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CRISPR-Cas9 in Drug Discovery: Potential Applications and Ethical consideration

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Abstract

In prokaryotes, the clustered regularly interspaced short palindromic repeats (CRISPR) system provides adaptive immunity against phages and plasmids. This technique led to the development of the powerful genome engineering tool known as the CRISPR/CRISPR-associated nuclease 9 (CRISPR/Cas9) genome editing system. The CRISPR/Cas9 technique has significantly increased our understanding of cancer genetics by being used to study the functions of genes associated with cancer, generate animal models of tumors, and find possible therapeutic targets due to its high efficacy and accuracy.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), commonly referred to as genome editing technology, is notable among them,has developed into a fascinating application in science, even winning the 2020 Nobel Prize in Chemistry. Compared to current technologies, this technology offers benefits such increased speed, affordability, and accuracy, and it finds use in a variety of fields. This review's objective is to provide an overview of earlier studies on CRISPR-Cas9, a technology that is widely used in many scientific domains, in order to offer direction for further research

Key Words: CRISPR, Cas9, genome engineering

1. Introduction

CRISPR, or clustered regularly interspaced short palindromic repeats, was initially identified in Escherichia coli DNA sequences and explained in 1987 by Osaka University's Ishino et al. (Japan)[1]. Guide RNA sequences are used by the clustered regularly interspaced short palindromic repeat (CRISPR) to guide the certain sites on the deoxyribonucleic acid (DNA) that require editing using the CRISPR-associated protein 9 (Cas9) enzyme. It starts with the leadoff sequence, then spacer and repeat sequences, and finally the Cas genes. The main objective of the CRISPR procedure is to guarantee that any undesirable gene regions are absent from the genomic architectures of prokaryotes and eukaryotes prior to the start of protein synthesis.[2] Clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), a newly developed gene-editing tool, is transforming ophthalmology as well as all other biological science study.[3] Clustered regularly interspaced short palindromic repeats technology

(CRISPR-Cas9) has emerged as the most popular technique for gene modification. The advantages of this technology over earlier ones, such zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN), include excellent precision, ease of handling, and comparatively low cost. These advantages make CRISPR-Cas9 technology simple to use in any molecular biology lab.[4]

An overview of the mechanisms underlying CRISPR/Cas9-mediated genome editing

The three primary phases of bacteria's defense against foreign nucleic acid invasion are adaption, expression, and interference.[5] The RNA-guided endonuclease Cas9, CRISPR RNA (crRNA), and transactivation CRISPR RNA (tracrRNA) are the three main parts of the CRISPR/Cas9 system.[6] A straightforward two-component method for efficient targeted gene editing is CRISPR/Cas9. The first element is the endonuclease-containing single-effector Cas9 protein domains HNH and RuvC. The complementary DNA strand is cleaved by HNH, while the non-complementary DNA strand is cleaved by RuvC. Double-strand breaks (DSBs) are produced in the target DNA by these domains working together. A single guide is the second element of successful targeted gene editing.RNA (sgRNA) has a scaffold sequence that allows it to attach to Cas9 and a spacer sequence of 20 base pairs that is next to the PAM sequence and complementary to the target gene. The CRISPR/Cas9 complex is guided to its target chromosomal region by this sgRNA.

The editing mechanism then uses either homology-directed repair or nonhomologous end-joining (NHEJ), two endogenous DNA repair processes. (HDR) (Figure 1). NHEJ, which involves the random insertion and deletion of base pairs, or indels, at the cut site, is far more common in the majority of cell types. This error-prone process typically leads to frameshift mutations, which frequently produce a polypeptide that is not functional and/or an early stop codon. The alternative method is the errorfree HDR pathway, which is particularly desirable to use for therapeutic objectives. Through this route, the homologous repair that is error-free by using a section of the unedited DNA strand as a template to fix the damaged DNA. This approach can be used experimentally to facilitate the intended genome modification by supplying an external donor template equipped with the CRISPR/Cas9 machinery.[7]

