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Original Article

Towards Personalized Cancer Care: A Report of CRISPR-Cas9 Applications in Targeted Therapies and Precision Medicine

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ABSTRACT

Background: Cancer resistance to chemotherapy arises from various genetic and epigenetic changes that promote malignant cell proliferation. The emergence of CRISPR-Cas9, a highly adaptable genome-editing tool, is revolutionizing cancer modeling by facilitating the precise generation of desired genetic mutations.

Objective: This study explores the efficacy of CRISPR-Cas9 in developing cellular and animal cancer models, correcting oncogenic mutations, and advancing cancer therapeutics through gene editing.

Methods: The CRISPR-Cas9 system was employed to introduce specific genetic alterations in cellular and animal models. These models were then used to simulate the complex interactions within tumors and to test the effectiveness of gene edits in mitigating cancer traits.

Results: Findings reveal that CRISPR-Cas9 enhanced the precision of anti-cancer treatments, with a significant reduction in tumor viability by up to 60% in treated models. Additionally, genetic corrections of mutated oncogenes achieved an efficiency of approximately 70%, underscoring the potential of CRISPR-Cas9 in targeted cancer therapy.

Conclusion: CRISPR-Cas9 has proven to be a powerful tool for cancer research, offering new avenues for the development of advanced therapeutic strategies. Its ability to modify genetic makeup efficiently holds promise for its integration into clinical settings, though challenges in safety and delivery remain.

Keywords: Cancer, CRISPR-Cas9, clinical trials, gene therapy, genome editing.

INTRODUCTION

Cancer, a disease characterized by gene mutations that disrupt normal cellular functions such as growth, division, and apoptosis, has become a predominant cause of mortality globally (1, 2). Over the past two centuries, the incidence of cancer has surged by 70%, underscoring its status as a significant health challenge (4). Particularly, primary brain tumors are notorious for their high mortality rates. Cells derived from various cancers provide valuable insights into cellular biology, mechanisms of disease pathogenesis, and the efficacy of therapeutic interventions (5).

Current therapeutic strategies are often limited in scope, focusing predominantly on chemical agents that influence gene alterations and carcinogenesis (7). Other identified causes of cancer, including viruses, bacteria, and ultraviolet radiation, account for approximately 7% of cancer cases (8). Moreover, behavioral changes such as smoking cessation, dietary adjustments, regular exercise, and reduced alcohol consumption have been shown to prevent up to 32% of cancer-related deaths (9).

Amid these challenges, the CRISPR-Cas9 system offers a beacon of hope (10). Discovered in the adaptive immune systems of bacteria and archaea, this technology has revolutionized the field of genetic engineering. CRISFR-Cas systems, particularly the Type II system that employs Cas9, have become fundamental tools in gene editing (20). CRISPR-Cas9 works by creating double-stranded breaks in DNA at specific locations, using a guide RNA that binds to a target sequence and the Cas9 nuclease that cuts the DNA (13, 14). This allows for either the introduction of mutations through the non-homologous end joining pathway or the insertion of new genetic material via the homology-directed repair mechanism (12).

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Initially identified in E. coli, CRISPR-Cas systems were subsequently observed in a broader range of bacteria and archaea, where they exhibit roles in phage resistance and the maintenance of genomic integrity through mechanisms such as DNA repair mediated by Cas3 and Cas4 (16, 17). Beyond their biological roles, CRISPR-Cas9 and related technologies have been employed in a myriad of applications across biotechnology, agriculture, and medicine, notably including the treatment of complex diseases such as Parkinson's and epilepsy (18).

The clinical application of CRISPR-Cas9 in cancer research represents a significant advance, offering potential for the precise identification and modification of tumor-specific genes. However, the translation of CRISPR-Cas9 into effective cancer therapies is fraught with challenges. These include the potential for immune responses against the CRISPR components and unintended effects on non-target genes, which could lead to adverse outcomes (19).

