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RESEARCH ARTICLE

CRISPR - CAS9 GENE EDITING: A REVIEW

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Abstract

CRISPR is an RNA guided genome editing technique of genetic engineering which works like genetic scissors. Based on simplified version of bacterial CRISPR-Cas9 antiviral defense system. It is more accurate, faster and cost efficient than other genome editing methods. There are two components in this system: First component includes a single guide RNA (sgRNA) of system which will identify target sequence in genome and Second component will include Cas9 nuclease of system which will act as a pair of scissors to spilt the double strands of DNA. CRISPR has promising therapeutic applications. This current review focuses on mechanism, therapeutic applications, delivery systems, limitations and different approaches used for gene editing using CRISPR.

Introduction:-

CRISPR's first description was given by Ishino in year 1987[1]. CRISPR which is also known as clustered regularly interspaced short palindromic repeats. For protection of bacteria and archea against encroaching nucleic acids of plasmids and phages CRISPR and CRISPR – Cas are a part of the acquired immune system of bacteria and archae. Cas9 is more widely used type because its faster, cheaper and more accurate then other genome editing methods. To cleave the DNA at certain sites Cas use single guide (sgRNA) to form complentary base pairs with the targeted DNA. Cas9/sgRNA is a two component system which is very accurate in gene editing. In this system the identification of specific sequence in genome is done by sgRNA and the DNA sequence will be cleaved with the help of Cas9 protein which will act like a pair of scissors.

There are two factors that decides its specificity:
1. Target Sequence
2. PAM Sequence

Target Sequence has a length of 20 bases as it's a part of each CRISPR locus in the crRNA array[2]. Cas9 identifies the PAM sequence present on the host's genome. To find the correct sequence in host cell's DNA Cas9 protein will take assistance of crRNA. After which it depends upon the Cas9 variant either to make a single or double stranded break at the correct location.

Mechanism:
Genome editing system occurs naturally in bacteria from which CRISPR – Cas9 is adapted.Cas9 nuclease is the main component of CRISPR which has two catalytic active sites RuvC and HNH, and single guide RNA (sgRNA) derived from the crRNA and trans acting CRISPRRNA [5,7].

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sgRNA directs the Cas9 target site with pairing of base in presence of PAM on the opposite strand sgRNA , which results in site specific DNA double strand breaks that are later repaired by HDR(homologous directed repair) if the homologous sequences are not available then they could be repaired by NHEJ(non homologous end joining) [3,4,5,7]. There is precision in gene correction or replacement when HDR are used compared to NHEJ which can induce small insert or delete (indel) mutations.

By the inactivation of either RuvC or HNH Cas9 can be reprogrammed into nickase (nCas9) [6,7]. And by inactivation of RuvC and HCH it can be reprogrammed into catalytically inactive Cas9(dcas9).

When S.pyogenes Cas9 taken for structural analysis it was revealed that additional insight are present into mechanism of CRISPR [7].The NHEJ pathway is highly effective thanHDR pathway as Gene Knock out is usually more effective than the Gene Knock in [7]. Crispr is new promising therapy of genetic disorders in which direct editing of disease related mutations is possible [7]. Lots of efforts are given to improve the specificity, efficacy of gene editing and efficiency of delivery in CRISPR [7].

Different approaches to edit genes using CRISPR-Cas9:

There are various approaches which are used when one has to edit genes. In case of CRISPR – Cas9 there are three main approaches that are used to edit genes. In the first approach multiple transfections in different components can be avoided by using plasmid based CRISPR – Cas9 system which will encode Cas9 proteins and sgRNA from the same vector [8,15]. Mixture of Cas9 mRNA and sgRNA is delivered as second approach [9,15]. Mixture of Cas9 protein and sgRNA is delivered as third approach [10,15].

Greater stability is seen in plasmid based CRISPR – Cas9 system approach compared to the approach that uses sgRNA mixture with Cas9 mRNA [8,15]. Cas9 mRNA mixture with sgRNA will be directly delivered to the target cells which expresses the Cas9 protein and will form the Cas9/sgRNA complex inside the cells after which it will edit the gene [11,12,15]. One of the advantages is that the duration of gene editing is fixed. For mRNAs to exert their effects have to enter cytoplasm [15]. Lower off targets are seen in the delivery of mRNAs compared to the plasmid based approach [15]. Lower levels of cytotoxicity are seen in primary cell and cell lines [13,15]. When mRNA encoding Cas9 protein is used.

Ribonucleoprotein complexes (RNPs) have some advantages on its direct delivery such as rapid action; high gene editing efficacy; reduced off target effects; reduced toxicity and many more [14,15]. When a purified Cas9 protein is positively charged and forms complex with sgRNA Cas9/sgRNA ribonucleoprotein complexes (RNPs) are formed.

Delivery systems:

With help of viral systems as well as non viral systems the Cas9,sgRNA, and other associated complexes can deliver to target cells. Electropermeabilization is most widely used method for delivery nucleic acids and proteins to mammalian cells [44,15]. With the help of electroporators

Electropermeabilization is produced through which permeability of cell membrane can be enhanced temporary which allows the nucleic acids and proteins to enter the cell. This method is used for delivery of RNP to fibroblasts, stem cells and CD4+ human t cells [17,15].

