International Journal of Endorsing Health Science Research
©Advance Educational Institute & Research Centre – 2017
Volume 5 Issue 1, March 2017
www.aeirc-edu.com
Print: ISSN 2307-3748
Online: ISSN 2310-3841

Review article

Optimizing CRISPR Cas9 Genome Editing System: A Review

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Abstract

CRISPR Cas9 is highly advanced genome editing technology extensively used for the modifications of genetic components in various sectors of living organisms. This technology has been adapted from the prokaryotic immune system, where it plays a vital role in protecting bacteria and archaea from virus attacks. This robust technology has currently been proven efficient in selective and precise editing, the genome of different living organisms for different purposes ranging from therapeutic, diagnostic to programmable gene regulation. This technology has been continuously upgraded, enhancing its performance thus reducing unfavorable outcomes. Customizing this technology is not a piece of cake. Hundreds of thousands of experiments have been conducted all around the world to optimize this highly intellectual technology to make it an error prone programmable technology to serve each and every living kind. In this review, we have summarized the modifications that have been made in different components of CRISPR cas9 system, engineering of CRISPR Cas9 for specific purposes, different external factors that has to be considered to obtain the best possible outcome minimizing the hazards.

Keywords

CRISPR Cas9, Cas9, genome-editing tool, optimizing CRISPR Cas9, off-target mutations

Introduction

The prokaryotic derived immunity, which has been successfully implemented in different sectors of life science, has gained recognition within few years of its discovery and has been able to maintain its position in thousands of scientific papers. This highly potential tool CRISPR-Cas christened in the year 2002, is a genome-editing tool, which is an abbreviation of Clustered Regularly Interspaced Palindromic Repeats and CRISPR-associated proteins[1]. Indeed, CRISPR/ Cas is an adaptive immunity of 40% of bacteria and 90% of archaea, which serve to protect against invading viruses, plasmids and foreign nucleic acids by recognizing and cleaving pathogen DNA[2]. It was first noticed as a strange structure in Escherichia coli during the study of the nucleotide sequence of iap gene[3]. However, just few years earlier than that stern MJ et.al had also encountered Repetitive extra-genic palindromic sequence (REP) in the genome of E.coli and S. typhimurium[4]. CRISPR consists of chain of highly conserved short-repeats, 23-44bp long sequences, separated by similarly sized spacers that originate from phage or plasmid DNA. The amalgamation of protein and short RNAs cleaves the target specific DNA sequence.

The bacteria cleaves the foreign nucleic acids by utilizing the protospacers that have been collected and incorporated into their genome which help in expressing the short guide RNAs to identify and destroy DNA sequence matching the protospacers[5,6]. Cas proteins are programmed with putative operons and contain domains that are characteristics of nucleases, helicases, polymerase and various RNA-binding protens[1]. Cas proteins are functionally categorized into four groups: nucleases and /or recombinases that are involved in spacer acquisition, ribonucleases catalyzing the processing of crRNA guides, nucleases responsible for degradation of DNA or RNA targets and proteins that along with RNA guides form crRNP complexes for target surveillance[7]. CRISPR-Cas has been broadly classified into three major types and several subtypes; distinguishable by the of the presence of individual signature genes: Cas3 in Type I systems, Cas9 in Type II, and Cas10 in Type III that use distinctive molecular mechanisms for recognition and cleavage of targeted nucleic acid[8]. The less common however, clearly distinct Type IV system has also been reported[9,10]. Type III and I are phylogenetically and structurally distinct which are found in various patterns among phylogenetically diverse bacteria and archaea, whereas Type II is restricted only to bacteria[7]. There are also evidences of CRISPR-Cas systems found in viral genome and plasmids suggesting that these systems are frequently swapped via horizontal gene transfer[7]. Among these highly diverse CRISPR-Cas systems, CRISPR-associated RNA guided endonuclease Cas9 has become a highly potential genome editing tool. CRISPR-Cas9 technology has proved itself efficient genome editing tool among other genome-editing tool such as homologous recombination (HR)[11], zinc finger nucleases (ZNFs) [12] and transcription activator-like effector nucleases (TALENs)[13].

