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CRISPER CAS9 TECHNOLOGY: THE FUTURE OF PRECISION HEALTH

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Abstract:

Genome edition can lead to a change physical trait of an organism. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. CRISPR/Cas9 system allows adding, altering, and deleting the genomic code in organisms. Cas9 protein has six domains. It is the most developed and widely used tool for current genome editing. It has been rapidly promoted and applied in the generation of animal models; gene function research; multiplexed mutations; chromosome rearrangements etc. It has certain limitations. This article focuses on the discovery, mechanism, applications, and limitations of CRISPR/Cas 9 gene editing.

Keywords: CRISPR/Cas9, genome editing, mutations.

Introduction:

Like every year, the 2020s made history when the Nobel in chemistry was announced. It was awarded to women scientists Jennifer Doudna and Emmanuelle Charpentier. They honored Noble for the "Development of a method of genome editing". It can lead to the emergence of novel biological applications. All human dreams may come true when this technology is utilized for curing inherited diseases.

In this article, we will discuss the discovery of gene technology's sharpest tool that is CRISPR/Cas9, it's concept, applications, and its limitations.

Origin of gene therapy:

The introduction of gene therapy into the clinic provided hope for thousands of patients with genetic diseases and limited treatment options. Initially, gene therapy utilized viral vector delivery of therapeutic transgenes for cancer treatment.[7]

Tragic setbacks for gene therapy [7]:

Jesse Gelsinger, an 18-year-old with a mild form of the genetic disease ornithine transcarbamylase (OTC) deficiency, participated in a clinical trial. This trial was related to delivering a non-mutated OTC gene to the liver through a hepatic artery injection of the recombinant adenoviral vector housing the therapeutic gene. Unfortunately, Jesse passed away 4 days after treatment. The adenovirus vector triggered a much stronger immune response in Jesse than it had in other patients, causing a chain of multiple organ failures that ultimately led to his death. At the time of the trial, adenoviral vectors were considered reasonably safe.[7]

CRISPR/Cas 9 and gene therapy[7]:

Gene therapy is the strategy to provide therapeutic benefits. It includes modifying genes via disruption, correction, or replacement. It has witnessed both early successes and tragic failures in a clinical setting.

Genome editing is a technique that leads scientists to change the DNA of many organisms such as plants, bacteria, and animals. Genome edition can lead to change physical traits of an organism.

What exactly CRISPR/Cas9 is?:

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats.[7] It is part of bacteria's immunological system that helps them to recognize threatening viruses. When bacteria sense to hide out the virus, bacteria produce RNAs which are translated into proteins. This also contains the Cas gene which is used to produce enzymes like Cas-9. This Cas-9 enzyme is used to cleave the DNA of a virus that has entered during infection.

CRISPR/Cas9 system allows for adding, altering, and deleting the genomic code in living beings as we do the addition, altering, and deletion of words in the computer. CRISPR are pieces of DNA that bacteria snip from viruses that once attacked them.

Surgery, radiation therapy, and chemotherapy are still the main treatment options for pancreatic cancer, but there is now a considerable effort in identifying better treatment strategies for pancreatic cancer, such as targeted therapy, immune therapy, and potentially CRISPR/Cas9-directed gene therapy.[8]

History of CRISPR/Cas9:

CRISPRs were first discovered in Archae by Francisco Mojica at the University of Alicante, Spain.

CRISPR repetitive sequences were first observed by Ishino et.al in 1987. They identified highly conserved nucleotide sequences with 14 bp dyad symmetry at 30 ends flanking region of alkaline phosphatase gene in *E. coli.*

The function and biological importance of CRISPR sequences were not fully understood until 2007 when Barrangou et al. exposed Streptococcus thermophilus to phage and sequenced the resultant phage-resistant variants. Analysis of the variant DNA revealed that the bacteria had gained new CRISPR spacers that were derived from the phage genome.[8,10]

The identification of CRISPR sequences in bacterial genomes led to the identification of a set of homologous genes referred to as CRISPR and associated (Cas) genes that together comprise the CRISPR locus.

Subsequent work by Jinek et. al(2012) proved that an endonuclease can be directed to cleave target DNA. From this discovery CRISPR/Cas9 technology has rapidly evolved, leading to incredible progress in research and clinical applications.

Compared to the other gene-editing technologies, such as meganucleases (MNs), zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs), CRISPR/Cas9 technology has lower cost, higher efficiency, and is less complex in its application [8]

CRISPR/Cas9 for gene editing:

Emmanuelle Charpentier (Director at Max Plank Institute for Infection Biology, Berlin) studied Streptococcus pyogenes, the bacteria that is associated with a range of illnesses like pharyngitis, scarlet fever, and tonsillitis. During this study, she discovered a previously unknown molecule tracrRNA. Her work showed that tracrRNA is part of bacteria's ancient immune system. Dr. Charpentier published her discovery in 2011.

