NIJBAS
Publications 2024

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES (NIJBAS)

Volume 5 Issue 1 2024

https://doi.org/10.59298/NIJBAS/2024/5.1.9297102

CRISPR-Cas9 Mediated Gene Editing for Targeted Cancer Therapy: Mechanisms, Challenges, and Clinical Applications

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ABSTRACT

The CRISPR-Cas9 system has revolutionized gene editing with its high efficiency and specificity, offering new avenues for targeted cancer therapy. This review focuses on the mechanisms through which CRISPR-Cas9 can be used to suppress oncogenes, restore tumor suppressor genes, and enhance immunotherapy by editing key genetic sequences. This review also explores how CRISPR-Cas9 targets DNA repair pathways such as homology-directed repair (HDR) and non-homologous end joining (NHEJ), emphasizing the precision required for successful cancer treatment. Despite promising in vitro results and ongoing clinical trials, challenges such as off-target effects and immune responses remain. This review also highlights advances in CRISPR technology, preclinical and clinical studies, combination therapies, and future directions in cancer therapy.

Keywords: CRISPR-Cas9, cancer therapy, gene editing, DNA repair pathways, oncogenes, tumor suppressor genes, immunotherapy, clinical trials

INTRODUCTION

The CRISPR-Cas9 system has emerged as a groundbreaking tool in the field of gene editing, offering unprecedented precision in modifying specific DNA sequences. Initially discovered as part of the adaptive immune system in bacteria, CRISPR-Cas9 has been adapted for use in mammalian cells, enabling targeted modifications to the genome [1]. This technology holds immense potential for cancer therapy, where genetic aberrations play a crucial role in disease progression [2]. Cancer is characterized by the accumulation of genetic mutations that lead to uncontrolled cell growth and metastasis [3]. Traditional cancer treatments such as chemotherapy and radiation therapy often lack specificity, leading to significant side effects. In contrast, CRISPR-Cas9 allows for the precise targeting of cancer-related genes, providing a more focused approach to treatment [4, 5, 6]. This review explores the mechanisms by which CRISPR-Cas9 can be used in cancer therapy, focusing on its ability to disrupt oncogenes, restore tumor suppressor genes, and enhance the efficacy of immunotherapies. We also discuss the challenges and limitations of CRISPR-Cas9, including off-target effects and immune responses, as well as recent advancements in the technology and its application in clinical settings.

Mechanisms of CRISPR-Cas9 in Cancer Therapy

The clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (Cas9) system has become a popular gene editing strategy due to its excellent efficiency and specificity [7]. According to basic research, gene engineering can precisely remove or suppress oncogenes, restore or

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ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

enhance tumor suppressor genes, and extend the application of immunotherapy by editing the sequence of the B2M or PDCD1 gene [8]. Additionally, this system is capable of eradicating Hepatitis B Virus (HBV) or Human Papillomavirus (HPV) by targeting the hybrid DNA. Based on these mechanisms, the system is expected to achieve a comparable, if not higher, therapeutic effect than other traditional drugs [9]. Despite most of the research being conducted in vitro, there are no reports of CRISPR-Cas9 system-related adverse events in clinical trials [10]. It is thus believed to have excellent safety. Currently, the CRISPR-Cas9 system is being tested in cancer patients with phase I clinical trials [11]. The clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (Cas9) system has induced a revolution in the field of biomedicine [12]. Based on the known mechanisms, we can modulate its properties, such as cell- or tissue-specificity and the pathway that mediates the repair of double-strand breaks, to optimize its application in cancer therapy [13]. Although many in vitro studies have reported that the CRISPR-Cas9 system is very effective for cancer treatment, a mass of barriers are obstructing it from being used in clinical trials. In clinical trials for cancer treatment, this system has thus far been mainly applied to patients with melanoma, synovial sarcoma, and non-small-cell lung carcinoma [14].

