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Crispr/Cas9 Technology and Cancer Therapy: A Brief Review on Therapeutic Methods and Challenges

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1. Abstract

Cancer genomes often harbor diverse genetic aberrations that are mainly related to carcinogenesis, cancer development, and metastasis. CRISPR/Cas9 technology is a bacterial immune system that can oppose plasmid in bacteria or exogenous viruses. CRISPR/Cas has been used in life science studies and its application has undergone notable changes and modifications. Lately, the CRISPR/Cas9 method has been broadly utilized in cancer therapeutic investigations as it is a highly efficient method. Several studies utilized CRISPR/Cas9 to directly target potential therapeutic genes in wide range of in vitro and in vivo cancer models. Furthermore, it can likewise be applied to battle oncogenic disease, discover anti-cancer drugs, design oncolytic viruses, and modify immune cells for cancer immunotherapy. It is a strong technology for the identification of mechanisms underlying tumorigenesis and it also a promising method for cancer treatment. Here, we provide a brief overview of current preclinical CRISPR-based studies involving therapeutic targets for the inhibition of cancer cells and discuss recent advances and cha llenges in bringing CRISPR/Cas9 from the bedside to the clinic.

2. Introduction

Cancer is one of the most lethal diseases in human life [1]. It is characterized by the 38 accumulation of genetic and epigenetic aberrations in the genome of a cell over time [2]. Despite recent advances in cancer therapy, tumor relapse and primary or secondary drug resistance eventually result in poor prognosis. Thus, additional advances and novel treatment strategies are required.

CRISPR was first found in the genome of archaea and bacteria as an adaptive immune response to foreign genetic components. It has been broadly used as an accurate genome editing tool to identify therapeutic targets for cancer therapy in several preclinical studies [3-5]. CRISPR/Cas9 can distinguish genomic loci through its Single Guide RNA (sgRNA) and edit the loci efficiently through the Cas9 protein. The precision and adaptively of CRISPR/Cas9 technology has been used in biomedical studies and has brought novel opportunities to the field of cancer treatment [6]. Utilizing sgRNA, the Cas9 endonuclease can be guided to the target site to create DNA Double- Strand Breaks (DSBS). This triggers a DNA repair mechanism resulting in site specific genomic modifications. The CRISPR/Cas9 technique has also been used as one of the most feasible and adaptive techniques for cancer modeling and treatment investigations [7-9]. Additionally, CRISPR/Cas9 has promising potentials in cancer studies including managing functional genomics/epigenomics, modeling the genesis and development of

in vivo and in vitro cancer models, targeting non-coding RNA, screening for new therapeutic targets [10], and identification of potential resistance mechanisms in different cancer types [3, 6, 11, 12]. This technique may also be utilized to employ oncolytic viruses and immune cells for cancer immunotherapy (Figure 1) *In vivo* gene editing treatment, the Cas9/sgRNA combination is systemic or targeted conveyed into the patient through either non-viral or viral vectors for gene editing in cancer treatment, for instance, transcriptional regulation of cancerous cells or (epi) genome alteration. In vitro gene editing immunotherapy, patient-obtained immune cells are separated and genetically altered with CRISPR-Cas9 technique, and afterward implanted back into the same patient; for oncolytic treatment, oncolytic viruses are checked and presented into patients to destroy cancerous cells [13, 14]. Depending on the transfection method, i.e., viral, or non-viral vectors, CRISPR/Cas9 can mediate accurate gene modifications or disruptions both in vivo and in vitro [6, 15-18]. CRISPR-based gene activation (CRISPRa) and inhibition (CRISPRi) are also effective approaches for the gain and loss of function studies, respectively. The CRISPRi system is a much stronger tool as compared to RNA Interference (RNAI) to induce loss of function (Figure 2) The CRISPR, CRISPRi, and CRISPRa technologies. (A) The CRISPR-Cas method comprises of a structured chimeric sgRNA and Cas9 protein complementary to the genomic target sequence (blue line). Upon binding to the specific DNA sequence by Cas9-sgRNA complex, the DNA is cleaved by Cas9 with endonuclease activity. CRISPR interference (CRISPRi) is caused by a catalytically dead Cas9 (D10A and H840A mutations indicated by red dots), denoted as dCas9. The dCas9-sgRNA complex binds to the upstream region of the gene of interest, resulting in that the process of RNA polymerase (RNAP) is inhibited and consequently transcription is blocked. CRISPR activation (CRISPRa) is applied for gene activation by the fusion of dCas9 and transcription activators such as the RNAP ω subunit in E. coli. (B) Double strand break formed by CRISPR/Cas system can be repaired by the error- prone non-homologous end joining (native NHEJ) pathway, resulting in the formation of random sized deletions around the targeted DNA sequence. With the expression of a ligase such as LigD, The NHEJ pathway creates precise editing (reconstituted NHEJ). With the presence of a homologous template, the gene deletion or replacement by homology-directed repair (HDR) is generated with high efficiency and the precision with near 100% frequency [19]. CRISPR libraries can help recognize vital cancer-survival genes leading to the identification of potential therapeutic candidates for targeted medicines [14]. The CRISPR/Cas9 technology has also been applied to alter cancer-causing gene deletions/mutations and to modify immune cells, includes chimeric antigen receptor (CAR) T cells, for cancer immunotherapy [20].

