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A Review on CRISPR/CAS9 And Its Application in Research, Industry and Health Biotechnology

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Abstract: *Genome editing is a process that target and break down DNA at specific areas in the genome. In this review we discussed about methods use in gene editing such as Zinc fingers (ZFs), transcription activator-like effectors (TALEs) and CRISPR /Cas9 but the main focused was CRISPR /Cas9 in which we discussed about its application in research, industry and health biotechnology. In conclusion CRISPR /Cas9 can be used in treatment of cancer, HIV, sickle cell, antimicrobial resistance and other diseases.*

Keywords: *Genome editing, CRISPR/ Cas9, germ line studies, antimicrobial resistance*

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I. Introduction

Genome editing is a powerful hereditary engineering strategy that utilizes 'molecular scissors', or artificially engineered nucleases, to target and break down DNA at specific areas in the genome. Homology-directed repair (HRD) or non-homologous end joining (NHEJ) are natural mechanisms that repair the double stranded DNA break (DSB) that was created by the engineered nucleases at the target site (Osakabe & Osakabe, 2014). Zinc fingers (ZFs) and transcription activator-like effectors (TALEs) were initially found in nature. For example, microorganisms secrete TALEs by means of their type III discharge system when they contaminate plants. They are portrayed by having generally large DNA target site that comprises of 12–40 bp (Smith, *et al.*, 2006). ZFNs and TALENs utilized for genome editing are engineered nucleases in which a ZF or TALE DNA-binding domain is connected to domain of the nuclease, for instance those gotten from the restriction enzyme FokI. The capacity of ZFNs to modify DNA through DSBs relies on a pair of DNA-binding zinc fingers made up of B30 amino acid modules that interact with nucleotide triplets, and the restriction endonuclease FokI, which oversees catalytic cleavage of DNA. 9–18 nucleotides are recognized by each ZFN. Since the nucleases to which they are connected to function as dimers, sets of ZFNs are required to target a specific locus; one that recognizes the upstream sequence and the other that recognizes the downstream sequence of the site to be altered. The fundamental restriction of this system is the difficulty and high cost related with the modular assembly and custom proteins development for the recognition of specific target sequence of DNA, notwithstanding the ZF modules not being 100% orthogonal (Kim & Kim, 2014).

TALENs require DNA-binding proteins called transcription activator-like effectors that are merged with a DNA cleavage area, for example, FokI. The specificity of TALE is decided by two hypervariable amino acids that are known as the repeat variable di-residues and it consist of multiple 33–35 amino acid recurrent domain, each recognizing a single base pair. TALENs has an advantage over ZFNs, including the likelihood to have a higher success rate in genome editing in human cells (Ain *et al.*, 2015; Ding, *et al.*, 2013). ZFNs are normally smaller than TALENs, which makes it uncomplicated to deliver and express them in cells. Certainly, a regular cDNA encoding a ZFN is roughly 1 kb, while for a cDNA encoding a TALEN is normally around 3kb. In addition, engineering ZFN does not involved gathering many highly repetitive components like TALENs. Nonetheless, both technologies have a disadvantage since they depend on protein engineering process to change specificity, which has hindered ZFNs and TALENs from being generally accepted for genome engineering applications in various fields (de la Fuente-Núñez & Lu, 2017).

The ability of Cas9/sgRNA complex to edit gene made ready for the utilization of Cas9 as a genome editing tool in various organisms as it offered a few extraordinary advantages over ZFNs and TALENs, including low cost, easy to use, simplicity, programmability and multiplexing. The CRISPR system is programmable, provided there is a PAM flanking the target DNA sequence, the Cas9 nuclease can be guided to any gene of interest by basically changing the guide sequence of the sgRNA. By introducing numerous sgRNAs into the cell CRISPR system can be multiplexed to target more than one gene concurrently (Cho *et al.*, 2013). Current technology for scalable barcoded combinatorial gene assembly, CombiGEM, made a library of 23409

barcoded double guideRNA (gRNA) combination, accompanied by a high-throughput pooled screen. This CombiGEM-CRISPR approach recognized pairs of gene that inhibit the growth of ovarian cancer cell when they were targeted for knockouts, alongside drug pairs that targeted these same pairs of gene and accomplished synergistic effect when utilized together (Wong, et al., 2016). Adding to these advantages, the specificity of CRISPR-Cas system towards its target sequence in human cells is like that of ZFNs and TALENs, with high efficiency rates of approx. 90% and moderate off target effect rates of approx.20% (Kim & Kim, 2014; Ain *et al.,* 2015; Ding, *et al*., 2013). Howeever, this review will focused on the method of CRISPR/Cas9 delivery and it applications in cell lines studies, animal models, antibiotic resistance and food biotechnology.

