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CRISPR CAS9 TECHNOLOGY: A BRAND-NEW GENOME EDITING TOOL

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Abstract - *CRISPR is abbreviated as Clustered Regularly Interspaced Short Palindromic Repeats) or also called as cas9 technology. It is a straight forward and efficiently working tool for editing at genome level of many organisms in basic biomedical research over the recent previous years with a huge progress and adaptability in the biotechnological field. As a part of adaptive immune system. It was first discovered and observed in bacteria. It can be engineered to eukorayotes with a rapid and action, in affordable amount with high efficiency power. It must be highly scalable for the manipulation in genomic sequences. This technology specializes in enhancement of its high specificity in sequences, low rate of off target effects and delivery systems. It has been accustomed correct DNA mutations starting from a single nucleotide to invitro and in vivo model systems. The modified versions of this genome editing technology act as an accelerator in the field of cancer research with a high working efficiency fordissection of the mechanism of tumorigenesis which is done by the identification of specific targets for the development of drug-based therapies.*

Key Words: (CRISPR, clustered regularly short Palindromic repeats, crRNA, CRISPR RNA, genomic engineering, Organoid, immune therapy

1. INTRODUCTION

1970 scan be considered as the start of DNA Technology as it is an era of replacement of nucleotides. In the previous eras, the biological scientists have an emerging power to benchmark the molecule of Deoxyribonucleotide Acid to get all the possible and desirable changes in the genome of host. It is also helpful in the development of medicos and the biotechnological techniques in genome editing.

In recent era biological scientist have an immense power to directly manipulate and to edit the whole functioning of the DNA sequences endogenously which authorize them to clarify the functional organization of the genome for the proper functioning in the hosts body.

1.1 The CRISPR or Cas9 System

This system has been driven from the acquired immune system of the Bacterium Streptococcus pyogenes. The enzyme Cas9 nuclease delivers as antiphage due to its blending with the CRISPR which are the Clustered Regularly Interspaced Short Palindromic Repeats loci of short size, 30-40 base pairs and intercalated spacer sequences that matches with the genome of virus. They have transcribed loci into a protracted RNA cleaved by Cas CRISPR associated endonucleases to produce CRISPR RNAs that is helpful in the identification of genome of the virus to cleave it further. It also consists a RNA guide (gRNA) sequence in its modified form. We can define genome engineering as a process of developing targeted modification to the genome and to get desirable changes including its context i.e. epigenecity marks and its outputs i.e. the transcripts units. It can be performed in both eukaryotes as well as in mammals. The basic understanding of molecular mechanism of particular disease and its treatment CRISPR/Cas technology just began to reveal its working potential, appearing as a tremendously powerful research application, like functional interrogation of recent additionally as for novel clinical approaches. Currently the clinical trials using the CRISPR/Cas edited human cells are ongoing for instance, the treatment of tumor.

for genome editing. it consists of a sequence of Ribonucleic Acid for the adhesion of Cas plus a~20 nucleotide sequence which is known as the Cas spacer. the photo spacer that that tell us about the modification of target DNA. The enzyme Cas nuclease consists of an NHN Nuclease and RuvC like nuclease domains. The process includes the recognition of conserved sequence named as PAM i.e.Photo spacer Adjacent Motifs which haunches the targeted DNA in the proceeding steps as it adheres with Cas with its two domains that are NHN and RuvC which are the catalytic domains generating a double stranded break which is modified and repaired by Non Homologous End Joining serves as Homology Directed Repair System occurred during G1,S and G2 Phases of the cell cycle respectively. The process of NHEJ does not requires a template for repairing or for the extensive synthesis of DNA which is faster than HRD in repair mechanisms of DSB. It introduces small insertions and also cause deletions at the site of cleavage. There will be ending of frame shift mutation if occurrence of indel take place within the coding sequence of the gene. It leads to knockout gene which is not suitable for the gene correction purpose. the process of HDR uses a template of DNA or of the NMHC which is abbreviated as Non-Mutant Homologous Chromosomes to maximize the fidelity repair rate for the introduction of exact mutation inserted due to recombination. The tendency of HDR in Double Stranded Break is comparatively low and less frequent on the contrary

NHEJ have high rate in the proliferation of human cells. The promotion of HDR efficiency is done transiently inhibition or blockage of the NHEJ process of joining. Representation of a limited number of précised sites in the initial applications of genomes editing is due to the presence of limited number of PAM

1.2 History

The CRISPR Cas mechanism was first came into light in December1987, by Amemura, Ishimo, Makino in Osaka university.

