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BIOLOGICAL BREAKTHROUGH IN GENOME EDITING : CRISPR Cas9 TECHNOLOGY - A BLESSING OR A DISTRESSING DEVELOPMENT

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ABSTRACT :

CRISPR/Cas9 technology, a genetic modification tool developed from certain bacteria's defence mechanism against viruses and plasmids, brought genome editing back into the public eye in 2012. This approach is simple to employ and has been tested on a range of experimental models, including cell lines, animals in the lab, plants, and even humans in clinical trials. The Cas9 nuclease is instructed to make a site-directed double-strand DNA break utilising the short RNA molecule as a guide in the CRISPR/Cas9 system. This technology has advanced significantly in recent years, and it is now often used to edit the genome to add, remove, or modify certain genes. Additionally, CRISPR/Cas9-based gene drive may have the ability to stop the spread of vector-borne illnesses. Although comprehensive testing is necessary to support these claims, the bacterial and archaeal adaptive immune systems may offer a therapeutic solution to previously incurable illnesses. using regard to disease modelling and therapy, we discuss current advances made using CRISPR/Cas9 in this review, as well as what future considerations should be made when utilising this technology.

KEY WORDS : CRISPR/Cas9, Genome editing, Cas proteins, Tumour infiltrating leukocytes (TILs), Nonhomologous end-joining (NHEJ), Homology-directed repair (HDR), Adenosine deaminase-severe combined immunodeficiency (ADA-SCID), off-target

1. INTRODUCTION :

By demonstrating biological principles, enhancing disease models, implementing gene treatments, and creating precision medications, genome editing has revolutionised gene therapy research. [1]The alteration of any gene at its own locus in a wide range of species and tissues, including cultured cells and animal organs, is now possible as a result of recent advancements in genome editing. A potent tool for scientific research, genome editing offers promise for treating certain hereditary disorders. Gene editing has so far showed potential in treating single-gene diseases including Huntington's disease, cystic fibrosis, and sickle cell anaemia. [2]

Genome engineering, which allows for the exact alteration and manipulation of desired DNA sequences, is at its height. The ability of genome engineering techniques to undertake accurate and efficient DNA sequence editing has attracted research committed to a several biotechnology disciplines, including as medicine, energy, and environmental research. The fascinating method of targeting and programming nucleases with site-specific DNA binding areas enables researchers to carry out experimental investigations with increased efficiency, quicker nuclease assembly, cheap cost of genome modification, etc. Mega nucleases (homing endonuclease engineered), transcription activator-like

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effector nucleases (TALENs), and zinc finger nucleases (ZFNs) are techniques created by modifying DNA endonucleases that aid in producing double-strand breaks (DSBs) at the target DNA sequence. [3]

The clustered regularly interspaced short palindromic repeats (CRISPR)/-CRISPR-associated nuclease (Cas) system is a typical acquired security system found in numerous bacteria and archaea that defends organisms against invading viruses and plasmids. [4] The CRISPR/Cas9 system is made up of the CRISPR association system (Cas) genes, which are positioned in an operon and encode nucleases that interact with single guide RNA (sgRNA), which is made up of the transverse acting CRIPSR RNA strand (tracrRNA) and the CRIPSR RNA strand (crRNA). [5]

The Cas9 protein is an effective tool for gene editing since it is an RNA-guided DNA endonuclease. It performs the function of biomolecular scissors to cut the desired DNA at a particular site indicated by the sgRNA. To find protospacer adjacent motifs (PAMs), which are short distinct sequences that enable additional binding of the Cas9 nuclease to the DNA, the CRISPR/Cas9 complex randomly searches DNA in the cells through three-dimensional collisions. When no PAM site is found, Cas9 quickly separates from the DNA. [6]

2. THE CRISPR/CAS9 SYSTEM'S DISCOVERY

Thousands of people with hereditary illnesses and few therapeutic choices now have hope due to the development of gene therapy in medical facilities. Initially, gene therapy delivered therapeutic transgenes for the treatment of cancer via viral vectors. [7] One of these revolutionary clinical trials included ex vivo retroviral delivery of a selective neomycin-resistance marker to tumour infiltrating leukocytes (TILs) isolated from patients with advanced melanoma. [8] This study was the first to provide evidence for both the feasibility and safety of viral-mediated gene therapy. The monogenic disease adenosine deaminase-severe combined immunodeficiency (ADA-SCID) was the subject of the first clinical experiment that utilised gene therapy with therapeutic attempt, which was approved in 1990. Retroviruses were administered to two young girls with ADA-SCID in order to ex vivo transfer a wildtype adenosine deaminase gene to autologous T-lymphocytes, which were subsequently injected back into the patient. [9]

