

Tbilisi, Crispr/Cas9 Technology in Cancer Treatment: Current Applications and Perspectives

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Abstract: Cancer remains a leading cause of mortality worldwide, with its incidence rising due to aging populations, environmental factors, and genetic predispositions. Traditional treatment approaches (surgery, chemotherapy, and radiation) offer limited efficacy and often cause significant side effects, highlighting the urgent need for more effective and targeted therapies. Presented article provides brief review about cancer treatment methods and potential of CRISPR/Cas9 technology in anticancer therapy. Recent advances in targeted therapy and immunotherapy have improved cancer treatment outcomes, yet challenges such as resistance, immune evasion, and high costs persist. CRISPR/Cas9, a revolutionary gene-editing tool, has emerged as a promising strategy in cancer research and treatment. This technology allows precise genetic modifications, offering potential applications in tumor suppression, overcoming drug resistance, and enhancing immunotherapy. CRISPR/Cas9 has been utilized to restore tumor suppressor gene function, disrupt drug resistance mechanisms, and engineer T cells to improve immune response against cancer. The first U.S. clinical trial of CRISPR-based cancer therapy, demonstrated its feasibility in modifying T cells for enhanced tumor recognition and destruction. Despite its promise, CRISPR-based therapies face significant challenges, including off-target effects, delivery inefficiencies, immune system responses, and ethical concerns. Researchers are actively working on improving delivery methods using viral vectors and nanotechnology, as well as refining high-fidelity Cas9 variants to enhance precision and safety. In conclusion, CRISPR/Cas9 holds great potential for transforming cancer treatment by offering more precise, effective, and personalized therapeutic strategies. While challenges remain, ongoing advancements in CRISPR technology may pave the way for its integration into clinical oncology, ultimately improving patient outcomes and advancing the field of cancer medicine.

Key words: Cancer, treatment, CRISPR/Cas9 technology.

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Introduction

Cancer is one of the main causes of disease-associated mortalities worldwide with ever-increasing incidence worldwide [1]. In 2020 alone, an estimated 19.3 million new cancer cases and 10 million cancer-related deaths were reported globally. The increasing aging population, chronic exposure to risk factors, and other environmental and genetic influences contribute to the rising incidence of cancer. There is an urgent need for more effective, accessible, and affordable cancer treatments, particularly in low- and middle-income countries where access to cutting-edge therapies is often limited.

Historically, cancer treatment options have included surgery, radiation therapy, and chemotherapy. These approaches aim to remove, shrink, or kill cancer cells but often come with significant side effects and limitations. Surgery is effective for localized cancers but may not be suitable for metastasized or advanced-stage cancers. Radiation therapy and chemotherapy, although widely used, can be toxic to healthy cells, leading to adverse effects such as nausea, immunosuppression, hair loss, and infertility. Furthermore, chemotherapy resistance is a major challenge, as tumors often evolve mechanisms to evade drug effects over time. This phenomenon, known as acquired resistance, leads to relapse even after initial tumor shrinkage, necessitating the development of more effective and sustainable treatments [2].

Cancer is not a single disease but a collection of related diseases, each with distinct genetic, molecular, and phenotypic characteristics. Even within the same tumor, significant cellular heterogeneity exists, complicating the development of universal treatments. This makes it difficult to develop "one-size-fits-all" treatments. Therefore, a more personalized or targeted approach is essential for effectively treating various cancer types and subtypes.

Advances in Targeted Therapy and Immunotherapy.

Recent advances in cancer treatment have focused on targeted therapies and immunotherapies. Targeted therapies aim to inhibit specific molecules involved in cancer cell growth and survival, whereas immunotherapies harness the body's immune system to recognize and destroy cancer cells. While immunotherapy, particularly immune checkpoint inhibitors and CAR T-cell therapy, has shown promising results, not all patients respond to treatment [3]. Tumors may evade immune detection, and in some cases, the immune system fails to recognize or effectively attack cancer cells. Moreover, the high cost of therapies, resistance development, and potential immune-related adverse effects pose challenges that necessitate further improvement.