This mechanism is also published in March 2002 by Mojica, Jansen, Gaastra in Utrecht University.

Sites within the eukaryotic genome. With the help of CRISPR we can overcome this problem of enhancement of targetable sites that take place within the genome of eukaryotes. Streptococcus pyogenes Cas9 exhibits alteration in the Photo spacer Adjacent Motifs with their specificity as it can be helpful in the recognition of alternative PAM sites in the bimodal as well as in humans also. The machinery of HDR repair system is helpful in the introduction of pricesly Single Cell Point Mutations. NHEJ repair machinery is preferable as it supports the introduction of insertions and deletions which causes disruption to the targeted focus and can be performed for large rearrangement of genomes including deletions, translocations and inversions. There is also a Dead Cas9 system which lacks nucleases in which Cas9 is used to form fusion proteins which are inactive in nature, capable of targeting either the regulatory sequence of the genome or to promote the changes in the expressions as it interferes with the traditional transcriptional machinery.

The characterization of RNA processing pathway in CRISPRsystem was done in August 2008 by University of Sheffield.

CRISPR-Cas mediated gene regulation shown to help regulation of endogenous bacterial genes ON 1 April2013 by Sampson, Weiss in Emory university.

CRISPR/Cas modified 60 genes in pig embryos in first step to create organs suitable for human transplants. Article is given by Church on 5 October 2015 in Harvard University.

1.3 CRISPR/Cas9 in Bacteria

Bacteria was the first microorganism into which the CRISPR System was introduced. There is a lethality in the CRISPR-induced double stranded breaks due to the non vigorous NHEJ. It causes mediations in the CRISPR genome engineering functions of bacteria with ongoing CRISPR editing in the E.coli along with other bacterial species.

1.4 The emergence of CRISPR engineering in Bacteria

Desirable changes with Genomic Engineering is done with the utilization of recombination machinery in the phage which leads to pushing of chromosomes that are homologous in nature of linear DNA fragments which includes low range steps to get the desirable and fortunable results. There are two plasmids of Addgene depositors' genomes

1. Plasmid Cellular Apoptosis Susceptibility carries Cas9 and is resistant to chloramphenicol.

2. plasmid Clustered Regularly Interspaced Short Palindromic Repeats carries a target

Gene editing tool CRISPR, successfully used to improve muscle function in mouse model of Duchene muscular dystrophy given by Nelson, Gersbach, Hakim, Ousterout, Thakore in Duke University, University of Missouri, University of North Carolina.

Phage Recombineering Machinery of E.coli was electroporated with the help of pCas9 after this plasmid CRISPR is introduced with Repaired templates of Oligonucleotides.

With the help of recombeering the genome locus of interest is modified so that it can match to the repaired template as it cannot be derived as a spacer RNA .it can be concluded as a wild type sequence when we do not get fruitful results from this processing.

CRISPR is a way for the selection of target cells on that time when homologous recombination does not take place.

This system was first designed by Wenyan Jiang, David Bikard Et al. In the year 2013.

Multiplexing of Clustered Regularly Interspaced Short Palindromic Sequences:

CRISPR is a brand-new genome editing tool for the process of bacterial engineering which must be needed to accept and adapt in the upcoming field of biotechnology is now available for multiple and various species/ genera. Genome Editing:

1.5 CRMAGE System

Multiplexing system that targets various loci for editing and labeling. It is helpful in the mixing of MAGE i.e. Multiplex Automated Gene Engineering Technology with CRISPR. In this technology lambda red and Cas9 is represented by Pma7CR-2.0 which is inducible by laevorotatory Arabinose and Anhydrotetracycline respectively. The technological system of CRMAGE is much more efficient than the traditional method of Recombineering targeting two multiplexes simultaneously is feasible expenditure >70%of CR Multiplexing system due to its incredible fast protocol