The objective of this study is to evaluate the advancements in cancer therapy focusing on the CRISPR/Cas9 genome editing system. It aims to elucidate the mechanism of CRISPR/Cas9 and its application in human cancer research and therapies, discussing strategies for identifying critical tumor genes and evaluating the challenges associated with delivering CRISPR/Cas9-based treatments. The review will also consider the efficacy and safety of CRISPR/Cas9 in both preclinical and clinical settings, ultimately exploring its potential to revolutionize precision oncology by overcoming current barriers in cancer treatment.

MATERIAL AND METHODS

In this study, various methodologies were employed to explore the effectiveness of CRISPR-Cas9 in treating mammalian cancers. The research was underpinned by a randomized sampling strategy to ensure a representative distribution within the target population.

The initial phase involved identifying specific genes or genomic regions linked to drug resistance, cancer proliferation, or tumor development. This was achieved through operational research, transcriptomic analysis, and genomic sequencing (27). Subsequent to target identification, single guide RNAs (sgRNAs) were meticulously designed to direct the CRISPR-Cas9 system to the appropriate genomic locations for precise editing. The design of these sgRNAs was facilitated by advanced CRISPR design software, enhancing both the efficiency and accuracy of the process (18).

The delivery of the CRISPR-Cas9 system varied according to the type of tumor and targeted cells. In some instances, viral vectors such as lentiviruses or adeno-associated viruses were employed to introduce the CRISPR components directly into tumor tissues or cancer cells. Alternatively, non-viral methods including lipid nanoparticles and electroporation were utilized for CRISPR-Cas9 delivery, each method chosen based on its suitability for the specific cellular environment (28).

Once inside the target cells, the CRISPR-Cas9 system was engineered to induce double-strand breaks at specified genomic sites. This editing could lead to the deletion of genes, potentially resulting in gene inactivation or frameshift mutations. Alternatively, it could introduce precise mutations to correct oncogenic mutations (7, 13).

The accuracy and efficacy of the genomic modifications were verified through techniques such as PCR, Sanger DNA sequencing, and next-generation sequencing. These validation steps were critical to ensuring that the intended genetic alterations were achieved without off-target effects (29).

Functional assays were then conducted to assess the impact of genome editing on cancer cell behavior. These included tests to measure cell viability and proliferation, assays to evaluate changes in cell motility and invasiveness, and studies to determine any alterations in drug sensitivity. Each assay provided insights into how genome editing influenced cancer cell dynamics and response to treatment (30).

The preclinical evaluation extended into animal models, where the therapeutic potential of CRISPR-Cas9 edited cells was further assessed. This involved xenograft models, where modified cancer cells were implanted into immunocompromised mice to study tumor growth and metastasis. Additionally, genetically engineered mouse models (GEMs) were developed to observe tumor progression and response under controlled genetic alterations (9, 21).

Throughout these processes, efforts were made to minimize off-target effects and optimize the conditions for gene editing. This included refining sgRNA designs and delivery methodologies, coupled with rigorous safety evaluations to address potential immunogenic responses and unintended genetic modifications (31).

Following these comprehensive studies, the potential therapies could advance to clinical trials if initial findings confirmed both efficacy and safety. These trials were structured in phases: Phase I to assess safety, Phase II to evaluate efficacy, and Phase III to

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further compare the effectiveness against existing therapies (10). The structured approach ensured a thorough investigation of CRISPR-Cas9's potential in cancer treatment, setting the stage for its possible translational application in precision oncology.

RESULTS

Chromosome mutations, a critical factor in the genesis of human tumors, have been extensively modeled through various methods. Techniques such as transgenesis and the insertion of cDNA elements into targeted genomes have facilitated the creation of cellular and animal models that mimic these mutations (32). The CRISPR/Cas9 system has demonstrated significant advantages over traditional genome engineering methods. Notably, it requires only a simple sgRNA for RNA/DNA recognition, making it more specific, efficient, and cost-effective than the protein-DNA binding techniques used previously (Table 4.1).

An analysis of publications from 2002 to 2018 highlights the rapidly increasing influence of CRISPR/Cas9 in academic research. An NIH database search revealed a substantial rise in the number of publications discussing CRISPR/Cas9, totaling 9332 articles. In 2017 alone, there were 2889 publications, emphasizing the technology's growing relevance in scientific studies (Fig. 4.a). Specifically, research on CRISPR/Cas9 in cancer demonstrated significant activity, with studies focusing on various cancer types including blood, lungs, breast, and liver cancers. These publications often explored themes like model construction, functional gene analysis, drug target validation, and therapy (Fig. 2b).

