Contemporary Evolution in CRISPR–CAS9 System Technology and their Uses in Treatment of Human Common Diseases: A Review

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Abstract

Gene therapy has proven its potential in treatment of several human diseases. Most recent method in a long line of the genome-editing-techniques is called "A Clustered-Regularly-Interspaced-Short-Palindromic-Repeat-associated-protein9" abbreviated as CRISPR-Cas9. The scientists and researcher have long sought to the control, and modification the DNA that consider "the code of life". The CRISPR-Cas9technology is offer very important improvements that differ about other gene-editing.technologies, it's uncomplicated to apply & low-cost, also have comparatively high degree of the efficiency and precision . This technology uses a ".ribonucleic acid.(RNA)-guided. Deoxyribonucleic. acid-DNA-endonuclease.", Cas9, which induces double-strand breaks (DSBs) in the target site, the DSBs was repaired by the use of a variety of cellular DNA-repair systems leading to changes in target sites, that technology have proven to be effective to investigation, prevention, and treatment of diseases.

This review summarizes contemporary evolution in CRISPR –Cas9 system technology and their uses in treatment of common diseases.

Keywords: *Genome editing, CRISPR/Cas9 technology and Gene therapy.*

Introduction

The.Genome-editing-technologies is included mutations caused by UV radiation and chemicals, the zinc-finger-nucleases (ZFNs), the DNA-recombinase mediated gene replacement, and the "transcriptional-activator-like-effector-nuclease (TALEN)" mechanisms, it have contributed significantly to both basic and clinical developments in the biological research [1,2].

Scientists have reformed or change the genes by using radioactivity or chemical materials. These procedures of modifying produce random outcomes. The development of the technology of the recombinant-DNA in a year (1970s) permitted to the researchers with adding a new fragment of DNA addicted to genes structure, but introducing a precise gene or sequence within the genome persisted large theoretically inaccurate and challenging [3]. Gene editing can be used to many process such as insert, eliminate, or transform DNA in a genome.

The CRISPR-Cas9 technology is one of the gene-editing-technology that longer provide the possibility of making significant improvements over other gene-editing-technologies[4]. That technology was described, easy to ably, efficacy, speed, also inexpensive[5]. the CRISPR-Cas9 technology have proven, it provide Revolutionary developments in the diagnosis, treatment and prevention of several diseases[6] .

Definition and description of CRISPR-Cas9 technology

The CRISPR-Cas9technology is one of a gene editing-technologies, its uses mixture of the enzyme called "a nuclease-Cas9 enzyme " to facilitate cuts DNA,

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and (guide-RNA) a guide piece of the genetic material to indicate a position in the genome [1, 2], Usually, the guide RNA has been target and bind to the special-DNA-sequence, while an attached-Cas9enzyme works on cleave both DNA strands at that location, that slash could be used to inserts, removes, or edits the DNA-sequence, After that a slash is repaired and the changes have been integrated, (Show fig1). CRISPR/Cas9technology is derived from the adaptive immunity of *Streptococcus pyogenes* bacterium [7], where Cas9-nuclease-enzyme was mediated antiphage action recognition to its mixture with clustered frequently interspaced to CRISPR-loci that is a short palindromic repeats. that loci is short consist of 30–40 bp repetitive sequences and also intercalates with the spacing sequences match a virus genome [8] . And a CRISPR-loci has been transcribed to the long RNA that consequently has been cleaved.via "CRISPR-associated endo-ribonucleases" Known a Cas9 to liberate small CRISPR-RNAs (crRNAs). The crRNAs after that in cooperation with tracrRNA form a Cas-RNA complex which recognize the target DNA and profits for cleaving it; in general, four strategies has been utilized for "double-strand break repair include geneknockout ordisruption, "gene deletion via NHEJ way", "gene correction", & "gene knock-in/insertion via HDR way"[9] .

