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Biochemistry

A REVIEW ON THE APPLICATION OF CRISPR/Cas9-GENOME EDITING TOOL

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ABSTRACT CRISPR-CAS9, a bacterial defensive genome editing mechanism against phages, introduced by Jennifer Doudna and Emmanuelle Charpentier as a system that can be programmed and reprogrammed in 2012, is a modern revolutionary bioscience tool with applications far beyond the present-day scientific boundaries. With its accelerating advancements in fields ranging from agriculture, horticulture, genetic disorders, biomedicines, live-imaging of genes, epigenetic editing, CRISPR technology has already altered the biological perspective in a great deal. In this review paper, an overview of the CRISPR/CAS9 technology and its applications in agricultural and bio-medicinal field of studies based on previously available works of literature, has been shortly summarized.

KEYWORDS : CRISPR/Cas9 , Agriculture, Biomedicine, Genome Editing

INTRODUCTION

Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR is a natural adaptive immune mechanism in bacteria against the viruses. CRISPR basically serves as a memory bank by recording a fragment of the viral genome sequence after it attacks the bacteria for the first time, in short, unique sequences called spacers which remains bounded by repetitive sequences on both sides. In a subsequent attack by the virus, the bacteria synthesis a guide RNA or gRNA which recognizes and binds to the target DNA by the help of identification of a PAM site (Protospacer Adjacent Motif) that remains adjacent to the target DNA. PAM is usually 3-6 NT long (NGG, NAG, etc.) This recognition is assisted by a nuclease Cas9, a 1368 residue protein obtained from *Streptococcus pyogenes* also commercially termed as SpCas9. Cas9 basically has a DNA binding domain and a cleavage introducing domain. Cas9 binds to the gRNA, facilitates the interaction of the gRNA with the target viral DNA, and introduce DNA double stranded breaks (DSBs). It must be mentioned that Cas protein in addition to Cas9 has multiple types, such as Cas3, Cas12a, Cas13a. The DNA molecule then attempts to repair itself but due to error prone repair mechanisms, is rendered defective and effectively destroyed. There are two possible mechanisms for repairing the DSBs, either by the Non-Homologous End-Joining Repair mechanism (NHEJ) or the Homology Directed Repair mechanism (HDR). NHEJ doesn't require a template strand and is a random insertion of base pairs to the broken DNA helix, but it is significantly more prone to errors than HDR often resulting in Indels (insertions and deletions), HDRs on the other hand essentially requires a homologous template for it to repair a DSB.

The point at which the target DNA is cleaved and repaired, is of interest in the scientific researches. Various experiments have been successful in knocking out previously present sequences by NHEJ as well as introducing new sequences during the repair mechanism through Homology directed repair (HDR) such that the targeted DNA can take up that homologous sequence and undergo effective expression. And for the sequence to be effectively expressed it has to be ligated to the target DNA at the particular site specified. But irreversible Off-target mutations is one of the common errors observed in CRISPR/Cas9 technology similar to those in Zinc Finger Nucleases (ZFNs) or in Transcription Activator-Like Effector Nucleases (TALENS), which results in undesired or unreliable products. Tools for reducing the off-target mutation rate and increasing the efficiency of the overall process includes BLESS, GUIDE-seq, DISCOVER-seq, CIRCLE-seq to name a few. CRISPR in comparison to ZFNs and TALENs which are both developed by science, is a natural mechanism that has a significantly lower number of off-target mutations in addition to being simpler, cost-effective and has higher efficiency even at multiplexed gene editing by using multiple guide RNAs (gRNAs).

CRISPR-Cas9 could also be utilized for transcriptional regulation through the CRISPRi or CRISPR interference mechanism where a dCas9 or dead Cas9 has been designed by researchers in which the Cas9 even though has an active DNA binding domain, lacks or has an inactivated cleavage domain. Effector molecules which could be either activators or inhibitors binds to the dCas9 complex which in turn binds to a PAM adjacent to a target gene and can be directed in such a way as to administer the effector's affect towards the gene of interest. In comparison to RNAi where it only affects the translation process or at the RNA level, CRISPRi has the added advantage of influencing the genome from the transcriptional or DNA level.

