IJCSPUB.ORG ISSN : 2250-1770
INTERNATIONAL JOURNAL OF CURRENT SCIENCE (IJCSPUB)
IJCS PUBLICATION (IJCSPUB.ORG) An International Open Access, Peer-reviewed, Refereed Journal

The Role of CRISPR-Cas9 in Science and Medicine in the Era of Gene Editing

[1]Sandra Krishnan, [2]Aiswarya Krishnan

[1]Student, [2]Student

[1]Department of Biotechnology, St Berchmans College, Changanassery, Kerala, India

[2]Department of life Science, University of Sussex, Sussex House, Falmer Brighton, BN1 9RH United Kingdom

Abstract

The exact manipulation of DNA sequences made possible by genome engineering, which includes genome and gene editing, transforms genetic research. In contrast to conventional techniques that introduce genetic material into genomes at random, contemporary gene editing technologies like CRISPR-Cas9 enable precise modifications at certain genomic regions. CRISPR-Cas9 was made possible by the development of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which replaced earlier methods such as restriction endonucleases. CRISPR-Cas9, which derrived from bacterial immune systems, has become a versatile tool for editing eukaryotic genomes since Jennifer Doudna and Emmanuelle Charpentier developed it in 2012**.** With the aid of a single-guide RNA (sgRNA), the Cas9 nuclease in CRISPR-Cas9 is able to facilitate targeted genetic alterations through either non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms by causing double-strand breaks at specific DNA sequences. Applications in a number of fields, such as correcting disease-causing mutations in human cells and enhancing disease resistance in crops, have been accelerated by its simplicity and programmability. The development of clinical translation and wider applications is hindered by issues including off-target effects and effective delivery systems, despite its potential. Base editing and prime editing are recent developments that seek to improve accuracy and increase the potential of CRISPR-Cas9 beyond traditional gene editing. This review provides a thorough analysis of the history, mechanisms, applications and challenges of CRISPR-Cas9 technology, highlighting both its transformative impact on genetic research and its potential applications in therapeutic and agriculture.

Introduction to gene editing and CRISPR Cas9

The field of designing and modifying genomic DNA sequences is known as genome engineering. DNA repair mechanisms are used in genome engineering approaches such as genome editing and gene editing to introduce site-specific alterations into genomic DNA. Because gene editing focuses on a single gene, it differs from genome editing. In contrast to conventional techniques that introduce genetic material into a host genome at random, modern gene editing technologies specifically target and modify specific genome sites. Enzymes, specifically nucleases designed to target a particular DNA sequence, are used in gene editing to create cuts in DNA strands that allow replacement DNA to be inserted in place of the original and existing DNA to be removed.

Early attempts to modify DNA with enzymes such as restriction endonucleases, which were identified in the 1970s (Smith and Wilcox, 1970). Targeted genetic alterations could be made possible by these enzymes' ability to cleave DNA at particular sequences. However, the challenge of creating recognition sites for

desired DNA sequences limited their usefulness. In the 1990s, zinc finger nucleases (ZFNs) were developed, which was a significant advancement. ZFNs are created zinc finger proteins that have been fused to the FokI restriction enzyme's cleavage domain (Kim et al., 1996). These proteins might be engineered to attach to particular DNA sequences, allowing for precise breakage and ensuing genetic changes. While ZFNs constituted a substantial advancement, their design was complex and labour-intensive, restricting their widespread use. Transcription activator-like effector nucleases (TALENs) have emerged as a substitute tool for genome editing in parallel. DNA-binding proteins generated from transcription activator-like effectors (TALEs) fused to the FokI nuclease domain are employed in TALENs, which were produced approximately 2010 (Christian et al., 2010). Similar to ZFNs, TALENs also allowed researchers to designate DNA binding sites, but more easily and adaptably than ZFNs. A major advancement in genetic engineering and molecular biology may be seen in the path from ancient genome editing methods to the creation of CRISPR/Cas9. The discovery of the CRISPR-Cas system, which was adapted from bacteria and archaea, marked a milestone in genome editing. In prokaryotes, CRISPR-Cas systems serve as adaptive immune systems that cleave foreign DNA sequences to protect against viral infections. CRISPR-Cas9 was proposed by Jennifer Doudna and Emmanuelle Charpentier in 2012 as a technique for eukaryotic cell genome editing (Jinek et al., 2012).