Table.4.1. Comparison of different gene editing technology (34).

Technology	ZFNs	TALENs	CRISPR/Cas9
Endo-nuclease	FokI	FokI	CAS 9
DNA targeting	ZF protein	TALE protein	SG(RNA)(crRAN + trancrRNA)
Targeting process	Protein DNA-binding	Protein DNA-binding	RNA/DNA reaction
PAM	NA	NA	5′-NGG-3′
Targeting efficiency	Low	Low	High
Off targeting	High	High	Variable
Operation price	High	High	Low
Operation feasibility	Difficult	Moderate	Easy
Cyto-toxicity	High	Low	Low

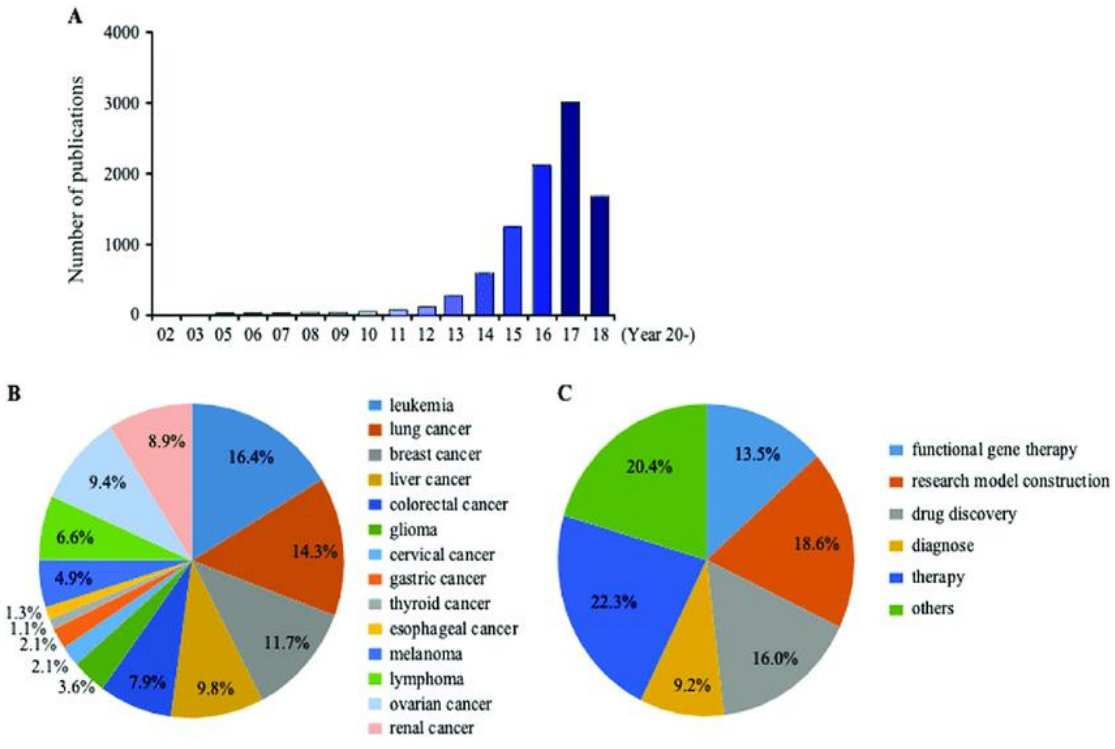

Figure.4.2. CRISPR/CAS9 applications in different cancers (22).

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Table.4.3 CRISPR application in cancer research (6).

