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A REVIEW ARTICLE ON: GENE EDITING TECHNOLOGY (CRISPR/CAS9)

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ABSTRACT

Genome editing reemerged in 2012 with the development of CRISPR/Cas9 technology, which is a genetic manipulation tool derived from the defense system of certain bacteria against viruses and plasmids. This methodis easy to apply and has been used in a wide variety of experimental models including cell lines, laboratory animals, plants, and even in human clinical trials. The CRISPR/Cas9 system consists of directing the Cas9 nuclease to create a site-directed double-strand DNA break using a small RNA molecule as a guide. A process that allows a permanent modification of the genomic target sequence canrepair the damage caused to DNA. In the present study, the basic principles of the CRISPR/Cas9 system are reviewed, as well as the strategies and modifications of theenzyme Cas9 to eliminate the off-target cuts, and the different applications of CRISPR/Cas9 as a system for visualization and gene expression activation or suppression. In addition, the review emphasizes on the potential application of this system in the treatment of different diseases, such as pulmonary, gastrointestinal, hematologic, immune system, viral, autoimmune and inflammatory diseases, and cancer.

KEYWORDS: A process that allows a permanent modification of the genomic target sequence canrepair the damage caused to DNA.

INTRODUCTION

Clustered regularly interspaced short palindromic repeats (CRISPR) were first discovered in Escherichia coli in 1987 and later found in other bacteria species. The role of these repeat sequences remained unclear until, in 2005, several researchers described the similarities of

the sequences DNA, leading to the hypothesis that the sequences are part of adaptive immune system in bacteria (CRISPR/Cas9 for cancer research and therapy).

As you know, CRISPR is also refers to an adaptive immune responsein bacteria and archaea that is cast-off to target and cut down viral DNA by using endonuclease in specific ways. By reengineering this immune response to target parts of genetic material, scientists could make extremely precise genetic alterations tailored to the type of cell. This is the basis of CRISPR therapeutic and diagnostic platforms.

CRISPR and CRISPR-associated proteins characterize the immune system of archaea and bacteria, and deliver protection against invasive nucleic acids, DNA, or RNA from phages, plasmids, and other exogenous DNA elements. At this time, two different classes, six types, and 21 subtypes of CRISPR-Cas systems havebeen identified. The length of the repeat sequences varies between 25 and 40nt, whereas the length of the spacer sequences varies between 21 land 71 nt.

Recent developments in CRISPR/Casgene editing have made it possible to introduce precise changes into a wide variety of genomes, including those of dsDNA viruses; in addition to this, it is pursuing relies on the co expression of a prokaryotic Casnuclease and an associated guide RNA (gRNA) sequence. Because, once gRNA targeted Cas, it creates a double-stranded break (DSB) in the genome, by making of two main repair processes compete to repair the damage, leading to with some modifications of from the original sequence.

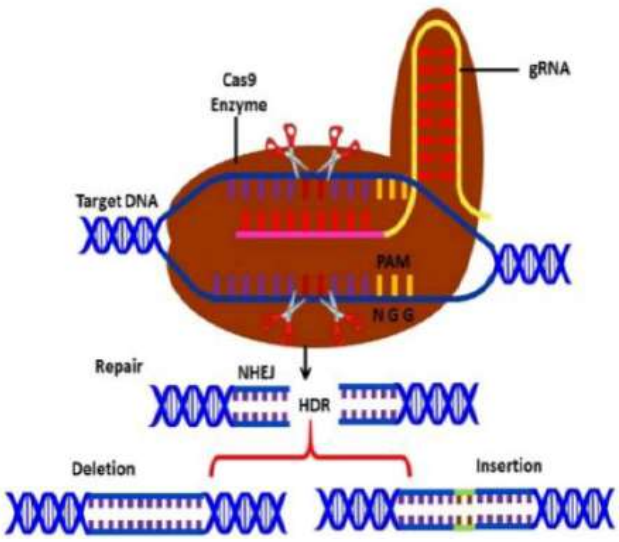
The study of gene function in the recent decades has trusted on the generation of systemic and conditional gene knockouts to disrupt gene expression especially in coronaviruses. For example, de Wilde et al. investigated the cyclophilin A (CypA) protein-dependence in the replication of three related nidoviruses in the same cell line (Huh7), in which CypA expression was knocked-out using CRISPR/Cas9 gene editing technology (Huhz-CypAKO cells). Therefore, even if successful studies and some innovation ideas and solution were done, but, it has been challenging to knockout a gene in specific cell types in the brain. The final target and goals of CRISPR was to revolutionize the treatment of hereditary diseases based therapeutics. Finally, the aim of this systematic review paper is to summarize the application of CRISPR/Cas and its overall immune signal transduction and also discussing gene editing technology against novel diseases like coronavirus (COVID-19).