In this brief review, we discuss applications of CRISPR/Cas9 technology in cancer preclinical studies. We also provide a short overview of the current advances and present obstacles in the application of CRISPR/Cas9 in the clinic.

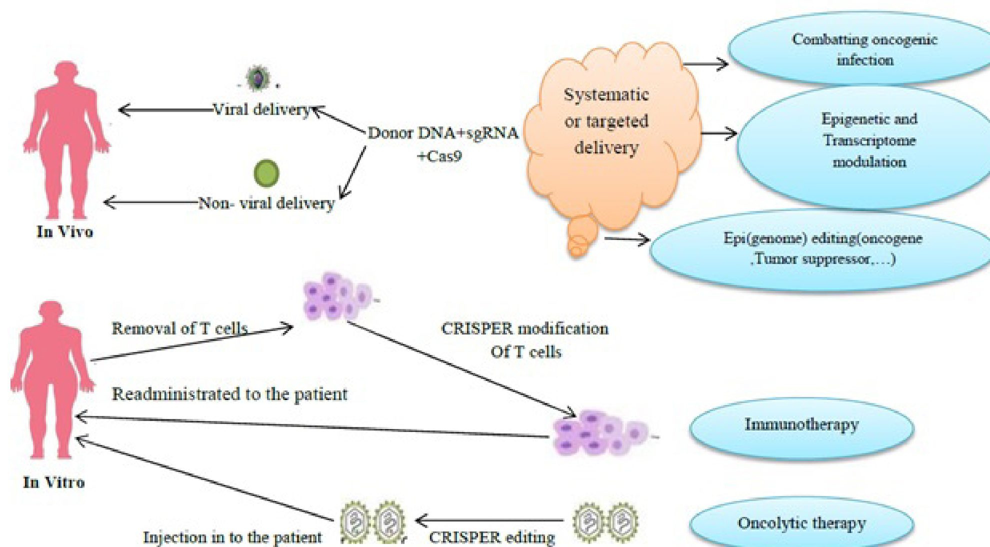

Figure 1: In vivo and in vitro gene editing therapeutic system for CRISPR-Cas9 cancer treatment