Significance of gene editing in biotechnology

The future of the environment, as it relates to agriculture, biodiversity, human society, and almost every aspect of our planet, is seriously threatened by climate change. The atmospheric build-up of greenhouse gases due to human activity is the main contributor to climate change. In general, more frequent heatwaves, droughts, floods, persistent sea level rise, and rises in global temperature are projected the extent of the consequences depends on the amount of emissions. As a response to these difficulties, the use of genome editing, also known as genome editing or genome engineering, has surfaced as a technique to help either lessen the consequences of climate change on agriculture or assist in helping species adapt to it. The process of creating DNA alterations at specific genomic sites is known as gene editing. Without causing any permanent insertion of foreign DNA, these alterations can lead to the knock out or deletion of one or more genes. Alternatively, a new feature can be knocked in by inserting genes into specific regions of the genome, either from other animals or from inside the organism's gene pool. To accomplish precise gene modifications, researchers have used CRISPR/Cas systems, Zinc Finger Nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). Although earlier gene editing technologies undoubtedly still have a place, the emergence of CRISPR/Cas systems has greatly increased the accuracy and efficiency of creating alterations. The use of gene editing methods has produced a lot of promise (Karavolias et al. 2021).

Brief History and development of CRISPR Cas9 technology

The discovery of clustered regularly interspaced short palindromic repeats (CRISPR) in bacteria as a component of their adaptive immune system is the source of CRISPR-Cas9 development. After discovering CRISPR sequences in Streptococcus thermophilus in 2007, scientists were able to explain how these sequences functioned to provide resistance against viral infections by cleaving DNA under the guidance of RNA (Barrangou et al., 2007).

The basis for CRISPR-Cas9's adaption for genome editing has been created by Emmanuelle Charpentier and Jennifer Doudna. They showed that Streptococcus pyogenes' CRISPR-Cas9 may be modified to target particular DNA sequences in vitro (Jinek et al., 2012). This discovery made it possible to use CRISPR-Cas9 as a flexible tool for editing the genome of a variety of organisms, including human cells. After making these first discoveries, scientists quickly improved the accuracy, effectiveness, and adaptability of CRISPR-Cas9 technology. To reduce off-target effects, they enhanced the selectivity of Cas9 nucleases and created variants such base editors and Cas9 nickases for more complex genetic alterations (Komor et al., 2016; Ran et al., 2013). The CRISPR/Cas9 genome editing method allows for the precise manipulation of functional genes implicated in the progression of disease, the correction of mutations that initiate disease, the activation of tumour suppressor genes, and the inactivation of activated oncogenes. Because of its simple design and capacity to alter numerous locations at once, CRISPR/Cas9 technology can be utilized to treat a wide range of infectious and genetic diseases, including metabolic disorders, cardiovascular diseases, Alzheimer's disease, and HIV infections (Allemailem et al. 2024).

The Versatile CRISPR/Cas9: Its Mechanism of Action and Predominance in Gene Editing Technologies

CRISPR/Cas systems are divided into two classes, Class I (which includes types I, III, and IV) and Class II (which includes types II, V, and VI), according to the composition and roles of the Cas-proteins. Class II systems are made up of a single Cas-protein, while class I systems are characterised by multi-subunit Cas-protein complexes. Because of its straightforward structure, the class II CRISPR/Cas-9 surface-exposed protein has received the greatest attention and has been used in genetic engineering applications more than any other (Liu Z et al ; 2020). The CRISPR-associated (Cas-9) proteins and guide RNA (gRNA) make up the CRISPR/Cas 9 system. Derived from Streptococcus pyogenes (SpCas-9), Cas-9 is the first Cas protein extensively utilised in genome editing. The aptly termed genetic scissor is a big (1368 amino acid) multidomain DNA endonuclease that, in conjunction with the guide RNA, splits the DNA at the target spot to generate a double-stranded break (Mei Y et al ; 2016). The recognition (REC) lobe and the nuclease (NUC) lobe are the two lobes that make up the Cas-9 protein. The two REC1 and REC2 domains, which make up the REC lobe, are responsible for binding the guide RNA with great force. The domains that interact with PAM, HNH, and RuvC are located in the NUC lobe. The PAM-interacting domain is in charge of PAM specificity and starts binding to the target DNA, while the RuvC and HNH domains cut each strand of single-stranded DNA (Nishimasu H et al ; 2017). Trans-activating CRISPR RNA (tracrRNA) and CRISPR RNA (crRNA) are the two components of the guide RNA that may be separated. The tracrRNA is a ring-shaped sequence that acts as a binding scaffold for the Cas-9 nuclease. The 18–20 base pair sequence of the crRNA links with the target region in the DNA to designate the target. The guide RNA in a bacterial cell recognises the viral DNA. The gene-editing technique is programmable: it combines tracrRNA and crRNA to form a single guide that can target any gene whose sequence scientists wish to change, whether it be in a human cell or a laboratory organism like yeast (Mengstie and Wondimu, 2021).

