity

CRISPR-induced DSBs frequently cause apoptosis instead of the desired gene change. Using this method in human pluripotent stem cells (hPSCs), it was shown that p53 activation in response to the harmful DSBs induced by CRISPR frequently causes subsequent apoptosis (. As a result, p53-suppressed cells are more likely to undergo effective CRISPR modifications, which lead to a bias in favour of oncogenic cell survival. Additionally, massive deletions spanning kilo bases and intricate rearrangements have been described in numerous cases as unintended results of on-target activity, emphasizing a significant safety concern for clinical uses of DSB-inducing CRISPR therapy. (10,11)

E. Immunotoxicity

CRISPR/Cas9, like conventional gene therapy, still raises questions about immunogenic toxicity in addition to technological restrictions. More over half of the human participants in Charles worth et a study.'s had anti-Cas9 antibodies against the two most often employed bacterial orthologs, SaCas9 and SpCas9 . Furthermore, CRISPR components for gene therapy are frequently delivered using AAV vectors. In order to find immunological orthologs that can be employed for secure recurrent administration of AAV-CRISPR gene therapy, a number of Cas9 orthologs and AAV serotypes were examined based on sequence similarity and projected binding strength to MHC class I and class II. (11)

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 1, January 2023
Impact Factor: 6.252

1.5 Precision Gene Editing With CRISPR

The potential of CRISPR gene therapy depends on precise genome editing. Despite the fact that HDR pathways can help with a desired edit, their low effectiveness severely restricts their use for precise gene editing for clinical intervention, with NHEJ being the standard mechanism that human cells use for repair. By chemically inhibiting important NHEJ modifying enzymes as Ku , DNA ligase IV , and DNA-dependent protein kinesis (DNA-PKcs), it has been possible to increase HDR efficiency . Utilizing single-stranded oligodeoxynucleotide (ssODN) templates rather than double-stranded DNA, which contain the homology arms necessary for recombination and the desired edit sequence, can increase HDR efficiency (dsDNA) (11)

REFERENCES

[1]. Gaoyuan Song , Meiling Jia , Kai Chen , Xing chen , Kong Bushra , Khattak Chuan , Long Mao Long Mao The Crop Journal Volume 4, Issue 2, April 2016, Pages 75-82 \
[2]. Personalized medicine ,precision-medicine-and-you
[3]. https://www.jax.org/personalized-medicine/precision-medicine-and-you/what-is-crispr
[4]. What Is CRISPR By Aparna Vidyasagar, Nicoletta Lanese May 13, 2022
[5]. https://www.livescience.com/58790-crispr-explained.html
[6]. what-is-crispr
[7]. www.newscientist.com/definition/what-is-crispr/
[8]. Fathema Uddin, Charles M. Rudin, and Triparna Sen , CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future , 2020 Aug7 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7427626/
[9]. Jocelyn Kaiser , May 2016 , the gene editor CRISPR https://www.science.org/content/article/gene-editor-crispr-won-t-fully-fix-sick-people-anytime-soon-here-s-why
[10]. Genome Editing Learning Center , CRISPR Technology Information , https://www.thermofisher.com/in/en/home/life-science/genome-editing/genome-editing-learning-center/crispr-cas9-technology-information.
[11]. CRISPR Technology – An Emerging Solution in Healthcare Published on 31 Oct, 2022. https://www.aranca.com/knowledge-library/articles/ip-research/crispr-technology-an-emerging-solution-in-healthcare.
[12]. Omodamilola and Ibrahim, J Biomed Pharm Sci 2018, 1:2 Journal of Biomedical and Pharmaceutical Sciences Department of Biomedical Engineering, Near East University, Mersin 10, Turkey
[13]. Yue Yang,1,† Jin Xu,2,† Shuyu Ge,3 and Liqin Lai1, Published online 2021 Mar03 doi: 10.3389/fmed.2021.649896 national library of medicines
[14]. Fathema Uddin,1 Charles M. Rudin,1,2,* and Triparna Sen1,2,*† Published online 2020 Aug 7.national library of medicine