DNA Repair Pathways and Gene Editing Precision

While targeting the DNA double-stranded breaks of the Cas9 system to cancer cells is quite straightforward, doing that safely and efficiently to target genes that the cancer cell uniquely depends on is the ultimate goal [15]. Generally speaking, blocking overactive proto-oncogenes or restoring functionality to inactivated tumor suppressors can provide exactly that type of dependency, and CRISPR-Cas9 has provided biologists with logical means to deploy that strategy [16]. However, the system that actually and uniquely causes cells to over-proliferate, metastasize, and resist programmed cell death - the hallmarks of cancer - often revolves around multiple regulatory layers with redundant backups or compensating expression profiles [17]. Considering the raw power of gene editing, this leads to the ever-growing question of cancer treatment and gene therapy in general: how safe is using CRISPR-Cas9 to perform fundamental changes to the genome that are intrinsically dependent on the biological pathways utilized to make them, and how could the choice between those two pathways - non-homologous end-joining (NHEJ) and homologous-directed repair - specifically minimize any concerns? A primary goal of cancer therapy research is to recognize and eliminate the cancerous populations of cells in the human body [18]. In the ever-analytical quest to create customized cancer therapies, the focus has primarily been on the wealth of genetic information that is compromised in the cancer genome. One popular research avenue into cancer targeting truncates proteins that are directly responsible for the survival of the malignant population or manipulates the cellular environment to enhance cancer cell susceptibility to apoptotic stimuli [19]. Dramatically, gene editing technology has recently provided biologists with a concrete method to edit the human genome directly [20]. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 is a system driven by an RNA-splice reaction that has quickly established itself as the foremost gene editing system and is doing just that with an unprecedented level of precision and portability.

CRISPR-Cas9 for Specific Cancer Types

Despite remarkable progress in developing anticancer drugs, cancer remains one of the main causes of death in countries worldwide. Cancer therapy remains the most dynamic field within molecular medicine, and presently, the most common cancer treatments still depend on methods such as chemotherapy, radiotherapy, and surgery, which are frequently associated with significant side-effects such as damage to healthy tissues [21]. Therefore, the development of novel targeted therapies is necessary to increase the likelihood of therapeutic success with decreased off-target effects and less risk of developing drug-resistant cancer cells. However, these novel agents are still not suitable for all patients due to the diverse tumor heterogeneity of almost all types of cancer [22]. As the targeted gene-editing CRISPR-Cas9 system can correct and inactivate abnormal genes, this technology is receiving more attention due to the potential of improving targeted cancer therapies. In this review, we detail the recent advances of CRISPR-Cas9 gene editing on treating breast cancer, lung cancer, and hematologic malignancies. Moreover, the key challenges and several clinical applications of CRISPR-Cas9 on cancer therapy are also discussed. In recent years, technological advances in gene editing tools derived from the CRISPR system have paved the way for developing novel targeted cancer therapies [9]. The CRISPR-Cas9 system allows the visualization and correction of abnormal genes by precisely breaking the DNA at the mutation site and promoting DNA repair in mammalian cells via homology recombination. Breast cancer is one of the most common types of cancer, and more than 10% of breast cancers are hereditary caused by mutations in BRCA1 and BRCA2. Therefore, in this review, we mainly focus on the use of CRISPR-Cas9 to target BRCA genes in breast cancer therapy [12]. Additionally, we also introduce the clinical potential of using CRISPR-Cas9 assisted by RNA guide sequences or adenovirus vectors to correct specific mutations of the

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ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

EGFR and KRAS genes in lung cancer therapy. Moreover, we present how CRISPR-Cas9 can impact targeted cell reprogramming to induce specific cell types such as chimeric antigen receptor T-cells for effective breast cancer, lung cancer, and hematologic malignancy treatment [23].