The initial appliance of the CRISPR-Cas9 coordination acts correspondingly to the immune systems in macroorganisms. After a bacterial or archaeal cell infected by virus, the fragment of the DNA viral categorization is incorporated into the CRISPR region in the bacteria [10]. Immune response to CRISPR–Cas9 and the presence of preexisting antibodies against Cas9 (a protein) could be a significant hurdle especially for *in vivo* gene editing. It is highly desirable to exercise more rigorous assessment of possible immunological responses to the microbial origin of the system[11]. However, delivery of RNP complexes and codon optimization may hold the key to overcome such hurdles[12]. The CRISPR/Cas9 technology have been proven effective in establishing a gene KO or knockdout that known knockin in human cells and is predominantly valuable for editing stimulated pluripotent stem cells or (iPSCs.)[9]. Despite that advances, several the ethical, technical & biological researches limiting about utilization that technology in analysis and treatment of common diseases.

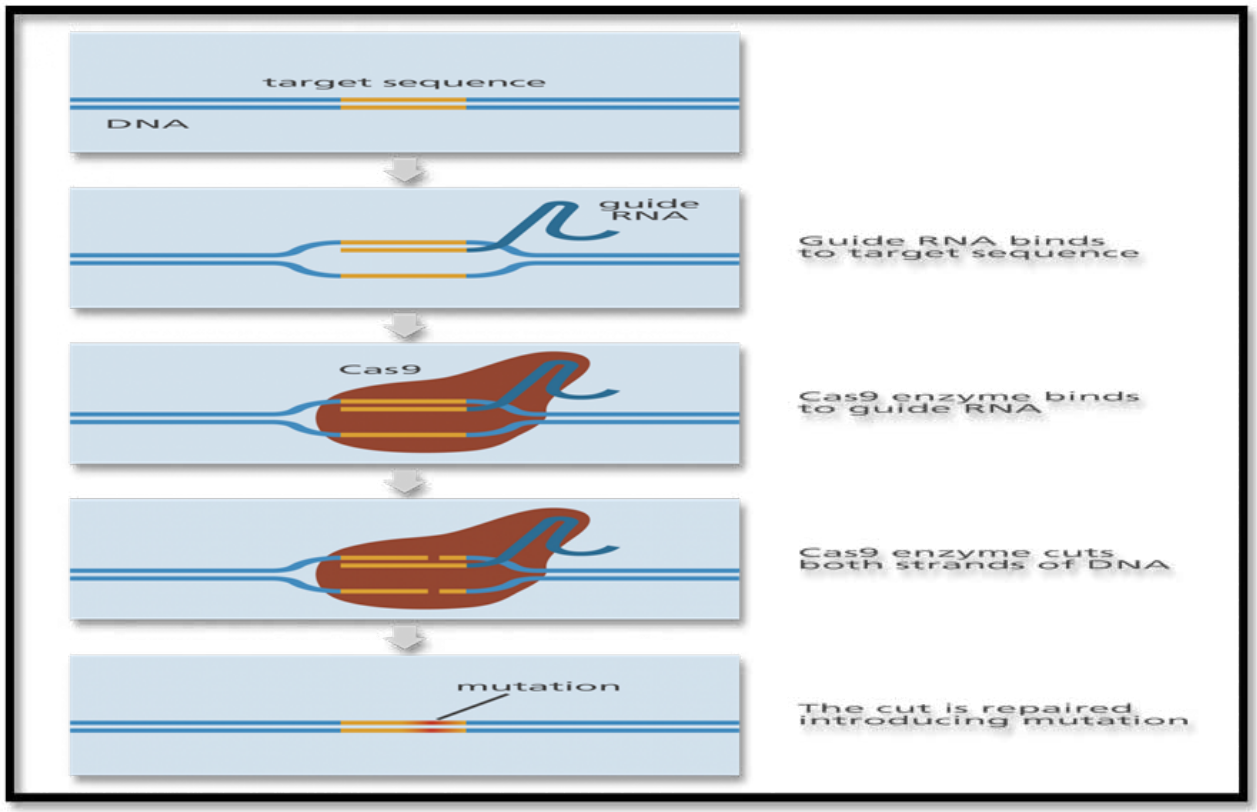

Fig. 1: "What Is CRISPR-Cas9?[13]," at http://www.yourgenome.org/facts/what-is-crispr-cas9

Compensations or Advantage of CRISPR Cas-9 technology: The genome editing system of CRISPR Cas-9 gives many benefits concluded the nuclease enzyme of zinc finger and transcription activator like effector nuclease in human iPSCs, and also stem cells of somatic origin .