In this paradigm, there are three main steps that bacteria go through while rejecting foreign nucleic acids: acquisition, expression, and interference. When a virus infects a strain of bacteria for the first time, the bacteria break down the viral DNA and store spacer sequences of a specific size in the CRISPR spacer region according to their associated PAM sequence, which helps the bacteria remember the virus. In the event that the same strain of virus infects someone again, the bacteria identify the information they have stored and transcribe spacer sequences into precursor crRNA. Following their binding to the tracrRNA, these crRNAs are processed by CnsI and RNaseIII to become mature crRNAs. crRNA is also known as guide RNA (gRNA) because it uses complementary sequences to bind and recognise foreign DNA. When a mature tracrRNA and crRNA join to create the ribonucleoprotein complex, Cas protein may be led by crRNA to cleave the invasive DNA by recognising its PAM site, allowing for the destruction of the foreign DNA and the achievement of self-defense. However, Cas nuclease cannot break the foreign DNA on its own.

The CRISPR/Cas system's capacity for gene editing is provided by the particular target sequence recognition capability of crRNA, the DNA cleavage activity of Cas nuclease, and the cell's DNA repair processes. In order to repair DNA damage and prevent cell death, cells will initiate their own repair processes when DSBs happen. Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are the two types of repair methods. In order to repair double-strand breaks (DSBs) by homologous recombination—the integration of homologous pieces into DNA—the cells often initiate the homologous sequence recognition (HDR) process when homologous sequences are available. By using this repair process, we are able to co-transform a donor DNA fragment with homologous arms into a repair template in an artificial manner. This allows donor DNA to be integrated into the genomic DNA of the cell, resulting in gene knock-in. The cells often start the NHEJ pathway, which immediately connects the damaged DNA, when homologous DNA is not available. Base pair insertions or deletions (indels) that result in frameshift mutations in genes that lead to gene knockout are common in this repair method.

In order to do gene editing, Cas9, gRNA, and a matching promoter, terminator, replicon, and selection marker can be built into one vector or two distinct vectors. The gRNA that was created intentionally is a chimaera RNA that combines all of the necessary tracrRNA and crRNA components. The recognition of the target site is carried out by the front section of the gRNA, or sgRNA. The subsequent section serves as a scaffold for attaching to the Cas9 protein. Every time sgRNA has to identify a new target, it must be updated. 20 nt is the ideal length for sgRNA, which makes building the CRISPR/Cas9 vector simple. With a possible target present in almost every 8 bp DNA sequence, there are a large number of targets that might be appropriate candidates for CRISPR/Cas9. The CRISPR/Cas9 system is now the most widely used gene editing technology due to these features. After target cells are modified with CRISPR/Cas9 vectors, they

will express the gene-editing tools Cas9 and gRNA. The CRISPR/Cas9 system is one of the greatest scientific discoveries of the past ten years due to the fact that its simplicity has drawn several experts to study it in-depth (Youmin Zhu,2022).

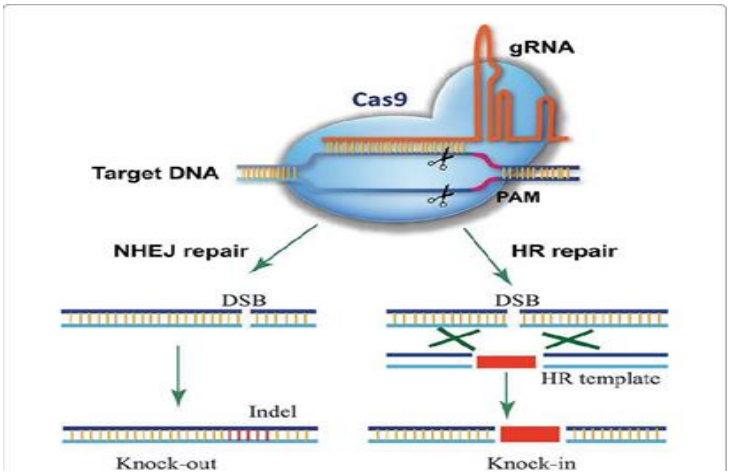

Figure 1: CRISPR/Cas9 mechanism (Razzaq and Masood, 2018)