II. Crispr/Cas9 Technology

Clustered regularly interspaced short palindromic repeats (CRISPRs) are recurring sequence of DNA that behave in agreement with CRISPR-associated (Cas) genes to give bacterial and archaeal resistance against foreign invading nucleotides, e.g phages and plasmid DNA (Wiedenheft *et al.,* 2012). Type 2 CRISPR/Cas system has been studied the most among the 3 types systems recognized up until this point. The attacking DNA first gets cut into tiny pieces and fused into the CRISPR locus during the immune reaction (Doudna & Charpentier, 2014). Presently the system obtained from Streptococcus pyogenes (Sp) is the most frequently used, it comprises of two small RNAs that are separated, called tracrRNA and crRNA and a Cas9 endonuclease (Deltcheva, et al., 2011), they can be joined with a tetraloop to form a single guide RNA (sgRNA) (Jinek et al., 2012). A blunt end is created at 3bp upstream of the NGG PAM (protospacer adjacent motif) when double stranded DNA is cut by Cas9 under the control of sgRNA, which particularly recognizes the chromosomal loci of interest with 17–20 nucleotides (nt) (Fu *et al.,* 2014; Zhang, *et al*., 2016).

III. Method of Delivery

There are different methods in which the CRISPR/Cas9 system can be delivered depending upon the application(Wang *et al.,2016).*Two components are required to achieve genome editing by CRISPR/Cas system, the Cas9 protein and a guide RNA (gRNA) consisting of 17-20 nt of identity to a target sequence near a protospacer adjacent motif (PAM). DNA double strand break will occur in the gene target specified by the gRNA, when it forms a complex with the Cas9 nuclease. For genomic control of cells in culture, they are normally transfected with a single plasmid vector expressing the gRNA from an RNA Polymerase III promoter gotten from human snRNA U6 gene and the Cas9 mRNA from an RNA Polymerase II promoter (Mali, et al., 2013). gRNA template and the Cas9 gene that are carried by individual plasmids are co-transfected into target cells for gene editing. With Cas9 protein available from commercial sources, the gRNA-Cas9 complex (mixture of *in vitro* transcribed gRNAs and purified Cas9 protein) is introduced into cells for gene editing (D'Astolfo, et al., 2015).

In gene therapy, delivery of the Cas9 system is more demanding and raises more security concerns. Adeno associate virus (AAV) vectors are the favoured candidates for somatic gene treatment because of their mild immune reaction, absence of pathogenicity, and capability to target non dividing cells (Gaj *et al.,* 2016). Nonetheless, the coding sequence of Sp Cas9, the extensively utilized Cas9, and sgRNA are moving toward the packaging limit of AAV-based gene therapy vectors. AAV-based gene therapy is disadvantageous due to the extensive size of Sp Cas9, particularly when promoter sequence, localization signals, donor DNA, or extra sgRNAs are required. The Sa Cas9 has roughly 1 kb smaller coding sequence than Sp Cas9, permitting Sa Cas9 to be effectively packaged into AAV-based vectors for gene editing (Ran, *et al*., 2015; Friedland, *et al.,* 2015).

Delivery of Cas9 and sgRNAs by Ribonucleoproteins (RNPs) can be accomplished through different procedures. Numerous procedures including microinjections, electroporation, and lipid-mediated transfection that are used traditionally for nucleotide transfection have been demonstrated to be valuable for Cas9 RNP delivery. Genome edited animals are generated by microinjection of purified Cas9 RNP complexes into embryos of animals (Cho *et al.,* 2013; Sung, *et al.,* 2014). Cas9 and sgRNA are delivered into primary and embryonic stem cells by electroporation, and these can prompt targeted gene mutations and huge deletion of chromosomes while limiting the off-target effects (Kim *et al.,* 2014; Lin, *et al.,* 2014; Liu, *et al*., 2015).