APPLICATIONS UTILIZING CRISPR/CAS9 SYSTEM IN AGRICULTURE AND BIOMEDICINES

CRISPR/CAS9 in Agriculture; With the global population expected to reach a staggering 9-10 billion between the years 2040 and 2050, a major concern of time has been increasing the agricultural produce with an expectancy of doubling the food requirements worldwide by 2050. But changing of environmental conditions, more so due to global warming and with an inadequate supply of nutrients in natural growth media's, agricultural demands though have increased, the rate of produce to substantiate those demands have not. In order to meet such high demands within the field, CRISPR-CAS9 has been utilized to introduce a number of mutations, genome disruptions, insertions and deletions in a number of plants to increase the yield of those crops.

With minor alterations in promoter sequence of *Solanum lycopersicum* (tomato) by CRISPR/CAS9, new alleles have been developed for improved physical attributes like fruit shape, size as well as plant makeup generating novel variants which are far more superior to the existing tomato variants. Similarly, in *Oryza sativa* (rice), mutations in the grain width and weight genes GW2, GW5 and TGW6 results in enhancement of rice seed size significantly up to a 30%. In *Triticum* (wheat), knocking out the entire homologs of TaGW2 which is an inhibitor of seed size in wheat results in size enlargement. CRIPR-CAS9 TaMLO gene knockout in wheat in another experiment introduces resistance against powdery mildew fungal disease which is favored by high humidity.

Phytoene desaturase (PDS) in banana, encodes several of the essential enzymes in the carotenoid biosynthesis pathway and mutations leading to disruption of the PDS genes like RAS-PDS1 and RAS-PDS2 have demonstrated in improved quality of bananas. In another study, controlling of GhMMYB25 homologous genes in *Gossypium hirsutum* (cotton) through CRISPR-CAS9 technology by either mutations or deletions causing gene disruptions effects the quality of the produce by improving resistance against external stresses.

Abiotic stressors like changing temperature, pH, drought or floods, and UV radiations play an important role in the growth and development of plants with each plant having their specific requirement. CRISPR-CAS9 can influence the genome to resist such stresses by certain modifications like in the MAPKKK gene in cassava or EgWRKY gene in oil palm that shows tissue-specific expressions.

CRISPR/CAS9 technology due to its several advantages over traditional plant breeding methods as well as Zinc-Finger Nucleases (ZFNs) or TALENs have possibly stabilized itself as the preferred editing bioscience tool in the last few years. With the various possible alterations that can be introduced directly into a plant's genome and a great reduction in off-target mutations compared to that in ZFNs and TALENs, CRISPR-CAS9 have demonstrated a wide range of qualitative as well as quantitative improvements in the agricultural field. Although the crops subjected to modifications via CRISPR technology is limited in the moment, with most researches centered around plants like tomato, wheat, rice and banana, future researches suggesting transformations in the genome of other organisms as well as generating hybrids characterized by multi-nutritive properties could possibly answer the growing demands within the agricultural field.

CRISPR studies in Genetic Abnormalities and Biomedicines; With an

estimated 50% childhood death being caused due to genetic disorders and abnormalities, researches on CRISPR-Cas9 based studies associated with these disorders have been underway in many labs globally and is establishing itself as a promising treatment for the near future. Since most of these researches are still in their preliminary stages, involving model studies, a very few CRISPR based therapeutics are actually in practice.

Sickle cell disease characterized by RBCs in sickle or crescent moon shapes is due to a single base mutation in the gene for beta globin in chromosome 11. In CRISPR sickle cell disease studies, CD34+ haematopoietic stem-progenitor cells (HSPCs) are used for the purpose in which the Cas9 system replaces the mutation by a gRNA and a ssDNA donor template. CRISPR-CAS9 edited cells demonstrates a higher level of wild type haemoglobin than the sickle cell mRNA-protein level haemoglobin. When edited cells are transplanted into mice, they have recorded a 16-week stability of the genes in the organism, suggesting possible therapeutics in future. Similarly, Cleidocranial dysostosis (CCD), a rare genetic disorder, affecting the bones and teeth has its mutations in the RUNX2 gene which upon subjecting to CRISPR/Cas9 genome editing demonstrates positive osteogenesis induction when evaluated in rat study models, in the two iPSC cell lines used for the experiment. Results from the research's predicts a possible CRISPR/Cas9 dependent therapeutics for CCD treatment.