Mode of delivery

The delivery of CRISPR/Cas9 is an extremely important issue. A targeted and effective genome editing may be achieved with precise delivery. Systems based on viruses and non-viruses are both utilised to deliver CRISPR/Cas9 to a target location.

a. Viral method

Over the past few decades, viral systems like as lentiviruses, adeno-associated viruses (AAV), retroviruses, and adenoviruses have been chosen for gene delivery. Systems for delivering viruses are separated into two groups. Firstly, viruses such as lentiviruses and retroviruses whose genetic material can be incorporated into the genetic material of the host. viruses such as adenoviruses (AAV) that persist in the host's cytoplasm and nucleus. There are benefits and drawbacks to viral delivery. For example, when a retrovirus integrates into the host, it might activate an oncogene that raises the probability of accidental mutations. However, the benefit is that the long-term expression is produced via the retroviral delivery mechanism.

Since AAV creates long-term expression, is safe, and has a minimal immunological reaction, it is the official gene therapy technique. However, several drawbacks are also evident, such as the poor packing ability (4.7 kb) and the 4.2 kb size of the Cas9 protein from Streptococcus pyogenes. Delivering SpCas9, sgRNA, and other essential components via the AAV delivery mechanism becomes difficult as a result. By employing little orthologs of Cas9, such as SaCas9 (~3.3 kb) or StCas9 (~3.3 kb), this issue is reduced.

Gene delivery is another use for lentivirus. To enable the delivery of genes into any kind of host, researchers have modified HIV-1 to create lentivirus. With 9.7 kb, it has a higher packing capacity than the others. Therefore, Cas9, sgRNA, and other essential components are readily delivered into present.

b. Non-viral method

The delivery of CRISPR/Cas9 to the target locations can also be accomplished by non-viral means. Liposomes, nanoparticles, and hydrodynamic injections are frequently employed in this method. There are several benefits to this strategy: it is easy to produce, safe, has a big packing capacity, and a low immune reaction.

The method that has been used most recently to administer CRISPR/Cas9 is called hydrodynamic injection. The injection volume is high—between 8 and 10 percent of the mice's total weight. This injection enters the circulatory system immediately of the host's body. Delivery occurs at a very fast pace (5-7 s). Researchers inject CRISPR/Cas9 into the liver using this technique in order to conduct research (Razzaq and Masood, 2018).

Further development

It is challenging to build on recent advancements since CRISPR/Cas9 is the dominant technique for genomic editing. Due to its accuracy and widespread usage, the system has a brief history effectiveness of genetic editing (Razzaq and Masood, 2018).

Combination of Cas9 with proteins

dCas9 fuses with methylation-related epigenetic factors to create desired alterations in the host's epigenomes. Cas9 was additionally coupled with a few fluorescent proteins for the purpose of labelling. It is also employed for academic purposes radioactive material's intricate arrangement (Razzaq and Masood, 2018).

Enhanced Precision Editing with CRISPR-Cas9: Base and Prime Editing

a. Base editing

Adenine, Cytosine, Guanine, or Thymine are the individual bases of DNA that can be precisely changed using base editing, a novel gene editing technique. Base editors immediately convert one base into another, in contrast to typical CRISPR-Cas9 editing, which depends on the cell's repair mechanisms to introduce alterations after a break. Base editors (BEs) are novel tools designed to convert nucleotides precisely. They work by combining deaminases with catalytically modified Cas proteins, including Cas9-D10A nickases. A single-stranded DNA (ssDNA) R-loop is formed when a Cas protein and its gRNA bind to and hybridize with the target DNA strand in response to recognition of target DNA sequences. Then, without creating DSBs, the deaminase domain reaches the ssDNA R-loop and initiates nucleotide conversion.