NON-HOMOLOGOUS END JOINING AND HOMOLOGOUS DIRECTED REPAIR

Double stranded breaks are repaired by cells by two procedures: homology-directed repair (HDR) and non-homologous end joining (NHEJ). Non-homologous end joining produces a knockout phenotype by presenting variable insertions or deletions (InDels) at the DSB, the HDR mechanism makes accurate deletions, base substitution, or insertion of coding sequence of interest in the company of a recombination donor flanked with right and left homology arms (HA). In this way, the HDR mechanism is utilize to ease editing of diseased genes, insertion of epitope tags or fluorescent reporters, and overexpression of genes of interest in a site-specific way (Zhang, et al., 2017).

APPLICATIONS OF CRISPR/CAS9

CELL LINES STUDIES

CRISPR/Cas9 Type IIB systems acquired from a few bacterial species with a couple of adjustments have been used in the mammalian cells. The *Sp*Cas9 was the first to be utilized application of CRISPR/Cas9 system for producing DSBs in mammalian genomes it was demonstrated by (Cong, *et al.,* 2013; Mali, *et al*., 2013). Cas9 enzyme from *Neisseria meningitides* (*Nm*Cas9) has an identical cutting capability as *Sp*Cas9, however the *Nm*Cas9 enzyme operate with a gRNA in the absence of a tracrRNA and utilizes a prolonged PAM site (NGATT) thus specificity is increased (Hou, *et al.,* 2013). As of late, a Cas9 model shorter by 1 kb than *Sp*Cas9 and gotten from *Staphylococcus aureus* (*Sa*Cas9) was identified and found to have comparable productivity to *Sp*Cas9 (Ran, et al., 2013).

Due to precision of Homologous direct repair, this mechanism can be utilized to recapitulate different diseases that causes mutation found in people in cell lines and can be valuable in rectifying mutations prompting to gene therapy. The HDR mediated knock-in utilizing CRISPR/Cas9 has been suggested as the mechanism that can be proficiently utilized in gene therapy. For instance, genetic lesion in the cystic fibrosis transmembrane conductor receptor (CFTR) gene bring about cystic fibrosis. The CFTR gene mutation in the patient-derived intestinal stem cells was corrected by CRISPR/Cas9 system and this amendment ameliorated the disease in an *in vitro* test (Schwank, et al., 2014).

ANIMAL MODELS

Large animals are engineered to study the process of immune rejection and disease transmission across species boundaries using CRISPR technologies. The presentation a year ago, that endogenous retroviruses could be obliterated from porcine cells by CRISPR targeting suggest entire animals could be designed with a decreased risk of transmitting disease, bringing xenotransplantation applications one stage closer (Yang, et al., 2015). The development of large animal models of human diseases, including in primates could be accelerated by CRISPR-mediated genome editing, and in this way, quicken the discovery of appropriate therapies (Niu, et al., 2014), although mosaicism issues must be tackled to guarantee the genotype of interest is regularly produced across tissues and cell types. Strong *in vitro* and *in vivo* models of human disease are established by using CRISPR-mediated genome editing (Barrangou and May, 2015).

ANTIBIOTICS RESISTANCE

A few new possible approaches have been created as of late to fight resistance in antibiotics, including the utilization of bioengineered and biologically inspired synthetic peptides (de la Fuente-Núñez, *et al.,* 2015; Reffuveille *et al.,* 2014), engineered bacteriophages (Lu & Collins, 2009) and others. CRISPR technology serve as a unique chance to fight resistance in antibiotics through its capability to specifically target any gene of interest in a specific pathogen. In fact, CRISPR-Cas has been lately exploited to designed antimicrobials that work by targeting a specific sequence of DNA. These sequence specific antibiotics work by utilizing the RNA-guided nuclease Cas9 to apply a poisonous eff ect in cells through chromosomal cleavage (Citorik, *et al*., 2014; Gomaa, *et al*., 2014). CRISPR-Cas antimicrobials can be arranged with a sgRNA matching a specific target gene of choice in comparison with current antibiotic agents that are broad spectrum regarding their action. Consequently, these antimicrobials could be able to selectively kill the organism that harbour this gene while leaving all other neighbouring microorganisms unaffected, even in complex bacterial populaces, for example, biofilms or the human microbiota (Bikard, et al., 2014).