Given the complexity of cancer biology and the limitations of existing treatments, researchers are exploring novel approaches to create more effective and less toxic therapies. These efforts focus on understanding the molecular basis of cancer, leveraging emerging technologies, and improving treatment strategies to enhance survival rates and quality of life for cancer patients.

The Role of Gene Editing in Cancer Treatment

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a natural defense mechanism found in bacteria, allowing them to recognize and cut viral DNA. Cas9 (CRISPR-associated protein 9) is an enzyme that acts as molecular scissors, making targeted cuts in DNA.

CRISPR/Cas9 is a powerful gene-editing tool and one of the most promising technologies in molecular biology that allows for precise modifications of DNA in living organisms. Originally discovered as a bacterial defense mechanism against viruses, CRISPR/Cas9 has been repurposed for precise genetic modifications in eukaryotic cells. This technology employs a guide RNA (gRNA) to direct the Cas9 enzyme to a specific DNA sequence, where it creates a double-strand break. The Cas9 enzyme binds to the gRNA and scans the genome. When it finds the matching DNA sequence, Cas9 cuts both DNA strands. The cell repairs the break either through non-homologous end joining (NHEJ - an error-prone repair pathway that often results in small insertions or deletions, leading to gene knockout), or homology-directed repair (HDR - a precise repair pathway that can introduce specific genetic changes by using a donor DNA template) [4].

The precision, efficiency, and relative affordability of CRISPR/Cas9 have made it a powerful tool in various fields, including agriculture, biotechnology, and medicine. CRISPR/Cas9 has been used in medical research to study genetic diseases and develop potential gene therapies, e.g. Gene knockout (disrupting the function of a specific gene to study its role), Gene correction (fixing mutations responsible for genetic diseases), Gene insertion (adding new genetic sequences for therapeutic or research purposes), Biomedical research (studying genetic disorders and potential treatments). In agriculture, CRISPR/Cas9 technology enhances crop resistance to diseases, pests, and harsh environments. In biotechnology it is used in synthetic biology for new biological systems.

CRISPR/Cas9 in Cancer Immunotherapy

Immunotherapy has revolutionized cancer treatment, particularly with immune checkpoint inhibitors like PD-1/PD-L1 antibodies and Chimeric Antigen Receptor T-cell therapies (CAR-T therapy where a patient's T cells are genetically engineered to target cancer cells). However, immune evasion and tumor heterogeneity can limit their effectiveness.

CRISPR/Cas9 has emerged as a transformative tool in cancer immunotherapy, enabling precise genetic modifications to enhance the immune system's ability to recognize and attack tumors. Its applications in oncology primarily focus on modifying immune cells, targeting oncogenes, and overcoming resistance mechanisms in cancer treatment [5].

In CAR T-cell therapy, CRISPR has been used to knock out the PD-1 gene in T-cells, enhancing their persistence and ability to attack tumors without being inhibited by immune checkpoint pathways. Furthermore, CRISPR/Cas9 can engineer T-cells to target multiple antigens simultaneously, reducing the risk of tumor escape due to antigen loss.

CRISPR/Cas9 enhances CAR-T therapy approach by:

- Improving T-cell persistence and function: Knockout of PD-1 (a checkpoint inhibitor) prevents immune exhaustion.
- Enhancing tumor recognition: Gene editing can optimize CAR-T cells to recognize more cancer-specific antigens.
- Universal CAR-T development: CRISPR enables the creation of "off-the-shelf" CAR-T cells from donor sources by eliminating rejection-causing molecules.
- Edit tumor cells to reduce their ability to suppress immune responses.

CRISPR/Cas9 can also be used to edit tumor cells to reduce their ability to suppress immune responses. One of the primary applications of CRISPR/Cas9 in cancer therapy is the direct modification of tumor suppressor genes (TSGs). These genes regulate cell growth, induce apoptosis, and maintain genomic integrity. In many cancers, TSGs are mutated or silenced, leading to uncontrolled cell proliferation. CRISPR/Cas9 can correct these mutations, restoring normal TSG function and potentially reversing tumor growth. Researchers have utilized CRISPR/Cas9 to restore p53 function in cancer cells (p53 gene, which is known as the "guardian of the genome," is mutated in over 50% of human cancers) increasing tumor cell death and enhancing chemotherapy sensitivity. Similar strategies have been explored for other TSGs, such as PTEN, BRCA1, and RB1, with promising results in preclinical models [6].

