

Applications of Three Gene Engineering Mechanisms in Breast Cancer Therapy

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Abstract. Breast cancer (BC) is one of the most common malignancies worldwide. Its complex molecular mechanisms make traditional treatments ineffective in some patients. In recent years, advances in genetic engineering technologies have opened up new possibilities for precision medicine in BC. Researchers have attempted to intervene in cancer cell proliferation and induce apoptosis through various genetic strategies. Preclinical studies are increasing, and therapeutic targets and delivery technologies are expanding. However, current research has largely focused on specific cases or single technologies, lacking a systematic integration and analysis of various genetic strategies from a mechanistic perspective. This paper reviews three major genetic engineering strategies for BC based on their therapeutic mechanisms: RNA interference, gene compensatory expression, and gene repair and deletion. Representative studies, key technologies, challenges, and development trends are analyzed for each. Furthermore, this paper summarizes common challenges across these different mechanisms regarding delivery efficiency, stability, and targeting, and proposes potential directions for future synergistic strategies. This paper helps clarify current research approaches in BC gene therapy from a mechanistic perspective, providing a reference for the design of subsequent cross-mechanism integration and precision intervention strategies. However, issues such as mechanism overlap and diversification of delivery platforms still need further in-depth exploration. Future research can focus more on multi-mechanism combination strategies, individualized treatment pathways, and feasibility assessment of clinical transformation.

Keywords: Breast cancer, gene engineering, gene interference, compensatory expression, gene editing

1. Introduction

Breast cancer (BC) is one of the most common cancers in the world, and its incidence rate continues to rise worldwide [1,2]. In 2022, about 2.3 million people worldwide were diagnosed with BC, resulting in 670,000 cases related deaths [1]. It is estimated that in 2040, there will be about 3.19 million cases of BC worldwide [2]. BC arises from a combination of hormonal, genetic, and environmental factors and is typically diagnosed through imaging and pathological examination [3].

The current treatments for BC mainly include surgical removal of breast tumors, chemotherapy, radiotherapy, etc. [4,5]. Although these methods have made progress in prolonging survival, they

also face a series of challenges. For example, chemotherapy and radiotherapy often lead to toxic reactions and affect normal cells; endocrine therapy is prone to hormone resistance and reduced efficacy; targeted therapy and immunotherapy may fail due to the molecular heterogeneity of tumors. In addition, most treatments cannot completely eradicate cancer cells, which can easily lead to tumor recurrence or metastasis [4,5].

In this condition, researchers have begun to try to use genetic engineering technology to develop more targeted and sustained treatments. Genetic engineering is a method of treating diseases by editing and modifying DNA or RNA to fundamentally intervene in pathogenic genes. Common methods include CRISPR/Cas9, TALEN, ZFNs, etc., which can accurately cut, repair or regulate genes. Compared with traditional treatment methods, genetic engineering is more likely to act fundamentally and directly on the causes of cancer, so it is considered an important means of treating BC and other diseases in the future.

At present, genetic engineering has been initially explored and attempted in the treatment of BC, including silencing abnormally expressed targets (e.g., siRNA-based inhibition of PI3K/Akt in triple-negative breast cancer, TNBC) [6]; using functional gene delivery strategies modulates key cell signaling pathways, enhancing anticancer effects or inducing cancer cell death (e.g., inducible caspase-9 system for apoptosis activation) [7]; and achieving oncogene knockout and interfering with its functional expression [8]. These attempts have made some progress in cell lines and animal models.

Despite promising progress, the current studies are relatively scattered, lacking a unified therapeutic framework. This hinders the systematic evaluation and optimization of gene-based strategies in BC treatment.

This paper aims to systematically summarize the existing genetic engineering research in BC through a proposed classification framework with therapeutic mechanisms. Specifically, this article summarizes the existing strategies into three types of therapeutic mechanisms: RNA interference (RNAi) mechanism, gene compensatory expression mechanism and gene repair and deletion mechanisms, and sorts out the representative research results. By systematically combing through relevant literature in recent years, this article integrates and compares the core therapeutic logic of these three mechanisms to provide a more holistic understanding perspective.

2. Mechanism I: RNAi

2.1. The introduction of RNAi mechanism

RNAi is a mechanism that regulates cell function by inhibiting the expression of specific genes and is widely used in tumor treatment research. In BC, certain oncogenes—such as human epidermal growth factor receptor 2 (HER2) and B-cell lymphoma 2 (Bcl-2)—are continuously abnormally expressed, promoting the proliferation and survival of tumor cells, and are often closely related to the occurrence of drug resistance. By implementing RNAi-based strategies, it is expected to achieve precise attenuation of oncogenic signaling pathway activity, thereby blocking tumor development and, to a certain extent, reversing resistance to chemotherapy or targeted therapy, thereby improving treatment efficacy.

