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Research on CRISPR-Cas9 Gene Editing Technology in Cancer Treatment

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Abstract:

Gene editing technology represents a pioneering biotechnology that leverages molecular tools to achieve efficient and precise modifications of the DNA sequences within a biological genome, enabling the regulation of gene functions or the enhancement of traits. As a component of bacterial adaptive immunity, CRISPR/Cas9 has swiftly emerged as a revolutionary gene editing tool in the life sciences, outperforming traditional zinc finger nucleases and TALEN technologies in terms of editing efficiency and cost-effectiveness. The CRISPR-Cas gene editing tool boasts not only robust functionality but also remarkable specificity and efficiency, facilitating accurate and rapid screening of the entire genome and enabling gene therapy for specific diseases. It has found widespread application in research pertaining to human disease treatment. In the realm of oncology, the CRISPR-Cas system can be employed to edit the genome and delve into the mechanisms underlying tumor initiation, progression, and metastasis. This article provides a comprehensive review of the advancements in CRISPR/Cas9 research pertaining to lung cancer, cervical cancer, pancreatic cancer, and bladder cancer, encompassing key target screening, elucidation of drug resistance mechanisms, development of gene therapy vectors, and clinical trial outcomes. Furthermore, it delves into its potential for clinical application and existing challenges, offering a theoretical foundation for future precision cancer research endeavors.

Keywords: Gene editing technology; CRISPR/Cas9; Cancer.

1. Introduction

Malignant tumors, characterized by their high invasiveness, drug resistance, and complex molecular mechanisms, remain a major challenge in clinical treatment and pose a significant threat to the global population [1]. For example, lung cancer is one of the most common malignant tumors. Additionally, the incidence and mortality rates of cervical cancer, pancreatic cancer, bladder cancer, and others are

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showing an upward trend year by year, severely threatening public health. These cancers often result in poor patient outcomes due to difficulties in early diagnosis and poor response to traditional radiotherapy and chemotherapy, necessitating the development of novel precision treatment modalities.

Gene editing is a technology that precisely modifies genomic sequences to induce insertions, deletions, or base substitutions in the genome. To date, the most widely used is CRISPR/Cas9 technology.This system originates from the adaptive immune mechanism of bacteria. Through years of research, its structure and function have been gradually clarified. Leveraging the precise cutting ability mediated by the Cas9 protein and sgRNA, as well as its simplicity, efficiency, and high precision, it has become a highly effective gene editing tool. By targeting mutated genes and repairing tumor suppressor gene, it provides a powerful tool for deepening our understanding of cancer mechanisms and developing new therapies, effectively addressing the shortcomings of first-generation ZFN and second-generation TALENs technologies. It is currently widely applied in gene knockout, targeted mutation, gene activation, disease model construction, and gene therapy.

As research continues to advance, CRISPR-Cas9 has demonstrated unique advantages in various tumor studies [2]. For example, in pancreatic cancer, it targets KRAS mutations and enhances therapeutic efficacy when combined with drugs; in lung cancer, it constructs models and explores resistance mechanisms; in cervical cancer, it knocks out KIFC1 or HPV oncogenes to inhibit proliferation; and in bladder cancer, it reverses malignant phenotypes by regulating UCA1. However, there is currently no systematic review of its application in different cancers. This article will briefly introduce CRISPR/Cas9 technology and its mechanism of action, primarily reviewing its application in various cancers, with the aim of promoting the development of CRISPR/Cas9-mediated therapy and providing guidance for treating other cancers.

2. CRISPR-Cas9 System

The CRISPR-Cas9 system originates from the adaptive immune defense mechanisms of bacteria and archaea, initially evolved to defend against exogenous nucleic acid invasions such as phages. Its discovery began in 1987 when Japanese scientists identified repetitive sequences in E. coli. In 2002, it was officially named “clustered regularly interspaced short palindromic repeats” (CRISPR), and the adjacent Cas gene sequences were discovered the same year. In 2007, experiments first validated that the system could defend against phage and exogenous plasmid invasion [3].