CRISPR-Cas9 in Brief

The Type II bacterial derived Cas9 protein based immunity system stands amongst others as the most efficient and the most potent gene, editing technology with its diverse application in wide range of biologic systems including yeasts, worm, insects, plants, aquatic plants and animals and mammals[14]. The CRISPR-Cas9 utilizes a 20-nucleotide gRNA as a guide to search the complementary protospacer DNA target in a genome, and Cas9 nuclease that brings about the double stranded DNA breakage precisely 3 base pairs upstream of the PAM sequence. The nuclease activity is locked in the HNH and RuvC like domains of the Cas9 nuclease, which cleaves target and the opposite non-target strands of DNA simultaneously. Thus formed DNA breaks is repaired either by NHEJ (non-homologous end joining) generating indels thus resulting random mutations or by HDR (homologous direct repair) with the introduction of donor oligonucleotides or DNA fragments leading to targeted gene mutations or corrections[15,72]. (Figure 1) CRISPR/Cas9 system is being adapted to a robust and multiplex genome-editing tool, which enables researchers to manipulate targeted genome. This technology has already shown to be effective in stem cells, induced pluripotent stem cells and somatic tissues of human[14]. The first successful application of CRISPR-Cas system was done in 2007 as infection experiments of lactic acid bacterium Streptococcus thermophiles with lytic phages where the natural CRISPR-Cas systems of cultured bacteria was used in dairy industry for harnessing immunization against phages[16,17]. A year later, mature CRISPR RNAs (crRNAs) were noticed to serve as guides in complex with Ccas

proteins to interfere with virus proliferation in E. coli[5] and in the same year, the DNA targeting activity of CRISPR-Cas system was reported in pathogen Staphylococcus epidermis [18]. A major breakthrough was the discovery of tracrRNA, a small RNA that was trans-coded in upstream of TYPE II CRISPR-Cas locus in S.Pyogens which has an integral role in crRNA maturation by ribonuclease III and Cas9, and tracrRNA-mediated activation of crRNA maturation was found to confer sequence–specific immunity against parasite genomes[19]. The DNA target site by Waston-Crick base pairing, and the double-stranded structure at the 3'side of the guide sequence that binds to Cas9[20].

fully functional CRISPR-Cas9 model was purposed in 2012 where the S.pyogenes CRISPR-Cas9 protein was discovered to be as a dual–RNA-guided DNA endonuclease that utilizes the tracrRNA:crRNA duplex to direct DNA cleavage[20]. The signature model of CRISPR-Cas9 with dual tracrRNA:crRNA was further engineered into a single guide RNA(sgRNA) preserving the two critical features of the 20-nucleotide sequence at the 5'end of sgRNA that determines the

