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CRISPR/CAS GENE THERAPY

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Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated enzyme (Cas) is a naturally occurring genome editing tool adopted from the prokaryotic adaptive immune defense system. Recently, Cas9/CRISPR has been reported to successfully induce targeted gene disruption and homologous recombination in both prokaryotic and eukaryotic cells with higher efficiency compared with ZFN and TALEN. Additionally, it is easier to design guide sequence and easy to use for Cas9/CRISPR system. This novel technology will be of great potential for application in both research field and clinical trials. This Review describes the development of technologies based on nuclease-deactivated Cas9, termed dCas9, for RNA-guided genomic transcription regulation, both by repression through CRISPR interference (CRISPRi) and by activation through CRISPR activation (CRISPRa).

Keywords: Gene therapy, CRISPR/Cas9, ZNF, Talen

1. Introduction

GENE THERAPY - The ability to make local modifications in the human genome has been the objective of Medicine since the knowledge of DNA as the basic unit of heredity. Gene therapy is understood as the capacity for gene improvement by means of the correction of altered (mutated) genes or site-specific modifications that have therapeutic treatment as target. Further on, diffrent strategies are described, which are often used for this purpose (Tebas et al., 2014). Gene therapy involves manipulating DNA or RNA for human disease treatment or prevention. The strategies of gene therapy are diverse, such as rectifying, replacing or deleting the culprit genes in genetic diseases, producing disabling mutations in pathogen genomes to combat infectious diseases or inducing therapeutic or protective somatic mutations. It is a promising therapy for a wide range of human diseases including hematological diseases, cancer, AIDS, diabetes, heart failure and neurodegenerative diseases (Xiao-Jie et al.)

Recently, a RNA-guided genome editing tool termed CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9) added to the list of programmable nucleases, offers several advantages over its counterparts and shows therapeutic potentials. Herein, we introduce the basic mechanisms and merits of CRISPR-Cas9 in genome editing, retrospect studies on CRISPR-mediated gene therapy in cell lines and animal models, discuss its challenges and possible solutions and prospect future directions. (H Kim et al.)

The CRISPR/Cas system was originally discovered as a prokaryotic adaptive immunity system used to recognize and cleave invading nucleic acids [6-8] (Mojica Fj et al.)

The aim of this review is the Cas9 targeting methodology, outline key steps toward enhancing the efficacy, specificity and versatility of Cas9-mediated genome editing and regulation, and highlight its transformative potential for basic science, cellular engineering and therapeutics (Shao-Shuai Wu et al.)

The gRNA-directed CRISPR-Cas9 nuclease technology has been a long awaited tool for manipulation of genomes at will. The successful application of this technology in a wide range of biological systems from yeast, worm, insect, plant, and mammals from rodent to monkey has made it the most popular genome editing

technology in history. (Weninger A et al .)

2. History of CRISPER

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) was first discovered in Escherichia coli and described as a 1,664-nucleotide sequence by Ishino and his colleagues in 1987 (Fig . 1). Six years later, Mojica and Van Soolingen found similar DNA fragments in Haloarcula and Haloferax archaea, and M. tuberculosis separately. By 2000, Mojica's group had identified CRISPR DNA sequences in twenty microbial species. (Mojica FJM et al ., Bological significance of family of regurarly spaced repeats in the genomes of Archeas,Bacteria and mitochondria.)
Following the development of DNA sequencing techniques, the broader existence of CRISPR was reported in 2005: the majority of archaea (90%) and certain bacteria (40%) contain CRISPR fragments However, compared with its wide discovery in the first two decades, the functions of the CRISPR still kept unknown until 2007.Before making clear its functions, two crucial research speeded up the development: the study of genes adjacent to the CRISPR locus in 2002, and the discovery of foreign viral DNA in CRISPR spacers in 2005, which directly propelled the proposing of the hypothesis that the CRISPR system is an innate immune system in archaea and bacteria to Fig ht against viruses. Finally, in 2007,the hypothesis was verified by experiments in Barrangou's lab, and the functions of the 'weird'CRISPR sequences were finally Fig ured out (Ning Guo et al .)