The eubacteria and archaea have a defence mechanism that can recognise and eliminate foreign DNA and RNA by adapting via RNA. As a result, acquired immunity against plasmids and viruses infiltrating the body is provided. [10] This system is likely to be present in roughly half of sequenced bacterial genomes and 87% of archaeal genomes. [11]Classification of CRISPR-Cas systems include two classes (Class 1 and Class 2), six types (I to VI), and several subtypes, with Class 1 systems (Types I, III, and IV) utilising multiple Cas protein effector complexes and Class 2 systems (Types II, V, and VI) utilising a single Cas protein effector.

By employing a conserved set of procedures, such as adaptation, expression, and interference, this system offers adaptive immunity. These three phases enable DNA-encoded RNA-mediated targeting of certain exogenous nucleic acid sequences. The adaption phase after the host has been infected starts with the invasion of the host cell by foreign DNA (such as phage DNA). The multiple cas genes cut the foreign DNA into brief DNA pieces known as protospacer.

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3. Overview of CRISPR/Cas9-Mediated Genome Editing's Mechanisms

For efficient targeted gene editing, CRISPR/Cas9 is a straightforward two-part method. The single-effector Cas9 protein, which has both the RuvC and HNH endonuclease domains, is the initial component. HNH splits the complementary strand of DNA whereas RuvC splits the strand that is not complementary to the spacer sequence. Double stranded breaks (DSBs) are produced by these domains together in the target DNA. The second element of successful targeted gene editing is a single guide RNA (sgRNA) bearing a scaffold sequence that facilitates its attachment to Cas9 and a 20 base pair spacer sequence that is complementary to the target gene and adjacent to the PAM region. This sgRNA directs the CRISPR/Cas9 system to the desired genomic region. Next, one of the two endogenous DNA repair pathways-nonhomologous end-joining (NHEJ) or homology-directed repair (HDR) [Figure 1]

NHEJ is often more effective than HDR because it participates in approximately 90 percent of the cell cycle and is independent of neighbouring homology donors. [12] Although NHEJ is typically more effective than HDR, it has drawbacks in that it can lead to non-specific gene disruptions such deletions, insertions, and translocations, resulting in frameshift mutations or nonsense mutations. [13]

4. CRISPR/Cas9-based targeted epigenetic gene regulation

The two pieces below have to be combined in order to use the CRISPR-Cas9 system to control the epigenome. First, alanine is substituted for the catalytic residues in the DNA endonuclease domain of wild-type Cas9 to allow for the targeting of the genomic loci by catalytically inactive variants of Cas9 (dCas9). As a result, even if dCas9 properly attaches to the target spot on the genome, no DSB is created. Second, for epigenetic regulation of the target gene expression, dCas9 fusion must attract epigenetic factors to the target region on the genome. According to the kind of epigenetic effectors used in the epigenome investigations, there are three categories.