Cytosine Base Editors (CBEs) and Adenine Base Editors (ABEs) are the two main types of base editing. CBEs allow for the targeted conversion of cytosine (C) to uracil (U) by fusing a modified Cas9 enzyme with a cytidine deaminase enzyme. The cell's repair mechanism then change uracil into thymine (T). Adenine deaminase (ABE) enzymes, on the other hand, are coupled with a modified Cas9 enzyme to facilitate the conversion of adenine (A) to inosine (I). Inosine is translated to guanine (G) during the processes of DNA replication and repair. When compared to conventional CRISPR-Cas9 technologies, these base editing techniques offer much less off-target effects because to their great precision. They allow for the targeted correction of point mutations linked to hereditary diseases such muscular dystrophy, cystic fibrosis, and sickle cell anaemia, possibly offering therapeutic advantages without interfering with other genes. Base editing has the potential to more precisely and effectively improve crop features like disease resistance and nutritional value in agriculture.

b. Prime editing

Prime editing is a novel two-step method for modifying the genome. A Cas9 nickase enzyme is first directed to the precise target location within the DNA by a pegRNA (prime editing guide RNA). The prime editor's designed reverse transcriptase component can use the pegRNA as a template since the Cas9 nickase produces a single-strand break. The precise insertion, deletion, or substitution of desired edits—such as insertions, deletions, or substitutions—into the genomic DNA strand is guided by this template. Compared to traditional CRISPR-Cas9 approaches, prime editing has a number of benefits, such as improved precision in editing particular genomic sites and less off-target consequences. Because of its versatility, it can be modified in ways that go beyond basic base changes, which makes it a potentially useful tool for fixing a variety of genetic abnormalities linked to complicated diseases. Prime editing has the ability to create tailored treatments in therapeutic applications that were previously challenging to accomplish with conventional gene editing methods. Furthermore, primary editing makes it easier to create accurate cellular and animal models in research settings, which is useful for understanding disease causes and developing treatment approaches.

Challenges and opportunities in translating CRISPR-Cas9 technology into clinical practice

As CRISPR-Cas9 technology advances toward therapeutic application, it will encounter a number of difficulties. The possibility of unwanted genetic alterations occurring in locations other than the intended target, or "off-target effects," is a major cause for concern. Reducing these off-target effects is still essential to ensuring the reliability and safety of CRISPR-based treatments in clinical settings, even with advances in

specificity. Another challenge is the effective distribution of CRISPR components into target cells. Biological constraints must be overcome by efficient delivery systems in order to attain enough editing efficiency in vivo. For CRISPR-based medicines to be successfully used in the treatment of genetic disorders, several obstacles must be overcome. Cas9 proteins were first produced by bacteria, and they have the potential to cause immunological reactions in patients, affecting the safety and efficacy of treatment. Therefore, it's essential to create Cas9 variants with lower immunogenicity to lessen immune responses and improve CRISPR-Cas9's therapeutic potential. The ethical implications of CRISPR-Cas9 are diverse, especially when it comes to heritable genomic alterations and germline editing. Imperfect DNA repair systems can lead to unwanted mutations in on-target regions in addition to off-target consequences. Maintaining the accuracy of gene edits and ensuring therapeutic efficacy require an understanding of and reduction in these mutations. CRISPR-Cas9 targeting to certain organs or tissues is still difficult, particularly in cases of disease affecting complex tissues like the brain or muscle. To treat localized genetic disease effectively and optimize therapeutic outcomes, it is imperative to develop accurate, tissue-specific delivery techniques.

Applications

1.CRISPR /Cas9 in Gene therapy

CRISPR/Cas-9 gene editing has been promising since its discovery in 2012, since it may be able to treat most known genetic diseases, including muscular dystrophy, β-thalassemia, sickle cell disease, and cystic fibrosis. Clinical trials have also utilised CRISPR/Cas-9 for β-thalassemia and targeted sickle cell disease (SCD) treatment. Sickle haemoglobin (HbS) is a genetic disorder that affects red blood cells and is caused by a single mutation in the β-globin chain of haemoglobin. It is an autosomal recessive disease. The two primary ways that CRISPR/Cas-9 is being utilised to treat sickle cell disease (SCD) are either directly correcting the haemoglobin S gene or increasing foetal γ-globin. On the other hand, increasing foetal haemoglobin is the most often utilised strategy in clinical trials. The gene known as B-cell lymphoma 11A (BCL11A), which inhibits the formation of foetal haemoglobin, is first removed from patients' bone marrow cells and then rendered inactive using CRISPR/Cas-9. The body is then reinfused with the gene-edited cells. The 200 base pair gene BCL11A is located on chromosome 2 and its product represses the expression of the γ-globin gene, causing γ-globin to change into the β-globin chain. After CRISPR/Cas-9 is used to silence this gene, red blood cells will produce more foetal haemoglobin containing γ-globin, which will lessen the severity and symptoms of sickle cell disease.

