Mechanisms Insights into the Role of CRISPR/CAS9 in Breast Cancer Development

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Abstract: The function of CRISPR/Cas9 gene editing technology in the evolution and therapy of breast cancer is discussed in this paper. Although existing therapy approaches have many flaws and breast cancer is a high-incidence malignancy in women worldwide, CRISPR/Cas9 technology offers a fresh approach with its precision editing capacity. This paper presents the fundamental idea, method of delivery, and application of CRISPR/Cas9 technology in the treatment of breast cancer together with targeted resistance mechanism, precision therapy, and immune cell modification. Simultaneously, the obstacles that the technology faces—off-target effect, immune response and delivery efficiency—are covered, and potential development paths are hinted at. Expected to be a major turning point in breast cancer treatment, CRISPR/Cas9 technology will provide accurate and successful treatment plans to patients and help to prevent and treat breast cancer to a new age.

1 INTRODUCTION

In contemporary society, cancer has emerged as a global health crisis, particularly as the second leading cause of death in the United States (Siegel et al. 2017). Among various cancers, breast cancer stands out as one of the most prevalent types and a significant contributor to female mortality (Curigliano 2012, Siegel et al. 2022). In Western countries, breast cancer is among the most frequently diagnosed diseases among women, with the highest incidence rates observed in the European region (Bodewes et al. 2022). Given these alarming statistics, enhancing the prevention of breast cancer and identifying novel therapeutic approaches are not only inevitable imperatives of our time but also hold profound scientific significance.

Although traditional cancer treatments, such as chemotherapy, cytotoxic therapy, and surgical resection, are widely used, they are often associated with significant drawbacks, including severe side effects and a high risk of complications (Yang et al. 2021). CRISPR/Cas9 is a highly innovative gene-editing technology that enables the correction, insertion, or deletion of genetic material in both vivo and in vitro settings (Karn et al. 2022). This technology employs the principle of primer targeting to identify specific DNA sequences and guides the Cas protein to a precise location in the genome by modifying a guide RNA sequence, thereby enhancing the efficiency of gene editing (Li et al. 2023). In breast cancer research, CRISPR/Cas9 has been instrumental in elucidating the mechanisms of drug resistance and immune evasion in tumor cells. It has emerged as an extremely important, effective, and direct tool in the treatment of breast cancer (Sabit et al. 2021).

Breast cancer can be induced by genetic mutations or familial inheritance, as well as alterations in the microenvironment of breast cells (Obeague & Obeague 2024). However, despite significant advancements in breast cancer research, its complex pathogenesis remains incompletely understood. Additionally, breast cancer is highly heterogeneous, with distinct subtypes exhibiting marked differences in biological behavior and treatment response (Wang et al. 2024). Therefore, systematically elucidating the role of CRISPR/Cas9 technology in the development and progression of breast cancer, and summarizing relevant research progress, are of great significance for deepening our understanding of its pathological mechanisms and identifying new therapeutic targets and strategies. Through a comprehensive literature review, the

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current state and limitations of the existing research can be better understood, providing guidance for future research directions and advance the development of breast cancer prevention and treatment strategies.

2 FUNDAMENTAL PRINCIPLES OF CRISPR/CAS9 TECHNOLOGY

As an epochal gene editing tool, CRISPR/Cas9 employs the complementary effects of gRNA and Cas9 endonuclinase to precisely change a genome. With 20 nucleotide sequences, gRNA's sequence is straightforward, and the 20-base spacer sequence is supplementary coupled with the target DNA during the working process so directing Cas9 to a specific genetic spot. This process reflects the high specificity of genes (Asmamaw&Zawdie 2021). After that, the single-stranded guide RNA, or gRNA, hooks itself to the Cas9 nuclease to create a CRISpen-Cas9 functional complex. To locate the proto-spacer sequence adjacent motif (PAM) at the 3' end of the target sequence, the complex glides down the DNA strand (Aljabali et al. 2024). The PAM sequence is the fundamental component for Cas9 to identify and bind the target DNA; its presence or absence affects whether the system can operate as it should. Usually a short-maintained sequence, the PAM sequence is "NGG," in the common SpCas9 system, where "N" indicates any base and "GG" is the complete PAM sequence. Although only DNA fragments which includes PAM sequences can be discovered and cut by Cas9, this conserved PAM sequence offers unambiguous guidelines for the aiming design of CRISPR/Cas9 systems and limits their targeting range. Cas9 starts the DNA cutting process when it identifies PAM. The conformation of Cas9 changes when sgRNA complements and pairs with the target DNA sequence to produce PAM. The alteration of Cas9 conformation boosts nuclease activity even further. The Cas9 protein is now creating a double-strand break (DSB), cutting two strands of the target DNA throughout the nucleic acid domain. Key to enabling gene editing using CRISpen/Cas9 is the formation of double-stranded breaks; thereafter, the DNA repair machinery within the cell is triggered to repair DNA via either homologous directed repair (HDR) or non-homologous end joining (NHEJ).

