Integrated Use and Research Progress of CRISPR/Cas9 in Cancer Therapy

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Abstract. Due to the complexity and recurrence of cancer, traditional therapies often face the dilemma of insufficient efficacy or obvious side effects. In recent years, breakthroughs in gene editing technology have opened up new possibilities for cancer treatment, with CRISPR-Cas9 acting as a pair of molecular scissors, capable of pinpointing and modifying the genetic errors that cause cancer. For example, it can turn off overactive cancer-causing genes, repair key genetic defects that inhibit tumour growth in the body and even modify immune cells to more efficiently identify and attack cancer cells. Currently, scientists are exploring combining it with precision drug delivery and immunotherapy to boost treatment effectiveness and reduce damage to healthy tissue. Although the safety and efficiency of the editing process still needs to be addressed, the technology has already shown potential in some leukaemia and solid tumour treatments. In this paper, we systematically review the core strategies, recent advances and challenges of CRISPR-Cas9 in cancer therapy, which will provide a reference for the development of safer and more personalized anti-cancer regimens.

Keywords: CRISPR-Cas9, guide DNA, immunotherapy, tumour disease, cancer-causing genes.

1. Introduction

CRISPR/Cas (clustered regularly interspaced shrot palindromic repeats/CRISPR-associated proteins) is a gene-shearing tool derived from bacterial self-protection system, originally designed for the transcription and translation of the Cas9 gene by the CRISPR sequences to perform matching cuts. There are two main types of naturally occurring CRISPR systems, the main difference being whether or not the effector module that cuts DNA or RNA has a multisubunit complex; type I involves multiple effector complexes, while type II contains only a single effector protein. The type II, which is most commonly used for genome editing, consists of structurally specific double-stranded DNA formed by complementary pairing of the Cas9 protein, CRISPR RNA (sgRNA), and tracrRNA. the DNA can be repaired by homology directed repair HDR or non-homologous end-joining (non-homologous end-joining). Genome editing was achieved by homology directed repair HDR or non-homologous end-joining (NHEJ). In addition, the dCas9 formed by inactivating the nuclease domain of Cas9 protein can bind target genes under the guidance of sgRNA, but it does not have the function of cutting DNA.CRISPR-Cas9 technology, as a cutting-edge technology of gene editing, has become a hot technology of science and technology due to its high efficiency, easy operation and low cost. It is widely used in various fields including but not limited to DNA genetic disease exploration, cancer treatment means [1]. It provides new ideas for scholars to explore and treat difficult and difficult diseases. At present, the use of CRISPR/Cas9 technology to enrich and optimize various cancer therapeutic means has become one of the important directions for future research and development [2].

2. CRISPR/Cas9-related technologies

2.1. Chimeric antigen receptor-T (T) cell therapy

As a new type of immunotherapy, its main principle is to transform T cells into CAR-T cells carrying chimeric antigen receptors through genetic engineering technology, and use the immune effect of T cell sparks to specifically kill cancer cells. The extracellular structural domain of CAR

can specifically recognize and bind to the antigen on the surface of the cancer cells, and then through the intracellular signaling domain to activate the T cells so as to activate the T cells, and the activated T cells can release the granule After activation, it can release granzyme, perforin, etc. to kill cancer cells directly, and activate other immune cells to play the role of profile killing at the same time. At present, this therapy is mostly applied to hematological malignant tumours, among which acute leukaemia and non-Hodgkin's lymphoma are the most widely researched and have the most remarkable efficacy [3].

2.1.1. Preparation of universal CAR-T cells using CRISPR/Cas9 technology

T cells used in clinical trials of CAR-T therapy are mostly collected and processed from the peripheral blood of cancer patients, which is costly, time-consuming and labor-intensive, and often many patients are unable to collect enough high-quality T cells, making it impossible for CAR-T therapy to be used on a wide scale, which greatly restricts the prospects for the use of this technology. Even though allogeneic T cells can make up for the lack of quantity and quality of autologous T cells, differences in the T cell receptor (TCR) of the major histocompatibility complex between the supplier and the recipient can lead to severe allogeneic reactions and graft versus host disease (GVHD)[4]. We can take advantage of the multiple genomes editing of CRISPR/Cas9 technology to simultaneously interfere with multiple loci, which can generate CAR-T cells defective in endogenous MHC and TCR expression, thus preventing the occurrence of GVHD and obtaining sufficient numbers of T cells to meet the therapeutic demand. At the same time, this technology can help to greatly improve the accuracy of CAR gene insertion into the TRAC site and efficiently import the target gene while knocking out the TCR gene.

