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Review

Crispr/Cas9 Enzyme Using For Editing Of Cancer Gene

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeats are DNA sequences that go by the acronym CRISPR. Scientists may now alter an organism's DNA thanks to a set of technologies known as genome editing, sometimes referred to as gene editing. Cancer. Mutations in various genes, including TP53, MLH1, BRCA1, and HLA-B, can result in cancer. Serious endometrial carcinoma is an extremely uncommon but aggressive kind of cancer that affects women, and it has been related to BRCA1 mutations. The use of CRISPR-Cas9 protein and other genome editing technologies to modify human genomes raises ethical questions. Somatic cells—those distinct from egg and sperm cells—are the only ones affected by the majority of the alterations brought about by genome editing. These alterations are exclusive to particular tissues and do not transfer from one generation to the next. The treatment and prevention of human diseases are highly interested in genome editing. These technologies make it possible to add, remove, or change genetic material at specific points along the genome sequence, including sickle cell disease, haemophilia, and cystic fibrosis, among other single-gene illnesses. Additionally, there is hope that it can help treat and prevent more complicated illnesses like cancer, heart disease, and mental illness.

Keywords: CRISPR CAS9 enzyme, Gene edition technology and cancer gene.

INTRODUCTION

CRISPR/Cas9 edits genes by precisely cutting DNA and then letting natural DNA repair processes to take over. The PAM sequence on the host genome is recognized by Cas9 and cannot be easily modified to recognize a different PAM sequence[1-3]. However, this is ultimately not too limiting, as it is typically a very short and nonspecific sequence that occurs frequently at many places throughout the genome The system consists of two parts: the Cas9 enzyme and a guide RNA.Targeted nucleases are powerful tools for mediating genome alteration with high precision[4-7]. The RNA-guided Cas9 nuclease from the microbial clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system can be used to facilitate efficient genome engineering in eukaryotic cells by simply specifying a 20-nt targeting sequence within its guide RNA. Here we describe a set of tools for Cas9-mediated genome editing via nonhomologous end joining (NHEJ) or homology-directed repair (HDR) in mammalian cells, as well as generation of modified cell lines for downstream

functional studies[8,9]. Methods to control genome editing with small molecules include an allosteric Cas9, with no detectable background editing, that will activate binding and cleavage upon the addition of 4-hydroxytamoxifen (4-HT).

MECHANISM OF CRISPR/CAS9 ENZYME

The CRISPR/Cas9 system is a heritable adaptive antiviral immune system of prokaryotes or Eukaryotes that targets infectious invading viruses and bacteriophages and uses RNA-guided nucleases to cut foreign genetic components. It contains two compartments, one for Cas9 endonuclease and one for single-stranded guide RNA The sgRNA directs the Cas9 endonuclease to cleave both DNA strands of the target gene in a sequence-specific manner. DNA cleavage occurs at a sequence 3 base pairs upstream of an "NGG" protospacer adjacent motif (PAM). The genome DNA is repaired by double-strand break repair mechanisms after the cleavage. Therefore, the utilization of the CRISPR/Cas9 gene editing system achieves genome modifications by the introduction of small insertions or deletions through the relatively error-prone non-homologous end-joining or the high-fidelity homology-directed repair.(1.sgRNA guide and recognise the damaged segment of DNA.