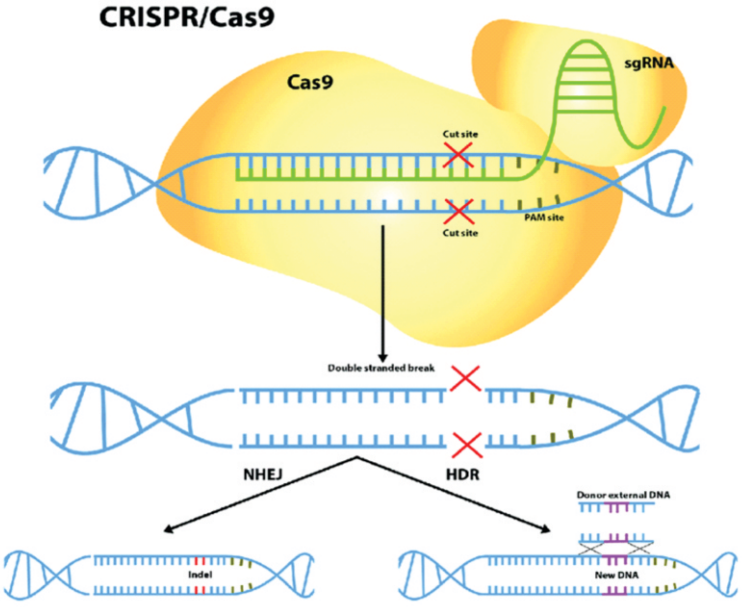

Figure 1:CRISPR-Cas9 mediated gene-editing mechanisms. A single guide RNA (sgRNA) recognizes a genomic region followed by 5'-NGG-3' PAM sequence, which recruits the Cas9 DNA endonuclease. This introduces a double-stranded break that is repaired by (i) non-homologous end joining (NHEJ), an error prone pathway that can result in the creation of Indels that can disrupt the gene, or by (ii) homology directed repair (HDR) in the presence of a donor construct.

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1) By fusing an activator like VP64 or VPR, CRISPR activator (CRISPRa) first stimulates transcriptional activation. [14]
2) Second, by joining a repressor like Krüppel-associated box repressor (KRAB) to dCas9, CRISPR interference (CRISPRi) causes transcriptional repression. [15]
3) Third, various epigenetic modifiers fused to dCas9, such as p300 or DNMT3a, cause changes in the epigenetic landscape at the targeted genomic loci. [16]

5. BASE EDITORS

Numerous DNA base editing methods have recently been created, enabling single base conversions, commonly known as base editing, in cells and creatures in a way that is dependent on guide RNA. Using a Cas9 protein in conjunction with a deaminase, the sort of editing that could be accomplished by CRISPR/Cas systems increased with the development of targeted base editing. Base editors have been created in two main categories: cytidine base editor (CBE) and adenine base editor (ABE), both of which offer a significant deal of promise for targeted base mutagenesis.

The first iteration of CBE, called CBE1, was created by fusing rat-derived cytosine deaminase with apolipoprotein B mRNA editing enzyme catalytic subunit 1 (APOBEC1). APOBEC1 is a cytidine deaminase. Through the deamination of a C to produce a U, which is then changed to T by the cell's DNA mismatch repair mechanism, CBE1 facilitates the direct conversion of a targeted C-G base pair to a T-A base pair. [17]

An uracil DNA glycosylase inhibitor (UGI) was fused to CBE1 to create CBE2, which blocks the removal of the U by base excision repair involving uracil DNA glycosylase. [17]

By substituting dCas9 for Cas9 nickase (nCas9; D10A mutation), a different version known as CBE3 was produced. Comparing this version to CBE1 and CBE2, one can see improved base editing efficiency. [17]

Additionally, uracil-DNA glycosylase has been fused to a CBE to create a glycosylase base editor (GBE). The DNA repair process is triggered when the uracil DNA glycosylase removes the U created by the CBE and produces an apurinic/apyrimidinic site. In E. coli, GBE [that is, AID-nCas9-uracil DNA glycosylase] demonstrated efficient C-to-A conversion. The GBE (APOBEC-nCas9-uracil DNA glycosylase) that allows C-to-G conversion in mammalian cells was produced when AID was replaced with rAPOBEC1. [18]

6. APPLICATIONS OF Cas9

In the field of biotechnology, CRISPR-Cas9 technology has recently gained widespread recognition for its significant applications in the editing of any organism's genome to treat a number of complicated illnesses as well as for other uses.

CRISPR-Cas9 is commonly used in non-human studies to modify the genome and transcriptome to produce epigenetic modifications. [19] [20] We concentrate on research that have a higher direct impact on patients even if these non-patient studies offer an essential foundation for understanding the challenging action mechanism of CRISPR-Cas9 components.

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The therapeutic effectiveness of applying CRISPR-Cas9 gene-editing technology to correct pathogenic mutations that causes genetic diseases has just recently been supported by research on cells and small animals. -thalassemia, Duchenne muscular dystrophy, [21] sickle cell disease, and hereditary tyrosinemia type I (HT1) are a few conditions where gene correction has been successful. Studies on big animals have provided some information on the effectiveness and safety of CRISPR-Cas9 gene editing, particularly for DMD.