CRISPR/Cas9 system

This system, which was discovered for the first time in the genome of Escherichia coli, is an acquired immunity mechanism in various bacteria and archaea against foreign agents, including plasmids and viral genomes. CRISPR/Cas system is categorized into three major classes (I, II, and III), with a gene family encoding specific Casenzymes and a special functional mechanism. Types I and II utilize various Cas enzymes for endonuclease activity, whilst type Il employs only Cas9 enzyme for this purpose. From an evolutionary point of view, the rate of changes and evolution in the structural and functional diversity is very high due to the constant competition between the parasite and the host in the CRISPR/Cas9 system. Accordingly, in recent classification, the CRISPR system is classified into two classes (I and II) and also six types (I-VID).

Most genomeengineering studies have utilized CRISPR system typel, derived from S.pyogenes, SpCas9 bacterium. The advantage of system typell is the dependence on a protein (Cas9) for nuclease activity.

Also, system II requires some kinds of RNAS, such as crRNA, pairing with the target Genome sequences and functioning as a Cas guide, and tracrRNA, which acts as activator for maturity and summoning up Cas9 to the desired site. TracrRNA and crRNA sequences have been integrated into a chimeric sequence, known as small guide RNA (sgRNA), containing the characteristics of both types of RNA, for use in genome editing (Figure 3.[65] Also, the CRISPR/Cas9 system can be used for treating genetic diseases. For example, the mutated site in the dystrophin gene leading to Duchene' smuscle.



CRISPR/Cas9

dystrophy disease (DMD) has been eliminated by CRISPR. Then the expression of dystrophin returned to a normal level, and the muscular functions were significantly improved. All CRISPR sites contain tandem repeats and spacers. Tandem repeats include identical and spacer sequences originating from the genome of foreignagents. CRISPR sites associated with proteins (Cas) can make an acquired immunity against invading DNA. If a microorganismsur pathogen, the CRISPR system will be able to insert a fragment of the invading DNA into the genome and use it to deal with future attacks.

Briefly, the immunity in bacteria via the CRISPR systemis operated in three stages: Admission: In this step, short fragments of virus or plasmid DNA are detected and inserted as a spacer between two adjacent repeats into the CRISPR sites; Expression: During this step, CRISPR sites are copied as a Pre-crRNA, which contains a complete complex of CRISPRrepeats andembedded sequences derived from invasive agents among them:; Interference: In this step, Pre-crRNA is separated into crRNA as the small guide sequence by a special endoribonuclease.

Cas9 Protein Structure

Characterizing the crystalline structure of the Cas9 protein in S. pyogenes, was helpful in understanding the interaction between the components of the complex, including Cas9, crRNA, tracrRNA, and the target DNA at the molecular scale. Generally, Cas9 protein includes a nuclease lobe (NUC) and a recognition lobe (REC). The REC lobe, which consists of a long alpha helix and RECI and REC2 domains, is considered the specific functional domain of Cas9. At the same time, the NUC lobe is composed of RuvCHNH and PAM-interacting (PI) domains.[74] HNH and RuvC domains cut the target sense and antisense strands, respectively. And create a DSB upstream of PAM Also, the PAM sequence can regulate the sgRNA recognition control system. If each nuclease domain is inactivated, Cas9 can only cut one strand as a nickase.

Specificity of the cleavage

Although specificity in targeting depends heavily on the gRNA sequence, the Cas9 protein nuclease activity in the CRISPR-Cas9 system is dependent on the PAM-specific sequence. In this system, Cas9 nuclease can cut any genomic sequence that is immediately located on the 5' side of the PAM sequence, and in other words, the target sequence is cleaved in downstream. The absence of a PAM sequence can change the affinity between Cas and the target DNA, and Briefly, the immunity in bacteria via the CRISPR systemis operated in three

stages: Admission: In this step, short fragments of virus or plasmid DNA are detected and inserted as a spacer between two adjacent repeats into the CRISPR sites; Expression: During this step, CRISPR sites are copied as a Pre-crRNA, which contains a complete complex of CRISPRrepeats andembedded sequences derived from invasive agents among them:; Interference: In this step, Pre-crRNA is separated into crRNA as the small guide sequence by a special endoribonuclease.