This system consists of the tracrRNA sequence region, the Cas gene sequence region, and the CRISPR sequence region. The core components include the Cas9 protein, which contains two nuclease domains—RuvC-like and HNH—that cleave the non-complementary and the complementary strands of DNA, respectively; tracrRNA, which participates in crRNA maturation; and crRNA, which is responsible for locating foreign DNA [1,4]. The PAM sequence at the 3’ end of the target DNA (e.g., 5’-NGG-3’ in Streptococcus pyogenes) is critical for Cas9 function [5].

In gene editing applications, CRISPR-Cas9 guides the Cas9 protein to precisely cleave viral DNA, forming double-strand breaks, by designing sgRNA (a fusion of pre-crRNA and tracrRNA, which is more efficient) that complements specific viral DNA sequences; Subsequently, non-homologous end joining (NHEJ) in cells leads to viral gene dysfunction, or homologous recombination-mediated repair (HDR) introduces specific sequences to modify viral characteristics [3]. This technology provides efficient tools for antiviral research, viral vector modification, and other applications. It also demonstrates promising efficacy for advanced, severe, and difficult-to-treat cancers.

3. Applications of CRISPR/Cas9 Gene Editing Technology in Different Cancers

3.1 Lung Cancer

Researchers used gene editing technology to edit genes associated with lung cancer (such as KRAS, p53, and LKB1) to construct a mouse model of lung adenocarcinoma. This model can simulate the genetic mutation characteristics of human lung cancer, successfully reproduce pathological changes associated with lung adenocarcinoma, and highly match the molecular characteristics of human lung cancer, providing a reliable tool for studying the mechanisms of lung cancer development and for evaluating the efficacy and resistance mechanisms of candidate drugs. Additionally, researchers utilized a gene editing-derived GecKo library to screen for genes associated with tumor growth and metastasis in NSCLC cell lines. Through lentiviral transduction of the sgRNA library combined with animal experiments, key genes influencing lung cancer metastasis have been identified.

For abnormally activated oncogenes in lung cancer (such as KRAS mutations), gene editing technology can be used for precise editing to inhibit their activity. For example, by introducing mutations via homologous recombination repair, the downstream signaling pathways of KRAS (such

as MAPK, PI3K/AKT) can be blocked. In cell experiments, this approach significantly inhibited lung cancer cell proliferation; in animal models, tumor growth slowed, and no significant toxicity was observed in normal cells [6].

Clinically, researchers are also exploring related combination therapy strategies, such as combining gene editing technology with targeted drugs. After editing drug-resistant genes (such as the EGFR T790M mutation), the sensitivity of lung cancer cells to EGFR-TKIs (such as osimertinib) can be enhanced. In drug-resistant cell lines, combination therapy increased drug sensitivity by 3–5 times; in animal models, tumor shrinkage rates improved by over 40% compared to monotherapy [3].

In recent years, treatment strategies have continued to be optimized, identifying "innate genetic defects" and targeting their "last line of defense" to achieve precise killing of cancer cells. For example, the combination of KRAS G12C inhibitors with MEK inhibitors, or EGFR TKIs with mTOR inhibitors, significantly enhances efficacy; developing mutation-specific editing systems, such as gene editing targeting KRAS G12S mutations, inhibits tumor proliferation and reduces off-target effects; the construction of precise animal models (such as EML4-ALK fusion mice) for evaluating drug efficacy and resistance evolution; and gene editing library screening to match optimal combination therapies for different mutation subtypes [7]. Gene editing technology, through the construction of precise lung cancer models, screening of key genes, and direct editing of oncogenic targets, provides new strategies for lung cancer treatment. After continuous optimization, it is hoped to become an important tool for personalized lung cancer treatment in the future.

3.2 Cervical Cancer Treatment Research

Cervical cancer poses a serious threat to women's health. Its incidence is closely associated with persistent infection by high-risk human papillomavirus (HPV). The E6 and E7 oncogenes of HPV interfere with normal cellular regulatory mechanisms, leading to cellular carcinogenesis. Although vaccines are available for prevention, traditional treatments for patients who have already developed the disease still carry risks of recurrence and drug resistance.