During the same year, she initiated collaboration with biochemist Jennifer Doudna (Now a Professor at the University of California, Berkeley). They both succeeded in the creation of bacterial genetic scissors in vitro. They simplified the scissors' molecular components. In the significant experiment, they reprogrammed genetic scissors in their natural form, scissor recognizes DNA from viruses but Doudna and Charpentier proved that they could be controlled so that they can be cut at a predetermined site and rewrite the sequence that we want to insert at the cut site.

Mechanistic overview of CRISPR/Cas9-mediated genome editing [9]

The key step in editing an organism's genome is the selective targeting of a specific sequence of DNA. Two biological macromolecules, the Cas9 protein, and guide RNA. These molecules interact to form a complex that can identify target sequences with high selectivity.

The Cas9 protein is responsible for locating and cleaving target DNA, both in natural and artificial CRISPR/Cas systems.

The Cas9 protein has six domains, REC I, REC II, Bridge Helix, PAM Interacting, HNH, and RuvC (Fig.1)[1,3,4, 5]

REC I domain:

Largest domain and responsible for binding guide RNA[2]

REC II domain:

The role of the REC II domain is not yet well understood.[2]

PAM (protospacer adjacent motif) domain:

The interacting domain confers PAM specificity and is responsible for initiating binding to target DNA

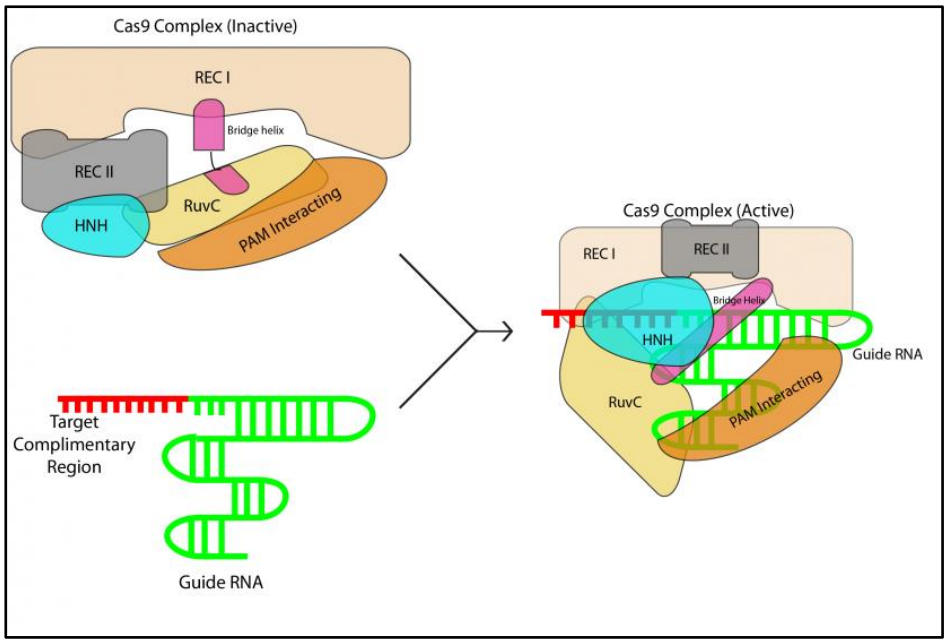

Fig. 1: Image credit: Cavanagh & Garrity, "CRISPR Mechanism", CRISPR/Cas9, Tufts University, 2014.