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Off-target effects

Off-target mutagenesis is one of the main problems in the CRISPR-Cas9 system, especially in gene therapy. Nevertheless, this issue does not seem to be serious in plants, which can be due to transformation efficiency, gene expression level, and codon usage bias (CUB) in plants. Off-target effects are defined as the acceptance or tolerance of Cas9 to awkward sequences in the sgRNA.

Off-target effects could be more likely when there is a high similarity of gRNA sequence with an off-target site up to three bps. Various studies show that the mismatched bps at the end of the 3' target sequence (usually 8 to 14bps upstream of the PAM sequence) are less tolerated, while the mismatched bps at the end of the 5' targetregionare better tolerated than 3' target sequence and they are more acceptable. Moreover, the value of gRNA and its ratio to Cas9 also affect the off-target effects.

Ingeneral, although Cas9 enzyme has different applications because of its high nuclease activity as well as wide targeting range, it has limitations due to high molecular weight and off-target effects. Nevertheless, some mutations occur in Cas9 variants, such as eSpCas9 and SpCas9-HF. Which reduce nonspecific interaction between Cas9 protein and the target sequence. In addition to various strategies to reduce off-target effects, there are numerous laboratory methods, such as digenome-seq, GUIDE-seq, and HTGTS, to identify off-target sites.

Decreasingoff-target effects in the CRISPR/Cas9 system

Selection of GUIDE-Seq with minimum potential sites

Off-targetis detected by whole genome homology search. Among them, some sequences are chosen whereby the mismatched pairs are concentrated in the region close to PAM since they are tolerated less for Cas9 functioning.[90]

Selection of truncated length GUIDE-Seq (trui-gRNA)

In this solution, gRNA with 2-3 nucleotide size is truncated at 5. It has been found that the selection of shorter sequences (17 or 18 nucleotides) reduces the editing efficiency to a small extent while significantly reducing undesired mutations, thereby mitigating off-target effects.[89]

Use of the pair need strategy (Cas9n)

Through inactivating one of the Cas9 domains, a variant has been created that functions as a demand. Using a pair of closely related sgRNAs, Cas9n can create two adjacent splices in single strands, consequently, a DSB. This solution reduces the off-target activity and can increase specificity by up to 50-1500 times. The pair need strategy has caused gene deletion in mouse eggs without reducing the cutting activity of the target sites.

dCas9-Fokstrategy

Dead-nuclease Cas9 (dCas9) is created by inhibiting the enzymatic activity of both RuvC and HNH domains. DSB is produced by combining the Fokl nuclease domain with dCas9 as a dimer and selecting a pair of sgRNA with suitable orientation and distance. Investigations have shown that this method significantly enhances specificity.[93]

CRISPR/Casl2a system

The Cas12a protein was known as an important discovery in 2015 at Feng Zhang' slaboratory, one of the pioneers of the CRISPR system. Cas12a protein is the smaller and simpler version of Cas9 belonging to class II and type V. This proteinisisolated from Staphylococcus Aureus and has a higher editing activity than Cas9. The small size of this protein has made it easier to transferit into cells and tissues. Furthermore, the specificity of Cas12a endonuclease is high, and thus its off-target effects, especially in human cells, are negligible.[94][95]

Some differences between Cas1 2a and Cas9 have caused the CRISPR/Cas1 2a system to overcome some limitations of the CRISPR/Cas9 system. For example, the Cas9 protein requires two molecules, crRNA, and tracrRNA, while Cas12a needs one RNA molecule (crRNA) for cutting DNA. Cas9 includes RuvC and HNH nuclease domains and cleaves both DNA strands at the same site, causing blunt ends, while, Cas1 2a only contains the RuvCnuclease domain, which creates two sticky ends at the target sites by cutting only one strand.[97] The Cas12a protein cuts DNA at different sites and provides more options while selecting a site for editing. The cleavage and detection sites in Cas9 are close together, while in Cas1 2a, they are further apart. The PAM sequence in Cas9 is richin' 5-NGG-3, while in Casl2a, it is rich in T (5-TTTN-3).