In this study, gene editing technology was used to knockout the KIFC1 gene in HeLa cells. Targeted sgRNAs were designed for this gene, and recombinant plasmids were constructed and transfected into cells. After sorting and screening, a monoclonal cell line was obtained, and the knockout effect was validated using RT-qPCR and Western blot analysis. The results proved that KIFC1 deficiency caused significant changes in HeLa cells (all $P<0.05$): morphologically, there was an increase in multinucleated and micronucleated cells, abnormal microtubule skeletons, and weakened adhesion ability; cell cycle disruption, increased proportions of cells in the G_0/G_1 and the G_2/M phases, reduced S phase, and inhibited DNA synthesis; decreased cell proliferation capacity, reduced EdU-labeled proliferating cells, and slowed growth; increased proportion of late apoptotic cells, and reduced normal cells. It is evident that KIFC1 gene knockout mediated by gene editing can inhibit cervical cancer cell proliferation by affecting microtubule assembly and cell division in HeLa cells, thereby inducing cell cycle arrest and the apoptosis [4].

Additionally, researchers established an editing system targeting the high-risk human papillomavirus oncogenes E6 and E7 using this technology and transfected the system into SiHa cervical cancer cell lines infected with the HPV-16 virus. Following editing of the E6 and E7 genes and their promoters using this system, p53 and p21 proteins accumulate within the cells, significantly reducing the proliferation capacity of cervical cancer cell lines in vitro. Furthermore, in mice experiments, tumor growth was also significantly inhibited [8].

3.3 Pancreatic Cancer

Pancreatic cancer is a highly malignant digestive tract cancer. Due to the early concealed symptoms and the lack of diagnostic tools, the disease is often diagnosed at a late stage, by which time patients are no longer eligible for radical surgery, and the efficacy of traditional radiotherapy and chemotherapy is limited, resulting in generally poor survival outcomes. Additionally, the tumor exhibits low sensitivity to traditional radiotherapy and chemotherapy, resulting in extremely poor prognosis.

Research targeting KRAS mutations using gene editing technology has provided a targeted treatment strategy. Through gene editing systems such as Cas9, Cas13a, and CasRx, approximately 90% of KRAS mutations present in pancreatic cancer (such as G12D and G12C) can be precisely edited. For example, using Cas13a to target KRAS-G12D mRNA achieves a knockdown efficiency of 94%; correcting KRAS point mutations via base editors (ABE) can also avoid genomic instability caused by double-strand breaks.

Current delivery methods for this technology include exosomes, viral vectors, and electroporation and transfection. Among these, exosome-mediated delivery involves loading gene editing plasmids into mesenchymal stem cell exosomes and targeting them to pancreatic cancer cells with high KRASG12D expression; viral vector delivery, such as the AAV8 vector carrying the CasRx-gRNA system, efficiently silences the mutated KRASG12D; Elec-

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troporation and transfection-based gene editing delivery enables multiplex editing of the pancreatic genome.

Strategies for restoring tumor suppressor gene function include gene editing-based homologous recombination repair to fix inactivated mutations in TP53, or base editing to correct point mutations, thereby restoring its regulatory functions in cell cycle arrest and apoptosis; For CDKN2A, gene editing can be used to activate its expression, or gene knock-in can be employed to mimic the function of the p16 protein it encodes, inhibiting the transition from the G1 to S phase of the cell cycle; for SMAD4, gene editing technology can be used to restore its role in the TGF-β signaling pathway, inhibiting tumor metastasis and epithelial-mesenchymal transition (EMT) [9].

Combination therapy strategies using this technology have also shown significant efficacy. For example, when combined with CDK4/6 inhibitors (such as palbociclib), gene editing screening has identified that targeted deletions of genes in the PI3K-AKT-mTOR pathway (such as CDK2 and BAP1) can enhance inhibitor sensitivity; combination with ERK inhibitors (such as SCH772984) or KRASG12C inhibitors (such as sotorasib) can enhance efficacy by synergistically inhibiting tumor proliferation signaling pathways; Editing T cells to enhance recognition of tumor cells, or downregulating PD-L1 expression in tumor cells via gene editing, improves the immune microenvironment [10].