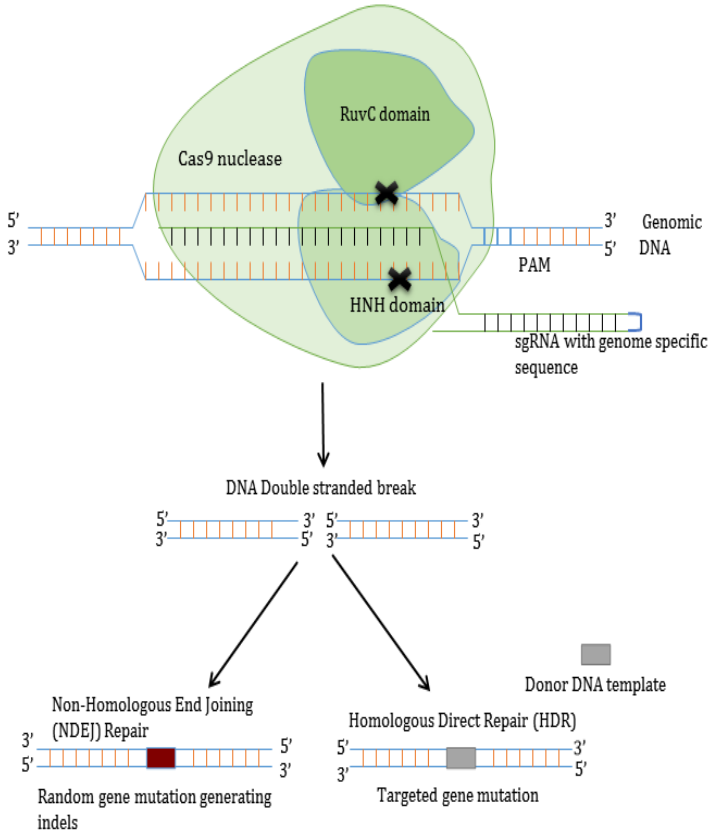

Figure 1. Basic mechanism of CRISPR Cas9 gene editing system. (a) Gene slicing of the target genome (b) guided by the guide RNA leading to the double stranded break (c) The double stranded break repaired by non-homologous end joining (NHEJ) repair mechanism resulting to the insertion or deletion (indels) of the DNA segment (d) or by homologous direct repair (HDR) mechanism with the introduction of the donor DNA template

CRISPR-Cas9 Modifications

The CRISPR-Cas9 technology has an advantage that it can be manipulated for various purposes. Accordingly, the specificity and efficiency of this technology is upgraded to the different level, thus signifying the flexibility in performances. The microbial defense system derived wild type (wt)-CRISPR-cas9, has been artificially engineered into several variants with widened horizon of functions and applicability of CRISPR-Cas9 system in genome editing[16]. (Figure 2) These engineered variants serve the purpose of specificity and multiplexing of CRISPR-Cas9 genome editing tools by which it stands above the other genome engineering tools such as HR, ZNFs and TALENs. HNH and RuvC like catalytic domains of wt-Cas9 cleaving the target DNA strand and the opposite strand respectively is artificially inactivated and reprogrammed into nCas9 (nickase) generating the single strand break (SSB). Engineered nCas9 can also be exploited to generate paired nicks directed by a pair of sgRNA targeting opposite strands of a target locus. This system mediates high efficiency NHEJ at levels comparable to those induced by wt-Cas9 suggesting that it can potentially reduce the likelihood of off-target modifications and thus suitable for genome editing with increased specificity. Furthermore, this system has proved to be efficient in multiplex nicking situating homology directed repair, micro-deletion and insertion along with the efficient modification of mouse zygote[21]. Inactivating both the HNH and RuvC domains, wt-Cas9 is modified into dCas9 which when fused to various effectors can act as a site-specific DNA binding vehicle. This can be exploited to target a protein at the targeted segment of the DNA, thus, providing a new dimension for the study of transcription, epigenetic regulation and both DNA replication and repair[22]. RNA scaffolds also known as scRNA can be incorporated to the tetraloop, stem loop or 3'end of sgRNAs and thus can be used to recruit protein effectors onto target genome loci[22,23]. Sets of scRNAs have been used to generate synthetic multi-gene transcriptional programs where one gene is activated whereas other gene is repressed. These systems can be exploited along with dcas9 proteins to act as a single regulatory control point which can be used to build synthetic gene expression programs for wide range of applications including rewiring cell fates or engineering metabolic pathways[23]. CRISPR-Cas9 also holds RNA cleaving property. By introducing an exogenous PAM-containing oligonucleotide (PAMmer) as an artificial complimentary DNA strand, CRISPR-Cas9 can be repurposed into RCas9, a programmable single stranded (ssRNA) cleaving tool [24]. This can be further modified into dRCas9 which can act as a site specific ssRNA binding domain that when fused to effectors may mediate diverse functions such as RNA splicing modulation, RNA editing and RNA imaging. Within a few decades of its discovery this bacterial adapted defense mechanism has gained much popularity as its implementation has been done in various fields. Modified CRISPR-Cas9 into several varieties has their own advantages over the conventional systems and has been successfully applied in various sectors apart from DNA modeling. CRISPR-Cas9 thus provides a robust technology for studying genomic rearrangements and the development and progression of cancers or other diseases.