CRISPR/Cas9 offers a potential solution to overcome drug resistance, a major hurdle in current cancer therapies. Cancer cells acquire resistance through various mechanisms, including the upregulation of drug efflux pumps, mutations in drug targets, or activation of alternative survival pathways. By knocking out genes associated with these resistance mechanisms, CRISPR/Cas9 can sensitize cancer cells to existing treatments. For example, in ovarian cancer, over-expression of ATP-binding cassette (ABC) transporters contributes to chemotherapy resistance by actively pumping drugs out of cells. CRISPR/Cas9-mediated knockout of the ABCB1 gene has been shown to increase the effectiveness of chemotherapy drugs like paclitaxel [7].

The first U.S. clinical trial utilizing CRISPR for cancer therapy began in 2019 at the University of Pennsylvania, partially funded by the National Cancer Institute (NCI). This study is exploring a CRISPR-enhanced immunotherapy designed to improve the ability of T cells, immune cells that combat cancer to recognize and eliminate cancerous cells [8].

The therapy involves four key genetic modifications to T cells. First, a synthetic gene is inserted to produce a receptor capable of recognizing **NY-ESO-1**, a molecule found on certain cancer cells. Then, CRISPR is used to remove three genes: two that could interfere with the NY-ESO-1 receptor's function and one that naturally limits the T cells' ability to attack cancer. The modified T cells, called **NYCE T cells**, are then expanded in number before being reinfused into patients.

This trial represents a significant step in evaluating CRISPR's potential as a cancer treatment. While results are still being assessed, the study provides valuable insights into how CRISPR-based therapies might be optimized for future clinical use.

In summary, CRISPR/Cas9 can be used to:

- Knock out oncogenes like KRAS (common in lung and pancreatic cancers).
- Restore tumor suppressor genes like TP53 to enhance apoptosis (programmed cell death) in cancer cells.
- Induce synthetic lethality, where targeting one pathway (e.g., DNA repair genes in BRCA-mutant tumors) causes cancer cell death.

Advantages of CRISPR/Cas9 are:

- High Precision (Targets specific DNA sequences with minimal off-target effects),
- Efficiency (Faster and cheaper than older gene-editing methods like TALENs and zinc-finger nucleases), and Versatility (Applicable to various organisms, including humans, plants, and animals).

Limitations of CRISPR in Cancer Research and Treatment

Despite its many advantages, CRISPR is not without its challenges. While it has become a widely used tool for cancer research and holds promise for therapeutic applications, scientists remain cautious about its use in human treatments due to several key limitations [9].

One significant concern is **off-target editing**, where CRISPR inadvertently modifies unintended sections of the genome. Such unintended edits can potentially lead to harmful mutations, including those associated with cancer. A notable case from 2002 involved gene therapy leading to unintended genomic alterations, raised concerns about similar risks with CRISPR-based interventions. Scientists have worked to refine the precision of CRISPR by modifying the Cas enzyme and guide RNA, reducing but not completely eliminating the risk of off-target effects.

Another challenge is **delivering CRISPR components effectively into cells**. In laboratory settings, CRISPR is relatively easy to introduce into cultured cells, but delivering it safely and efficiently within a human body presents additional complexities. One common delivery method involves using viral vectors to transport the CRISPR system. However, some of these viruses may inadvertently target unintended cells, leading to undesired genetic modifications. To address this, researchers are investigating more selective viral vectors and developing nanocapsules that can direct CRISPR to specific organs or cell types. Advances in high-fidelity Cas9 variants have helped minimize these unintended edits, but further research is required to enhance accuracy and ensure safety.

A further complication arises from **potential immune system reactions**. Since CRISPR's Cas enzyme originates from bacteria, the human immune system might recognize it as a foreign substance and mount an immune response, potentially eliminating CRISPR-edited cells. Past incidents with gene therapy have

demonstrated the dangers of severe immune reactions, but newer approaches using safer viral vectors may mitigate this risk.