2. CRISPR /Cas9 in Plant Biology

One of the main uses of CRISPR/Cas9 is to create gene knockouts in plants to use gene mutation to clarify the function of one or more gene targets (such as enzyme genes or microRNAs).

2.1. MicroRNAs:

In plants, microRNAs (miRNA) act as regulators to either promote or suppress gene expression. Two soybean miRNAs (miR1514 and miR1509) were subjected to CRISPR/Cas9 application The scientists verified that CRISPR/Cas9 could be used to target miRNA in soybean, which significantly expanded the applicability of CRISPR/Cas9 in plants. Vectors carrying sgRNA and Cas9 were delivered by particle bombardment for temporary expression. Using CRISPR/Cas9 targeted the miR156 recognition site in rice IPA1 (ideal plant architecture 1) and discovered that the phenotype of the mutant miR156 was comparable to that of IPA1 plants. The mutants' disruption of the miR156 recognition site, caused by deletions of either 12 or 21 base pairs, was shown by analysis.

For examining the role of enzyme genes and promoting the production of miRNAs, NHEJ-mediated CRISPR/Cas9 is an extremely powerful solution. The target genes, research species, techniques, and outcomes of CRISPR/Cas9 should all be integrated into a database. When using CRISPR/Cas9 to modify target genes in comparable or related species, this kind of database may be utilised to compare differences between the two (Liu et al., 2017).

2.2. Enhance Diseases Resistance

For example, making wheat resistant to powdery mildew can increase disease resistance.

Numerous crops, including wheat, are susceptible to powdery mildew, a common fungus. The wheat MLO (mildew resistance locus O) gene has been effectively deleted by scientists employing CRISPR/Cas9. Plants

prone to powdery mildew are those bearing the MLO gene. Reducing output losses and lowering the requirement for chemical fungicides is made possible by the modified wheat plants' increased disease resistance. This encourages more environmentally friendly agricultural methods in addition to increasing productivity.

3.CRISPR /Cas9 in Gene Activation and Silencing

Beyond its ability to change the genome, CRISPR/Cas-9 may be used to precisely control (repress or activate) a particular gene target by sophisticatedly modifying the Cas-9 protein.15 Researchers have created dCas-9 nuclease, a sophisticated modified Cas-9 endonuclease, by deactivating its HNH and RuvC domains. The dCas-9 nuclease has little activity in cleaving DNA, yet it does not exhibit any impairment in binding DNA. To create the CRISPR/dCas-9 complex, transcriptional activators or inhibitors can then be fused with dCas-9. Consequently, it is possible to employ catalytically inactive dCas-9 to either suppress (CRISPRi) or stimulate (CRISPRa) the expression of a particular gene of interest. Additionally, by combining the dCas-9 enzyme with a marker like green fluorescent protein (GFP), it is possible to use CRISPR/dCas-9 for subcellular localization, which would allow for site-specific labelling and imaging of endogenous loci in living cells for future use (Mengstie and Wondimu, 2021).

4. CRISPR/Cas9 in Therapeutic Oncology

The traditional treatment for cancer, a disease with high incidence and fatality rates, involves surgical excision, radiation, and chemotherapy. Nevertheless, the last two therapies have significant adverse effects. The development of cancer is typically accompanied by aberrant expression of several genes, including PD-L1, Notch, and P53. In tumor cells, aberrantly expressed genes may be silenced or overexpressed utilising CRISPR technology

One characteristic of cancer development that sets it apart is rapid cell proliferation, which uses a lot of oxygen and leaves the tumour in a hypoxic environment. Photothermal therapy is the application of infrared light externally to produce localised heat in the body and ablate tumours, combined with the delivery of infrared light-responsive nanomaterials to the body. Nonetheless, tumours have the ability to withstand temperatures as high as 50 °C, which could cause paracancerous tissue damage. Consequently, it may be possible to successfully prevent tumour formation by lowering the temperature tolerance of tumour cells and using photothermal treatment at moderate to low temperatures. A screen was used to acquire the protein heat shock protein 90α (HSP90α), which is overexpressed in tumour cells and linked to cellular heat tolerance. Researchers created a gold nanorod-based hypoxia-responsive nanoparticle that had a CRISPR/Cas9 system to knock down HSP90α.Following the vector's entry into the tumour cells, Cas9/sgRNA RNP was produced, which silenced HSP90α and made the cells incapable of withstanding heat. Ultimately, the gold nanorods were triggered by infrared light to ablate the tumour (Li et al., 2023).