The non-homologous end-join repair process (NHEJ) is most notable for without relying on the homologous template, hence it may be used in most cell types and occurs during any cycle of cell division. Although NHEJ maintains DNA rather quickly, since it does not rely on homologous templates, the repair results are generally less precise and can generate either insertion or deletion modifications. Conversely, homologous directed repair (HDR) has greater significance in procedures requiring exact manipulation such as gene insertion, knockout, and replacement since it utilizes homologous templates for specific repair. The technological advances in the complexity of the HDR technique are higher, nonetheless, and operating methods and examination circumstances are more strictly requested. Usually occurring only in the S and G2 phases of the cell, HDR results from sister chromatids present within a cell fit for homologous templates. Moreover, HDR is somewhat ineffective, particularly in some non-dividing cells, which restricts its utility in specific application situations. One can treat breast cancer using the fundamental ideas of CRISPR/Cas9 technology. Its exact positioning ability assists it to repair or knock out the genes linked to the development and progression of breast cancer, therefore attaining the effect of precision treatment. Alternatively use its technologies to change immune cells to improve immune system performance, thereby preventing the emergence of breast cancer. The development and creativity of CRISPR/Cas9 technology offer a wide range of opportunities for managing the worldwide sickness of breast cancer.

3 DELIVERY METHODS FOR CRISPR/Cas9

Once the fundamental ideas of CRISpen/Cas9 technology are grasped, its delivery in the body has also become a big issue. The most often used methods of delivery nowadays are plasmids, mRNA, ribonucleoprotein complexes (RNP), and viruses. Among multiple forms, the plasmid delivery technique is the simplest and most reasonably priced; nonetheless, the transfection efficiency is rather poor and can cause an immune response or insertion mutation. Although mRNA delivery can be expressed quickly and helps to avoid genome integration, its stability and delivery efficiency still have to be further improved. Although the flaws of this delivery technique are that it has poor in vivo stability and restricted delivery efficiency, ribonucleoprotein

complex (RNP) delivery can enable fast gene editing and lower the chance of off-target effects. Although viral vectors—such as adenoviruses—have great transduction capability and adaptability to a broad spectrum of cell types—there are vector capacity limits and possible immunological concerns. Thus, carrying out the use of CRISPR/Cas9 technology in breast cancer treatment depends mostly on its intended distribution form. This paper will extensively address in the sections that follow how to distribute CRISPR/Cas9 within cells to generate novel concepts and strategies for the exact treatment of breast cancer.

3.1 Plasmid DNA Delivery System

By containing the coding sequence of Cas9 nuclease and gRNA, direct transfection of plasmid DNA is the easiest approach to introduce external genetic material and attain long-term stable expression. The drawback of this method, though, is that Cas9 expression can cause more off-target effects and insertion mutations. Conversely, random integration of plasmid DNA can cause insertion mutations, which would subsequently influence the normal physiological operation of cells. The researchers will thus seek to place a degradation signal sequence or activity inhibition domain on Cas9 in order to lower these hazards. This ensures exact control of Cas9 protein activity, therefore lowering the possibility of off-target repercussions and insertion mutations. Simple and inexpensive to manufacture, plasmids can be extensively utilized in laboratory research. Nevertheless, the process of plasmid subsequent generations and purification has dangers of endotoxin contamination and host immunological reaction; so, the presence of endotoxin will set off the host immune response and influence the outcome of the experiment. Researchers have lately solved these issues by creating microcyclic plasmids (Ahmed et al. 2021). Non-viral methods of distribution call for additional components—physical means (electroporation, microinjection) or lipid nanoparticles (LNP)—to aid across the cell membrane. Under the physical strategy, the electroporation method uses a brief pulse of high voltage to generate reversible pores in the cell membrane, consequently facilitating the entrance of plasmid DNA. Microinjection is the direct injection of plasmid DNA into the cytoplasm under a microscope using microneedles. Though theoretically the physical technique may significantly boost the delivery efficiency, it damages cells dramatically and is usually suitable for single-cell level operation. Chemical techniques distribute plasmid DNA primarily through lipid nanoparticles. Including lipid, lipid nanoparticles are nanometers carriers that can wrap plasmid DNA and shield it from nuclease breakdown. By means of fusing with the cell membrane, lipid nanoparticles can efficiently introduce plasmid DNA into the cell, hence improving the efficiency of transport and decreasing immune response.