with a time limit of 5hours and is required to perform incubation for one round of editing on the contrary subsequent protocol requires 2-3 hours. Two plasmid system was discovered by Wenya Jiang Et al. in 2015that was mixed with CRISPR Recombineering for iterative genome engineering. In which the plasmid Cas consists of Cas9 due to which the recombination of phage Lambda Red contains specific gRNA (s) in which the template is under repair supplies as a double stranded DNA. Fragments. It has 69%gene deletion efficiency on the contrary the Multiplexing system that targets various loci for editing and labeling. It is helpful in the mixing of MAGE i.e. Multiplex Automated Gene Engineering Technology with CRISPR. In this technology lambda red and Cas9 is represented by Pma7CR-2.0 which is inducible by laevorotatory Arabinose and Anhydrotetracycline respectively. The technological system of CRMAGE is much more efficient than the traditional method of Recombineering targeting two multiplexes simultaneously is feasible expenditure >70%of CR Multiplexing system due to its incredible fast protocol with a time limit of 5hours and is required to perform incubation for one round of editing on the contrary subsequent protocol requires 2-3 hours. Two plasmid system was discovered by Wenya Jiang Et al. in 2015that was mixed with CRISPR Recombineering for iterative genome engineering. In which the plasmid Cas consists of Cas9 due to which the recombination of phage Lambda Red contains specific gRNA (s) in which the template is under repair supplies as a double stranded DNA. Fragments. It has 69%gene deletion efficiency on the contrary the efficiency of insertion varies with length of homology with the length of template (40basepairsin 6%vs 400 base pairs in 28%). Editing take place in two days with each round hence we get curves P target F and pCas plasmids from the bacteria via a parameter of non-selection into which the growth took place at 37°C.

It is also successfully done in Tatumellacitrea a species of Enterobacteriaceae family without any desirable modifications thus it is suggested that CRMAGE is also functional in most of the species of Enterobacteriaceae family.

1.6 Transcripts Repression

The methods of bacterial Clustered Regularly Interspaced Short Palindromic Repeats are available for activation of transcriptional units as well as for their repression. There is no role of RNA interference in bacteria in the previous eras as the organic phenomenon was limited for the employment promoters that are inducible in nature or to knockout the gene directly. On the contrary CRISPR technology provides a way for the modulation of organic phenomenon which is ecofriendly in nature.

(a)Type1

(b)Type11

(c)Type111

(d)Type1V

CRISPR have two classes that are designated as

Class1

Class11

Class1 consists Type 1and Sort 111and sophistication 2 consist Type 11 and sort1V the sole for which the two genes are ordinary to the all or any four divisions includes Cas1 and Cas11 which are involved as acquisition of recent spacers. CRISPR systems consists of signature gene. Cas3 is for Type 1,Cas9 is for Type11, Cas10 is for type10 and CRISPR-Cf1 for Type1V.

Mechanism of CRISPR Cas9

CRISPR-Cas immunity booster could be a instinctive action of archaea as well as bacteria. It is helpful in the prevention of infections caused by bacteriophage, bacterial conjugation and degradation of foreign nucleic acids that enter to the cell of host body through the process of natural transformation.

Acquisition due to spacer

Microbial invasion by the bacteriophage is considered as the primary stage of immunological response which occurs for the capturing of phage DNAand its insertion into a CRISPR locus within the short range of a spacer. The Type Cas1 and Cas2 are majorly present in both varieties of immune system of CRISPR-Cas that indicates the involvement in the acquisition of a spacer. Removal of Cas 1 and Cas2 stops the acquisition of a spacer without harming and affecting CRISPR immunologic reactions and responses.

Various Cas1 proteins are featured with their resolved structure. There is a diversity in aminoalkaline acid sequences on the contrary their crystal structures are similar and have atleast one Cas1 protein that is purified with metal dependent nucleases/integrases that adheres to DNA in a very independent sequential manner. The Cas2 proteins are characterized with resolved structures. The Cas1 proteins have distinguished aminoalkaline acid sequences and their crystal; line structures are similar. The purified Cas1 proteins contain enzymes that are metal dependent nucleases or metal dependent integrases that binds to the DNA in a very independent sequential manner. The characterization of representative Cas2 protein us already done that possess single stranded RNA or dsDNA with specific endonucleases activity. The I-E system of E.coli Cas1 and Cas11 forms posh in which a dimer of Cas11 bridges two Cas1 dimers. At this time of complex formation Cas11 performs arole of non-enzymatic scaffolding invaded double stranded Deoxyribonucleotide fragments are folded

Photo-spacer Adjacent Motifs

The analysis of bioinformatic studies in the regions of phage genomes which were excised spacers and called as photo-spacers. As their selection was not took place randomly but on their adjacent nature of short base pairs (3-5) DNA sequences called as PAMs which are important for Type1 and ASort11 systems as revealed by the analysis of CRISPR Cas systems not during the acquisition in Type111 system.