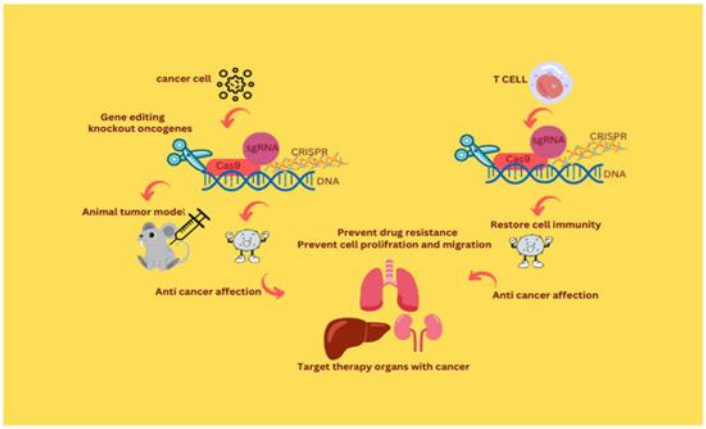

Figure 2: CRISPR/Cas9 in cancer treatment (Rabaan et al., 2023)

Case studies and success stories

Developing crops with improved characteristics has been made possible in part by CRISPR-Cas9. For example, CRISPR-Cas9 was effectively utilized by researchers to genetically modify wheat plants to withstand powdery mildew, a fungal disease that severely reduces wheat production globally. Without inserting foreign DNA into the genome, scientists were able to significantly boost plant resistance by focusing on particular genes linked to disease resistance. This method not only shows how precise CRISPR-

Cas9 is in improving crops, but it also shows how it might be used to solve issues with food security (Wang et al., 2020).

CRISPR-Cas9 has transformed the development of disease models in genetic research, especially in the understanding of complicated genetic disease like Alzheimer's disease. These diseases are defined by complex genetic connections and diverse disease pathways that have proven difficult to study using conventional means. With CRISPR-Cas9, disease-causing mutations may be precisely and effectively inserted into animal and cellular models, simulating the genetic diversity found in human populations. The development of cellular models of Alzheimer's disease, which duplicate important features like the build-up of tau protein tangles and amyloid-beta plaques, has been made possible in large part by CRISPR-Cas9. These models facilitate the analysis of the molecular pathways responsible for the pathophysiology of Alzheimer's disease and the testing of novel treatment approaches meant to prevent or cure the disease's progression (Chen et al., 2018).

Cystic fibrosis is a genetic condition caused by mutations in the CFTR gene. CRISPR-Cas9 has proven essential in increasing research on this disease. Scientists have constructed disease-specific cellular models by inserting CFTR mutations using CRISPR-Cas9 into epithelial cells produced from patient iPSCs. The creation and testing of innovative therapeutic techniques, such as gene therapy approaches aimed at rectifying CFTR mutations, are made easier by these animals, which accurately recreate the abnormal ion transport and mucus production found in patients with cystic fibrosis (Schwank et al., 2013).

Impact of these case studies on the field of genetics and biotechnology

The potential of CRISPR-Cas9 technology to accomplish precise genome editing has completely changed the field of genetics and biotechnology. This potential was proven by a scientific study that was published in science in 2013, highlighting CRISPR-Cas9 as an innovative tool in genetic research (Jinek et al., 2013). CRISPR-Cas9 has enabled precise genetic alterations in agricultural biotechnology, revolutionizing crop improvement. Researchers have been able to successfully modify crops to have higher nutritional value and more resistance to pests and diseases. CRISPR-Cas9 has been used to create disease-resistant rice varieties, demonstrating the technology's potential to solve issues with food security (Shimatani et al., 20). In biomedical research using animal models, researchers have shown that it is efficient in repairing genetic abnormalities linked to disorders such as Duchenne muscular dystrophy. This discovery holds potential for upcoming therapeutic approaches in addition to expanding our knowledge of disease mechanisms (Long et al., 2016). CRISPR-Cas9 has had a significant impact on genetics and biotechnology in a number of areas, ranging from fundamental studies to possible therapeutic uses. While it presents hitherto unseen prospects, its responsible and advantageous application in improving agriculture and human health depends on continued research and careful consideration of the ethical, legal, and societal ramifications.