In both vivo and vitro experiments, the effects are equally prominent. After KRAS editing, pancreatic cancer cell proliferation capacity decreases by over 70%, and sensitivity to chemotherapy drugs such as gemcitabine is enhanced (IC50 reduced by approximately 10-fold); Following TP53 repair, cancer cell apoptosis rates increased, DNA damage repair capacity improved, and sensitivity to radiotherapy increased; after SMAD4 function was restored, tumor cell migration and invasion capabilities significantly decreased, and expression of EMT-related markers (such as Zeb1) was downregulated. Additionally, studies have shown that KRAS targeted editing reduces tumor volume in mouse xenografts by 50%–70% and extends survival by 33%–50%; after SMAD4 repair, the number of lung metastases in mice decreases by 60%, and fibrosis in the tumor microenvironment is reduced; gene editing combined with CDK4/6 inhibitors accelerates tumor shrinkage by twofold compared to monotherapy and reduces the incidence of resistance.

Preclinical model validation also showed that in patient-derived organoid (PDO) experiments, the response of gene-edited organoids to drugs was consistent with clinical efficacy in patients at 80%, providing a basis for personalized treatment; the gene-edited organoid model successfully simulated the progression of pancreatic cancer from PanIN to invasive cancer, aiding in early intervention research. Gene editing technology precisely targets driver mutations such as KRAS and repairs tumor suppressor genes, significantly inhibiting pancreatic cancer proliferation and metastasis while enhancing treatment sensitivity in vitro and in animal models. Despite challenges such as delivery efficiency and off-target effects, its combination with combination therapy offers a new direction for precision treatment of pancreatic cancer, and further optimization of the system is needed to advance clinical translation [9].

3.4 Bladder Cancer

Muscle-invasive bladder cancer (MIBC) is highly malignant, prone to metastasis, and exhibiting high chemotherapy resistance, resulting in poor patient prognosis. Long non-coding RNA UCA1, abnormal activation of oncogenes, and disruption of the immune microenvironment all contribute to bladder cancer progression.

One of the core treatment strategies for bladder cancer involves using gene editing technology to target and inhibit lncRNA UCA1. The study designed eight specific gRNAs and constructed a gene editing-UCA1 system, with two intervention approaches: when intervening singly, the two highly efficient gRNAs selected were transfected individually, resulting in 85% and 80% inhibition of UCA1 expression, respectively. In combined intervention, UCA1-1 and UCA1-8 were co-transfected, producing a synergistic effect that further reduced UCA1 expression.

In vitro experiments showed that, through proliferation inhibition assays, the MTT assay indicated a significant decrease in cell viability 48 hours after combined intervention, with more pronounced inhibitory effects observed after 72 hours; cell cycle analysis revealed that UCA1 downregulation caused cells to arrest at the G0/G1 phase; flow cytometry showed a significant increase in the proportion of early and late apoptotic cells; wound healing was delayed in the scratch assay, and Transwell assays and gelatinase spectrum assays confirmed reduced cell invasion capacity and decreased MMP-2/9 activity.

In vivo experiments (nude mouse xenograft model) showed that after inoculation with gene-edited UCA1-(1+8)-treated 5637 cells, tumor volume and weight were significantly smaller than control group within 33 days, with the difference being particularly pronounced after 27 days; In tumor tissues, the expression of pro-invasive proteins MMP-2/9 and anti-apoptotic protein Bcl-2 was reduced, while the pro-apoptotic protein Bax was increased, consistent with in vitro results. Additionally, a meta-analysis showed that UCA1 in urine serves as a non-invasive diagnostic marker with sensitivity and specificity of 0.83

(95% CI = 0.80–0.86) and an area under the curve (AUC) of 0.83, indicating high diagnostic accuracy [11]. Gene editing technology has also driven the development of various novel therapeutic strategies with notable efficacy. For example, in reversing chemotherapy resistance, targeting SLFN11, genome-wide gene editing screening revealed that its deletion leads to cisplatin resistance. By upregulating SLFN11 through epigenetic regulation, tumor sensitivity to cisplatin can be enhanced, resulting in a twofold increase in chemotherapy response rates in animal models; Inhibiting HNRNPU, gene editing knockout can restore T24 cells' sensitivity to cisplatin by upregulating the tumor suppressor gene NF1, increasing apoptosis by 30%; repairing MSH2 function, whose deletion leads to resistance by weakening the DNA damage response, gene editing-mediated repair can increase cisplatin killing efficiency by 40%.