3.2 RNA Delivery

Gene editing efficiency and safety render RNA delivery options based on CRISPR components—such as Cas9 mRNA in conjunction with gRNA—better than traditional plasmid DNA delivery. Short-term studies might consider RNA delivery appropriate since it uses cellular translation procedures to rapidly start editing without waiting for plasmid transcription. Its brief action cycle lowers the likelihood of genome insertion and off-target alterations. Additionally, RNA has a brief action cycle in the cell, which helps significantly decrease the risk of off-target and undetected risks of genome insertion mutations, thus ensuring the safety of gene editing. But the low temperature storage and transportation demand as well as the great purity modification of mRNA raise the production cost. While their mature technology and effective delivery capability have made lipid nanoparticles (LNP) the preferred choice as the principal carrier of RNA shipping, their greater requirements for targeting accuracy have led to their marginal acceptance. Studies have revealed, for instance, that package delivery vectors (EDVs) can greatly lower the required CRISPR-Cas9 RNP dose and are at least twice as quick and more efficient than electroporation delivery CRISPR-Cas9 RNP. Safe and more efficient delivery technology for gene therapy has resulted from research on RNA delivery methods. Chemically modified GRnas can enhance CRISpen-Cas gene editing efficiency in basic human cells. Furthermore looked into are the use of biodegradable lipid nanoparticles for Cas9 mRNA delivery to increase gene editing efficiency and safety criteria. All things considered, CRISpen component RNA delivery methods are somewhat common in the world of gene editing but also have to balance technical maturity against safety. More research will maximize the RNA delivery mechanism to forward the clinical application of gene therapy.

3.3 RNP Delivery System

Apart from the above mentioned delivery techniques, the most effective approach to provide CRISpen/Cas9 components is the ribonucleoprotein complex (RNP). RNP is a compound pre-assembled by Cas9 protein and guide RNA (gRNA), so Cas9 protein and gRNA can straight enter the nucleus and rapidly start gene editing without the need of transcription and translation. By entering the nucleus straight and starting rapid gene editing, the Cas9 protein and gRNA greatly shorten the start-up time for gene editing. This approach reduces off-target effects and minimizes the possibility of foreign genes merging into the host genome as compared with RNA delivery. But RNP has higher technical operating requirements and is less stable than plasmid and mRNA as protease readily breaks it. Strict oversight of the concentration, delivery its duration, which stem and cellular environment of RNP during delivery is required, for instance, to guarantee its stability and function in the cell. RNP should thus be investigated case-by-case since cell type and delivery vector affect its delivery efficiency and impact it.

4 APPLICATIONS OF CRISPR/Cas9 TECHNOLOGY IN BREAST CANCER THERAPY

Due to its unique and innovative working mechanism, CRISPR/Cas9 technology has shown great potential in tumor therapy applications since its invention. More especially, a discovery has been made about triple negative breast cancer (TNBC). Triple-negative breast cancer (TNBC) is one of the most aggressive tumor subtypes; its high recurrence rate, difficult prevention, and lack of therapeutic response have long perplexed people. Therefore, it is essential to innovate a new type of tumor treatment. Academician Cao Xuetao's team discovered the existence of CD28 in cancer cells and its role in tumor therapy and immunity (Yang et al. 2025).

4.1 Targeting Drug Resistance Mechanisms

Though in clinical practice some individuals still display drug resistance at the initial stage or secondary drug resistance after therapy, at present with the development of several medications, breast cancer patients have many alternatives for treatment. The application of CRISPR/Cas9's targeted recognition DNA for gene editing in the treatment of breast cancer is conducive to the construction of stable cell lines carrying target gene mutations, and provides a good platform for screening drug targets.

