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CRISPR Cas 9 and Gene Editing

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Abstract:

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 system offers a strong and multiplexable genome editing tool, permitting researchers to manipulate precise genomic elements, and facilitating the elucidation of target gene function in biology and diseases.

Introduction:

Over the years Genome editing has provided scientists with the ability to change an organisms DNA. A number of strategies for genome editing have been developed such as Homologous recombination, Transcription activator like effector nucleases (TALENs), and Zinc finger nucleases (ZFN), all of which have been associated with challenges most important of which is their restricted application and difficulty in construction.(1)

Clustered regularly interspaced short palindromic repeats (CRISPR) unlike its predecessors is a straightforward technology with little assembly needed. CRISPRs are classes of repeated DNA sequences that act simultaneously with CRISPR-associated (Cas) genes towards foreign invading nucleotides such as phages and plasmids meditating bacterial and archaeal immunity. There are three forms of CRISPR/Cas systems recognized thus far, the type II system being the most extensively studied.

Throughout the bacterial immune response, the invading DNA is first segmented into small portions and integrated into the CRISPR locus, to permit the CRISPR system to mediate self and nonself recognition the integrated portions of DNA are arranged in the form of spacer sequences interspaced via conserved repeated sequences. The locus is then transcribed as a single noncoding precursor CRISPR RNA (pre-crRNA) that is further processed into short stretches of mature crRNA. Together with a second noncoding RNA, the trans-activating CRISPR RNA (tracrRNA), the crRNA subsequently forms a ribonucleoprotein complex with the endonuclease Cas9 once bound, the Cas9 protein cleaves the “crRNA complementary” and opposing strand via its HNH and RuvC1-like nuclease domains breaking down the invading DNA.

In 2012, a research group led by Emmanuelle Charpentier and Jennifer Doudna used Streptococcus pyogenes for genome editing by adapting its type II CRISPR system. They created a single guide RNA (sgRNA) via fusing the crRNA to the tracrRNA which recruits the Cas9 nuclease to a precise genomic location through standard DNA base pairing. The CRISPR/Cas9 complex results in the formation of site-specific double-strand breaks (DSBs) which then induce genome editing by 2 distinct mechanisms. First, in the absence of a homologous DNA template DSBs can be repaired through nonhomologous end joining (NHEJ), which can lead to small insertions or deletions frequently ending in gene silencing, hence an error-susceptible process. Second, DSBs can be repaired by means of homology-directed repair (HDR) in the presence of an artificial fix, which permits the formation of any preferred base-pair modifications.(2)

The aim of this report is to discuss some of CRISPR/Cas9 applications, the obstacles and challenges faced and their possible solutions.

Materials and methods:

CRISPR/Cas9 plasmids encoding the sgRNA and cas9 are constructed, afterwards the transport of the plasmid is carried out by viral and non-viral vectors.

Non-viral vectors:

Chemical Non-viral Gene delivery

Chemical methods utilize polycationic polymers or lipid particles which facilitate the entry into the cells through endocytosis. The use of these chemical carriers offers three functions: (a) masks the negative charge on DNA, (b) compresses the DNA molecule into a smaller size and (c) protects the DNA from being degraded by intracellular nucleases.

Physical Non-viral Gene delivery

Such as microinjection, electroporation and gene gun, uses physical force to disrupt the cell membrane in order to allow the gene to enter the cell.

Viral vectors:

Gene delivery by using viral vectors has been a successful way of entrance in a wide range of cell types both in vivo and vitro. It is an active receptor-dependent method which has greater performance and much less cytotoxicity in comparison to physical and chemical delivery methods. Therefore viral vectors including adenoviruses, lentiviruses and adeno associated viruses are widely used to deliver CRISPR systems. (3)

Results/Discussion:

The CRISPR/Cas9 has many advantages in contrast to TALENs and ZFNs. First of all, sgRNA is the only requirement in order to target a new DNA sequence, which is easier and simpler in comparison to TALENs and ZFNs which require the synthesis of a cumbersome guiding protein. Moreover, within the case of CRISPR/Cas9 several sgRNAs may be used to target distinctive genomic loci concurrently this is called “multiplexing”. (4)

CRISPR/Cas9 has been efficient in the correction of the mutations in some genetic disorders induced in iPSCs including Duchene muscular dystrophy (DMD), Beta thalassemia, Sickle cell anemia, and Tyrosinemia

In addition a number of viral diseases have been treated with CRISPR/Cas9 including:

Human papillomaviruses (HPV)

Human papillomaviruses (HPV) cause warts in humans; furthermore, they are also oncogenic in nature. The main contributors towards the oncogenic characteristics of the viruses are the viral proteins E6 and E7. The oncogenes E6 and E7 encode these proteins. CRISPR-associated editing of the E6 and E7 genes of HPV was able to cause mutations within these genes inactivating them and enhancing the anti-tumor effect of p53 and Rbp.