Suitability to any cells, higher efficiency for both in vivo in vitro are major advantages of electropermeabilization.It also has some disadvantages like cell death, non specific transfection etc.Another method is chemical transfection in which complex of Cas9 with lipids is produced through which sgRNA can be delivered to cells [18,15].

The third method is viral method in which there is use of plasmid based nucleic acids which are delivered to mammalian cells both in vitro and in vivo [15,19-21]. Adeno virus,lenti virus are mainly used for this kind of delivery.

Another approach for the delivery is use of Cas9 encoded mRNA. But mRNA based delivery are temporary in function and leads to removal of nuclease from the cell and overcome the risk associated with integration in host genome [22,45].

There could be also use of protein based delivery in which Cas9 protein is complexed with sgRNA using synthetic delivery vehicles which provides direct pathway for delivery.

Applications and Future Prospects:
Note: Some of the applications including the therapeutic ones are still under development. The information in these applications are the proven potentials of CRISPR gene editing and also shows what it's capable to do so take a note of that.

Derived from the inner cell mass (ICM) of blastocytes embryonic stem cells are Pluripotent stem cells (PSC) and hence shows difficulty in genome editing [46]. CRISPR - Cas system can efficiently control the genome without complex process [23,46]. This accelerates the stem cell study. Reversible and efficient genome editing can be enabled by combining CRISPR - Cas system and reverse system for comprehensive gene analysis [24,46]. Simplification of knock in, knock out, transgene and reversible genome engineering which has been achieved in stem cells is done by CRISPR - Cas system [25,24,26,27,46]. There is a acceleration seen in the generation of stem cell lines specially in disease models because of genome editing with CRISPR - Cas system.

The cause of neurogenerative diseases are neuronal impairments, stem cells models shows appropriate insights in study and cure of neurogenerative disease [28-30,46]. Recent study shows that modelling of clinical pathogenic mutations can summarize the specificity of neurogenerative disease for example amyloid β (Aβ)'s generation in Alzheimer's disease (AD) [31-33,46]. Validity and safety before clinical applications are lightened up by direct gene correction with CRISPR - Cas system [34,35,46]. Researchers have used Cas9 to inactive a endogenous retrovirus in pigs [36,47], and also to engineer T cells as a start to develop advanced immunotherapies to target cancers [37,47].

Merits:
Over conventional protein guided genome editing tools such as ZFN and TALEN, CRISPR – Cas9 RNA guided genome editing gives many advantages. ZFN or TALEN based tools requires de novo synthesis which is way complex than CRISPR – Cas9 which only requires design of complementary sgRNA to target a new site [7]. With the multiple sgRNAs that can target different genomic loci, CRISPR – Cas9 has capability to edit these genomic loci in parallel this property is called multiplexing [3,5,7]. TALEN also has some advantages over CRISPR – Cas9.

CRISPR – Cas9 has restricted targets because of presence of PAM sequence and a guanine at the 5′end 11, when compared to TALEN targets in presence of thymine at the 5′end is the only restriction [38,7]. Which means CRISPR – Cas9 has less target genome sites than TALEN.

Challenges using CRISPR - Cas9:
High frequency of off target effects is major challenge for CRISPR Gene therapy the observed frequency is ≥50% [39,48]. One method is used to overcome this is use of Cas9 nickase(Cas9n) this is the variant of Cas9 which induces single stranded Break. It can induce double strand breaks when it is combined with sgRNA which can target both DNA strands at intended locations [40,48]. Another challenge is presence of PAM near to target site.

Mostwidely Cas9 from the bacteria streptococcus pyogenes(SpCas9) it has 5'NGG3' a short canonical pam recognition site in this N can be any nucleotide. Packaging of these SpCas9 in AAV vectors is difficult [42,43,48] where staphylococcus aureus Cas9 (SaCas9) is smaller and can be easily packed to AAV vectors but it also have longer pam sequence which further narrows targeting sites.

Different engineered variants of SaCas9 are made which can recognize 5'NNRT3'pam widening the targeting sites by 2-4fold off target effects that noticed in similar frequencies to wildtype SaCas9 also it needed to be considered in designing any therapeutic application [41,48].

CRISPR induced double strand breaks can trigger apoptosis instead of intended gene edit [48,49]. In human pluripotent stem cells when this tool is used there are some safety concerns are present while demonstration, it was seen that due to the activation of p53 in response of toxic double strand breaks induced via CRISPR often triggers the subsequent apoptosis [48,50].

In catalytically inactive endonuclease dead where deactivation of nuclease domain carried out it can provide therapeutic utility while reducing the risk of double strand breaks [48,51].Precise genome editing is one of the

essential prospect of CRISPR Gene therapy. HDR pathway can make this desired edit easy but it's lower efficiency renders it's use of precise Gene editing for highly limiting clinical intervention and NHEJ can be used as default pathway for human cells repairing. Because of chemical inhibition ofNHEJ modulating enzymes like DNA ligase IV [48,52], DNA dependent protein kinases [48,53] and Ku [48,54] it causes suppression of NHEJ pathway through which enhancement in HDR efficiency has been achieved [48].

Conclusion:-

CRISPR – Cas9, the RNA guided genome tool has many advantages over protein guided counterparts. It has various therapeutic benefits but before it is used for patients benefit there are some challenges that needs to be overcome. As there are rapid advancements happening in CRISPR – Cas9 technology we can still believe that in future it can revolutionize gene therapy research and become the convenient tool for human gene therapy.

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