Previous work has demonstrated that this approach can be utilized for selective killing of bacterial populations (Citorik et al., 2014; Bikard, *et al*., 2014). Their reviews demonstrate effective delivery of RNA-guided nucleases by utilizing phagemids, which are plasmids that contain signals for bacteriophage packaging. These phagemids were built to contain genes that encode for both cas9 and gRNAs. For instance, Citorik, *et al.,* (2014) demonstrate effective delivery of RNA-guided nuclease Cas9 into *E. coli* by utilizing phagemids with packaging signals for the filamentous phage M13. This technique gives on to selective killing of pathogenic *E. coli* strains that expressed specific target genes including those that encode for antibiotic resistance.

FOOD AND INDUSTRIAL BIOTECHNOLOGY

Applications of CRISPR systems in microorganisms involved genotyping, vaccinating industrial cultures against viruses, controlling spreading and uptake of antibiotic resistance genes by microorganisms, and probiotic cultures engineering (Selle and Barrangou 2015).The commercial success of native CRISPR–Cas immune systems for the vaccination of *Streptococcus thermophilus* starter cultures utilized in dairy fermentation (yogurt and cheddar) has made way for CRISPRs in food (Barrangou, *et al*., 2013; Barrangou & Horvath, 2012). Previous work has demonstrated evidence of the idea that beneficial microorganisms might be produced that are immunized against spreading and uptake of genes that encode antibiotic resistance (Garneau, et al., 2010).

CRISPR technologies will broadly influence all industries associated with microorganisms, as we are on the cusp of extensive use of genome editing in these life forms (Barrangou & van Pijkeren, 2016). CRISPR–Cas9 is

probably going to be utilized to design industrial bacteria, yeast and fungi to make green chemicals, including biofuels and biomaterials (Ryan, *et al.*, 2014). Past report demonstrated that CRISPR-mediated vaccination procedures can be exploited as molecular recording events, with the potential to expressed synthetic DNA sequence, valuable for data storage, into bacterial, and perhaps other, genomes (Shipman *et al*., 2016).

IV. Summary and Conclusion

Numerous breakthrough was found in the brief history of CRISPR/Cas9-mediated genome editing. For instance, authorities in the United Kingdom have given consent to the lab of Kathy Niakan to perform genome editing on human embryos using CRISPR/Cas9. Since CRISPR/Cas9 provide simplicity, flexibility, and accuracy in gene targeting numerous breakthrough choices in the period of CRISPR/Cas9 based genome editing is expected. CRISPR/Cas9 technology can propel research in the life sciences field, particularly in biomedical research on cancer and other significant human illnesses and for the development of crop plants for enhancing food security. For instance, guidelines laid out for genetic modified crops in view of traditional plant gene transfer technology utilizing *Agrobacterium*-mediated modification and will clarify engineering plants for some agronomically vital attributes are redefine by CRISPR/Cas9 (Ceaser *et al.,* 2016). Future research utilizing CRISPR-Cas antimicrobials will probably concentrate on targeting biofilms, which are multicellular groups of bacteria that are evaluated to bring about no less than 65% of every persistent infection in humans (de la Fuente-Núñez, *et al*., 2013; Cohen *et al.,* 2013).

CRISPR/Cas9 system simplicity and specificity has allowed its applications in understanding transcriptional control, and in the improvement of potential treatment for diseases by disturbing genes or rectifying gene deformities in both germ line and somatic cells in vivo. In view of the multiplexing ability of CRISPR/Cas9 system, which permit concurrent editing of multiple genes, we imagine a future when cardiovascular disease could be cured by administering a cocktail of CRISPR/Cas9 based therapeutic agents, which function like a vaccination instead of drugs that must be taken daily (Li *et al*., 2017)

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