Several synthesizing primers essential to be designated for expending this technique, since its specificity is associated only to ribo-nucleotide multifaceted development [14]. Additional, these system is further economical, low cost for plasmid-mediated of that system. Another uses as the wildest presently obtain able technique of gene editing, it is using of these system can characteristically be achieved in present time[15]. The CRISPR/Cas9 technology have been established to be valuable & effective for an editing various human .cells [16-18]. Recently, researchers have been reveal a higher editing capability of the transcription-activator-like-effector-nuclease in human stem cells is more efficiency about 79%, of using for editing human iPSCs[19]. Furthermore, CRISPR/Cas9 also utilizing to monitor DNA noncoding sequences for recognizing regulatory elements to understand how a genetic variation were associated with cause the many of human diseases[20-21]; in this context, Fulco et al. recognized nine distal enhancers, and their target-gene via CRISPRi [20], and Also the CRISPR/Cas9 have been utilize to set up DNA changes in the noncoding locations, and established a association between transcriptional function and intronic SNPs in PHACTR1gene in that location [22]. Where there is Another concern was raised with CRISPR/Cas9 technology relates to editing efficiency sgRNAs. induce Cas9-mediated DSB. by the side of favorite target position, a DSB. induces "DNA-repair by HDR. way", conversely, the alternative-"DNA-repair-mechanism-NHEJ." be able to induce at low frequencies, giving rise to unpredictable events of diminutive insertions & deletions [23].

Application of CRISPR-Cas9 technology in treatment of the human common diseases: The previous scientists were able to create "alternative-cell-models" via induced pluripotent stem cell (iPSC), for monitoring the molecular mechanisms of several diseases including cardiovascular disease**,** in the context of CRISPR/Cas9 system has been provide a straight forward mechanism for elucidating how cells mishandled via allowing with reversing of a causal mutation to the disease [24-26]. A Previous study of Wang *et al*. [27]. showed that persons with usually happening loss of thefunction mutation or what is called with "proprotein convertase subtilisin/kexin type 9" abbreviated as PCSK9.", they had lower of serum LDLC concentration, and thus edition of the "PCSK9.gene via CRISPR/Cas9technology" was a hopefully therapeutic board to avoidance of the cardiovascular disorder. While the study of Xie, *et al*. [28] indicated that utilized the CRISPR/Cas9technology in the postnatal mice to correct the mutation that cause to PRKAG2 cardiac syndrome, while the study of Ma *et al*. [29] utilized a CRISPR/Cas9 technology for correction a pathogenic mutation, in the MYBPC3 gene that causes hypertrophic cardiomyopathy in human embryos.