Common RNAi tools include interfering RNA (siRNA), short hairpin RNA (shRNA), and microRNA (miRNA). They achieve specific inhibition by complementary binding to the target mRNA, guiding its degradation or preventing its translation. Among them, siRNA is widely used in BC models because of its simple design and rapid action. Beyond classical RNAi tools, recent

studies have also explored the use of CRISPR interference (CRISPRi) systems to inhibit gene transcription, although their application in BC remains in the early stages [9].

Considering that common oncogenes in BC are abnormally expressed and some patients are resistant to traditional treatments, RNAi-based strategies provide a precise, efficient and controllable supplementary treatment method, especially with potential value in reversing drug resistance and improving treatment prognosis.

2.2. The applications of siRNA in the BC

By binding complementarily to target mRNA, siRNA induces its degradation or inhibits translation, ultimately achieving selective and efficient silencing of oncogenic genes. In recent years, this technology has been widely used to target key pathways such as HER2, Bcl 2, and the phosphoinositide 3-kinase/v-akt murine thymoma viral oncogene homolog (PI3K/Akt) pathway. These interventions aim to inhibit BC cell proliferation, induce apoptosis, and reverse drug resistance [10]. The following two representative siRNA-based studies are selected to highlight, respectively, its targeted gene-silencing capacity and the therapeutic relevance of optimizing delivery systems.

In a 2023 study on HER2+ BC, researchers designed siRNA to target the mRNA of the ERBB2 (erb-b2 receptor tyrosine kinase 2) gene, which encodes the cancer-promoting protein HER2 [11]. After introducing siRNA into the SKBR3 (a human BC cell line with high HER2 expression) cell line via a liposome delivery system, the experimental results showed that HER2 protein expression was significantly downregulated, cell viability decreased by about 67%, and obvious cell apoptosis was induced; in contrast, similar effects were hardly observed in control cells with low HER2 expression, showing good targeting [11]. This study demonstrated that siRNA can be used to achieve efficient and specific inhibition of BC driver genes. However, there are still some technical challenges, such as the difficulty of directly converting in vitro experimental results to in vivo applications, the easy degradation of siRNA in the blood, and the need to improve delivery efficiency [11].

In the application of siRNA in BC treatment, delivery efficiency and in vivo stability are key factors affecting its therapeutic effect. Therefore, scientists developed a nanoparticle platform encapsulating chemically modified siRNA to target the PI3K/Akt signaling pathway in TNBC [6]. The platform significantly inhibited tumor growth in animal models and reversed resistance to conventional chemotherapy drugs in experiments, showing the broad prospects of siRNA intervention technology in BC treatment [6]. This study effectively improved the in vivo stability and therapeutic efficiency of siRNA, but also revealed that the interference strategy based on the nanoplatform still has challenges in biocompatibility, safety assessment and large-scale production [6]. These issues directly affect the feasibility of the interference mechanism from the laboratory to the clinic, and become an important direction for subsequent research.

2.3. Challenges and future aspects of the RNAi mechanism

In general, RNAi mechanism, especially tools represented by siRNA, shows good targeting and regulatory efficiency in the treatment of BC. By precisely silencing the expression of oncogenes, this strategy offers a feasible path for personalized therapy under complex molecular backgrounds.

Multiple studies have verified the anti-cancer effect of siRNA in vitro and animal models, and improved its stability and targeting ability through the optimization of the delivery system, indicating that it has preliminary feasibility in clinical transformation. However, the current

interference strategy still faces some technical bottlenecks, such as siRNA is easily degraded in the blood, the biocompatibility and immune safety of the delivery system need to be improved, and most studies are still in the cell and animal experiment stage, lacking systematic human clinical verification.

Future research should focus more on the coordinated optimization of interference tools and carrier platforms, while improving treatment efficiency, reducing off-target effects and systemic toxicity; at the same time, it is also possible to consider combining RNAi with other mechanisms (e.g., gene compensation, immune activation) to achieve multi-pathway and multi-level tumor regulation, opening up new directions for the comprehensive treatment of BC.

3. Mechanism II: gene compensatory expression

3.1. The introduction of gene compensatory expression mechanism

Gene compensatory expression is a therapeutic strategy that restores or enhances key cell functions by introducing exogenous functional genes. In BC, tumor suppressor genes such as TP53 and PTEN often malfunction due to mutation or deletion, resulting in blocked cell apoptosis and uncontrolled proliferation. By introducing therapeutic genes that induce apoptosis, inhibit proliferation, or activate immune system, it is expected to compensate for the loss of key signaling pathways and inhibit tumor progression from the source.

This strategy is often used to activate programmed cell death pathways or initiate the suicide mechanism of cancer cells. For example, Pathak et al. constructed a system that expresses inducible caspase-9 and successfully triggered selective apoptosis of cancer cells in a mouse model of BC, verifying its potential in precision killing [7].

3.2. Applications of gene compensatory expression mechanism in the BC

Specific cases are presented in this section, which respectively demonstrate the expression effects of induced apoptosis and immune-related factors.

