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CRISPR-Cas9 Technology in Cancer Therapy: A Review of Recent Advances and Future Perspectives

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Abstract. Cancer is still one of the most serious public health problems worldwide.Although the existing treatment methods are constantly developing, there are still some problems such as large side effects, strong drug resistance and significant differences in efficacy. In recent years, with the rise of gene editing technology. CRISPR-Cas9 has gradually become an important tool for precision cancer treatment due to its simple operation, high efficiency and wide application. In this review, we summarize the research progress of CRISPR-Cas9 technology in cancer treatment.focusing on three aspects: first, directly modifying oncogenes or tumor suppressor genes to block tumor growth or restore key gene function; Second, applied to there construction of immune cells, such as modified CAR - T or NK cells and knock out immune suppressor genes, to enhance the immune effect; Third, the use of large-scale genetic screening found new potential targets, provide a foundation for subsequent drug development. Although promising, the clinical application of this technology still faces challenges in terms of delivery system, off-target effects, and ethical regulation future research is expected to focus on improving the accuracy of editing, exploring combination treatment strategies, and integrating artificial intelligence to develop individualized regimen design. In general, CRISPR-Cas9 is gradually promoting cancer treatment into a new stage of more precision and efficiency

Keywords: CRISPR-Cas9 Gene Editing, Cancer Therapy, Genome Targeting, Delivery Systems

1. Introduction

Cancer is one of the most serious public health threats in the world, and its morbidity and mortality remain high for a long time. According to the statistics of the World Health Organization, there were more than 20 million new cancer cases and nearly 10million cancer-related deaths worldwide in 2024. This trend poses a great challenge to the medical system and socio-economic development [1]. Although modern medicine has made significant progress in cancer treatment, such as chemotherapy, radiotherapy, targeted drugs, immune checkpoint inhibitors and other strategies have been widely used in clinic, these methods still have obvious limitations. For example, chemotherapy and radiotherapy are often accompanied by serious toxic and side effects; Targeted drugs are easy to cause drug resistance; Although immune therapy has shown breakthrough efficacy in some patients,

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its efficacy is often limited due to individual differences and immunosuppressive microenvironment [2].

In this context, the concept of precision medicine has been gradually proposed and widely adopted. Its core is to develop individualized treatment plans for patients through molecular level analysis and intervention. The rapid development of gene editing technology, especially the emergence of CRISPR-Cas9 system, has provided a new way for tumor treatment. Since it was reported in 2012, CRISPR-Cas9 has rapidly become an important tool in the field of life sciences with its advantages of convenient operation, high efficiency and wide applicability, and is regarded as a key force to promote the development of precision medicine [2]. Compared with traditional gene editing technologies such as ZFN and TALEN, CRISPR-Cas9 is more flexible, economical and efficient [3].

This paper systematically reviews the latest research progress of CRISPR-Cas9 technology in tumor treatment. This paper first introduces the basic principle and mechanism of this technology, and focuses on its three application strategies in tumor therapy: direct editing of tumor related genes, engineering of immune cells, and discovery of new therapeutic targets. Finally, the future development of this technology is summarized and prospected. By combing and analyzing, we hope to provide a theoretical basis and reference for the research of gene editing applied to tumor treatment.

2. Introduction to CRISPR technology

2.1. Origin and discovery

Clustered Regularly Interspaced Short Palindromic Repeats(CRISPR) Originally found in the bacterial genome, it is an adaptive immune system used by bacteria to resist phage invasion. This system can rapidly initiate the immune response when encountering similar pathogens again by recognizing and cutting foreign DNA and recording infection information [4]. In 2012, Jinek and others successfully applied CRISPR-Cas9 system to in vitro gene editing for the first time, marking that the technology has moved from basic microbiology research to molecular biology and medical applications. Subsequently, the research of Doudna and Charpentier et al not only revealed the molecular mechanism of Cas9 endonuclease to specifically recognize and cleave DNA under RNA guidance, but also further proved that the system has efficient and programmable gene editing ability in mammalian cells [5,6]. These works laid the theoretical and experimental foundation of CRISPR-Cas9 as a general gene operation tool, and provided a solid support for its subsequent rapid development in gene regulation, functional genomics, disease model construction and potential clinical therapeutic applications.