Conclusion

In biological research, medicine, agriculture, and other fields, CRISPR-Cas9 gene editing has shown to be a significant technique. Its development is an outcome of decades of molecular biology and genetic engineering research, ranging from the use of simpler methods like as restriction endonucleases to more complex methods like zinc finger nucleases and transcription activator-like effector nucleases (TALENs). The adaptability and ease of use of CRISPR-Cas9 are among its distinguishing characteristics. In contrast to other genome editing techniques, CRISPR-Cas9 employs a guide RNA to target particular DNA sequences with the Cas9 nuclease, allowing for extremely accurate and efficient alterations (Doudna and Charpentier, 2014). The simplicity of usage of genome editing has made research into genetic diseases more accessible to scientists globally, enabling them to investigate genetic pathways and create new treatments. Since it was first adapted for eukaryotic systems, the technology has swiftly improved, showing constant gains in specificity, efficiency, and adaptability. Further expanding the toolkit are techniques like base editing and prime editing, which allow for more complicated genomic modifications without requiring double-strand breaks, in addition to precise nucleotide changes (Anzalone et al., 2019; Gaudelli et al., 2020).

Reference

[1] Allemailem KS, Almatroudi A, Rahmani AH, et al. (2024) Recent Updates of the CRISPR/Cas9 Editing System: Novel Approaches to Regulate Its Spatiotemporal Control by Genetic and Physicochemical Strategies. International Journal of Nanomedicine, 19, 5335–5363. doi:10.2147/IJN.S455574

[2] Barrangou R, Fremaux C, Deveau H, et al. (2007) CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315(5819), 1709–1712.

[3] Chen Y, Niu Y, Ji W, et al. (2018) Application of CRISPR/Cas9 in understanding tissue specificity and treatment of Alzheimer's disease and related neurodegenerative diseases. Journal of Alzheimer's Disease, 65(2), 283–302.

[4] Frangoul H, Bobruff Y, Cappellini MD, et al. (2021) CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. New England Journal of Medicine, 384(3), 252–260.

[5] Jinek M, Chylinski K, Fonfara I, et al. (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science, 337(6096), 816–821.

[6] Karavolias NG, Horner W, Abugu MN, Evanega SN (2021) Application of Gene Editing for Climate Change in Agriculture. Frontiers in Sustainable Food Systems, 5, 1–23. doi:10.3389/fsufs.2021.685801

[7] Khalil AM (2020) The genome editing revolution: review. Journal of Genetic Engineering and Biotechnology, 18, Article 78. doi:10.1186/s43141-020-00078-y

[8] Komor AC, Kim YB, Packer MS, et al. (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533(7603), 420–424.

[9] Li T, et al. (2023) CRISPR/Cas9 therapeutics: progress and prospects. Signal Transduction and Targeted Therapy, 8(1), Article 36. doi:10.1038/s41392-023-01309-7

[10] Liu X, et al. (2017) Application of CRISPR/Cas9 in plant biology. Acta Pharmaceutica Sinica B, 7(3), 292–302. doi:10.1016/j.apsb.2017.01.002

[11] Long C, McAnally JR, Shelton JM, et al. (2014) Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science, 345(6201), 1184–1188.

[12] Mengstie MA, Wondimu BZ (2021) Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. Biologics: Targets and Therapy, 15, 353–361. doi:10.2147/BTT.S326422

[13] Razzaq A, Masood A (2018) CRISPR/Cas9 System: A Breakthrough in Genome Editing. Molecular Biology, 07(02). doi:10.4172/2168-9547.100021

[14] Rabaan AA, et al. (2023) Application of CRISPR/Cas9 Technology in Cancer Treatment: A Future Direction. Current Oncology, 30(2), 1954–1976. doi:10.3390/curroncol30020152

[15] Ran FA, Hsu PD, Lin CY, et al. (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell, 154(6), 1380–1389.

[16] Schwank G, Koo BK, Sasselli V, et al. (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell, 13(6), 653–658.

[17] Shimatani Z, et al. (2017) Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nature Biotechnology, 35(5), 441–443.

[18] Wang Y, Cheng X, Shan Q, et al. (2020) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology, 38(4), 1–5.