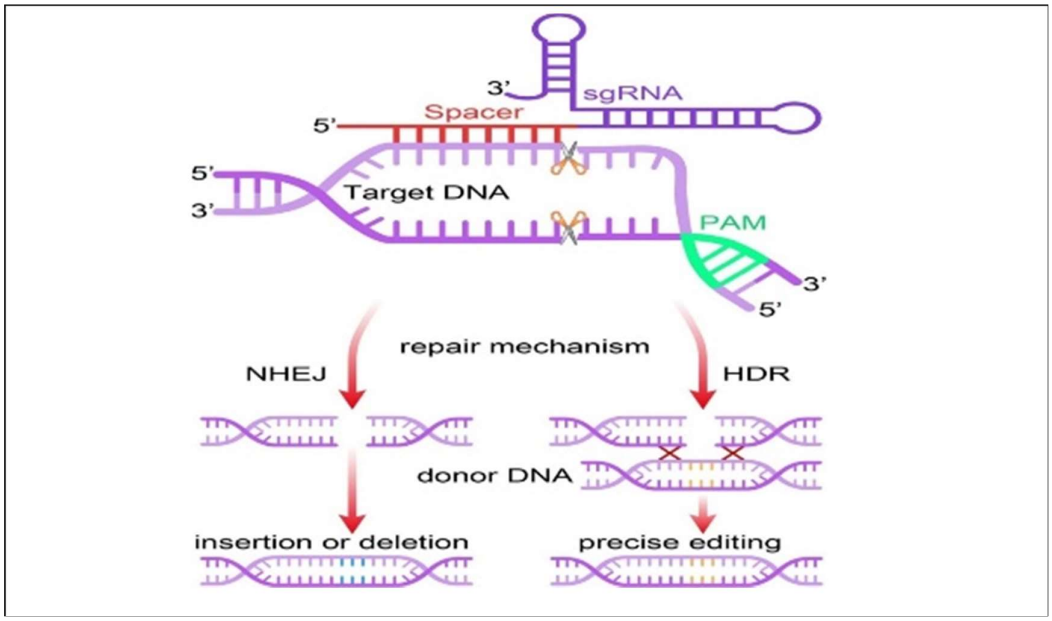

Fig 1: Mechanism Of Crispr/Cas9 Enzyme

Various steps involved in gene Editing By CRISPR/cas9 enzyme

Steps:-I

The first step in designing a CRISPR experiment is choosing the appropriate CRISPR-associated (Cas9) enzyme. The protospacer-adjacent motif (PAM) sequence is crucial in choosing which Cas enzyme to use, because it determines potential target sites for genome editing.

Step:-II

To deliver Cas9 enzyme and guide RNA to cells, we recommend complexing the enzyme and gRNA to form a ribonucleoprotein (RNP). The RNP can then be delivered to cells by either electroporation or lipofection. We offer separate electroporation enhancers for Cas9 to increase transferring efficiency.

Step:-III

RNP containing either Cas9 cuts the genomic DNA, forming a double-strand break (DSB). Cells naturally undergo non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways. The NHEJ pathway is useful for making knockouts to study gene function. HDR requires not only RNP to make the DSB but also a template (i.e. a donor oligo containing the insertion sequence) to direct the repair and can be used to knock in specific mutations (e.g., insertions, deletions, single-nucleotide polymorphisms).

Step:-IV

Mutation identification can be assessed by several methods based on your experimental needs. For non-specific determination of the presence of a change in a genomic sequence, gel-based methods can be used. For determining the success of on-target editing and for investigating off-target effects

APPLICATIONS OF CISPR/CAS9 ENZYME

1. CRISPR is poised to revolutionize medicine, with the potential to cure a range of genetic diseases, including neurodegenerative disease[10].
2. CRISPR technology also has the potential to transform medicine, enabling us to not only treat but also prevent many diseases[11].
3. 3.CRISPR is being used for all kinds of other purposes too, from fingerprinting cells and logging what happens inside them to directing evolution and creating gene drives[12].
4. Cas9 genomic modification has allowed for the quick and efficient generation of transgenic models within the field of genetics. Cas9 can be easily introduced into the target cells along with sgRNA via plasmid transfection in order to model the spread of diseases and the cell's response to and defense against infection[13].
5. CRISPR-Cas technology has been proposed as a treatment for multiple human diseases, especially those with a genetic cause. Its ability to modify specific DNA sequences makes it a tool with potential to fix disease-causing mutations.[14]
6. The CRISPR treatment for LCA10 (the most common variant of Leber Congenital Amaurosis which is the leading cause of inherited childhood blindness) modifies the patient's defective photoreceptor gene[15].
7. CRISPR has also found many applications in developing cell-based immunotherapies. The first clinical trial involving CRISPR started in 2016. It involved taking immune cells from people with lung cancer, using CRISPR to edit out the gene expressed PD-1, then administrating the altered cells back to the same person[16].
8. Type 1 Diabetes is an endocrine disorder which results from a lack of pancreatic beta cells to produce insulin, a vital compound in transporting blood sugar to cells for producing energy. Researchers have been trying to transplant healthy beta cells. CRISPR is used to edit the cells in order to reduce the chance the patient's body will reject the transplant[17].
9. Human immunodeficiency virus or HIV is a virus that attacks the body's immune system. While effective treatments exist which can allow patients to live healthy lives, HIV is retroactive meaning that it embeds an inactive version of itself in the human genome. CRISPR can be used to selectively remove the virus from the genome by designing guide RNA to target the retroactive HIV genome.

CONCLUSION

In conclusion, CRISPR and other genome editing technologies have opened up unprecedented possibilities for altering DNA sequences and have the potential to revolutionize the treatment and prevention of various diseases, including cancer. The identification of cancer-causing mutations such as TP53, MLH1, BRCA1, and HLA-B highlights the importance of understanding the genetic basis of diseases. The association between BRCA1 mutations and serious endometrial carcinoma underscores the significance of genetic research in elucidating disease mechanisms. However, the use of CRISPR-Cas9 and similar tools raises ethical considerations, particularly regarding their application in modifying human genomes. While these technologies predominantly affect somatic cells and exhibit tissue-specific alterations, concerns about unintended consequences and long-term effects persist. Nevertheless, the ability to precisely edit genetic material holds promise for addressing single-gene disorders like sickle cell disease, hemophilia, and cystic fibrosis, as well as more complex illnesses such as cancer, heart disease, and mental disorders. Moving forward, a balanced approach that considers both scientific advancements and ethical implications is essential to harnessing the full potential of genome editing for the benefit of humanity.

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