Additionally, ethical considerations regarding gene editing, especially germline modifications, remain a topic of debate. While somatic gene editing holds great promise for cancer treatment and other medical applications, unintended alterations in germline cells raise important ethical questions about the long-term consequences for the human genome. If CRISPR is used within the body and inadvertently alters sperm or egg cells, these genetic changes could be passed on to future generations. Most current studies aim to avoid this issue by using an **ex vivo** approach, where cells are edited outside the body before being reintroduced. However, some ongoing research, such as a study targeting Leber congenital amaurosis (a genetic cause of blindness), is testing in vivo CRISPR applications [9].

Conclusion.

1. CRISPR/Cas9 technology holds immense potential for revolutionizing cancer treatment by enabling precise gene editing facilitating cancer drug discovery.
2. The some key points that align with the current understanding of CRISPR/Cas9 in cancer treatment are:
 - Gene Editing for Tumor Suppression: CRISPR/Cas9 can modify tumor suppressor genes (TSGs) to restore their function and potentially reverse tumor growth. Examples include restoring the p53 gene function in cancer cells.
 - Overcoming Drug Resistance: CRISPR/Cas9 can knock out genes associated with drug resistance mechanisms, making cancer cells more sensitive to existing treatments. An example given is knocking out the ABCB1 gene in ovarian cancer to improve chemotherapy effectiveness.
 - Enhancing Immunotherapy: CRISPR/Cas9 can be used to engineer immune cells, such as T-cells, to better recognize and attack cancer cells. The document mentions the use of CRISPR to knock out the PD-1 gene in T-cells for enhanced tumor recognition.
3. Challenges and Limitations: The key challenges are off-target effects, delivery inefficiencies, immune system responses, and ethical considerations, all of which are crucial areas of ongoing research.
4. While challenges remain, including delivery efficiency, off-target effects, and ethical considerations, ongoing advancements in CRISPR/Cas9 applications are paving the way for more effective cancer therapies. With continued research and development, CRISPR/Cas9 could become an integral part of oncology, offering new hope for cancer patients and advancing personalized medicine. [10]

References

1. J. A. Doudna и S. H. Sternberg, *A Crack in Creation: Gene Editing and the Unthinkable Power to Control Evolution*. Houghton Mifflin Harcourt, 2017.
2. D. Baltimore и др., «A prudent path forward for genomic engineering and germline gene modification», *Science*, т. 348, вып. 6230, сс. 36–38, 2015, doi: 10.1126/science.aab1028.
3. N. Vasan, J. Baselga, и D. M. Hyman, «A view on drug resistance in cancer», *Nature*, т. 575, вып. 7782, сс. 299–309, 2019, doi: 10.1038/s41586-019-1730-1.
4. A. A. Rabaan, «Application of CRISPR/Cas9 Technology in Cancer Treatment: A Future Direction», *Curr. Oncol.*, т. 30, вып. 2, сс. 1954–1976, 2023, doi: 10.3390/curroncol30020152.
5. W. H. Organization, *Cancer*. World Health Organization, 2021. [Онлайн]. Доступно на: <https://www.who.int/news-room/fact-sheets/detail/cancer>
6. R. L. Siegel, K. D. Miller, и A. Jemal, «Cancer statistics, 2020», *CA. Cancer J. Clin.*, т. 70, вып. 1, сс. 7–30, 2020.

7. H. Sung и др., «Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries», *CA. Cancer J. Clin.*, т. 71, вып. 3, сс. 209–249, 2021.
8. Y. Shiravand и др., «Immune checkpoint inhibitors in cancer therapy», *Curr. Oncol.*, т. 29, сс. 3044–3060, 2022, doi: 10.3390/curroncol29050247.
9. S. L. Gupta, S. Basu, V. Soni, и R. K. Jaiswal, «Immunotherapy: An alternative promising therapeutic approach against cancers», *Mol. Biol. Rep.*, т. 49, сс. 9903–9913, 2022, doi: 10.1007/s11033-022-07525-8.
10. F. Bray, M. Laversanne, E. Weiderpass, и I. Soerjomataram, «The ever-increasing importance of cancer as a leading cause of premature death worldwide», *Cancer*, т. 127, вып. 16, сс. 3029–3030, 2021.