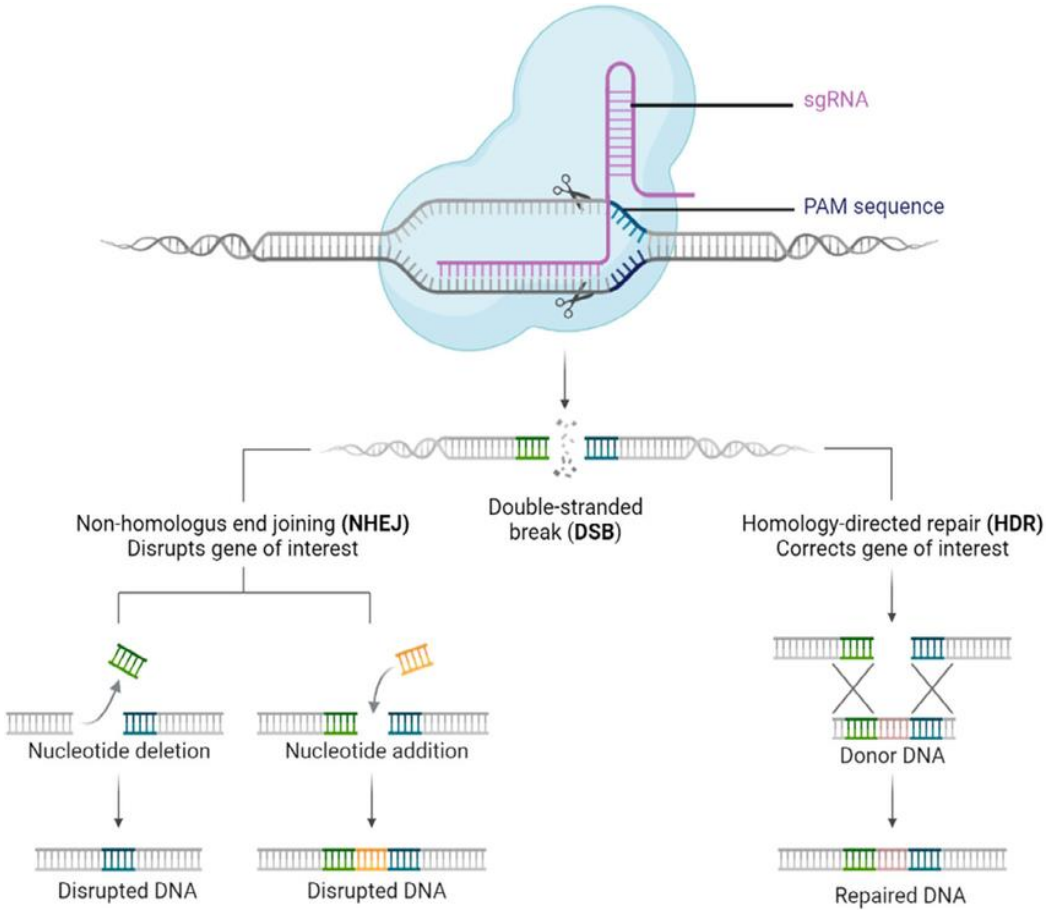

FIGURE .1: Diagrammatic representation of gene editing in eukaryotic cells using CRISPR/Cas9. Both sgRNA and PAM are used by the CRISPR/Cas9 enzyme to identify target DNA and cause DSB. This technique initiates targeted genomic editing mediated by NHEJ or HDR. Used BioRender.com to create. https://www.researchgate.net/figure/Schematic-illustration-of-CRISPR-Cas9-mediated-gene-editing-in-eukaryotic-cells-The_fig1_363600224

CRISPR/Cas9 genome editing approaches **[1]Presently used nonviral delivery methods for the CRISPR/CAS9 system**