2.2. Core components

CRISPR-Cas9 system is mainly composed of two core components: Cas9 protein and guide RNA (sgRNA). Cas9 protein is a kind of "molecular scissors" with endonuclease activity, which can cut DNA double strands at specific sites. The sgRNA is designed by researchers, one end of which binds to Cas9, and the other end guides Cas9 to precisely locate to the target by complementary pairing with the target DNA sequence [7,8]. In addition, Cas9 needs to rely on the presence of Protospacer Adjacent Motif (PAM) sequence when recognizing DNA, which is a short conserved sequence that plays a key role in the specificity and efficiency of editing [9].

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2.3. Mechanism of action

At present, it is generally believed that the working principle of CRISPR-Cas9 can be roughly divided into three main steps. The first step is targeted localization: the sgRNA is paired with the target DNA sequence, and the Cas9 protein confirms the binding site by recognizing the PAM sequence. The second step is cleavage: after correctly recognizing the target, Cas9 uses its two endonuclease domains to introduce double strand breaks at specific sites of the target DNA, thereby activating the cell's own DNA repair mechanism [10]. The third step is repair: broken DNA is repaired mainly through three paths. Non homologous end joining (NHEJ) is an error prone repair method, which often leads to insertion or deletion mutations, so it is widely used in gene knockout; Microhomology mediated end joining uses a small range of homologous sequences at the broken ends to mediate repair, often resulting in specific deletions; While homology directed repair requires the provision of exogenous repair templates, which can achieve precise gene knock in or repair, and is often used to restore the function of tumor suppressor genes or introduce specific mutations [11]. At present, NHEJ is still the most important repair mechanism in cells.

3. Major application strategies of CRISPR in cancer therapy

Leveraging its highly efficient gene-editing capability, CRISPR-Cas9 technology is gradually transitioning from basic research to the clinical exploration stage for cancer treatment. Its main application strategies can be categorized into three types: direct targeting of cancer-related genes, engineering immune cells, and systematic discovery of new therapeutic targets.

3.1. Direct targeting of cancer-related genes

Carcinogenesis is primarily driven by the activation of proto-oncogenes and the inactivation of tumor suppressor genes. CRISPR-Cas9 technology provides a means for precise genomic intervention directly targeting these elements. In terms of oncogene knockout, researchers use this technology to target key driver genes such as KRAS, MYC, and EGFR, inactivating them by introducing gene deletions or insertional mutations, thereby effectively inhibiting the proliferation and migration of cancer cells [12]. For example, in mouse models of lung cancer, CRISPR editing targeting specific mutations like KRAS G12D, G13D, and G12S significantly suppressed cancer cell growth [13]. For tumor suppressor gene restoration, the approach primarily relies on the HDR pathway to recover gene function. For instance, the TP53 gene, frequently mutated and inactivated in various cancers, can have its normal function and DNA repair capacity restored by co-delivering CRISPR-Cas9 components with a corrective donor template [14]. Similarly, restoration of PTEN gene function has been shown to reduce the malignancy of PIK3CA-mutant breast cancer and PTEN deficient gliomas.

However, the in vivo application of direct gene editing faces several challenges, with the efficiency and specificity of the delivery system being a key bottleneck. Existing viral vectors, such as AAV, face issues like limited cargo capacity and immunogenicity. Non-viral vectors, such as lipid nanoparticles (LNPs), offer higher safety profiles but still require optimization for stability and editing efficiency. Additionally, off-target effects and potential genomic instability pose risks for clinical translation [15].

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3.2. Engineering immune cells

Driven by the rapid progress in cancer immunotherapy, CRISPR-Cas9 technology has found promising applications, especially in engineering T cells and NK cells [16].

