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Review

CRISPR-Cas9 therapeutics in cancer: promising strategies and present challenges☆

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ABSTRACT

Cancer is characterized by multiple genetic and epigenetic alterations that drive malignant cell proliferation and confer chemoresistance. The ability to correct or ablate such mutations holds immense promise for combating cancer. Recently, because of its high efficiency and accuracy, the CRISPR-Cas9 genome editing technique has been widely used in cancer therapeutic explorations. Several studies used CRISPR-Cas9 to directly target cancer cell genomic DNA in cellular and animal cancer models which have shown therapeutic potential in expanding our anticancer protocols. Moreover, CRISPR-Cas9 can also be employed to fight oncogenic infections, explore anticancer drugs, and engineer immune cells and oncolytic viruses for cancer immunotherapeutic applications. Here, we summarize these preclinical CRISPR-Cas9-based therapeutic strategies against cancer, and discuss the challenges and improvements in translating therapeutic CRISPR-Cas9 into clinical use, which will facilitate better application of this technique in cancer research. Further, we propose potential directions of the CRISPR-Cas9 system in cancer therapy.

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Abbreviations: AAVs, adeno-associated virus vectors; AdV, adenoviral; BL, Burkitt lymphoma; CAR, chimeric antigen receptor; Cas9, CRISPR-associated protein 9; CPPs, cell-penetrating peptides; CRISPR, clustered regularly interspaced short palindromic repeat; crRNA, CRISPR RNA; dCas9, catalytically inactive Cas9; DSBs, double strand breaks; EBV, Epstein-Barr virus; FnCas9, Francisella novicida Cas9; GOF, gain of function; HBV, hepatitis B virus; HCV, hepatitis C virus; HDR, homologous-directed repair; HPV, human papillomaviruses; HR, homologous recombination; HSV, herpes simplex virus; LOF, loss of function; MDR1, multidrug resistance gene 1; NHEJ, nonhomologous end-joining; OVs, oncolytic viruses; PAM, protospacer adjacent motif; RNAi, RNA interference; sgRNA, single guide RNA; ssODNs, single-stranded DNA oligonucleotides; TALENs, transcription activator-like effector nucleases; TK, thymidine kinase; tracrRNA, trans-activating crRNA; VV, vaccinia virus; XPO1, exportin-1; ZFNs, zinc finger nucleases.

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1. Introduction

Cancer currently remains one of the most serious diseases challenging human life and public health [1]. Although important progress has been made in cancer therapy, including surgery, radiation therapy, and/or chemotherapy, the high tendency of relapse and the primary or acquired chemo/radiation resistance often result in poor prognosis. Therefore, further improvements and novel therapeutic approaches for cancer are urgently needed.

It is well established that cancer is characterized by the accumulation of multiple genetic and epigenetic alterations in the cancer cell genome, which drive cancer pathogenesis and development. These alterations include activated oncogenes (e.g. *ErbB*, *RAS*), inactivated tumor suppressors (e.g. *p53*, *PTEN*), mutations in epigenetic factors and their control loci (e.g. *DNMT1*), mutations in genes that confer chemoresistance (e.g. multidrug resistance gene1, *MDR1*), and others [2]. Therefore, the ability to correct or disable one or more sections of the cancer cell genome, for instance, restoring tumor suppressor genes function, may provide an attractive approach for cancer therapy [3]. In recent years, many therapeutic studies have been conducted with the rapid development genome engineering techniques that can precisely target any gene in the cancer cellular genome and establish knock-in and knockout alterations. These targeted gene therapeutic strategies lead to long-term activation or repression of cancer related molecular targets, and thus can avoid the numerous cycles of therapy. Ideally, such anticancer treatments require a tool that can specifically and robustly induce correcting genetic changes with limited off-target toxicity.

Conventional nuclease-based gene targeting technologies, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), have been utilized for generating animal and cellular cancer models and for therapy explorations [4–8]. However, their widespread use has been restricted by the complexity and time consuming engineering of these nucleases. RNA interference (RNAi) is a convenient approach for gene expression engineering and has been increasingly used in cancer therapy research; however, the knockdown effect of RNAi is temporary and incomplete, and must be repeatedly administrated to achieve an acceptable level of knockdown [9]. Moreover, RNAi often has pervasive unpredictable off-target effects [10].

More recently, a new RNA-guided endonuclease-based genome editing technique termed the clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (Cas9) system has been developed [11]. Using a single-guide RNA (sgRNA), the Cas9 endonuclease can be precisely guided to target sites to produce DNA double-strand breaks (DSBs), which initiate DNA repair processes that give rise to site-specific genomic modification. Since it was first applied in mammalian cells and animals three years ago, the CRISPR-Cas9 system has been used as the most facile and versatile platform for cancer modeling and treatment explorations [12–14]. In this brief review, we summarize and discuss the potential of the preclinical CRISPR-Cas9-based therapeutic strategies against human cancers, as well as the challenges and improvement in translating therapeutic CRISPR-Cas9 into clinical use. Further, we propose the potential directions of the CRISPR-Cas9 system for future cancer therapy research.

