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CONTENT

A BRIEF OVERVIEW ON ORIGIN, MECHANISM AND THERAPEUTICAL APPLICATIONS OF CRISPR-CAS9 TECHNOLOGY

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Abstract:

Clustered Regularly Interspaced Short Palindromic Repeat or CRISPR-Cas9 technology has redefined our approach to gene editing techniques and gene therapy. Apart from being expeditious, highly efficient, and categorical, it has emerged as a potentially potent implement in the treatment of lethal diseases such as cancer and AIDS. Cas9 technology with CRISPR enables precise and effective cleavage of a desired target DNA sequence, and given the relative facileness and simplicity of building sgRNAs, it has greatly facilitated genome editing. Leaving chemotherapy and radiotherapy for cancer behind, CRISPR has played a crucial role in the development of immunotherapy techniques for cancer. CRISPR-engineered oncolytic viruses additionally show optimized tumor selectivity and enhanced immune stimulation to cure cancer. It has also provided an adaptable gene-editing approach that has been effectively applied to AIDS prevention and reduction.

Keywords: CRISPR-Cas9, Gene editing, Gene therapy, Cas9 Technology, AIDS, Cancer, Medical Therapeutics.

Introduction:

Since its discovery in 2012, CRISPR-Cas9 technology has been responsible for a revolution in gene-editing techniques. This applied science has fueled the targeted gene editing methods that involve the application of engineered nucleases. Clustered Regularly Interspaced Short Palindromic Repeat technology, or the technology better hailed as CRISPR by genome editors and geneticists is a new important platform for generating RNA-guided nucleases (RGNs) such as Cas9, along with customizable specificities. Not only can CRISPR/Cas9 technology effectively mediate gene editing, but it can also confer biological activities. Inactive Cas9 (dCas9) is produced by mutations in two nuclease domains of Cas9, which is used as a locus-specific DNA-binding protein (Gilbert *et al.*, 2013; Qi *et al.*, 2013).

Apart from being rapid, RGN-mediated genome editing is facile and has enabled us to efficiently manipulate and modify endogenous genes in a wide variety of important cell types and novel organisms. CRISPR technology has redefined our approach to gene editing techniques

and gene therapy. This review focuses on the study of the mechanism of CRISPR-Cas9 and the revolutionary changes brought about by it in gene therapy for cancer and AIDS. CRISPR-Cas9, as a consequence of its high efficiency and accuracy, has emerged as a potentially powerful tool in the treatment of cancer. It has shown an unparalleled clinical potential to detect novel targets for cancer therapy and to dissect chemical-genetic interactions. CRISPR has provided insight into how tumors respond to drug treatment. Furthermore, CRISPR-Cas9 has the potential to rapidly engineer immune cells as well as oncolytic viruses for cancer immunotherapeutic applications. The (Cas9) system is also being developed as a powerful gene-editing tool to treat HIV/AIDS. It can be utilized to treat HIV-1 infection and clear the provirus by targeting cellular co-factors or the HIV-1 genome, as well as to induce transcriptional activation of latent virus in latent viral reservoirs for elimination. Also, in human cells and animal models, this adaptable gene-editing method has been effectively applied to HIV/AIDS prevention and treatment.

Mechanism:

CRISPR-Cas9 technology has originated from an immune defense mechanism that is found in archaea and bacteria, which provides immunity to the organism against invading viruses and plasmids. This adaptive immune system relies on ribonucleoprotein effector complexes. By storing the memory of encounters with foreign DNA in distinct spacer sequences, it allows the bacteria to eradicate invading phages, conjugative plasmids, and mobile genetic elements into CRISPR arrays. CRISPR systems naturally integrate foreign DNA molecules into CRISPR arrays. These subsequently produce crRNAs containing protospacer regions that are antigenic to the invading DNA molecules. This process is followed by the hybridization of each crRNA molecule with non-coding tracrRNA. This hybridization forms a crucial hybrid of crRNA-tracrRNA which forms a complex with Cas nucleases that tend to cleave target-DNA sequences nearby to short sequences called protospacer adjacent motifs (PAMs). This CRISPR/Cas system can be efficiently manipulated to target genes of interest to regulate their functions effectively in any eukaryote. Further studies in the molecular biology of the CRISPR/Cas reveal the way it can be used while using synthetic guide RNAs (gRNAs) and other components to the target gene of interest in DNA molecule for the desired application and discoveries of disease-causing genetic variations.