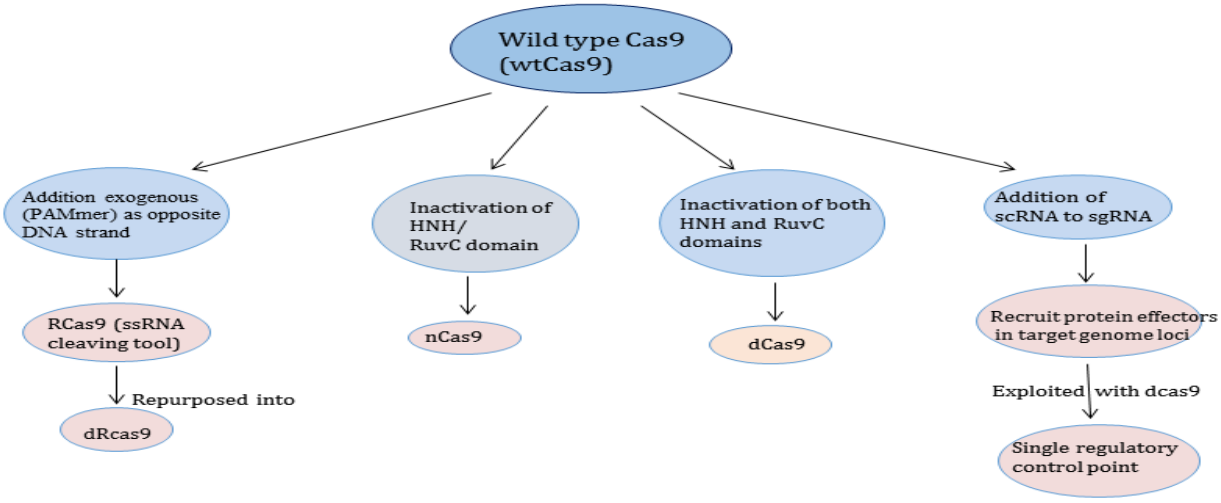

Figure 2. Modifications of wild type Cas9(wtCas9) into various purposes

Cas9 in Cancer Research, Diagnosis and Therapeutics

Cancer is a result of the alteration of normal genetic pattern, which is brought about by multiple genetic and epigenetic modifications in oncogenes and tumor suppressor genes[25]. The study of role of genes associated in cancer initiation, progression and therapeutic response has been possible with the development of genetic engineering technologies. These technologies have been successfully utilized in precisely deleting or otherwise modifying the specific DNA sequence in the genomes of cells in culture or of animal models. Among the different genome editing tools available, HR is found to be relatively less efficient in gene targeting as well as is time consuming whereas, the application of ZNF and TALENS are limited by the cost and the complexity of designing the custom-built endonuclease[26]. Difficulties of protein design, synthesis, and validation remained a barrier to wide spread adoption of these engineered nucleases for routine use. These limitations in gene modeling have been addressed by the introduction of Streptococcus pyogenes-derived type II CRISPR-Cas9 system. This technology has been successful in modifying, regulating and marking genomic loci in diverse cells and organisms[27]. CRISPR Cas9 is highly efficient in cleaving the target DNA by the combining the expression of Cas9 with a sgRNA complimentary to the target DNA that is rapidly repaired via NHEJ or HDR.