Challenges and Limitations

All studies involving CRISPR-Cas9 involve an immune response that can limit effectiveness of therapy. Dosing of CRISPR components, and the optimal promoter for long-term expression are active areas of study but resolving the immune response remains a significant impediment [24]. Future CRISPR therapies will need to account for the impact of their delivery methods and develop approaches to avoid or suppress potential transgenic immune responses. Achieving immune tolerance, inducing marker gene expression by combining transcription or translation inhibitors, and local delivery of CRISPR components are all effective methods for avoiding the activation of innate immunity by CRISPR/Cas9 components [16]. However, the latest progress in the study for immune regulation improves the efficiency of CRISPR/Cas9 gene editing and avoids inducing immune responses. We summarize the most recent studies for immune regulation that enhance the in vivo delivery of CRISPR components leading to the successful treatment of genetic disorders [19]. In addition to generating site-specific double-strand breaks, Cas9 also causes off-target single-strand nicks at sites with imperfectly homologous sequences, potentially allowing for a greater risk of off-target mutations. The accuracy of CRISPR-Cas9 is essential for its safe application in clinical settings, especially for gene therapy. However, off-target effects limit the applicability of CRISPR-Cas9 for biomedical research and therapeutic applications. Thus, CRISPR-Cas9 technology should be further refined and CRISPR-Cas9-induced off-target editing should be characterized before its translation to therapeutic use [20]. Off-target effects could be minimized by carefully designing the guide RNA, modifying the Cas9 protein, or using the high fidelity Cas9 protein. In this review, we discuss a number of these potential off-targets due to CRISPR-Cas9, and the cutting-edge strategies that could mitigate these off-targets.

Advances in CRISPR Technology

The efficiency and specificity of the CRISPR-Cas9 system endure considerable restrictions that hinder the completion and success of CRISPR-based clinical treatments [10]. To achieve better gene editing efficiency and targeting specificity, multiple strategies have been and are continuously being discovered. The selection of highly efficient Cas9 proteins, the development of analysis tools, and the increase in gRNA activity are pivotal strategies to improve the efficiency of the CRISPR system. Additionally, new genome-editing components enable efficient, precise, and targeted gene editing [13]. Advanced CRISPR system application studies and clinical trial processes suggest that CRISPR-mediated gene therapies are closer to the cure of diseases. This review focuses on the latest strategies to improve the CRISPR-Cas9 system and deepen the understanding of its applications in hematopoietic stem cell editing for the therapy of hematopoietic diseases and cancer. Furthermore, methylation-mediated CRISPR systems (Cas9, Cas12, and Cas13) have been developed for new applications in gene therapy, such as DNA methylation modification, base editing, and precision and universal prime editing [15]. As the field of gene therapy matured and human genomes were subject to increasing scrutiny, the limitations of CRISPR-Cas9 gene editing technology began to emerge, such as off-target effects, consequential indels, and inefficient HDR-based precision editing. In order to address these shortcomings, substantial efforts have been placed on developing safer and more precise next-generation CRISPR-Cas systems that can be used to treat cancers [8].

Preclinical and Clinical Studies

The ongoing advances in CRISPR-based technologies, genetic and epigenetic investigations, bioinformatics and delivery systems further reinforce the promise and potential of CRISPR treatment on the road to precision medicine [15]. Animal models have helped researchers gain a greater understanding of cancer progression, as well as identifying novel targets for developing new strategies for cancer therapy. Recently, CRISPR-Cas9 has emerged as a useful tool for generating animal models of cancer by the introduction of genetic and epigenetic changes in various cellular compartments [16]. Despite successful initial studies, several challenges still need to be addressed to optimize the use of CRISPR-based technologies in cancer research. The next generation CRISPR systems such as CRISPR-CasΦ and CRISPR-Cas12a along with the new delivery systems with enhanced safety and tumor targeting capabilities would be instrumental to address the current challenges and facilitate their in vivo application. Additionally, innovative designs and future improvements in bioinformatics analysis could facilitate the identification of the most reliable gene targets and loci for therapeutic intervention [18].