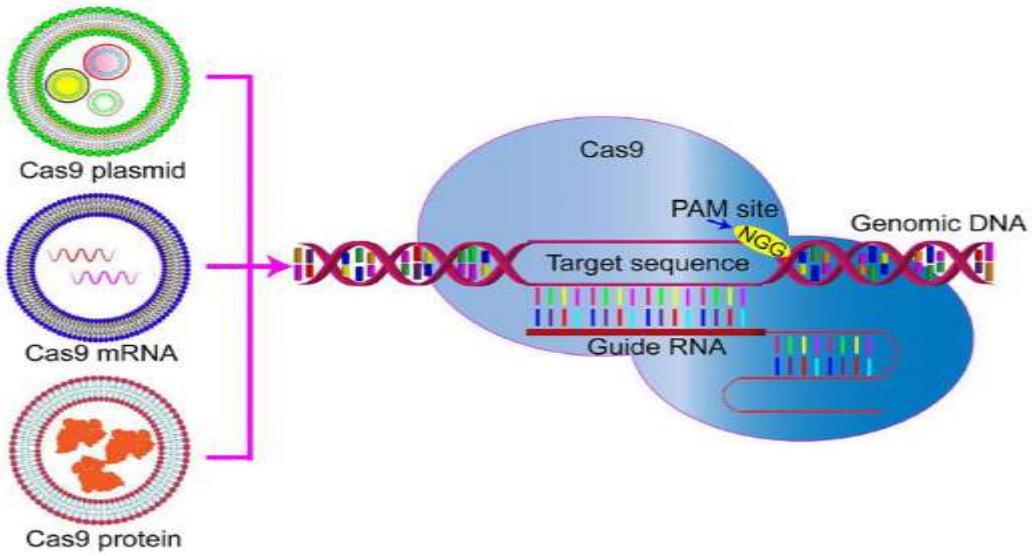


Fig. CRISPRtransfer method

CRISPRtransfer methods

According to the specific, precise, and effective editing, particularly in gene therapy, transferring the CRISPR system into the cells is highly important. The CRISPR construction transfer can be performed as DNA, mRNA, or ribonucleoprotein. The transformation strategies can also contain microinjection, electroporation, and viral and non-viral vectors suchas nanoparticles, liposomes, and agrobacterium-mediated vectors.

Important Applications of CRISPR Technology

Injust a few shortyears, CRISPR has had a massive impact on scientific research, contributing to breakthroughs in medicine and biotechnology. Let' s take a closer look at some of thekey applications of this technology.

1. Cell and gene therapies

CRISPRispoised to revolutionize medicine, with the potential to cure a range of genetic diseases, including neurodegenerative disease, blood disorders, cancer, and ocular disorders. As we mentioned earlier, the first trial of a CRISPR cell therapy was performed in 2019, treating patients with sickle cell disease. The treatmentrestored fetal hemoglobin, eliminating the need for a functional copy of adult hemoglobin In 2021, a significant CRISPR trial for transthyretin amyloidosis, a neurodegenerative disease, showed very promising results. It is also revolutionizing pediatric cancer treatment, described in this podcast interview with Shondra Miller from St Jude' s.

CRISPRcan also be used to generate chimeric antigen receptor (CAR) T cells, a form of immunotherapy used to treat cancer. The T cells are extracted from patients and engineered to express chimeric antigen receptors before being re-injected into the body. The receptors allow the Tcells to more efficiently target and destroy the specific type of cancer the patient suffers from.

While we are still in the early years of clinical trials, this technology could be used to treat thousands of genetic conditions in the future, including breast and ovarian cancer linked to BRCA mutations, Huntington' sdisease, Tay Sachs, beta-thalassemia, cystic fibrosis, and early-onset Alzheimer' s. For all the latest medical developments and clinical trials using this technology to cure a range of human diseases, you can take a look at the CRISPR Medicine News website.

2. Diagnostics

During the COVID 19 pandemic, CRISPR was used as both a potential therapeutics tool and as a diagnostic tool for the coronavirus. The SHERLOCK T™ CRISPRSARS-CoV-2 test kit was granted Emergency Use Authorization from the Federal authorities to be used in laboratory settings. You can learn more about SHERLOCK and the more recently developed STOPCovid diagnostic test here and in this podcast. Mammoth Biosciences has also developed a CRISPR-based Covid-19 diagnostic method, known as DETECTR. Like SHERLOCK and STOPCovid, DETECTRutilizes Cas9' s search function to detect genetic material from the virus, employing naturally occurring Cas nucleases, like Cas12 and Cas13. For more information on DETECTR, you can listen to this interview with Trevor Martin, CEO of Mammoth Bioscience. Similar diagnostics utilizing the search function of Cas9 have also been engineered to identify other diseases, both infectious and genetic. Early in 2021, Dr. Kiana Aran of Cardea Bio published a study which combined three different Nobel Prize-winning technologies graphene, transistors, and CRISPR- into a tiny chip that can detect pathogenic single nucleotide polymorphisms (SNPs). Since 50% of disease-causing mutations in humans are SNPs, this is a significant breakthrough in medical diagnostics.