4.2 Precision Therapy

By identifying and screening gene targets, CRISPR/Cas9 technology increases the likelihood of precisely treating TNBC by identifying tumor inhibitors, oncogenes, and associated drug resistance genes. In 2016, Chinese scientists used CRISPR/Cas9 to knock out ST8SIA1, a gene that affects TNBC metastasis and recurrence, in the clinical treatment of lung cancer, which can effectively inhibit the growth and spread of its cancer cells. In terms of drug combination therapy, CRISPR/Cas9 can target various drug resistance genes, such as knocking out PARP1 to make cells sensitive to adriamycin, gemcitabine and other drugs (Vaghari-Tabari et al. 2022). So CRISPR can be combined with drugs to treat TNBC.

4.3 Future Directions

Many clinical studies are now assessing the safety and efficacy of CRISpen/Cas9 technology in breast cancer treatment; a major obstacle is how to prevent the immune response generated by Cas9 protein in the clinical process, which can influence the treatment impact. Studies have shown, for example, that the Cas9 protein from Staphylococcus aureus and Streptococcus pyogenes may trigger an immune reaction in the body, therefore affecting not only the efficacy of gene editing but also maybe generating adverse effects. If this issue is to be tackled, future research should focus on ways to stop immunological reactions produced by CRISpen/Cas9 technology. Changing the Cas9 protein or choosing a more immunocompatible vector could help to lower immunogenicity. For instance, avoiding humoral and cellular immunological responses in mice has been demonstrated by delivery of modified Cas9 proteins via an AAV (adeno-associated virus) vector. Moreover, crucial directions for next study are refining the delivery mechanism to lower untargeted editing and raise the precision of gene editing. Simultaneously, the future enhancement of CRISPR/Cas9 technology requires increasing the efficiency and safety of gene editing tools. The growth of high-fidelity Cas enzyme shifts and

optimization of editing methods, for instance, can minimize off-target effects and thus raise editing accuracy. It also has fresh gene-editing procedures including Prime Editors and Base Editor. Consequently, in next research, the main challenge to be solved is how to prevent the immune response caused by this technology influencing the therapeutic effect. The original goal of inventive future development of CRISPR/Cas9 technology is to maximize gene editing tools and increase the efficiency and safety of editing in future studies so as to deliver fresh cure hopes to breast cancer patients.

5 THE FUTURE OF CRISPR/Cas9 TECHNOLOGY

As a revolutionary technology in the field of life sciences, CRISPR/Cas9 is poised to demonstrate broad development prospects and potential across medicine, agriculture, and basic research. In medicine, its high-precision gene-editing capabilities and targeting effects will play a pivotal role in treating genetic diseases, neurological disorders, cancer, and more. For instance, in late 2023, the US FDA approved Casgevy, the first CRISPR-based gene-editing therapy, for treating sickle cell disease (SCD). In 2024, it was also approved for transfusion-dependent beta-thalassemia (TDT) (Parums 2024, Singh et al. 2024). In agriculture, CRISPR/Cas9 can enhance crops through gene editing, improving yield, resistance, and environmental adaptability. However, the widespread application of this technology will inevitably raise ethical and moral concerns. As research progresses, it is essential to strengthen the ethical guidelines and regulatory frameworks governing its use. Overall, the future of CRISPR/Cas9 technology is full of both promise and uncertainty.

6 CONCLUSION

In addition to discussing possibilities for curing a number of illnesses, this article examines the mechanism of action and technological developments of CRISPR/Cas9 in the occurrence and progression of breast cancer. In the modern world, breast cancer is a high-incidence malignancy that presents a threat to women's lives and health. When compared to conventional treatment gets closer, the advent of CRISPR/Cas9 technology reduced numerous faults, thanks to its precision treatment features, offering a novel approach to the treatment of breast cancer. This work clarifies in great detail the mechanism of CRISPR/Cas9 technology in vivo. Perfect gene editing is made possible by the cooperative activity of Cas9 protein and gRNA. Furthermore to the mechanism of action, the advantages and limitations of three CRISPR/Cas9 delivery methods—plasmid DNA, RNA, and ribonucleoprotein complex (RNP)—are discussed. Despite being a novel cancer treatment method, CRISPR/Cas9 faces significant obstacles due to the high consistency and complicated biology of breast cancer.

The precise editing of genes by CRISPR/Cas9 technology may target the knocking out or repair of genes connected in the development of tumors, so drastically stopping the spread of cancer cells in the research of breast cancer treatment. By means of precision therapy and targeted resistance mechanisms, this paper shows that CRISPR/Cas9 technology greatly increases the sensitivity of drug therapy through triple-negative breast cancer (TNBC), so offering creative solutions for subtypes hard to overcome by traditional therapies. In addition, the method can be applied to change immune cells to improve their capacity of recognizing and eliminating cancerous cells, therefore activating the immunological defense system.