The basic mechanism of tumorigenic process is the gain of functions of oncogenes whereas loss of functions of tumor suppressor genes. So the treatment of cancer should aim at inactivating the gain of functions and loss of functions of oncogenes and tumor suppressor genes respectively. This phenomenon can be achieved by the precise application of CRISPR-Cas9 genome editing technology. CRISPR-Cas9 technology guided by the guide RNA can efficiently cut the desired loci of a genome by the Cas9 nuclease. DNA breaks so obtained can be repaired by either NHEJ or HDR, which results in generation of indels or insertion of HDR template carrying the desired mutations respectively[28]. It has

successfully been applied in various genome-scale loss of function screening which is a fundamental strategy for investigating physiological and pathological functions of a defined gene[27]. Genetic screening done in human cell lines with a library containing more than 70,000 sgRNAs has been performed and been successful in establishing Cas9/sgRNAa screens as a powerful tool for systematic genetic analysis in human cells[29]. Genome scale CRISPSR/cas9 knockout library has been developed and successfully used to screen genes associated with resistance to verurafenib, a cancer therapeutic agent for late state melanoma[30]. CRISPR-mediated repression (CRISPRi) and activation (CRISPRa) has been demonstrated as robust tools for functional genome screening in gene expression modulation. CRISPRi has been efficiently exploited to inhibit the transcription of target genes in E. coli and mammalian cells when dCas9 is recruited to transcriptional inhibitory domain [22], whereas to activate the expression of target endogenous genes when dCas9 is tethered to a transcriptional activator domain [31,32]. Genome-scale CRISPRi/a libraries have been successfully employed in identification of mediators for cellular sensitivity to a cholera-diptheria fusion toxin, as well as essential genes for proliferation, differentiation and tumor suppression. Additionally, CRISPRa helps in identification of a novel gene with its gain-of-function properties. dCas9 when complexes with sgRNA has shown to successful in simultaneous activation of multiple genes, up regulation of long non-coding RNA transcripts and identifying genes conferring resistance to a BRAF inhibitor in melanoma[32] signifying the versatility of this system in discovering crucial genes in various biological processes.

There are evidences of rapid generations of animal models with the application of CRISPPR-Cas9 models which are potent tools for understanding human disease pathogenesis and developing novel therapeutics. It has been widely used for therapeutic purposes in variety of pathologies by correction of aberrant genetic pattern. Genetically modified KO/KI mice models, somatic genome editing models and large scale genome modified mice have been generated[33,34,35,27,70]. Introduction of nCas9 to target multiple gene at the same time promotes the study of multiple gene interactions. The generation of precision cancer mouse models provides a rapid avenue for functional cancer genomics and paves the way for precision cancer medicine[35]. Somatic genome editing in vivo in different models demonstrates rapid functional identification of putative human disease. Hydrodynamic injection of CRISPR plasmid DNA to the liver targeting the Pten and p53 tumor suppressor genes resulted in insertion or deletion mutations of the genes including bi-allelic mutations of both genes in tumors. Thus demonstrates the feasibility of direct mutation of tumor suppressor genes and oncogenes in the liver[36]. Lung adenocarcinoma has been induced by somatic activation of oncogenic Kras (G12D) where pSECC, a lentiviral–based delivery system delivered CRISPR system and Cre recombines to selectively target lung tissues[26]. The CRISPR/Cas9 technology has been proved to be effective in large mammalian animal models, such as pigs and non-human primates. Gene modified pigs have been generated successfully by the co-injection of Cas9 mRNA and target gene sgRNA into one-cell stage embryo[37]. Recently Yang L demonstrated that pigs are almost perfect alternative for engineering human

transplantation organs where she was a successful in knocking out of all copies of the PERV pol gene in pigs and triggered a 1000-fold reduction of PERV infectivity of human cells[38]. Non-human primates are considered the best animal model for studying human neurodegenerative diseases. An important milestone was the first genetic modifications carried out in primate embryos. Here, CRISPR was introduced in one-cell embryos to successfully generate modified cynomolgus monkey. In 2015, Chen et al.[69] first achieved precise gene targeting in cynomolgus monkeys by co-injection of Cas9 mRNA and sgRNAs into one-cell-stage embryos. Owing to a closer relationship of tree shrew to primates and humans and their superiority in manipulating, maintaining and propagating, CRISPR cas9 can be introduced to generate tree shrew model in breast cancer research and drug development[39].