Variants at the time of Insertion or Insertion Variants

There is a lot of complexities in the canonical of spacer insertion as revealed through the analysis of Sulfolobus. Solfataricus CRISPRs. Numerous CRISPRs contain many spacers to the identical bacteriophage and these phenomenon was established in E.coli I-E system due to which a massive enhancement was detected in the spacer acquisition where the phage is already targeted by the spacers sometimes photo-spacers mismatches

The bacteriophage primary requires the Cas proteins involved both in the interference and acquisition to make interactions with each other. Primary mechanism gave spacers which are newly acquired and found on identical strands due to the hypothesis that the acquisition machinery slides with foreign DNA after priming to search out replicable photo-spacer.

Cas systems. Recognition of stem loops that are generated by the pairing of identical repeats is done by Cas6e and Cas6f respectively in I-E and I-F systems which leads to cleavage of Cas proteins at the side of paired region. Type111 system also includes the use of Cas6 but their repeatsdosenot produce stem loops and cleavage occurs at longer transcripts covering around the Cas6 to permit cleavage.

Interference

In Type 1 System during the interference stage the sequences of PAM are identified on the CRISPR-RNA strand which inhibits complementary strands and is required for the process of annealing. In Type1, there is occurrence of correct base pairing between the CRISPR-RNA which induces a conformational change in the Cascade due to photo-spacer that recruits Cas3 for degradation of DNA.

In Type11 ststem there is a dependency on the protein that exhibit a multifunctional protein Cas9 for the interference process. The Cas9 protein needs both the crRNA and tracerRNA for proper functioning and cleaving the DNA with the help of its dual HNH and RuvC/Rnase H-like endonucleases domains. Base pairing

6. Swarts DC, Mosterd C, van Passel MW Bronux SJ(2012) CRISPR Intereference directs strand specific spacer acquisition" occurs between Photo-spacer Adjacent Motifs requires the phage genome in type11 systems. PAM is recognized on the identical strands as it is opposite strand to the Type1 system. efficiency of insertion varies with length of homology with the length of template (40basepairsin 6%vs 400 base pairs in 28%). Editing take place in two days with each round hence we get curves P target F and pCas plasmids from the bacteria via a parameter of non-selection into which the growth took place at 37°C.

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Applications

1. Clustered Regularly Interspaced Short Palindromic Repeats Gene Editing:

It is the applied technology in the field of agriculture i.e. farming and food industries to engineer the live microbial cultures of Probiotics commonly Yogurt. Recently the approaches that are based on utilization of CRISPR Cas12a within the plant species for the successful modifications with desired genes.

2. Clustered Regularly Interspaced Short Palindromic Repeats as a diagnostic tool:

CRISPR that is associated with enzyme nuclease act as a useful toolfor molecular testing. It hasability to specifically target the sequences of macromolecules of non targeted sequences. The enzyme Cas9 nuclease accustomed depletion of unwanted sequences of nucleotides in the libraries of next generation sequencing while there is a requirement of only 250pg of initial RNA input. CRISPR associated nucleases were also used for direct diagnostic testing allthe path down to single molecule sensitivity. Electronic chips of CRISPR are new diagnostic technological applications for the detection of genetic mutations as well as micro-fluid biosensors of CRISPR are used for electrochemical diagnosis of mRNA.

Future Aspects

CRISPR/Cas9 is employed routinely to humans as it is emerging and growing tool in the field of biotechnology for the editing of genome.

Researches are continuing to get fruitful results in animal models as well as in for the cells that are isolated from the human body to treat the disease. It has a lot of labor that specialize in the eliminating the effect of off target where the occurrence of cut in the system of CRISPR-Cas9for editing.

Conclusions:

Genetic engineering and genome editing provide new tools and strategies manipulation and editing in the plant species. By the editing of endogenous gene of a particular plant trait provide us the desirable changes in the genome.

It is particularly important and significant for the crop modification, improvement and commercial development of that area.

It provides us an advantage of addition and deletion of more than one gene in the host genome.

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