2.1.2. Enhancement of CAR-T cell function by CRISPR/Cas9

Programmed death 1 (PD-1) regulates T cell activity by binding to its ligand PD-L1, and as an important immune checkpoint in the organism, it plays a significant role in normal immune regulation and tumour immune escape. Blocking the PD-L1/PD-L1 signaling pathway to reactivate T cells is a major approach in tumour immunotherapy. Currently, multi-clock-targeted PD-1/PD-L1 inhibitors have been approved for clinical use, but immune-related adverse reactions throw a probability of occurring, and the incidence of adverse reaction outbreaks is especially high with PD-1 inhibitor therapy as the mainstay. In contrast, PD-1-deficient CAR-T cells constructed using CRISPR/Cas9 are able to zengqian their anti-tumour activity and reduce the ability of systemic immune checkpoint inhibition-related toxicity while effectively improving cell survival. For immune checkpoints such as lymphocyte activation gene-3 (LAG-3), the use of CRISPR/Cas9 technology to block the expression of related genes in CAR-T cells also has significant therapeutic effects. It is worth noting that traditionally prepared CAR-T cells usually use γ-retroviral vectors to transfer CAR into T cells, and random insertion usually occurs. Thus, the anticancer potential of T cells is realized through the reduction of insertional carcinogenesis, endogenous control of CAR expression, reduction of constitutive signalling, and delayed T-cell exhaustion. An additional benefit is that the surface expression of endogenous TCRs can be eliminated by integrating CARs into the TRAC motif, thereby reducing the risk of TCR-induced autoimmune and allogeneic reactions.

2.1.3. Shortcomings and drawbacks of CRISPR/Cas9 in the use of CAR-T cell therapy

Despite the excellent achievements of CAR-T cell therapy in cancer treatment, it is often associated with apoptosis, depletion, or even "self-destruction", i.e., lack of cell viability and persistence; or it can lead to serious adverse effects, such as cytokine release syndrome (CRS) and neuroinflammatory disorders of potential toxicity, which are of great concern. which are of great concern and can be fatal if not handled properly [5]. Lentiviral vector-mediated CAR transgene insertion disrupts the gene encoding the methylcytosine dioxygenase TET2, generating potent CAR-T cells with the characteristics of transient memory cells that amplify the effector cell response and display a central memory phenotype associated with greater in vivo antitumor activity. There are no better means for prevention and intervention.

2.2. Advances in CRISPR/Cas9 gene editing systems in antitumor drug development

2.2.1. CRISPR gene editing system whole gene screening method

CRISPR gene editing system, as a high-throughput gene screening tool, screens genome-wide for genes that play an important role in phenotype and also analyses hundreds or thousands of genetic and cases at the same time, including, but not limited to, protein-coding genes, miRNAs, long non-coding RNA genes, or enhancers. CRISPR system in the screening, the sgRNA targeting of the genome in all genetic originals, then it is called a genome-wide screen; weak-in targets oncogenes, tumour suppressor genes [6]. Co-regulatory genes and other genetic elements become genomic screens. Cas9 nuclease introduces DSBs into constitutive exons through specific sgRNAs to achieve mutations [7]. Incomplete repair of non-homologous end-joining usually results in DSB site insertions or deletions that lead to gene inactivation by mutation of the sgRNA target site. High-throughput screening can be used to reduce the probability of sgRNA target site mutations through two different forms: hybrid library screening, in which single or several sgRNAs with known gene editing sequences are arrayed in microarrays or multiwell plates, and microarray library screening, in which a computer is used to design a library of the sgRNAs to be selected and enrich positive clones that are subsequently transferred to the host cell to introduce a variety of gene mutations. The microarray library is designed by computer and positive clones are enriched [8]. In contrast, hybrid screening has the advantages of low cost, simplicity, and availability for in vivo studies.

2.2.2. Advantages of CRISPR gene editing system in antitumour drug development

CRISPR whole genome screening method can effectively help researchers screen the whole genome, providing a new method for drug target development. The Cas9 expression cassette is integrated into the sgRNA or a separate plasmid is used to express Cas9; the sgRNA is then cloned into a lentiviral vector, and LV is applied to infect the cells to be tested. The SgRNA expression element can be stably integrated into the genome, effectively providing an identity for each cell, thus facilitating post-drug screening of mixed cell pools[9]. Detection of sgRNA expressions in LV-infected cells using second-generation sequencing allows identification of enriched or ablated sgRNAs from a mixed pool of cells based on the relative abundance values of sgRNAs, and determination of the relationship between genotype and drug. Also single-cell CRISPR screening can be used in combination with transcriptome or open chromatin sequencing to decipher RNA or chromatin interference-related changes [10].

2.2.3. Impact of CRISPR gene editing system in antitumour drug development

CRISPR genome-wide screening technology achieves rapid identification of key targets in tumour biology by systematically knocking out or activating all coding genes in the human genome, which uses the CRISPR-Cas9 system to construct genome-wide sgRNA libraries, combined with high-throughput sequencing and bioinformatics analysis. In the drug target discovery stage, large-scale loss-of-function (LofF) screening identifies tumour cell-specific dependent genes, while gain-of-function screening is used to reveal the compensatory mechanism after oncogene inactivation. For example, CRISPR screening identified the synthetic lethal effect of BRCA1/2-deficient tumours on PARP inhibitors, which directly promoted the clinical translation of the PARP inhibitor olaparib.In the analysis of drug mechanism of action, CRISPR technology can accurately construct gene-edited organoid models or PDX models to simulate specific driver mutations, which can be used to assess the efficacy and off-target toxicity of targeted drugs. Through genome-wide screening combined with drug treatment, researchers are able to map the drug-gene interaction network and reveal the key regulatory nodes of drug resistance-related signalling pathways. Recent studies have found that acquired resistance to CDK4/6 inhibitors is significantly associated with secondary mutations in the RB1 gene, a finding that stems directly from CRISPR-mediated conditional knockdown experiments of RB1.