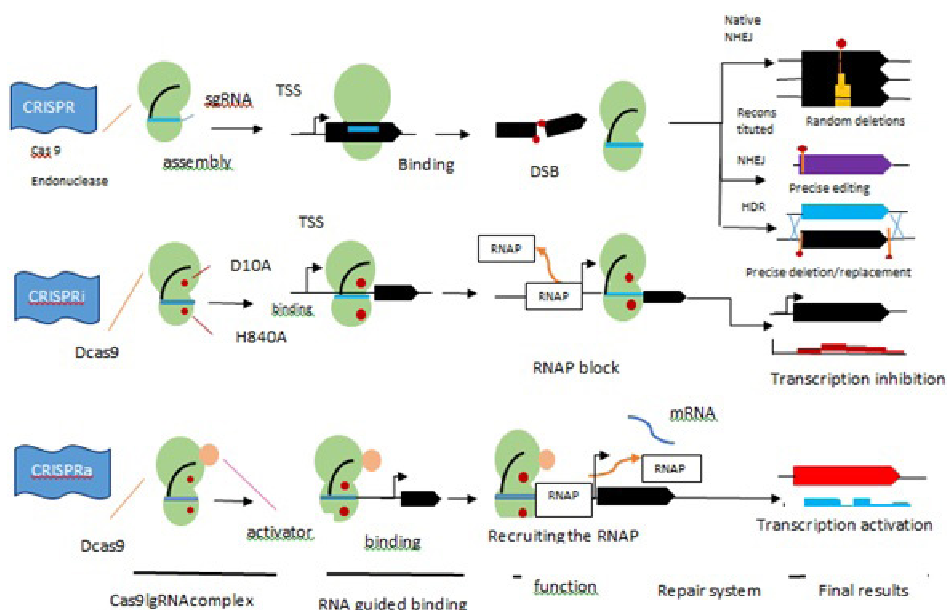

Figure 2: The CRISPR, CRISPRi, and CRISPRa technologies

3. The Structure and Advantages Of CRISPR/Cas9

The CRISPR/Cas system was discovered, for the first time, as a type II adaptive immune system of Streptococcus pyogenes which protect bacteria against phages and viruses. This system consists of three core components endonuclease Cas9, trans activating crRNA (tracrRNA) and a single guide RNA (sgRNA). The RNA-guided CRISPR/Cas9 has many benefits over standard genome engineering tools (Table 1) [21-23]. It requires a short complementary sgRNA for targeting DNA. Synthesizing sgRNA for a specific target is generally simple and cost-efficient. In addition, CRISPR/Cas9 technology can be used to simultaneously target independent genomic loci by utilizing a pool of several sgRNAs for each target [24]. This makes it a high-throughput approach for genomic modifications [24, 25]. The Cas9 can also be used to target the epigenome by the catalytically deactivated Cas9 (dCas9) [26]. The Specificity of CRISPR is the main concern, as several studies have shown CRISPR off-target effects [27]. Therefore, various techniques have been improved to reduce off-targets. One such technology is to interchange plasmid conveyance for the cellular conveyance of in vitro-assembled RNP compounds resulting in longer-lasting sgRNA and Cas9 expression as well as enhancing the proportion of on-target in mammalian cells providing an extremely effective editing [28-32]. Other techniques have been developed include using Cas variants which are inducible by light or small particles [33-35].

Table 1: Comparison of between standard gene editing (engineered) nucleases and CRISPR/Cas9

Feature	ZFNs*	TALENs**	CRISPR-Cas9***
Time first introduced into mammalian(year)	2000	2011	2013
Target sequence recognition	Zinc fingers protein, protein-DNA interactions	Repeat variable diresidues (RVDs) repeats, protein- DNA interactions	sgRNA, RNA-DNA interactions via Watson- Crick base pairing
Recognition sequence	Sequence containing G base as follows: 5'-GNNGNNGNN-3'	Sequence starting from 5'- T and ending with A-3'	Sequence immediately followed by an adjacent protospacer motif 5'-NGG-3'
DNA binding	Zinc finger protein	TALE protein	Guide RNA
DNA sequence recognition size	(9 or 12 bp) × 2	(8–31 bp) × 2	17–20 bp + NGG × 1
Endonuclease/DNA cleavage and repair	Double-strand break induced by FokI	Double-strand break induced by FokI	Single- or double-strand break induced by Cas9
Endonuclease construction	3–4 Zinc fingers domains	8–31 RVD repeats	sgRNA synthesis or cloning
Targeting efficiency	Low	Moderate	High
Off-targeting effect	Moderate	Low	Variable
Cytotoxicity	High	Low	Low
Easy of experiment	Difficult and time consuming	Moderate and time consuming	Easy and rapid
Cost of experiment	High	High	Low

Clinical development	Phase 1/2	Phase 1	Preclinical
Design	Challenging. zinc finger motifs assembled in arrays can affect specificity of neighboring zinc finger motifs, making the design challenging.	Easy. TALE motifs with target specificities are well defined.	Easy. SgRNA design based on complementarity with the target DNA.
Cloning	Requires engineering linkages between zinc finger motifs.	TALEs do not require linkages. Cloning of separate TALE motifs can be done using Golden Gate assembly5.	Expression vectors for Cas9 available. SgRNA can be delivered to cells as a DNA expression vector or directly as an RNA molecule or pre-loaded Cas9-RNA complex.
Advantages	Sequence-based module engineering Small protein size (<1 kb)	High specificity Accurate recognition by 1 bp Relatively easy selection of target region	Free selection of target region Simple synthesis of guide RNA Multiplexing ability
Limitations	Difficult sequence selection and zinc finger engineering Expensive and time- consuming	Not applicable to methyl cytosine Expensive and time- consuming Large protein size (>3 kb)	Large protein size (>4 kb)

4. The CRISPR/Cas9 Technology and Genetic Screening in Cancer

The CRISPR/Cas9 technique is a strong tool for gene knockout that has transformed gene editing in single genes and multiplex loss-of-function screening [36, 37]. A computational technique, CERES correction was developed to evaluate the gene-dependency degree from CRISPR/Cas9 essentiality screens while accounting for the copy number-specific impact. The outcomes proposed that CERES correction reduced false-positive in copy number-amplified regions. Furthermore, cancer-type-specific vulnerabilities might be recognized after CERES correction [37]. Cancer genomes harbor numerous genetic aberrations that are involved in carcinogenesis and may be used as potential therapeutic targets [33].