There are three types of CRISPR/Cas systems according to popular classification out of which the type II CRISPR/Cas system is commonly used. It consists of three components: an endonuclease (Cas9), a CRISPR RNA (crRNA), and a transactivating crRNA (tracrRNA). . The crRNA and tracrRNA molecules comprise the guide RNA (gRNA), which can be replaced by a synthetic fused chimeric single gRNA (sgRNA). The sgRNA possesses a unique 20-base-pair

(bp) sequence that is meant to complement the target DNA site, and it must be followed by a short DNA sequence known as the "protospacer-adjacent motif" (PAM), which is required for Cas9 protein compatibility. When the sgRNA and Cas9 nuclease are both produced in the cell, they create a ribonucleoprotein (RNP) complex, which is guided to a target DNA site by the sgRNA. Cas9 accurately cleaves the DNA to induce a DSB after the sgRNA attaches to the target sequence using Watson-Crick base-pairing. The cleavage takes place within the protospacer, three nucleotides upstream of the PAM, resulting in blunt ends. Cas9's RuvC and HNH active-site motifs act on the (-) and (+) strands, respectively, and are responsible for the cleavage of opposing DNA strands. The cell machinery heals the DSB using one of two basic processes, depending on the cell state and the presence of a repair template: homology-directed repair (HDR) or non-homologous end joining (NHEJ). The HDR pathway works by recombining a donor DNA template at the DSB location, resulting in precise repair. Specific sequences or mutations can be introduced into a target section of the genome via homology-directed repair. The more common NHEJ mechanism is an error-prone system that generates frameshift mutations by randomly inserting or deleting nucleotides at the DSB site (indels). It can thus be utilized to induce specific gene knockouts (KO). Cas9 technology with clustered regularly interspaced short palindromic repeats enables accurate and effective cleavage of a desired target DNA sequence, and given the relative ease and simplicity of building sgRNAs, it has greatly facilitated genome editing. The use of distinct sgRNAs allows this method to be multiplexed, which is an extra benefit. Only the CRISPR/Cas9 system, among genome editing nucleases, can edit several loci at the same time by introducing sgRNAs to distinct sites. When two sgRNAs are used in the same cell, minor deletions, complicated rearrangements, and even full chromosomal suppression can occur.

CRISPR-cas9 in cancer therapeutics

Cancer is considered a complicated disease. Dysregulation of cancer can be done by acquiring effective immunity against cancer cells. This immunity solely depends upon the interactions that occur between the host, tumor, and the environment that surrounds them. Enhancing the immune response to cancer cells by applying immunotherapy has emerged as a hopeful option in the treatment of cancer. Such immune responses are enhanced either by applying synthetic chimeric antigen receptor (CAR) therapy or by targeting the programmed death receptor 1 (PD-1).

Using CRISPR/Cas9, catalytically inactive dCas9 can be recruited by gRNAs to specific target DNA sites and can be exploited to activate or repress specific target genes by fusing them to transcriptional activation or inhibition domains. This advantage is used in cancer therapy for

the regulation of endogenous gene expression. On the other hand, targeted epigenome editing can also be a boon. Multiple kinds of cancer involve epigenetic factors. In these cases, if we target the epigenetic regulatory machinery such as histone modifiers as well as proteins involved in altering DNA methylation, cancer dysregulation can be carried out efficiently. Another approach in CRISPR/Cas9-based cancer therapeutics may involve the exploitation of oncolytic viruses. Genetic modifications can help viruses lose their virulence against normal cells but their capability to attack and lyse cancer cells possessing deficiency of antiviral defenses can be maintained. Direct cellular lysis, one of the various mechanisms concerned with the viral-induced destruction of cancer cells also directly triggers further immune stimulation through tumor antigens released from dying cancer cells. CRISPR-engineered oncolytic viruses also show optimized tumor selectivity and enhanced immune stimulation.

Along with these and many more CRISPR-Cas9 mediated genome editing applications, cancer therapeutics appear to have a promising future.

CRISPR-Cas9 in HIV-1/AIDS therapeutics

AIDS remains a severe threat to worldwide human health, despite the tremendous efforts of researchers in the prevention and treatment of HIV-1 infection. The CRISPR-Cas9 system has recently been developed as a promising gene-editing method that could be used to cure AIDS. To minimize the infection caused by HIV-1, CRISPR can be used to target cellular co-factors or viral DNA. It's being tinkered with to eliminate the provirus and stimulate transcriptional activation of latent virus in latent viral reservoirs. In human cells and animal models, this adaptable gene-editing approach has been effectively applied to AIDS prevention and reduction.

In 2013, CRISPR/Cas9 was used for the first time to prevent HIV-1 infection by disrupting latent HIV-1 provirus (Ebina *et al.,* 2013). By targeting HIV-1 LTR using CRISPR/Cas9, researchers were able to decrease the production of HIV-1 genes in Jurkat cell lines (Ebina *et al.,* 2013). Soon after, researchers used Cas9/gRNA to target conserved sites in the HIV-1 LTR U3 region, which resulted in inactivating viral gene expression and restricting virus replication in an HIV-1 latently infected T cell line, pro-monocytic cell line, and a microglial cell line with little genotoxicity and no detectable off-target editing (Hu *et al.,* 2014). Furthermore, combining two successful sgRNAs that target separate sections of the HIV genome had become able to stop the virus from replicating and escaping (Lebbink *et al.,* 2017). A single sgRNA-driven CRISPR/Cas9 editing triggered mutational inactivation of HIV-1 provirus has also been described (Wang *et al.,* 2018). In this way, CRISPR-Cas9 has brought about revolutionary changes in the therapeutics of HIV-1/AIDS.

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