Hepatitis B virus

Hepatitis B virus causes liver cirrhosis and hepatocellular carcinoma, making it one of the most significant viruses of health concern. A research crew designed eight gRNAs towards HBV and confirmed that the CRISPR/Cas9 system drastically decreased the formation of HBV core and HBsAg proteins.

Human immunodeficiency virus (HIV)

The causative agent of acquired immunodeficiency syndrome (AIDS) in humans. HIV gene expression is inactivated by the usage of CRISPR/Cas9 through two possible mechanisms: 1. Cas/9 can inactivate viral gene expression before its integration into the host genome, 2. Cas9 can lead to the disruption of the proviral element already incorporated into the host genome.

Another important prospect against HIV is modifying essential host cell factors such as replication and infection within the T-cells. Examples of such host cell factors include CXCR4 (Chemokine receptor type 4) and CCR5 (Chemokine receptor type 5). For efficient access of the virus into the cell, the envelope (Env) must bind with these two receptors. (5)

In spite of current advances, there are yet many obstacles to conquer for the final applications of CRISPR-Cas9 to clinical gene therapy, such as the specificity and efficacy of CRISPR-Cas9 in therapeutic genome editing in vivo delivery strategies, and potential immunogenicity of CRISPR-Cas9 and the transport vehicles.

Specificity of CRISPR-Cas9

One of the main fundamental obstacles to the clinical applications of CRISPR- Cas9 is its unpredictable and uncontrollable off-target effects that can result in complications such as malignant transformation

Attempts including improving sgRNA design, adjusting Cas9 construction,and the nickase Cas9 approach have been made to decrease the off-target effects.

Before its application in humans they must be thoroughly examined in big-animal models, due to its potentially devastating nature of off-target effects.

Efficacy of HDR-mediated gene correction

HDR-mediated gene correction in comparison to NHEJ-mediated gene deletion or inactivation has broader application due to the presence of far more diseases whose treatments require specific gene correction rather than those requiring only culprit gene deletion or inactivation. In the case of hereditary tyrosinemia type I, for instance, to cure the disease-inducing point mutation (G→A) in FAH gene needs to be rectified. Nevertheless, NHEJ is much more effective than HDR in that it takes vicinity in post mitotic adult tissues and doesn't requires a donor template. Consequently, it remains difficult to enhance the efficacy of HDR to degrees which are adequate for gene therapy. The increase of HDR efficacy has been suggested through many techniques including the inhibition of NHEJ pathway, and increasing the degree of similarity amongst the donor templates and the double-strand break sites.

Immunogenicity of CRISPR-Cas9 and delivery vehicles

Cas9 proteins or delivery vehicls may induce an immune response thus constituting another layer of hurdles in vivo therapeutic applications of CRISPR-Cas9. A common challenge encountered in gene therapy attempts is the immunogenicity towards viral vectors. Other possible immunogenicity is against Cas9 proteins or peptides due to their bacterium origin.

Host immune responses attenuate therapeutic outcomes and result in side effects, hence have to be circumvented or minimized.

One of the ways developed to avoid the immunogenicity of viral vectors, is the formation of non-viral vectors such as nanoparticle- and lipid-based vectors. Another promising approach to reduce the immunogenicity is humanizing Cas9 proteins which is accomplished via changing portions of the bacterial protein with those of human origin. Humanized proteins may also nonetheless preserve some degree of immunogenicity and hence, may not, completely circumvent immune surveillance.

Fitness of edited cells

CRISPR-Cas9 therapeutic genome editing can modify the fitness of edited cells, which in turn can have an effect on the duration and efficacy of gene therapy. In instances where therapeutic genome editing causes a growth advantage, the disorders phenotype can be rescued by the editing of a small amount of cells, hence the therapeutic efficacy is less difficult to obtain and maintain. For example, in a study where CRISPR-Cas9 was used in hereditary tyrosinemia in a mouse model, it genetically corrected only 0.25% of liver cells, yet after 33 days, the amount of genetically corrected cells reached 33.5%, which was adequate to rescue the disease phenotype.

On the other hand, there are also cases where therapeutic genome editing causes a growth disadvantage. If CRISPR-Cas9 is used to inactivate oncogenes in cancer cells, for instance, the genetically edited cells will be out numbered quickly via the unedited malignant cells. As a result, excessive editing efficiencies and repeated episodes of therapy would be needed to be therapeutic, this is rather difficult and still beyond the ability of present day CRISPR-Cas9 technology. (4)

Conclusion:

An ideal genome editing tool should have simple, efficient and low-cost assembly of nucleases that can target any site without off-target mutations in genomes. CRISPR/Cas9 has the potential to become a reliable and facile genome editing tool. However For the final application of CRISPR-Cas9 to the clinic, there are still many hurdles to overcome. With the rapid advances in CRISPR technology, we can optimistically anticipate these hurdles to be overcome in the foreseeable future to pave the way for the final application of CRISPR-Cas9 to human gene therapies.

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