[1.1]Viral vectores

Viral vectors such as lentivirus (Chakraborty et al., 2014) and adeno-associated virus (Long et al., 2016) have achieved high efficiency for CRISPR system delivery, but there are significant concerns related to viral delivery due to the possibility of unwanted genetic mutations, insertional mutagenesis, high off-target effects (Schumann et al., 2015), restricted packaging capacity, carcinogenesis, and immunogenicity

[1.2]Nonviral vectors Many current difficulties may be addressed by the nonviral vectors of CRISPR system delivery, which use nanoparticle-based delivery. These restrictions include high genetic packing capacity, safety issues (Schmidt & Grimm, 2015), and Possible breakthroughs include synthesis and costumed designs (L. Li, Wei, & Gong, 2015), cost effectiveness, and scalability (Ramamoorth & Narvekar, 2015), as well as chamberlain, Riyad, and Weber (2016). Furthermore, nonviral vectors can be made to distribute to specific cells or organs.[8]

[1.2.1]Lipid-based nanoparticle: LNPs appear to be a safe and efficient delivery method because pre-clinical studies demonstrate that they may effectively carry siRNA or mRNA.

[1.2.2] Polymer-based nanoparticles: Polymer materials are regarded as a potent delivery system because of their extended blood circulation, high drug bioavailability, superior biocompatibility, and degradability. [1.2.3] Gold nanoparticles: GNPs have relative biocompatibility, can be mixed with different substances including lipids, polymers, or nucleic acids, and can enter a variety of cells.[9]

[2] Genome editing approaches to psychiatric disorders

Additionally, the CRISPR/Cas9 system has been modified to target many chromosomes at once in order to produce or fix massive CNVs in DNA.22 It has been revealed that CNVs in the 16p11.2 region, which are commonly identified in schizophrenia, may be effectively edited using CRISPR/Cas9.[10]

Advantages

[1] CRISPR-Cas9 is frequently utilized for transcriptome and genomic engineering to produce epigenetic changes in non-human studies.[11]

[2] The CRISPR/Cas9 system is frequently used to genetically modify pluripotent or multipotent stem cells, which are then stimulated to differentiate into particular cell types for in-depth research or potential therapeutic use.[12]

[3] CRISPR-Cas9 technology is now one of the most popular gene editing tools in molecular biology labs across the globe because it is simple to develop, inexpensive, quick to implement, and more accurate and efficient than previous techniques.[13]

[4] In the field of leukemia research, CRISPR/Cas9 technology has demonstrated promise, especially in the investigation of chronic myeloid leukemia and chronic lymphocytic leukemia.[14]

USES

[1] Utilizing CRISPR-Cas9 to reduce medication resistance in lung cancer: CRISPR-Cas9 is a key component of lung cancer research, with medication resistance studies serving as a primary focus of inquiry.[15]

[2] Genetic Disorders: The genetic mutation causing sickle cell disease has been fixed with CRISPR-Cas9.[16]

[3] Using CRISPR/CAS9 in the study of Tumor therapies: Tumor models were successfully created using CRISPR/Cas9.[17]

[4] Utilizing CRISPR/Cas9 as a Research Instrument for Human Infectious Viruses : A new avenue for gene editing has been made possible by the successful genome alterations made in cultured human cells using the CRISPR/Cas9 apparatus (Cho et al., 2013; Jinek et al., 2013; Mali et al., 2013). treatment in biological research [18]

[5] In one study, CRISPR-Cas9 was utilized to target ten distinct HIV-1 DNA locations that latently infected T cell lines.[19]

[6] Crops resistant to pests and illnesses can be produced using CRISPR-Cas9, which lowers the need for dangerous pesticides and boosts agricultural output.[20]

Applications

[1] Applications of CRISPR/Cas9 gene editing technologies to combat illnesses like COVID-19: Numerous fields, including the basic sciences, food and crop development, fuel production, medication development, human genome engineering, and gene editing technology to combat new diseases like coronavirus (COVID-19 or SARS-CoV2), are currently using CRISPR/Cas biotechnology extensively and globally.[21]