Secondly, it can optimize immunotherapy. After CTLA4 knockout, gene-edited cytotoxic T cells (CTLs) exhibit a twofold increase in in vitro killing capacity against MIBC cells, with tumor volume reduced by 50% in mouse models. When targeting both PD1 and UCA1, gene-edited combined knockout can reshape the tumor microenvironment (Th1-type immune activation), extending survival by 40% in mouse models.

Additionally, it can be used for synthetic lethality and cell cycle regulation. Targeting CBP/p300, gene editing-mediated bladder-specific dual gene silencing downregulates c-MYC to inhibit cell proliferation, increasing MIBC cell apoptosis by 60% without toxicity to normal urothelial cells. For CDK4/6 inhibitor resistance, gene editing screening identified activation of PI3K-AKT pathway as the primary cause, and combination inhibitors increased the drug response rate from 15% to 45%.

Finally, this technology can target oncogenes and tumor suppressor genes. When activating KLF4, gene editing activation technology upregulates this gene, inducing G1 phase arrest through the AKT-p21 pathway, resulting in 50% decrease in T24 cell proliferation rate and 70% decrease in invasive capacity; When inhibiting EZH2, gene editing validated it as a synthetic lethal target for UTX-deficient MIBC, and inhibitors reduced xenograft tumor volume by 60% [12]. Gene editing technology offers new insights into precision therapy for MIBC by elucidating resistance mechanisms, optimizing immunotherapy, and developing synthetic lethal targets. However, most strategies are currently in the cellular and animal experimental stages, and it is hoped that clinical translation can be achieved in the near future to better improve patient outcomes.

4. Conclusion

With the advancement of genome editing technology, research on using CRISPR/Cas9 in cancer-related therapies is rapidly developing, demonstrating significant potential in the field of precision cancer therapy, particularly achieving breakthrough progress in the study of malignant tumors such as pancreatic cancer, lung cancer, cervical cancer, and bladder cancer. Using tools such as the CRISPR/Cas system, researchers can precisely target driver mutations (e.g., KRAS mutations in lung and pancreatic cancer), repair tumor suppressor genes (e.g., TP53, CDKN2A), and significantly inhibit tumor proliferation and metastasis through combination therapy strategies (combined with targeted therapy and immunotherapy), thereby enhancing treatment sensitivity. In terms of delivery methods, the application of technologies such as exosomes, viral vectors, and electroporation has further enhanced the targeting and efficiency of editing systems. In vitro and in vivo experiments, as well as preclinical models, have validated their significant therapeutic effects, providing strong support for personalized treatment.

However, the clinical translation of this technology still faces key challenges, with off-target effects being the primary risk—sgRNA may bind to and cleave non-target DNA sequences, leading to random mutations, disrupting genomic stability, and potentially triggering normal cell carcinogenesis. Although off-target rates have been reduced through sgRNA optimization and the use of high-fidelity Cas protein variants, risks persist in complex genomic environments. Additionally, the targeting, efficiency, and safety of delivery systems, as well as the assessment of long-term biological effects after editing, remain the major challenges to be overcome. In the future, efforts should focus on reducing off-target risks and enhancing the precision and safety of the technology through optimizing sgRNA design, developing highly specific editing tools (such as base editors), and improving delivery systems (such as enhancing the targeted recognition capabilities of exosomes). Concurrently, synergistic innovation between gene editing and existing therapeutic modalities should be strengthened. By integrating preclinical models (such as patient-derived organoids), the application mechanisms of gene editing across different cancer types should be thoroughly explored to facilitate the transition from basic research to clinical application. As technology continues to mature, gene editing holds promise as a core modality for precision cancer therapy, offering new hope to patients.

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