Human diseases due to fault in the genetic elements can be treated by editing the defective genes with the introduction of new genes by the CRISPR cas9 system. It has been found that Cas9mRNA and sgRNA when injected together along with HDR template targeting the mutant Crygc allele into zygotes, corrected the dominant Crygc mutations in a cataracts mouse models[40]. Similarly, Duchenne muscular dystrophy (DMD) has shown to be corrected by effective gene modification of dystrophic gene in the germ line of mdx mice[41]. This robust technology can be implemented as a promising antiviral therapy. Significant reduction in cccDNA and other parameters of viral gene expression and replication was observed when Cas9 and sgRNA was introduced targeting cccDNA[71]. Thus, directly targeting viral episomal DNA is a novel therapeutic approach to control the virus and possibly cure patients. The latent reservoir of HIV-1 virus which provides a threat to re-emerging of infection at any time when transfected with CRISPR-Cas9 system showed effective cleaving and mutation of LTR (long terminal repeats) target sites and also was able to remove internal viral genes from the host cell chromosome. This suggests that the CRISPR-Cas9 system can be a therapeutic agent in curing HIV-1 infection[42]. CRISPR/Cas9-based gene therapy offered a new tool to modify the targeted intervention points, such as CD4+ receptor and the CCR5 which aim at establishing HIV-1 resistance[43]. Cas9/gRNA shown to inactivate HIV-1 viral gene expression and replication in latently infected microglial, promonocytic, and T cells and also prevented HIV-1 infection suggesting that Cas9 can provide a specific, efficacious prophylactic and therapeutic approach against AIDS[44]. Further, it can perform as a long term adaptive defense against new viral infection as engineered human IPS (Induced Pluripotent Stem Cells) expressing Cas9 and HIV-targeted sgRNA was differentiated into HIV reservoir cell types providing resistance to HIV-1 challenge[45]. In cells derived from human Burkitt's lymphoma with latent Epstein-Barr virus infection, treatment with CRISPRCas9 targeting the viral genome resulted in cell proliferation arrest and decreased viral load demonstrating the specificity in targeting the genomes of latent viral infections, which cannot be eradicated by standard antiviral therapies[46].

The CRISPR-Cas9 technology is more precise and effective in gene editing overcoming the limitations caused by the transgenic technologies. This system is able to create both germ line and somatic animal models with point mutations, deletions and complex chromosomal rearrangements and thus could be directly applied to pain related

genes. CRISPR Cas9 has shown efficient to alleviate nerve injury-induced pain hypersensitivity when targeted to genes encoding opioid receptors and voltage gated potassium channels[47].