1. **The diabetes mellitus disease:** The "California Institute for Regenerative Medicine (CIRM)" awarded a scholarship to the scientists. who work in the "Children's Hospital Los Angeles", they have been utilized the CRISPR-Cas9 technology for developing the .modified approach to treat a genetic variations of the diabetic disease (for example Type I diabetes mellitus) via replacement the insulin producing cells in diabetic patients, and scientists suppose that the technique may also ultimately suggest treatment for non autoimmune diabetic disease (for example Type II diabetes mellitus)[30,31]. These study's findings were indicated that utilization of the "patient's own cells" reduced risks of the transplant rejection, also the patients wouldn't be dependent on a limited availability of external donors.
2. **The respiratory diseases:** The DF508 mutation have been recognized in (CFTR) is one respiratory disease and its recognized in nineteen percent of patients with a cystic fibrosis, and previous study indicated that improvement of the DF508mutation in pluripotent stem cell (iPCS) by the use of "CRISPR/ Cas9-mediated HDR" way to treat CFTR disease [32]. In same the context, The "Alpha-1 Antitrypsin Deficiency (AATD)" that cause a type of respiratory disorder that corrected by utilization of CRISPR/ Cas9technology by way of the NHEJ. mediated gene-disruption & the HDR. based precise gene-correction [33].
3. **The hematologic diseases:** Recently, the researchers have been utilized CRISPR/Cas9 technology to treatment the sickle cell disease (SCD) via two strategies : correction the mutation that cause sickle cell and stimulation the fetal hemoglobin (HbF) expression. In 2018 FDA established the submission of two bio-technology establishments—CRISPR

and Vertex for an investigational management with gene therapy for (SCD).

The new Method of treatment by using system of CRISPR-Cas9 for modification of stem cells that separated from blood of patients and then rein-fused for production of F Hb. The advanced intensities of F Hb are predictable to stabilize the pain that caused by the mutation of sickle cell [34] **.** In the gene correction technique have been used the hematopoietic stem cells that derivative from the SCD patients & also corrected the Ex. Vivo. by the use of CRISPR/Cas9technology, after that transplanted into the SCD patients [35]

The study of Canver *et al*. [36] indicated that the BCL11A." is a transcriptional controller which serve as a strong Hb F silencer & suppress to expression of cglobin, So this is suppression of BCL11A. could be utilized to treatment both beta-thalassemia & sickle cell anemia, while the study of Liu *et al*. [37] indicated via utilization of the CRISPR/Cas9system, the beta-thalassemia causing mutation in patients derivative induced pluripotent stem cells (iPSCs) could be corrected & after that transplants a corrected iPSCs into that same patient . Also by the CRISPR/Cas9-mediated HDR have utilized to correction a mutation in patient with haemophilia-B[38] . Majority a strict haemophilia-A were resulted via large-chromosomal inversions (600 or 140 kb) within coagulation Factor VIII (F8), & correction of those inversions in the patient-derived-iPS cells were created by the transfer of RNP-complex of Cas9, & a couple of sgRNAs for targeting two dissimilar locations for stimulating rein version of those mutations[39] .

5. **The severe combined immunodeficiency disease:** Currently, the study of Chang *et al*. [40]. indicated that using the "CRISPR/Cas9-mediated HDR approach" able to correction a point mutation at "the exon14 of JAK3gene" in patient-derived iPSC for treating severe combined immunodeficiency. Also the study of De Ravin *et al*. [41] indicated Patient-derived iPSC of "the X-linked chronic granulomatous-disease-X-CGD- patients" that cause by a point mutation at the CYBB gene, could be corrected via "CRISPR/ Cas9-mediated HDR approach" and after that, transplants into the same patient. The Knockout of miR-155 is imperative pro-inflammatory controller in the rheumatoid arthritis disease,via CRISPR/ CAS9 technology have been revealed that the inhibition of pro-inflammatory-cytokine creation in "macrophage cell line" & presented a optimistic therapeutic strategy to treatment of arthritis disease [42] .

6. **The neurological diseases:** This is a comparatively new and more advanced technology of gene editing but there are several setbacks: first being the host genome itself, and Targets to modify may have enough sequence resemblance (similarity) with other part of genome that leads to unintended cuts in host genome. Thus, undesired mutations are generated, which may affect overall health and even survival of host organism adversely. *In vivo* delivery is another limiting factor. Crossing blood–brain barrier is an utmost challenge for components of this gene-editing technology [43,44].