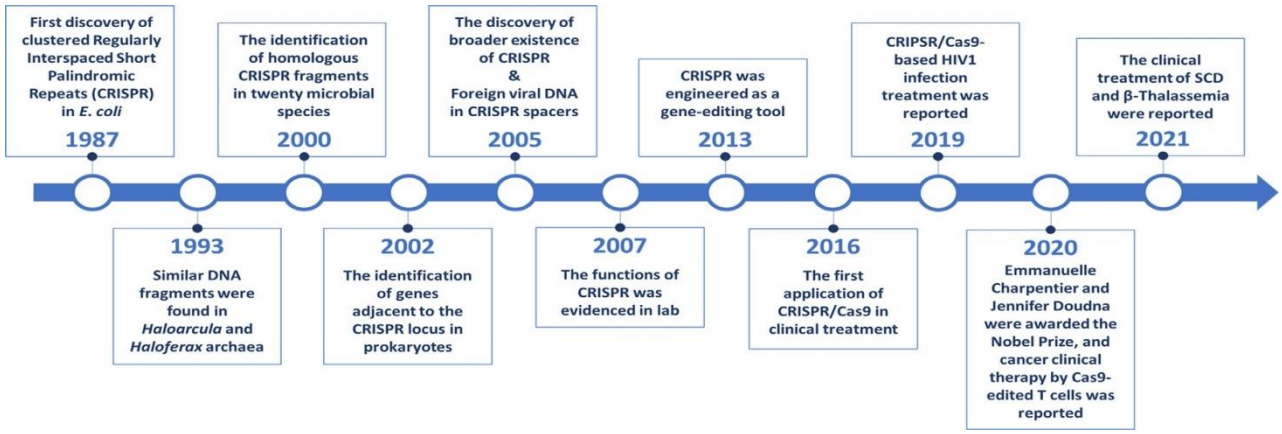


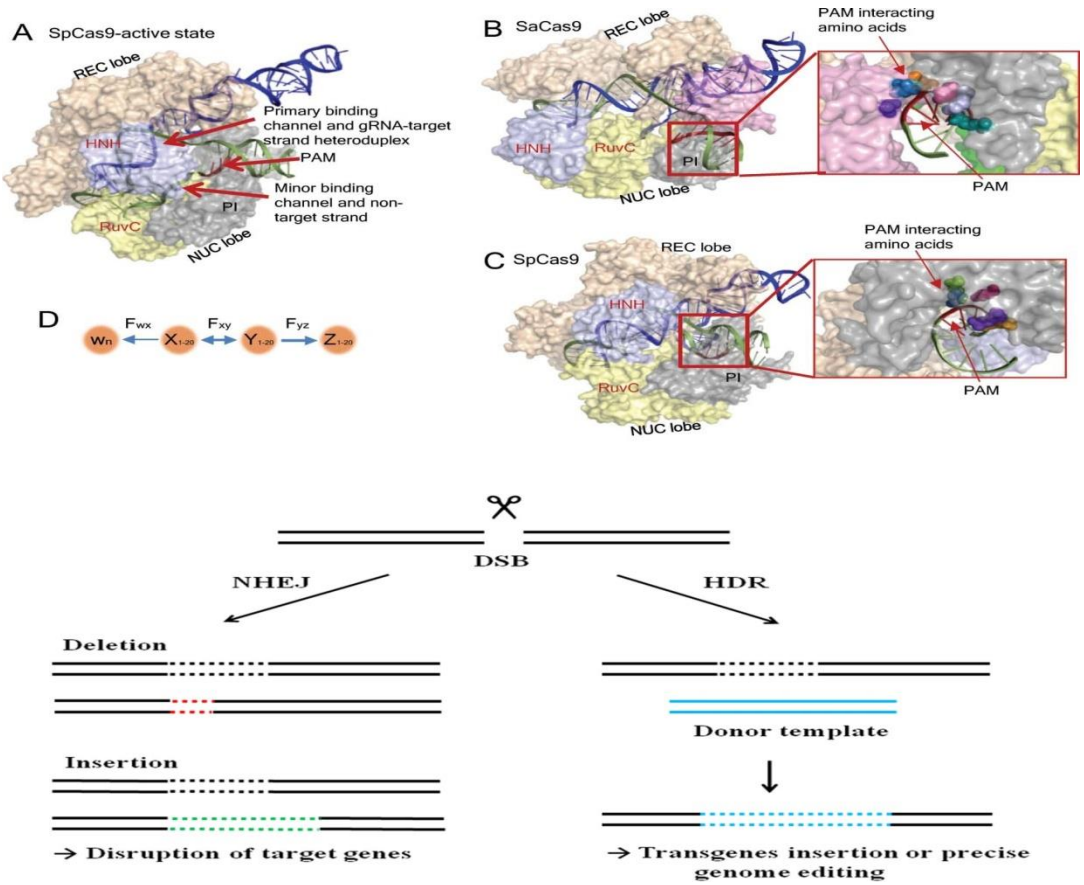
Fig 1. The history of CRISPR techniques. CRISPR was firstly reported in E. coli in 1987, then was discovered widely from 1993 to 2005. After identification of genes adjacent to the CRISPR locus in 2002 and foreign viral DNA sequences in CRIPSR spacers in 2005, the functions of CRISPR was proven in 2007. In 2013, two labs simultaneously engineered CRISPR to become the most effective gene-editing tool available. The first CRISPR/Cas9 application in clinical treatment occurred in 2016 to treat lung cancer in China. In recent three years, several clinical treatments based on CRISPR/Cas9 techniques were reported. To recognize their contributions of CRISPR/Cas9 techniques, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in 2020. (Mojica FJM et al .)