2. Mechanisms and advantages of CRISPR-Cas9-mediated genome editing

The CRISPR-Cas system originated from the prokaryotic adaptive immune system which provides immunity defense against invading viruses and phages [15]. The most commonly used CRISPR-Cas9 system belongs to the type II CRISPR-Cas system, which consists of three core components: the endonuclease Cas9, CRISPR RNA (crRNA), and trans-activating crRNA (tracrRNA) [16]. Moreover, the crRNA-tracrRNA duplex can be fused to form a chimeric sgRNA for ease of use in genome engineering (Fig. 1) [16]. Specifically, the sgRNA contains a 20 nucleotide guide sequence complementary to the target site. When recognizing the presence of a protospacer adjacent motif (PAM, typically 5′-NGG for *Streptococcus pyogenes*), the sgRNA binds to the target sequence by Watson-Crick base-pairing and guides Cas9 to precisely cleave the DNA strand, forming a DSB at the target site (Fig. 1) [16,17]. Subsequently, the breaks are typically processed via two major repair mechanisms: nonhomologous end-joining (NHEJ) and homologous-directed repair (HDR) [18]. In general, NHEJ is error-prone and joins the break sequences directly, frequently introducing random insertions/deletions (indels) at the DSB site, and thereby disrupting or eliminating target genes function by inducing frame shifts (Fig. 1). More precise genetic modifications can be achieved using the HDR pathway with a homologous DNA template, which can be exploited to correct genetic changes and induce intended mutations. Recently, the single-stranded DNA oligonucleotides (ssODNs) represent the most usable and active repair templates in programmable nuclease-mediated genome editing, which provide a more effective and simple method for making small edits in the genome [19–23]. By combining the expression of sgRNA and Cas9, high-efficiency cleavage of any target sequence can be easily achieved [24].

The RNA-guided CRISPR-Cas9 has several advantages over conventional genome engineering tools (Table 1) [25–27]. Briefly, CRISPR-Cas9 simply requires a short complementary sgRNA for DNA targeting, which is relatively easy and cost-efficient to synthesize. Notably, by using multiple sgRNAs for different target sequences, CRISPR-Cas9 can be used to edit multiple independent sites simultaneously, providing a high-throughput approach for genomic modification [28,29]. Furthermore, Cas9 has several variants that can be used for various purposes, for example, the epigenome can also be targeted by the catalytically deactivated Cas9 (dCas9) [30]. Compared to RNAi techniques, the CRISPR-Cas9 system functions at the DNA level, resulting in the knockdown or permanent inactivation of a gene, with fewer off-target effects.

The merits of CRISPR-Cas9 make it a robust and attractive approach for various genome editing purposes. Since most cancers involve a multistep mutation process, the ability to edit multiple genes in parallel and directly target the causes of cancer makes the CRISPR-Cas9 system widely used in cancer research. CRISPR-Cas9 is a convenient laboratory tool for targeted interrogation of cancer-related genomic alterations both *in vitro* and *in vivo* [12,31,32], and may contribute to expanding the available armamentarium for cancer treatment.

3. Potential therapeutic application of CRISPR-Cas9 in cancer

The rapid development of the CRISPR-Cas9-mediated genome editing tool has revolutionized the field of gene therapy, which not only holds extensive application potential for therapeutic manipulations of cancer (*epi*)genomes, but also can be used to fight oncogenic infections, modulate gene expression, and explore anti-cancer drugs (Fig. 2A). In addition to targeting cancer cell genomes directly, the CRISPR-Cas9 system can also be applied for precise engineering of immune cells and oncolytic viruses for cancer immunotherapeutic applications (Fig. 2B).

3.1. Cancer (epi)genome editing for anticancer therapy

Given that cancer accumulates multiple genetic alterations and that CRISPR-Cas9 can efficiently induce precise loss of function (LOF) and gain of function (GOF) mutations *in vitro* and *in vivo* (Fig. 1), it is reasonable to predict that CRISPR-Cas9 can be used to correct gene aberrations that drive cancer pathogenesis and development or to target knockout necessities for cancer cell survival and chemo-resistant genes as a promising anticancer strategy.

Mutated oncogenes and tumor suppressor genes in cancer cells are attractive therapeutic targets in cancer [2]. One proof-of-concept study for cancer gene therapy based on CRISPR-Cas9 system was conducted in bladder cancer [14]. By applying CRISPR-Cas9-mediated genome