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ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

Combination Therapies

CRISPR-Cas9 technology can be combined with the immune system to initiate a powerful anti-tumor immune response [9]. This strategy has high specificity, few side effects, and is applicable to many different types of cancer. Here, we review the current status for using CRISPR-Cas9 in conjunction with the immune system for cancer treatment [12]. A high mutation rate is a hallmark of cancer cells, resulting in the generation of tumor-specific antigens that can be recognized by the patient's endogenous immune system. However, mammalian cells inherently display few cancer-specific antigens. Chimeric antigen receptor (CAR)-T cells recognize and attack target cells, overcoming this limitation. By utilizing CRISPR-Cas9 to eradicate patient immune system activating compounds, researchers can produce these cells with much greater accuracy and efficiency [16]. This dramatically improves the success of CAR-T cell therapy. In the future, when the technology is better refined, we can expect CAR-NK and CAR macrophages to become mainstream. CRISPR-Cas9 mediated gene editing is a new class of therapeutics that is poised to revolutionize the treatment of inherited diseases and cancer. In the context of cancer, CRISPR-Cas9 technology can not only be used to knockout oncogenes to stop tumor growth, but also be used to activate tumor suppressor genes to stop tumor growth [19]. There are ongoing clinical trials and pre-clinical studies that have shown promise for cancer patients treated with CRISPR-Cas9 technology [20].

Future Directions

Cancer cells characteristically sustain critical mutations in precise genes known as oncogenes and tumor suppressor genes. The differentiation between cancer and normal cells by these mutations can be utilized to design a cure that mainly targets cancer cells while leaving normal cells unharmed with general evolution witnessed in cancer therapy [5]. The intention of personalized medicine based on patient tumor profiling as quickly accomplished or possibly in the future when patient mapping becomes low-priced, opens new approaches to distinct patient therapeutic vaccination against individual recognized mutations. This plan is in the most advanced phase, with rigorous ex vivo expanded and reprogrammed TCR T-cells described during the implementation of immunotherapy involving therapy for individual point mutations and personal neoantigens in patients [8]. Only a small number of affected patient group were those found with the tumor tissue somatic mutation due to the mutation. The advancement of next-generation sequencing has identified a number of somatic mutations found particularly in cancer genomes. Tumor-specific mutations harboring small insertions or deletions have opened ways to develop personalized cancer therapeutic vaccines [9]. The CRISPR-Cas9 mediated cancer targeting presented in this review has further extended the concept of personalized therapy. Both autologous and allogeneic approaches are found with some specific precautions required to take care of the contra selective growth of on-target edited cells [10]. The immunological response due to the CRISPR-Cas9 Protein/sgRNA edited peptides, their processing, and presentation to the immune system and future cancer therapy survivors due to the peptide edited in normal tissues require further prophylactic studies.

CONCLUSION

The CRISPR-Cas9 system represents a significant advancement in the field of cancer therapy, offering a precise and efficient method for targeting genetic mutations. While in vitro studies have demonstrated the potential of CRISPR-Cas9 to effectively treat various types of cancer, translating these findings into clinical practice remains challenging due to issues such as off-target effects and immune responses. Ongoing research is focused on refining CRISPR-Cas9 technology, developing better delivery methods, and conducting comprehensive preclinical and clinical studies to address these challenges. The future of CRISPR-Cas9 in cancer therapy is promising, with potential applications extending beyond gene editing to include combination therapies with immunotherapies and personalized medicine approaches. As the technology continues to evolve, it is expected to play a pivotal role in the development of more effective and targeted cancer treatments, ultimately improving patient outcomes.

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ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

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CITE AS: Ugwu Okechukwu Paul-Chima, Anyanwu Chinyere Nkemjika, Alum Esther Ugo, Okon Michael Ben, Egba Simeon Ikechukwu, Uti Daniel Ejim and Awafung Emmanuel Adie (2024). CRISPR-Cas9 Mediated Gene Editing for Targeted Cancer Therapy: Mechanisms, Challenges, and Clinical Applications. NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES,5(1):97-102.
https://doi.org/10.59298/NIJBAS/2024/5.1.9297102