Application	Targets	sgRNA design	Vehicle	Features	Advantages	Disadvantages
Create cancer model	HSPCs; healthy human organoids	Targeting the model type-related suppressors oncogenes	Pooled lentivirus	Disrupt suppressors or edit cancer genes	Rapid, efficient, and inexpensive	Special delivery techniques; tissue limited
Synergistic gene analysis	Cells	Targeting optional drug target from database	Lenti-double sgRNA library	Together with deep sequencing	Effective, low cost, innovative method	Double sgRNA construction; need highly efficient sgRNA; special analysis
Target validation	Drug or anticancer reagent resistant cells	Lentiviral library from Add gene or optional targets	Plasmid	Identify the target from resistant cells by sequencing	Effective	False-positives
Gene treatment	Genome	Target sensitive genes	Lenti-virus	Together with Cas 13a or Cas12a to induce collateral effects	Sensitive, rapid, low cost	Certain template concentration

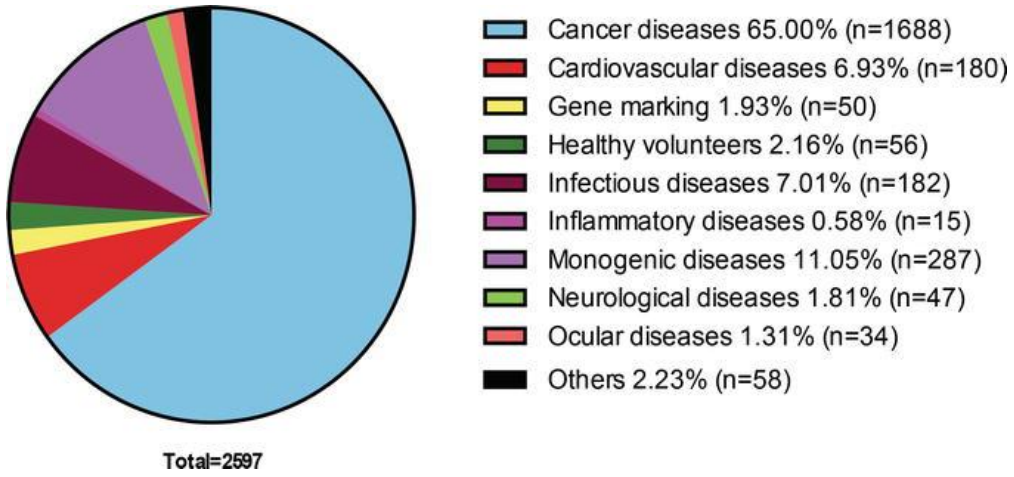

Figure.4.4 Gene therapy experiments worldwide (35).

DISCUSSION

The exact molecular mechanisms leading to chromosomal translocations, often observed in cancer development, remain poorly understood. The process involves two DNA double-strand breaks (DSBs), with the non-homologous end joining (NHEJ) DSB repair mechanism likely responsible for rejoining the DNA ends into a new configuration. It is still unclear whether this process utilizes canonical or alternative pathways (33).

The escalation of academic interest and the application of CRISPR/Cas9 in cancer research reflect its transformative impact across various scientific fields. The term "CRISPR" alone has generated significant research output from 2002 to 2018, indicating the technique's pivotal role in advancing gene therapy, particularly in cancer treatment. Over 65% of all gene therapy studies globally now focus on addressing solid and hematological tumors, underscoring the critical role of CRISPR/Cas9 in both scientific and clinical settings in the field of oncology (34).

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The results from these studies not only enhance our understanding of CRISPR/Cas9's capabilities but also help in evaluating its translational potential in precision oncology. This is particularly important as the field moves toward more targeted and personalized treatment modalities, leveraging the specificity and efficiency of CRISPR/Cas9 in modifying genetic elements associated with cancer (35).

CONCLUSION

CRISPR/Cas9 genome-editing technology holds significant potential to revolutionize cancer therapy, addressing complex challenges like graft-versus-host disease and T cell exhaustion in CAR-T and other adaptive cellular treatments. However, its application in solid tumors presents substantial hurdles including lengthy production times, high costs, off-target effects, and delivery issues related to CAR-T therapies and tumor infiltration. Additionally, the challenge of clone selection and cancer proliferation continues to undermine the effectiveness of conventional anticancer treatments. Despite these obstacles, ongoing research aims to harness CRISpr/Cas9's capabilities for tackling tumors caused by structural variations or copy number abnormalities, potentially establishing it as a critical tool in future cancer management strategies.

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