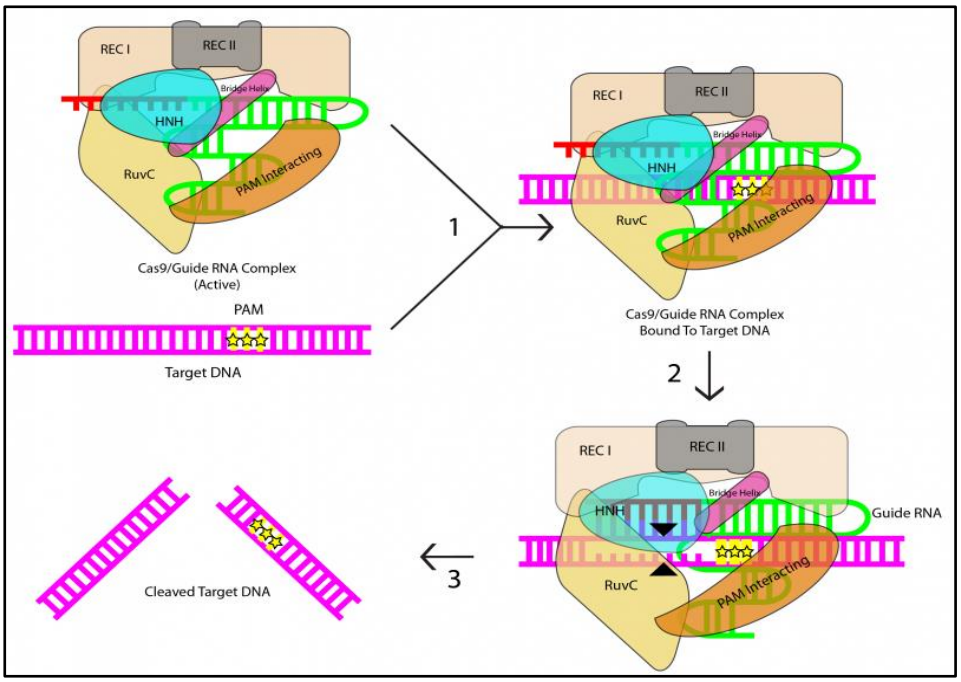

Fig. 2: Image credit: Cavanagh & Garrity, "CRISPR Mechanism", CRISPR/Cas9, Tufts University, 2014.

HNH and RuvC domains:

Nuclease domains that cut single-stranded DNA.

They are highly homologous to HNH and RuvC domains found in other proteins

The Cas9 protein remains inactive in the absence of guide RNA[3]. In engineered CRISPR systems, guide RNA is comprised of a single strand of RNA that forms a T-shape comprised of

one tetraloop and two or three stem-loops [3,5] (Figure 2). The guide RNA is engineered in such a way that it should have a 5′ end that is complimentary to the target DNA sequence.

This artificial guide RNA binds to the Cas9 protein and, upon binding, induces a conformational change in the protein. The conformational change converts the inactive protein into its active form (Fig. 1) The mechanism of the conformational change is not completely understood, but Jinek and colleagues hypothesize that steric interactions or weak binding between protein side chains and RNA bases may induce the change [3].

Once the Cas9 protein is activated, it searches for target DNA by binding with sequences that match its protospacer adjacent motif (PAM) sequence (Sternberg et al. 2014). A PAM is a two- or three-base sequence located within one nucleotide downstream of the region complementary to the guide RNA. When the Cas9 protein finds a potential target sequence with the appropriate PAM, the protein will melt the bases immediately upstream of the PAM and pair them with the complementary region on the guide RNA (Sternberg et al. 2014). If the complementary region and the target region pair properly, the RuvC and HNH nuclease domains will cut the target DNA after the third nucleotide base upstream of the PAM (Anders et al. 2014)[9]

Applications:

CRISPR/Cas9 technology has been rapidly promoted and applied in the generation of animal models, gene function research, multiplexed mutations, and chromosome rearrangements.

Cancer immunotherapy:

The first CRISPR Phase 1 clinical trial in the US opened in 2018 with the intent to use CRISPR/Cas9 to edit autologous T cells for cancer immunotherapy against several cancers with relapsed tumors. These include multiple myeloma, melanoma, and synovial sarcoma. This trial was approved by the United States Food and Drug Administration (FDA) after careful consideration of the risk-to-benefit ratios.[7]

CRISPR Cas12-based assay:

Recently, a CRISPR Cas12-based assay named SARS-CoV-2 DETECTR was developed for the detection of COVID-19 with a short turnaround time of about 40 min and a 95% reported accuracy. This method is easy to apply and has been used in a wide variety of experimental models such as cell lines, laboratory animals, plants, and human clinical trials.[6]

CRISPR/Cas9 in other diseases:

The CRISPR/Cas9 system has been applied in cellular and animal models to study and search for treatments for different neurological disorders, such as Parkinson's disease.

CRISPR/Cas9 in disease models:

The CRISPR/Cas9 system has an extraordinary therapeutic potential for treating different diseases in which the genetic cause of dysfunction is known.[6]

Apart from pancreatic cancer research, it is also used in other cancer settings to further understanding of disease progression, identify mechanisms of drug resistance, and uncover potential therapeutic vulnerabilities.

Limitations:

In addition to technical limitations, CRISPR/Cas9, like traditional gene therapy, still raises concerns about immunogenic toxicity

Human subjects in their study possessed pre-existing anti-Cas9 antibodies against the most commonly used bacterial orthologs, SaCas9 and SpCas9 .[7]

While viral vectors continue to be essential for current gene therapy, the concerns and limitations of viral-mediated gene editions have broadened the diversity of gene-editing approaches being considered. The discovery and repurposing of nucleases for programmable gene editing made this possible, beginning with the development of various methods and most recently, the CRISPR/Cas system. Of the CRISPR/Cas systems, CRISPR/Cas9 is the most developed and widely used tool for current genome editing.[7]

Having studied both the applications and limitations of CRISPR technology it can be concluded that it should be the better option for precision medicine in the future. But as a coin has two sides, how we will use this technology will take time to watch.

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