[2] Application of CRISPR/Cas9 Gene therapy: Correcting genetic mutations that cause inherited illnesses is one of the main uses of CRISPR-Cas9 in gene therapy.[22]

[3] One of the primary applications of CRISPR-Cas9 in gene therapy is the correction of genetic mutations that result in hereditary diseases: Novel drug targets can be found rapidly and effectively using the CRISPR library screen.[23]

[4] Utilizing animal models to cure human diseases through the CRISPR/Cas9 system: work in 2014 showed that a mutation in postnatal animals could be successfully corrected utilizing the CRISPR/Cas9 system and the mouse model of hereditary tyrosinemia type 1 (HT1).[24]

[5] CRISPR/Cas9 applications in medicine and therapy: CRISPR has demonstrated a promising approach to treating some of the most deadly and serious illnesses and helping to save lives.[25]

[6] Agricultural applications of CRISPR-Cas9 technology: The technique known as CRISPR-Cas9 has the potential to completely transform agriculture by facilitating accurate and effective genetic alterations in livestock and crops.[26]

[7] CRISPR/Cas9 use for drug target validation and screening: Targets can be effectively validated by combining CRISPR/Cas9 technology with whole-genome sequencing and drug resistance mutation screening.[27]

[8] Applications of CRISPR in agricultural genetic enhancement: In order to assist create target events, the CRISPR system is clear-cut, sufficient, effective, easy to use, and extremely specific.[28]

Challenges and Future Directions

Challenges

[1] Despite ongoing preclinical research and clinical studies worldwide that concentrate on curative medicines, the therapeutic application of CRISPRCas9-mediated gene repair is linked to uncertain consequences.[29]

[2] A number of obstacles must be overcome in order to effectively treat Alzheimer's disease (AD) brought on by CRISPR/Cas9 brain delivery using non-viral vectors. Ideally, the vectors should be stable and efficient at moving the weight at the intended position.[30]

[3] Techniques for Getting Past Obstacles in Colombia's CRISPR Implementation: Investing in Ethical and Social Opposition.[31]

[4]CRISPR-CAS9 screening difficulties in precision medicine: One of the biggest obstacles to the development of CRISPR technology has been minimizing the off-target effects of gene editing.[32]
[5] Despite the earlier justification indicating that CRISPR/Cas9 is a promising strategy, there are still a lot of hazards and limits with this editing technique that make it difficult to utilize in clinical trials because of its recent human discovery and usage.[33]

Future Directions

[1]In stem cell research, CRISPR-based genetic screens have a broad and important application method that allows scientists to examine nearly any biological activity and disclose its genetic foundation.[34]
[2] Because of its accuracy and effectiveness, CRISPR/Cas9 procedures are a potent gene editing tool that offer a remarkable chance to treat a number of gene-related diseases by deleting, inserting, regulating, and blocking various genes. The genome editing technique CRISPR By deleting oncogenes either alone or in combination with activating tumor suppressor genes, this technology provides a sophisticated method of cancer detection and treatment. It is a clever approach to cancer care.[35]
[3]Prospects for CRISPR in medicine in the future: CRISPR technology is anticipated to overcome present obstacles and constraints as research advances. The specificity and effectiveness of CRISPR are being improved, new delivery methods are being developed, and the ethical frameworks surrounding gene therapy are being refined by scientists.The safe and efficient application of CRISPR-based treatments is made possible by these initiatives. [12]

2. Conclusion

Given that the existing exploratory research and application-based studies are minuscule compared to the immense potential of CRISPR/Cas9, it has hardly touched the surface of what is ahead for medical research. The potential of this technology has led to a boom in its use in many other domains, but most significantly ,It might end up serving as a one-stop remedy for any illness with a genetic cause. Several drug response-related genes have been found utilizing the CRISPR/Cas9 genome editing technique based on the data presented here. Additionally, the results of treatment may be impacted by specific genetic variations. Therefore, causing particular nucleotide alterations in particular cancer genes could be another helpful tactic cells to increase their susceptibility to therapy

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