Rauscher designed MINGLE, a computational method to examine and analyze information from CRISPR/Cas9 screening of human cancer cells to discover more than 2.1 million gene basis connections. Novel genotype-specific vulnerabilities of cancer cells, PRKCSH, and GANAB were identified as novel positive regulators of the Wnt/β-catenin signaling pathway that might be used as potential therapeutic targets [38].

5. Screening Anti-Cancer Drugs Target and Drug Resistance Genes

Identification of novel cancer therapeutic targets is critical in cancer treatment. One main issue in 108 the development of anti-cancer medicine is the validation of drug targets and identification of drug resistance genes. The CRISPR/Cas9 technology can be utilized to identify and validate genetic aberrations in anti-cancer drug resistance. For example, ispinesib, a suppressor of kinesin-5 is under assessment in clinical trials as an anti-cancer agent for osteosarcoma treatment [39]. Kasap showed that A133P cells gained resistance to ispinesib after long-term treatment, confirming that kinesin-5 is the direct target of ispinesib in cancer cells [40]. Selinexor is a promising anti-cancer drug that suppresses exportin-1 and is currently being assessed in clinical trials for the treatment of patients with prostate and myeloma cancer [41]. Utilizing CRISPR-Cas9, Neggers showed that the exportin-1 cysteine residue is the main target of Selinexor [42]. In addition, CRISPR-based ablation of SMARCB1 protected cancer cells from the chemotherapeutic doxorubicin [43]. The CRISPR/Cas9 technology can also be applied to identify novel targets by focusing on exons that encode functional protein domains [44]. CAR T-cell therapy focusing on the CD19 antigen has been the most studied and successful approach because of the expression of CD19 in B cell leukemia. In 2016, for the first time, CRISPR altered T-cells were infused to a patient with aggressive lung cancer to deactivate PD-1 [45]. Currently, CAR T-cell therapy has just gotten an endorsement from the FDA for the treatment of relapse and resistant B-cell acute lymphoblastic leukemia in children and adults [46].

6. Future Perspective and Conclusions

CRISPR-associated nucleases have revolutionized genomic engineering by providing a simple method for the mammalian genome modifications. They are utilized in basic research as well as pre-clinical and clinical studies as a powerful approach to reverse disease-causing mutations in inherited genetic disorders. Commercial and non-commercial advancements have allowed consistent improvements in innovations which started only 20 years ago. They will be used more widely in the future in biomedical sciences. The recent developments in the CRISPR/Cas9 technology combined with conventional therapy might be beneficial for cancer patients.

Radiotherapy is one of the most effective treatment methods for cancer therapy. However, tumors with different gene mutations such as p21 and p53 mutations have poor radiation affectability and

frequently results in radiotherapy failure [6]. Using CRISPR/Cas9 to particularly correct these mutations in cancer cells or to hinder the cellular radiation damage repair signaling pathways might be a potential treatment strategy to improve tumor response. A combination of CRISPR mediated gene therapy and radiotherapy with synergistic anti-cancer agents might be a promising treatment option for patients with cancer. Editing genomic DNA in cancer cells using CRISPR/Cas9 might be essential in personalized treatment soon. Functional evaluation of the mutations with unknown effects or assessment of the proposed resistance mechanisms may help to attain successful treatment for a given patient. For instance, CRISPR/Cas9 technology may be used to modify the mutated EGFR in patients with lung cancer [47]. Despite remarkable improvements in technology, we are still a long way away to use CRISPR/Cas9 as a therapeutic method in the clinic. In addition, more fundamental studies are required to minimize its off-target effects and to find an efficient delivery method to the target cells. Furthermore, the potential host immune responses against the CRISPR/Cas9 elements remain unknown [48]. Although important challenges are still present, a meticulous validation in animal models for therapeutic application of CRISPR/Cas9 is crucial. CRISPR/Cas9-based technologies have great therapeutic potential for the development of anti-cancer drugs that may provide precise genomic alterations and gene editing for transcriptional regulation and epigenetic modulation or modifications of immune cells for cancer treatment [49].

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