3. Biological mechanism of CRISPR

Following discovery of the native CRISPR system function in bacteria, researchers set out to understand the mechanism of the adaptive immune system. (Mojica et al.)
CRISPR systems were further classified into six types that were additionally grouped into two classes (Wiedenheft et al., 2011; Wright et al., 2016). Types I–III are the most studied, while types IV–VI were more recently identified (Makarova & Koonin, 2015; Makarova et al., 2015; Shmakov et al., 2015). Type I and Type III CRISPR systems both utilize sets of Cas proteins. In Type I systems, a multi-protein CRISPR RNA

(crRNA) complex known as Cascade recognizes the target DNA, which is then cleaved by Cas3. In Type III systems, Cas10 assembles into a Cascade-like complex that recognizes and cleaves the target. Type II CRISPR systems require only one protein, Cas9, to scan, bind and cleave the target DNA sequence (Makarova & Koonin)

3.1. CRISPR/Cas9 for gene editing: At the core, gene gain/loss-of-function therapy comprises the generation of double-stranded breaks (DSBs) in defined regions of the genome, correction of the defective endogenous genes or introduction of exogenous genes, and DSB repair. (Hefferin & Tomkinson AE.)Therefore, introducing desired changes into genomes, i.e., "genome editing", has been a long sought-after goal in molecular biology. (Mazhar Adli 2018) CRISPR-Cas9 uses a 20 nucleotide gRNA as a guide to find the complementary protospacer DNA target in a genome where it cuts the double stranded DNA precisely 3 base pairs upstream of the PAM sequence, a process that requires CRISPR-Cas9 to undergo several complicated but finely-tuned conformational changes.(Fig .2A-C). (Jinag F et al.)



Traditional CRISPR/Cas9 protein-RNA complexes localize to a target DNA sequence through base pairing with a guide RNA, and natively create a dsDNA break (DSB) at the locus specified by the guide RNA. (Cui Zhang et aal.) Moreover, the RNA components of the CRISPR/Cas9 system can be used separately as crRNA containing the targeting guide sequence and constant tracrRNA molecules, or as single guide RNA (sgRNA) chimera, consisting of a fusion of a crRNA and a tracrRNA facilitates rapid implementation of the CRISPR/Cas9 system for genome engineering. Cas9 target recognition requires both the PAM sequence in the target DNA and RNA–DNA complementarity base pairing between the 20-nt guide RNA sequence and the complementary target DNA sequence. (Jinek et al.) Cas9-generated site-specific DNA double-strand breaks induce endogenous cellular DNA repair processes, which can be exploited to engineer the genome. DSBs are

generally repaired by one of two pathways, homologous directed repair (HDR) if the homologous template is available or otherwise by nonhomologous end joining (NHEJ). NHEJ is an error-prone process that can rapidly ligate the broken ends but generate small insertions and deletions (indels) at targeted sites, which often result in the function of target genes being disrupted or abolished. Alternatively, DSB may also be repaired via HDR, which is able to recombine exogenous DNA, and can be used to introduce transgenes or precise genome editing (Fig .3). (Magdalena Hryhorowiez et al.)