Though difficulties related to human trials including off-target effects, easy immune response and delivery efficiency still exist to CRISPR/Cas9 technology, its enormous future potential in the treatment of breast cancer has been typically acknowledged. Future studies ought to focus more on ways to maximize the technology of gene editing to raise their efficiency. At the same time, it enhances the ethical and legal structure to guarantee the legitimacy and safety of newly developed technology. With the ongoing development of technology, CRISPR/Cas9 technology is expected to be a major breakthrough point in the field of breast cancer treatment, bringing more accurate, efficient and personalized treatment plans to patients, and so promoting breast cancer treatment to a new era.

REFERENCES

Ahmed, M., Daoud, G. H., & Mohamed, A., et al. 2021. New insights into the therapeutic applications of

CRISPR/Cas9 genome editing in breast cancer. Genes 12(5):723.

Aljabali, A. A., El-Tanani, M., & Tambuwala, M. M. 2024. Principles of CRISPR-Cas9 technology: Advancements in genome editing and emerging trends in drug delivery. Journal of Drug Delivery Science and Technology 92:105338.

Asmamaw, M. & Zawdie, B. 2021. Mechanism and applications of CRISPR/Cas-9-Mediated genome editing. Biologics 15:353–361.

Bodewes, F. T. H., Van Asselt, A. A., & De Bock, G. H. 2022. Mammographic breast density and the risk of breast cancer: a systematic review and meta-analysis. The Breast 66：62-68.

Curigliano, G. 2012. New drugs for breast cancer subtypes: targeting driver pathways to overcome resistance. Cancer Drug Resistance 3(2):179–198.

Karn, V., Sandhya, S., & Hsu, W., et al. 2022. CRISPR/Cas9 system in breast cancer therapy: advancement, limitations and future scope. Cancer Cell International 22(1): 234.

Li, T., Yang, Y., & Qi, H., et al. 2023. CRISPR/Cas9 therapeutics: progress and prospects. Signal Transduction and Targeted Therapy 8(1):36.

Obeagu, E. I. & Obeagu, G. U. 2024. Breast cancer: A review of risk factors and diagnosis. Medicine 103(3):e36905.

Parums, D.V. 2024. Editorial: First Regulatory Approvals for CRISPR-Cas9 Therapeutic Gene Editing for Sickle Cell Disease and Transfusion-Dependent β-Thalassemia.Medical Science Monitor 30: e944204.

Sabit, H., Abdel-Ghany, S., & Tombuloglu, H., et al. 2021. New insights on CRISPR/Cas9-based therapy for breast Cancer. Genes and Environment 43(1):15.

Siegel, R. L., Miller, K. D., & Jemal, A. 2017. Cancer statistics. A Cancer Journal for Clinicians 67(1): 7–30.

Siegel, R. L., Miller, K. D., & Fuchs, H. E., et al. 2022. Cancer statistics. A Cancer Journal for Clinicians 72(1): 7–33.

Singh, A., Irfan, H., & Fatima, E., et al. 2024. Revolutionary breakthrough: FDA approves CASGEVY, the first CRISPR/Cas9 gene therapy for sickle cell disease. Annals of Medicine and Surgery 86(8):4555–4559.

Vaghari-Tabari, M., Hassanpour, P., & Sadeghsoltani, F., et al. 2022. CRISPR/Cas9 gene editing: a new approach for overcoming drug resistance in cancer. Cellular & Molecular Biology Letters 27(1):49.

Wang, J., Li, B., & Luo, M., et al. 2024. Progression from ductal carcinoma in situ to invasive breast cancer: molecular features and clinical significance. Signal Transduction and Targeted Therapy 9(1):83.

Yang, Z., Zhang, Q., & Gao, X., et al. 2021. The signaling pathways and targets of traditional Chinese medicine and natural medicine in triple-negative breast cancer. Journal of Ethnopharmacology 264:113249.

Yang, Z., Liu, X., & Cao, X. et al. 2025. Inhibiting intracellular CD28 in cancer cells enhances antitumor immunity and overcomes anti-PD-1 resistance via targeting PD-L1. Cancer Cell 43(1):86–102.