In modulating immune checkpoints, CRISPR technology can be used to knock out genes like PD-1 or CTLA-4 in T cells, thereby releasing the immunosuppressive brakes imposed by the tumor microenvironment and enhancing the cytotoxic function of T cells. This approach may potentially lead to more durable immune effects compared to traditional antibody-based methods [17]. In Chimeric Antigen Receptor T-cell (CAR-T) therapy, CRISPR-Cas9 enables the precise insertion of the CAR gene into specific designated "safe harbor" sites in the genome. This avoids the genomic instability risks associated with random integration, which can occur with conventional viral vector methods. This strategy not only improves treatment safety but may also enhance the persistence and anti-tumor activity of CAR-T cells in vivo. Clinical studies have already reported that CRISPR-edited CAR-T cells exhibit promising safety profiles and preliminary efficacy in patients with relapsed/refractory hematological malignancies. Furthermore, genetic engineering of NK cells is also gaining momentum. Researchers are knocking out inhibitory receptor genes or enhancing the expression of cytotoxic molecules in NK cells to improve their ability to recognize and kill solid tumors, offering a potential strategy to address the limitations of CAR-T therapy in solid malignancies [18,19]. Despite the promising outlook, immune cell editing still confronts challenges related to in vivo persistence, potential immune rejection, and ethical scrutiny, necessiting validation through more clinical trials and long-term follow-up.

3.3. Discovery of novel therapeutic targets

Beyond direct intervention on known genes, CRISPR technology also demonstrates unique advantages in the systematic exploration of new cancer targets [20]. Using genome-wide CRISPR knockout or screening libraries, researchers can simultaneously knock out thousands of genes to identify those essential for cancer cell survival, proliferation, or drug sensitivity. This strategy has achieved significant progress in various cancer types. For example, screens have revealed that the loss of certain DNA repair pathway genes significantly sensitizes cancer cells to PARP inhibitors, uncovering new synthetic lethal interactions. Furthermore, CRISPR screens in models of pancreatic cancer, lung cancer, and melanoma have identified previously unknown cancer dependency genes, providing candidate targets for subsequent targeted drug development [21-23].

CRISPR screening technology not only aids in discovering individual targets but also can reveal complex regulatory networks and interactions between genes and signaling pathways. This type of systemic analysis will powerfully propel the development of precision medicine, making cancer treatment strategies more personalized and targeted.

4. Conclusion and outlook

CRISPR-Cas9 technology, with its highly efficient and flexible gene-editing capabilities, has brought unprecedented opportunities to the field of cancer therapy. From directly targeting oncogenes and tumor suppressor genes, engineering immune cells, to systematically discovering new therapeutic targets, this technology has penetrated multiple levels of cancer research and clinical translation, demonstrating immense potential in inhibiting tumor growth, enhancing immune efficacy, and revealing cancer vulnerabilities. The clinical application of this technology, however, is still hampered by considerable hurdles, ranging from the efficiency and specificity of delivery

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vehicles and the precise control of off-target effects to risks linked to genomic instability and associated ethical and regulatory dilemmas. Optimistically, with the discovery and optimization of novel Cas proteins and the ongoing development of non-viral nano-delivery systems, these technical bottlenecks are being gradually overcome [24].

Looking forward, the future development of CRISPR technology will likely concentrate on several key areas: first, developing editing tools with higher precision and lower off-target rates; second, forming multimodal combination therapy strategies with existing modalities like radiotherapy, chemotherapy, and immune checkpoint inhibitors; and third, integrating big data and artificial intelligence technologies to advance the design and implementation of personalized treatment regimens [25]. In summary, although still in the exploratory stages, CRISPR-Cas9 technology is regarded as a significant breakthrough point for achieving precision cancer therapy. As the technology matures and clinical evidence accumulates, it is poised to become an indispensable core component of the future cancer treatment arsenal.

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