Another important CRISPR associated study is in the cancer treatment, where it targets inactivation of HIV-1 in Jurkat cell line or removal of the entire HIV-1 genome by gRNAs directed towards the long terminal repeats of the HIV-1 genome in dormant infected cells. Presence of inactive or latent viral reserves that remains undetected by various cancer treatment is the major hurdle in achieving a thoroughly successful HIV cure, which upon CRISPR/Cas9 dependent removal of the HIV whole genome could give much improved outcome. Researchers also suggest that HIV could possibly be controlled through reactivation of the provirus genome in latent cells and utilizing the 'shock and kill' method to induce cell death by the application of dCas9 reprogramming mechanism.

WHO estimates 17.9 million per year deaths worldwide due to cardiovascular pathologies, a staggering number which marks it as one of the very urgent and essential field of studies of present times. CRISPR with its highly advance applications has been invested in understanding cardiovascular diseases as well by developing a large number of models for studying various cardiovascular disorders, from cardiomyopathies, arrhythmias, rheumatic heart disease to stroke which are fairly common globally. These study models are generated by utilizing the CRISPR/Cas9 tool to influence and edit the genome in the embryos of rats and rabbits, the common model organisms. In Titin cardiomyopathy, analysis of the TTN gene and subsequently the protein which eventually weakens and prevents the heart from pumping blood, has also been studied by generating contractile deficit iPSCs which differentiates into cardiomyocytes. Successful studies have also been conducted in analysing hypertrophic cardiomyopathy by generating models which was then utilized to demonstrate pharmacological treatments success rates.

Studies have proposed stem cell regenerative therapy based on genome editing of iPSCs through CRISPR/Cas9 for diabetes and cancer as well. CRISPR has also been demonstrated to generate biological models for furthering the researches associated with understanding the underlying epigenetic and genetic factors influencing a number of diseases in addition to suggesting effective bioscience tools that could be utilized in the treatments. With continued expansion of CRISPR based stem cell researches and their high effectiveness within the biomedical field, CRISPR proposes a future with an in-depth knowledge of genetic diseases, their diagnosis and possible solutions.

CONCLUSION

In the recent studies CRISPR genome editing technology has also extended its application in being used as a diagnostic tool for COVID-19; credited to the high time and cost efficiency, precision and speed of the technique, as compared to other available techniques. Multiple Cas classes can be used in the diagnosis of the SARS-CoV2 like Cas3, FnCas9, Cas12 although class II Cas12 having the subclasses 12a, 12b are the popular. These studies show successful implementation of CRISPR gene editing in plant and animal cell models, with the technology's full potential yet to be uncovered. But CRISPR is not an error-proofed technique and has its own challenges and limitations as well. Off-target random mutation being a primary drawback resulting in possible unstable effects, a risk which is being investigated and tools have been developed to mitigate such mutations. Delivery of the CRISPR/Cas system into a target cell presents another challenge. Stability of the vehicle in cellular environment and appropriate size to hold the CRISPR/Cas system within it should be considered while selecting a delivery vector. Both virus-based and non-virus-based delivery system are currently under investigation. Adeno-associated Virus (AAV) may be a favoured one but non virus-based alternatives like lipid and calcium phosphates are being studied too. Ethical restrictions are another limitation in application of CRISPR, increasing the flexibility of such restrictions and boundaries could help in furthering CRISPR researches. Stability of the introduced gene edits in cells and vertical transfer of these mutations is a challenge which is currently being studied as well. Overall, CRISPR presents an opportunity for us to solve the many unanswered questions in science and provides us with a vehicle which if carefully programmed could possibly result in a biological revolution never seen before.

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