In addition, CRISPR screening has demonstrated unique value in combination therapy development. By systematically screening for the synergistic effects of kinase inhibitors and

epigenetic modulators, the researchers found that EZH2 inhibitors enhanced the anti-tumour activity of PD-1 blockers in a T-cell depletion model. In the field of precision medicine, the CRISPR-based in vitro drug sensitivity testing platform is able to predict individualised drug regimens based on the gene editing phenotypes of patients' tumour cells, which significantly improves the scientific design of clinical trial enrolment criteria.

3. Challenges of CRISPR gene editing technology in clinical treatment

Currently, CRISPR-Cas9 technology has been widely carried out in clinical trials and research worldwide, but CRISPR-Cas9 technology still possesses many uncontrollable factors and unknown risks. The editing accuracy of the CRISPR-Cas9 system relies on the specific complementary pairing of sgRNA and target DNA sequences, however, the existence of a large number of homologous sequences in the genome (e.g., pseudogenes or low However, the presence of a large number of homologous sequences in the genome (e.g., pseudogenes or low-complexity regions) may lead to the binding of sgRNAs to non-target sites and trigger off-target editing [11]. Off-target effects may activate proto-oncogenes (e.g., MYC) or inactivate oncogenes (e.g., TP53), triggering secondary tumour risk. The use of high-fidelity Cas9 variants (e.g., SpCas9-HF1 or HypaCas9) to reduce off-target rates may come at the expense of editing efficiency, which in turn increases the load demanded on the delivery system. Effective delivery of CRISPR components requires overcoming multiple biological barriers, including serum nuclease degradation, vascular endothelial permeability, cell membrane penetration, and nuclear localization efficiency. For example, adeno-associated virus (AAV) is widely used due to its low immunogenicity but is limited by packaging capacity (<4.7 kb) to carry SpCas9 (4.2 kb) and regulatory elements; lentiviral vectors, although large in capacity, are at risk of insertional mutagenesis. Then, for example, lipid nanoparticles (LNP) encapsulate Cas9 mRNA/sgRNA complexes by charge adsorption, but their liver-targeting propensity (due to ApoE protein-mediated LDL receptor binding) limits delivery efficiency to organs such as the lungs and the brain; exosomes or gold nanoparticles can be modified with targeting ligands (e.g., anti-EGFR antibodies), but large-scale production and stability are still challenges. These problems may even co-exist in the clinic, e.g., viral vectors (e.g., AAV) are prone to triggering anti-coat protein antibodies, while non-viral vectors (e.g., LNP) may trigger allergic reactions through complement activation; lowering the dose to avoid immunotoxicity will weaken the efficiency of the editing process, while raising the dose increases the risk of off-targeting and inflammation. Currently, researchers are actively looking for ways and strategies to deal with these problems, such as adopting single-base editors (e.g., ABE8.8) or pilot editors (PE4max) to avoid DSBs and reduce the off-target risk of NHEJ-dependent editing; humanizing Cas9 variants (e.g., HiFi-Cas9) to reduce the exposure of exogenous epitopes; adopting mRNA transient expression systems instead of DNA carriers to shorten the survival of Cas9 proteins; and adopting mRNA expression systems instead of DNA vectors to reduce the risk of off-targeting. , shortening the survival time of Cas9 protein, lowering the immune recognition window and other countermeasures have greatly improved the clinical response ability in the face of unexpected situations [12].

4. Conclusion

CIn the field of cancer treatment, the breakthrough progress of CRISPR gene editing technology is driving a revolutionary shift in the treatment paradigm. In the next decade, its clinical application may achieve the leap from "disease control" to "molecular eradication" and reshape the underlying logic of human fight against malignant tumours. In terms of therapeutic vision, CRISPR technology is expected to break through the limitations of traditional therapies and drive cancer treatment into a new era of "functional cure". By targeting and modifying driver mutations, remodeling the immune microenvironment, or eliminating tumour stem cells, it may be possible to intervene in the root cause of malignant tumours, rather than just alleviating phenotypic symptoms. This trend suggests that in

the future, cancer treatment may move away from reliance on radiotherapy to long-term disease management through precise regulation at the genetic level. This kind of "prognostic therapy" thinking may overturn the existing clinical practice and promote the transformation of tumour treatment from passive response to active intervention.

However, technological leaps always need to be viewed with scepticism. Currently, most clinical trials are still in the early stages, focusing on evaluating safety and efficacy to optimise and improve the molecular mechanisms involved in gene editing. Thus, in the future, CRISPR-Cas9 technology may be able to bring about cures for patients in the treatment of difficult diseases.

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