3. Agriculture

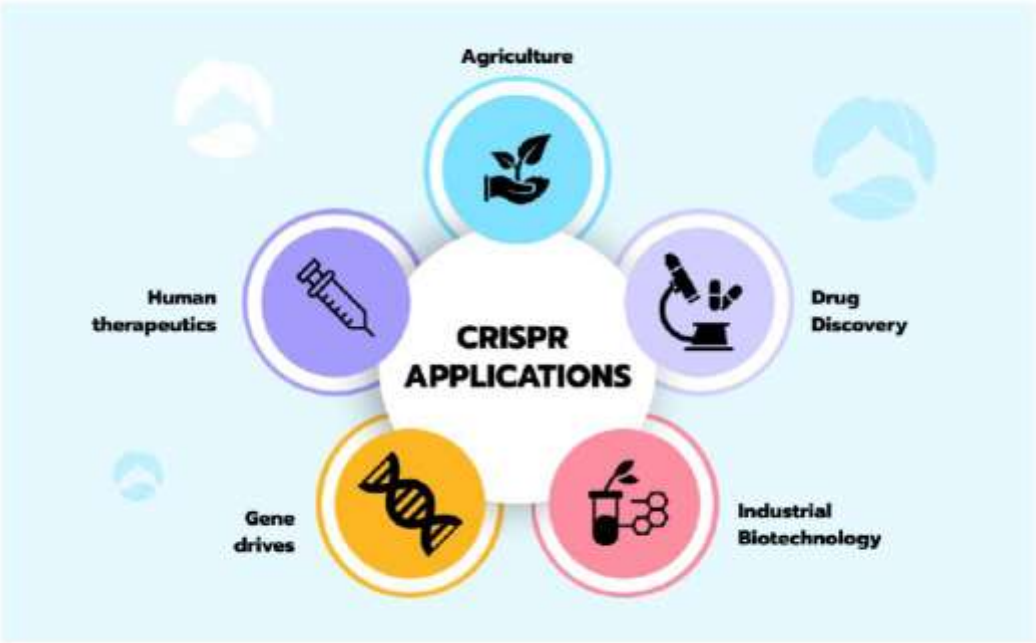
Gene editing technology has huge potential in agriculture, and experts Suggest that CRISPR-modified foods will be available within 5-10 years. This is primarily because it can be used to create crops that are disease-resistant and drought-resistant. For example, scientists from the University of Berkeley and Innovative Genomics Institute have partnered with Mars, Inc. to

create disease-resistant cacao plants. It can also be used to prolong the shelf life of other perishable foods, reducing food waste and allowing access to healthy foods at relatively low cost. For moreinformation on these applications, you can read our overview of CRISPR' s use in agriculture.

4. Bioenergy

As one of the leading alternatives to fossil fuels, bioenergy has been under the spotlight for a while now. However, there are several hurdles to producing biofuels at scale. By using CRISPR, Scientists have recently been able to make some significant advances in thisa area.

For example, KO of multiple transcription factors that control production of lipids in algae has led to a huge increase in lipid production for generating biodiesel. Similarly, gene editing can improve the tolerance of yeast to harsh conditions during the production of biofuels. It has also increased editing efficiencies in bacterial species that are used to produce ethanol. For more details, you can check out this blog on how CRISPR is helping the biofuel industry.



CONCLUSION

Form these systems review; we can appreciate the most important components of CRISPRRNAs (crRNAs) and Caseffector proteins. CRISPR will be a very important and crucial study area for disease diagnosis and treatment in the future, with the best potential for research in the scientific community. Beside this, it will be important for correcting the mutations and hereditary diseases concerning immune cells and their system disturbances; it

will be important even for immunological tolerance to correct wrongly activated immune cells that fail to identify self and non-self, leading to cancerous disturbances of immune balance It is very crucial in facilitating the etiology of diseases and checking their causative agents. From its adaption, it has revolutionized molecular biology and genetics ingenera.

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