4. The CRISPER-Cas9 perspective

As a tool of great promise for the treatment of inherited human diseases, the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 nuclease system has captured public imagination, with its precision and versatility being likened to genetic scissors and the letter-by-letter editing capabilities of word processing software. (Harry L. Malech)The greatest advantages of the CRISPR-Cas9 system are its simplicity and wide applicability in genome manipulations of almost all biological systems tested to date, including cell lines, stem cells, yeasts, worms, insects, rodents, and mammals. (Quetier F et al.) CRISPR/Cas has already shown great potential in generating disease models and correcting monogenic disease mutations. The CRISPR disease models can accelerate the discovery and development of drug targets (Hsu et al.). Before the application of CRISPR for human disease correction, efforts are needed to optimize and maximize the editing efficiency as well as minimize off-targets and develop novel tools to specifically deliver the CRISPR components to the target tissue for gene editing. As CRISPR/Cas-based gene therapy enters clinical trials, this technology holds great potential for treating genetic diseases particularly for the present incurable ones and enhancing cell therapies. (Jayavaradhan R et al.) The history of molecular biology will place CRISPR-Cas9 among the major tools that enabled breakthrough discoveries and methodological advancements in science. CRISPR applications have already expanded our vision of genome regulation and organization in living cells across diverse biological kingdoms. In this regard, CRISPR is not only transforming molecular biology but also medicine and biotechnology. The therapeutic applications of the CRISPR technologies are particularly exciting. (Dunbar et al.)

References

[1]. (Xiao-Jie1 et al., 2015). n.d.
[2]. Cui Zhang et aal., 2018. "Development and application of CRISPER Cas/9 technologies in genomic editing." Human Molecular Genetic (2018): 80. print.
[3]. Dunbar et al., 2018. "Gene therapy comes of age." science (2018): 359. print.
[4]. H Kim et al., 2014. "A guide to genome engineering with programmable nucleases." nat rev genet (2014): 321-34. print.
[5]. Harry L. Malech, 2021. "Treatment by CRISPER -Cas9 Gene Editing- A proof of Principle." The New England Journal of Medicine (2021): 286. print.
[6]. Hefferin & Tomkinson AE., 2005. "Mechanism of DNA double-strand break repair by non-homologous end joining. DNA Repair." Taylor&Francis group (2005): 639-48. print.
[7]. Hsu et al., 2013. "DNA targeting specicity of RNA guidet Cas9 nuclease." Nat Biotechnol (2013): 827-832. print.
[8]. Jayavaradhan R et al., 2019. "CRISPER Cas/9 function to dominant-negative 53BP1enhances." Nat communication (2019): 2866. print.
[9]. Jinag F et al., 2016. "Structures of CRISPER -Cas/9 R-loop complex primed for DNA cleavage." Science (2018): 351:867-71. print.
[10]. Jinek et al., 2012. "A programmable dual ARN-guiddet Dna endo nuclease in adaptive bacterial imunity." Science (2012).
[11]. Magdalena Hryhorowiez et al., 2017. "CRISPER/Cas9 Immune System as Tool for Genome Engineering." Springer (2017): 235. print.
[12]. Makarova & Koonin, 2015. "Annotation and classification of CRISPRCas systems. Methods ." Mol. Biol (2015): 1311:47–75. print.

[13]. Mazhar Adli 2018. "The CRISPER tool kit for genome editing and beyond." nature communication (2018): 1. print.
[14]. Mojica et al., 2005. "Intevening sequences of regulary spaced procaryiotic repeat derive froms forein genetics elements." Mol Evol (2005): 174-82. print.
[15]. Mojica Fj et al., 2005. "Intervening sequences of regulary spaced procariotic repeates derive from forein genetic elements." J Mol evol (2005): 174-82. print.
[16]. Mojica FJM et al . " Bological significance of family of regurarly spaced repeats in the genomes of Archeas,Bacteria and mitochondria." Mol.Microbiol (2000): 244-6. print.
[17]. "Intervenig secuences of regulary spaced procaryotic repeats derive from forein genetic elements." Mol. Evol (2005): 174-82. print.
[18]. Ning Guo et al . "The power and promise of CRISPER Cas/9 genome editing for clinical application with gene therapy." Journal of advenced research (2021): 1. print.
[19]. Quetier F et al., 2016. "the CRISPER Cas/9 technology:closer to the ultimate toolkit for target DNA recognition." Science (2016): 242. print.
[20]. Shao-Shuai Wu et al. "Advences in CRISPER/Cas-based Gene Therapy in Human Diseases." Theranostics (2020): 4374. print.
[21]. Weninger A et al . "Combinatorial optimization of CRISPER Cas/9 expression enables precision genome engineering in the methylotrophic yeast." J.jbiotec. (2016): 30134. print.
[22]. Xiao-Jie et al. "CRISPER-Cas9: a new and promising player in gene therapy." J Med Genet (2015): 1-2. Print.
[23]. Xiao-Jie et al., 2015. "CRISPER Cas/9: a new and promising player in the gene therapy." Med Genet (2015): 1. print.