Optimizing CRISPR-Cas9 delivery

Optimizing the amount of nucleases to be introduced and at the same moment minimizing the potential undesired results is a major difficulty while introducing this technology. As optimum level of nucleases and donor DNA templates are required to obtain desired genetic modifications. Low level of nuclease encoding plasmids and donor DNA leads to insufficient HDR rate to have a reasonable level of gene correction where as if the amount of nucleases in cells is too high, a large amount of cell death occurs due to cytotoxicity[48]. The established methods for gene delivery include lipofection, microinjection and electroporation. The effectiveness of the gene editing technology is dependent on concentration and identity of transfection material thus necessitating the selection of appropriate delivery method and medium. Cationic lipid materials are commercially available which have a wide range of RNAs encoding Cas9 system[49]. Microfluidic membrane deformation method to deliver sgRNA and Cas9 into various cell types such as hard to transfect lymphoma cells and embryonic stem cells while maintaining high cell viability have been developed and have demonstrated to be highly efficient in genome editing and successful in generation of gene-knockout cell lines. This approach results in rapid cell mechanical deformation to generate transient membrane disruptions to facilitate diffusion of biomaterials into the cytosol[50]. Electroporation is widely accepted technique that allows high efficient genome editing via. Delivery of purified Cas9 ribonucleoprotein[51]. Ribronucleoprotein (RNP), which is formed when recombinant Cas9 protein was complexed with SgRNA or dual RNA consisting of crRNA and tracrRNA has been directly delivered into cells via electroporation and showed efficient genome editing in human cells (human leukemia K562 cell line), primary human cells and embryonic stem cells reducing the incidence of off-target effects and avoiding unwanted integration of plasmid DNA in the host genome. To target several other loci in K562, new CRISPR-Cas9 system can be prepared by simply replacing sgRNA in the RNP complex[51]. Plasmid has been serving as a vector for transfecting cells in culture where plasmid can function as a single vector[52] expressing both gRNA and Cas9 mRNA or individually carrying gRNA template and Cas9 gene to the cells to be transfected[53]. The widely accepted method for gene editing in mice is microinjecting gRNA and Cas9 mRNA or protein into the pronuclear or the cytoplasm of the zygote where the gRNA is transcribed in vitro from a T7 bacteriophage promoter[54]. Cationic lipid based vectors could act as a delivery method as anionic nature of Cas9-gRNA could be integrated into the cationic liposome. Cationic liposomes have shown to be successful in introducing Cas9-sgRNA to achieve genome editing in the mouse inner ear in vivo[55]. The feasibility for delivery of the CRISPR/Cas9 system is enhanced by the cationic nature of cationic polymer nanoparticles. Polyethylenemine (PEI) is the most commonly used cationic polymer which prevents DNA and endosomal escape through proton sponge effect by the secondary amine. The transfection efficiency and toxicity of PEI is further dependent on the structural properties, degree of branched linearity and its molecular weight[56]. Cell penetrating peptides (CPP) based delivery methods offers precise gene alteration with minimum

off target mutations in human cells like embryonic stem cells, HEK-293T cells, Hela cells etc. In this technique sgRNA was complexed with CPP to form condensed and positively charged nanoparticles and Cas9 nuclease is fused by the thioether bond. This technique has an advantage that it transfers Cas components directly without reagents[57]. The Adeno-Associated Virus (AAV) is another, promising to deliver the Cas system into the targets. It has demonstrated to be successful in delivering Cas system in adult mice brain cells via stereotactic injection and in cardiac muscle in mdx mice by tibialis anterior muscle injection[58]. Lentivirus, a virus of retroviridae family is a promising tool for delivering CRISPR-Cas which is capable of supporting stable expression of large transgenes[59].

CRISPR Cas Challenges

The bacterial derived immunity against the invading viruses has been implemented successfully as a robust editing tool for modifying gene, investing gene function and as therapeutics in living cells. However, it is not devoid of pitfalls. Off-target cleavage of the genome is the major disadvantage of this editing tool that is governed by several factors. The off-target effects of CRISPR Cas9 genome editing tool is governed by several factors. Both Cas9 and sgRNAs can cause CRISPR-Casa9 off target mutations. NGG trinucleotide is a prerequisite for a guide RNA to drive Cas9 nuclease to cleave the specific portion of DNA. However non-canonical PAMs such as NAN, NTG and have also been recognized that can potentially drive nuclease to cleave the off target portion of genome thus leading to off target mutations[60]. Cas9 has been found to bind to a sequence with as many as 10 mismatches in the spacer of crRNAs[61], yet reported to cleave and induce mutations at sequences with 3-5 mismatches[62]. Furthermore, Cas9 off target cleavage at similar gene sequence with a base pair mismatch may lead to gross chromosomal deletions with high frequencies, as demonstrated by the deletion of 7-kb sequence between two cleavage sites in HBB and HBD[63]. sgRNAs with extremely high or low GC content (i.e >80% or < 20%) are ineffective against their targets thus not achieving the desirable outcomes[29]. The overexpression of both cas9 protein and sgRNA by plasmid DNA has also shown to induce off-target mutagenesis when higher amounts of DNA are transfected[64]. Non-specific off-target DNA cleavage is found to be brought about by partially matching on and off target sites which can be either due to same length with base mismatches or deletions of one or more bases or insertions of one or more extra bases in off-target sites of the genome[65].