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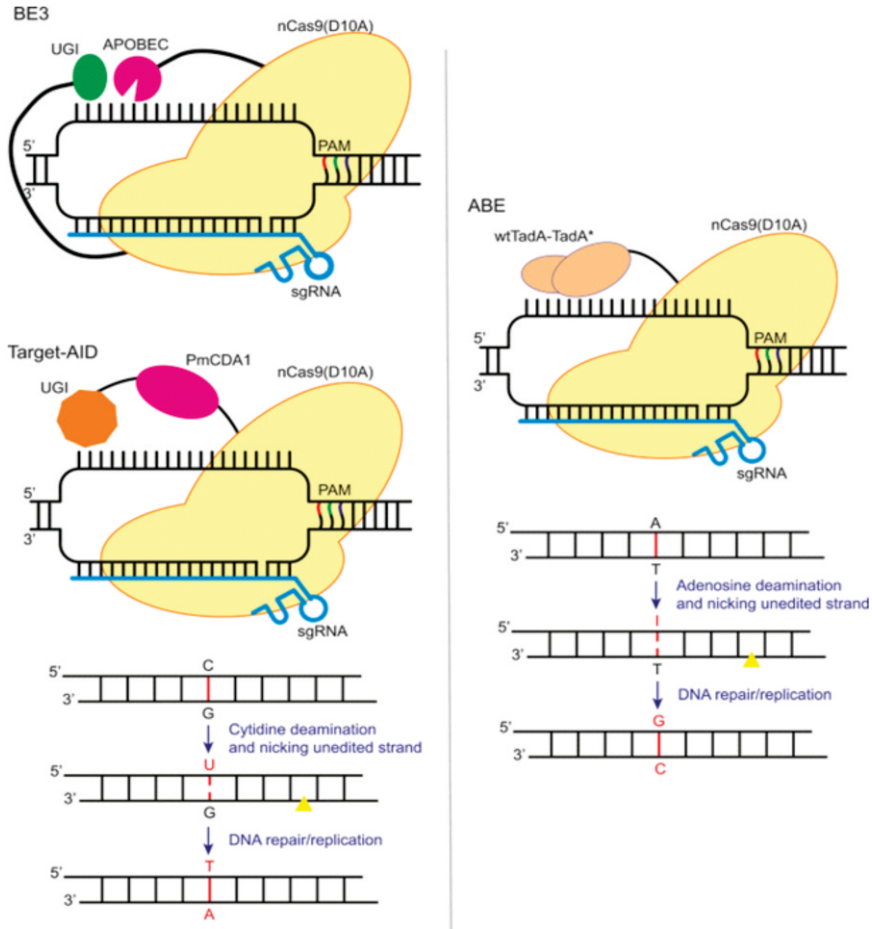

*Figure 2: Deaminase based Cas9 base editing. A schematic model of deaminase based Cas9 base editing systems: cytosine base editors (CBE, for example, BE3 & Target-AID) and adenine base editor (ABE). **CBE** consists of rat apolipoprotein B mRNA editing enzyme (APOBEC), and uracil glycosylase inhibitor (UGI) fused to N and C terminus of nCas9(D10A), respectively. Target-AID consists of activation-induced cytosine deaminase ortholog PmCDA1 and UGI fused to N terminus of nCas9(D10A). CBE involves deamination of cytosine by the deaminase which converts it into uracil, making UG wobble base. UGI prevents its conversion back to C. Mismatch repair machinery (MMR) recognizes it forming UA which is then converted to TA by replication machinery leading CG-to-TA substitution. **ABE** consists of heterodimeric wtTadA-TadA* fused to nCas9(D10A). ABE performs deamination converting TA-to-TI which is then recognized by DNA repair and replication machinery and converted to CG base pair (Yellow triangle: nick site).*