   There is two strategies have been done designed for gene therapy to treat the progressive degenerative neurological disorder, include: "deletion of pathogenic mutations and targeted gene correction". The CRISPR/Cas9technology could be ably for targeting deletion of the CAG repeats via the sgRNA/Cas9collection which flank this domain and creation a DSBs that consequently stimulate "non-homologous end joining (NHEJ) process" [45]. And also the "(deletion of open-reading-frame of HTT)" could reduced mutant-huntingtin-masses[46].Also the previous studies indicated that "Friedreich Ataxia and Amyotrophic lateral sclerosis (ALS)" were anther disease which have targeted via CRISPR/ Cas9technology [46-48.]

The Malaria Disease: This is mosquito infected human and other animals**,** one of the greatest pervasive and mortal disease in the biosphere. Operative revision, decrease, or dismissal of the *Anopheles* mosquito is initial route for communication of the malaria can significantly condense expenditures also opened up original way for commercial occasions in domain's unfortunate nations.

CRISPR-Cas-9 permitted attitudes that including use of driving genes in a improved gene being differently approved to children. This powerfulness proposal a incomes by which all *Anopheles* mosquitos might be prepared sterile [49]**.** Also consequence in all young male [50]**.** If effective, these methodologies will, considerably diminish or-even perhaps eliminate the populace being embattled. Additional method of CRISPR Cas-9 permitted attitude pursues for making the *Anopheles mosquito* impervious to the malaria.

7. **The Resistance for antibiotics:** Conferring to the center of disease control and prevention about two million of persons are infested yearly with microorganisms (bacteria) was appear resistance for antibiotics, also about thirty two thousands of individuals will decease or die every year and the consequence of such contaminations [51] . The new system of CRISPR/Cas9 had been revealed to successfully board and reduce of bacteria with different types, as well as the strains of bacteria that have resistance to antibiotics from a public of microorganisms . This accurate training permits removal of injurious of bacteria, but circumvents positive of bacteria . Furthermore, unlike old-fashioned or customy of antibiotics will be challenging for the bacteria to progression their resistances to the CRISPR -based antimicrobials since such as the resistance will probable destroys defense of the bacteria, permit to reviewers and researchers of the major problem to improvement of CRISPR-based antimicrobials are categorizing an efficient provision way [52] .

Conclusion:

1. Genome-editing-technology is very important technique that empowers productions of the genetically-modified-cells & organisms beings important to clarify gene role and human diseases mechanisms, And CRISPR/Cas9 system is one of Genome-editing-technology has been utilized for correcting DNA mutations that ranging from only single-base-pair into large deletion in the model systems of in vitro. & in vivo..
2. This "CRISPR–Cas9 technology" uses a "ribonucleic acid (RNA)-guided deoxyribonucleic acid (DNA) endonuclease", Cas9, which induces "double-strand breaks (DSBs)" at target location, the DSBs. corrected by the use of a variety of cellular DNA-repair systems leading to changes in target sites, that technology have proven to be effective to investigation, prevention, and treatment of diseases.
3. The CRISPR-Cas9 technology has been quickly become a one of mainly important systems for genome-editing during essential biomedical investigate due to its adaptability & simplicity.
4. The CRISPR Cas-9 technology have been given several benefits that accomplished the "zinc finger enzyme nuclease & transcription activatorlike effector nuclease in human pluripotent stem cells & stem cells of somatic origin"
5. Recently, researchers have been reveal a higher editing capability of the "transcription-activator-like-effector-nuclease" in human stem cells is more efficiency about 79%, of using for editing human iPSCs. Furthermore, CRISPR/Cas9 also utilized to monitor DNA noncoding sequences for recognizing the regulatory fundamentals to acknowledgment how the genetic variation were associated with cause the many of human diseases.
6. The CRISPR/Cas9 technology relates to editing efficiency "sgRNAs induce Cas9- mediated DSB" at favorite target position, "DSBs induces DNA-repair by HDRway"; conversely, an alternatives "DNA-repair mechanism NHEJ" be able to induce at low frequencies, giving rise to unpredictable events of diminutive insertions & deletions.
7. Several recent studies have made clear importance of the CRISPR-Cas9 technology for the diagnosis and treatment to the many common diseases.

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