Reducing off-target mutations

Point identification of the target segment in the genome is the first and foremost criteria for reducing off target effects, wherein the target site that differs from any other sites in the genome by at least 2-3 nucleotides in a 20-nt sequence is selected[66]. Web based computer algorithms are available which are highly efficient in searching potential off target sites and unique target sequence in the genomes of humans and other organism of research interest, also introducing a scoring system for choosing sgRNAs with minimum off target sites and providing user-friendly sgRNA designer program[67]. Modifications in sgRNAs length and designs have found to considerably reduce the off target cleavage of the genome which has been demonstrated by adding two extra guanine nucleotide at the 5'terminus or by creating truncated sgRNAs(tru-sgRNAs) with 17-nt rather than 20-nt to increase the specificity of RNA guided genome editing[66,68]. Cas9 nuclease

can be modified into paired nickases (nCas9) which can generate two separate breaks or nicks in the two strands of DNA .These breaks created has been repaired with high fidelity and has demonstrated to reduce off target activity by 50-1000 folds in cell lines and facilitated gene knockout in mouse zygotes without sacrificing on-target cleavage efficiency[21]. Prolonged expression of Cas9 and sgRNAs into the cells can lead to the accumulating mutations and thus increasing the off-target mutagenesis. CRISPR-Cas9 delivered as a Ribonucleoprotein (RNP) complexes have found to induce mutations at target sites more efficiently than that delivered encoding plasmids. Plasmids are shown rapidly integrated into the host genome. Unwanted insertion of plasmid DNA sequence at undesired sites are difficult to identify and therefore have more serious complications than those at on–target sites. RNPs can cleave chromosomal DNA almost immediately after delivery and the mutation reached the plateau the other day of electroporation whereas plasmids transfection took 3 days to reach the same level of mutations and also have shown to express in cells for several days thus, giving rise to accumulation of off target mutations[51]. This wise to choose those delivery methods, which limit the time required to obtain the desired outcome thus minimizing off target mutagenesis.

Conclusion

CRISPR Cas9 has already proved to be the most efficient genome-editing tool. The journey of CRISPR Cas from the discovery in prokaryotes to its implementation in the human cells is very short but the modifications made for optimizing this technology is enormous. Different factors have found to be contributing to the efficiency and precision editing of targeted gene with CRISPR Cas technology. CRISPR Cas9 is supposed to provide the best outcome which has been brought about by the modification of nuclease for the specific purpose, optimization of sgRNA for efficient targeting and optimizing CRISPR delivery assisted by the availability of the highly innovative designer programs. The biggest challenges of this technology are in implementing it in human cell. The genetic alterations which can even be brought about by the point alteration in genetic element can be very devastating as this unusual alteration not only has the present affect but also can be inherited to the future cell line resulting to the long term genetic alteration. Owing to the fact that this technology can be exploited to different parts of life and also turned to be the most appreciated technology, one should be very peculiar in implementing this technology. Indeed, various levels of optimizations made so far in different elements of this technology has driven this technology to be the highly sophisticated technology which is serving human mankind in its best possible way. However, considerations are still being made before introducing this intellectual technology in human live cells.

Conflict of interests

No authors declared any competing interests.

Acknowledgement

N/A

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