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1. Human embryos with MYBPC3 mutations linked to hypertrophic cardiomyopathy were effectively corrected using CRISPR-Cas9 gene editing, with a targeting effectiveness of 72.2%. Embryos showed no mosaicism, no off-target gene editing, and no additional problems. [22]
2. CRISPR/Cas9 has been utilised to identify crucial host components for HCV infections in the instance of the hepatitis C virus (HCV), which causes chronic hepatitis, liver cirrhosis, and hepatocellular carcinomas. [23]
3. The establishment of genetically altered animal and cell models of several human illnesses, including gene knockout models, exogenous gene knock-in models, and site-directed mutagenesis models, is the most significant use of CRISPR-Cas systems in the medical profession. [24]
4. Oncogenes and tumour suppressor genes have undergone several genetic and epigenetic modifications in a variety of illnesses, including cancer. The systematic study of the genes involved in the initiation, development, and treatment response of cancer requires the manipulation of normal and malignant cell genomes through experimentation. Due to the necessity to clarify the significance of genetic mutations found in human tumours, identified by large-scale sequencing of the cancer genome, the quick modelling of genetic events has assumed considerable relevance. [20] One of the most promising cancer treatment modalities is adoptive gene-engineered T cell immunotherapy, which includes chimeric antigen receptor (CAR)- and T cell receptor (TCR)-T cell therapy. While the latter has been successful in treating haematological malignancies, it has failed to do so with solid tumours. [25]
5. Zebrafish genome editing using CRISPR/Cas9 highlights the CRISPR Cas9 genome editing technology's potential. [26]
6. A research showed that cystic fibrosis might be cured in vitro by homologous recombination of the gene locus responsible for the illness, the CFTR (cystic fibrosis transmembrane conductance regulator), using organoids of cultivated intestinal stem cells. [27]
7. Genome editing using CRISPR-Cas technology has proven successful in treating conditions like Fanconi anaemia. [28]
8. This approach was used to efficiently modify the genomes of industrially essential bacteria, especially Streptomyces spp. and Clostridium spp., to produce biofuels, anticancer medications, and antibiotics. [29]
9. According to studies, CRISPR-Cas9 used in antimicrobial therapy is superior to conventional antimicrobials and medicines. [30]
10. CRISPR can delete the incorrect exon, therefore the duplicator system can generate a smaller amount of dystrophin protein as effective as its natural form and sgRNA. Modified Cas9 were delivered by using unique viral career; adenovirus into mouse muscle cells; and CRISPR system was applied to delete the incorrect exon. [31]

7. THE CHALLENGESOF CRISPR Cas9

The CRISPR/Cas system is undoubtedly a helpful tool for gene editing, but there are still a few obstacles in the way of its full potential. One of the principal concerns is off-target effects, or undesired genome

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editing at unanticipated locations. The ability of the sgRNA to tolerate mismatches with target DNA sequences (outside of the seed area positioned 1 to 5 nucleotides from the PAM) may be the source of these effects. [32]

Studies revealed that Cas9 also makes it easier for sgRNA to bind to off-target sites, whereupon Cas9 cleaves the off-target sites to produce DSBs. [33]

Another critical challenge for CRISPR/Cas9-mediated genome editing is gRNA development. It is currently challenging to use RNA polymerase II for the generation of gRNA due to the significant posttranscriptional processing and modification of mRNA produced by RNA polymerase II. gRNA is currently produced in vivo using RNA polymerase III, U3 and U6 snRNA promoters. U3 and U6 snRNA genes, which are housekeeping genes that are universally expressed, cannot be exploited to produce gRNA unique to certain tissues and cells. [34]

Concerns persist over how CRISPR/Cas9 is introduced into living things. Delivery of CRISPR/Cas9 involves methods based on DNA and RNA injection, such as the injection of plasmids expressing Cas9 and gRNA. [35] The target cells and tissues will affect how well a delivery strategy works. It is important to focus more on creating innovative, reliable CRISPR/Cas9 delivery systems.

8. DISCUSSION AND FUTURE PERSPECTIVES

The field of genome engineering has undergone a revolution thanks to the introduction of CRISPR/Cas technology. The most advanced and adaptable editing techniques may be employed in a variety of settings, from fundamental science to the development of new medicinal treatments. Additionally, the CRISPR/Cas system enables scientists to run genome-wide analyses to investigate the effects of altered gene expression on cellular function and to establish a relationship between genotypes and phenotypes.

It is crucial to improve CRISPR tools in order to effectively minimise Cas9's activity in cleaving targets. The precise use of CRISPR-Cas9 delivery techniques to the cells of higher class species, such as plants and mammals, is essential for advancement. The identification of pharmacological targets through the application of CRISPR-Cas9 technology can help with the creation of new medications for the terrible diseases that are now developing. By inserting modified TEs, this method has the potential to enhance crops and produce new attractive plants. Since the CRISPR-Cas system is located in bacteria, it is undergoing rapid evolution. As a result, it may produce new cas genes that will encode new proteins, which may one day be used for genome editing or other purposes.

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