Scientists designed an inducible apoptosis system with adeno-associated virus (AAV6) as a vector to introduce exogenous caspase-9 (iCasp9) into a BC mouse model [7]. Under the regulation of small molecule drugs, the system can specifically activate the apoptosis program in cancer cells, significantly inhibit tumor growth, and no obvious off-target toxicity was observed in the experiment, maintaining good selectivity for normal tissues [7]. This study not only verified the potential efficacy of gene compensation expression strategy for BC subtypes, but also highlighted the key role of vector system in expression control and tissue specificity. However, the system is still mainly in the animal experiment stage, and AAV itself also has certain challenges in immune response and long-term expression regulation. Its clinical adaptability and safety still need to be continuously optimized [7].

Researchers used attenuated vaccinia virus (VG9) as a delivery system to introduce the IL-24 gene, a cytokine with anti-tumor properties that can induce apoptosis of tumor cells and promote CD8⁺ T cell-mediated immune attacks, into the TNBC model [12]. Experimental results showed that the system successfully achieved efficient expression of IL-24 in tumor tissues, significantly enhanced anti-tumor immune responses, and had no obvious toxicity to normal tissues [12]. The study further demonstrated that local efficient expression of therapeutic genes with the help of suitable vectors can drive tumor cell death while mobilizing the body's immune defenses.

Nevertheless, the expression stability, immune adaptability, and response differences of viral vectors in different patients still need to be continuously evaluated.

3.3. Challenges and future aspects of gene compensatory expression mechanism

In general, the gene compensatory expression mechanism provides a feasible approach for BC treatment that is independent of traditional targeting pathways, and is particularly suitable for cancer subtypes that lack effective targets or are resistant to conventional therapies. Through the precise expression of exogenous genes, this strategy is expected to restore cell apoptosis, inhibit proliferation, or activate immune responses, and achieve comprehensive intervention on tumors at multiple pathway levels.

Existing studies have preliminarily verified the therapeutic potential of compensatory expression in animal models and demonstrated the optimization effects of various delivery systems in improving expression efficiency and tissue specificity. However, this mechanism still faces challenges in clinical transformation, including key issues such as the immune adaptability of the vector, the long-term controllability of expression, and the therapeutic response under individual differences. Most of the current results are still in the *in vitro* or animal experimental stage, and lack systematic human trial data.

Future research can focus on the design of safer and more efficient delivery platforms, and enhance the controllability and individual adaptation of therapeutic effects. At the same time, the compensatory expression mechanism can be used in combination with other treatment strategies (immunomodulation, signal pathway blocking) to construct a comprehensive treatment system integrating multiple mechanisms, further expanding its application prospects in the precision treatment of BC.

4. Mechanism III: gene repair and deletion

4.1. The introduction of gene repair and deletion mechanisms

Gene repair and deletion are interventional mechanisms that act directly at the DNA level, permanently altering the gene sequence. They aim to correct oncogenic mutations or knock out abnormally expressed genes, thereby restoring normal cellular function. In BC, the accumulation of driver mutations is a key factor in tumorigenesis. Mutations in genes such as PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) and TP53 are closely associated with multiple subtypes. Gene repair and deletion can fundamentally address these mutational targets.

Gene repair and deletion mechanisms typically rely on molecular tools capable of targeted manipulation at the DNA level, such as CRISPR/Cas9, TALENs, and ZFNs. These methods use engineered nucleic acid recognition modules to induce double-strand breaks in DNA. These mechanisms then utilize Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR) mechanisms within the cell to achieve gene knockout or sequence repair. The CRISPR/Cas9 system, due to its high targeting and ease of use, is particularly widely used in BC research. This system uses a specific guide RNA to direct the Cas9 enzyme to cleave a target DNA segment, enabling sequence deletion or precise repair.

Compared to expression regulation and interference mechanisms, gene repair and deletion can directly correct disease-causing mutations at the DNA level, resulting in more thorough and long-lasting therapeutic effects. These mechanisms are particularly suitable for BC patients with well-

defined mutation sites and stable genetic alterations, effectively blocking aberrant signaling pathways and reducing the risk of recurrence and drug resistance.

4.2. Therapeutic applications of CRISPR/Cas9 in BC

CRISPR/Cas9 can permanently delete or repair disease-causing genes at the DNA level, offering radical therapeutic potential. The following two representative case studies demonstrate the value of this system in targeting oncogenes and reshaping the immune microenvironment, respectively. They also highlight key challenges in delivery and off-target control.

In a 2023 study, researchers used an adenoviral system to deliver Cas9 and a sgRNA targeting EZH2 (Enhancer of Zeste Homolog 2) into MDA-MB-231 cells, achieving knockout of the EZH2 gene [13]. This gene is highly expressed in TNBC and promotes tumor proliferation and migration. Sanger sequencing and Western blot analysis confirmed the significant knockout effect, and functional experiments revealed decreased cell viability, migration, and healing abilities [13]. Xenograft models further validated that EZH2 depletion significantly inhibited tumor growth [13]. This study provides clear experimental evidence that disruption of epigenetic regulators by CRISPR/Cas9 can directly inhibit BC progression. However, this study is limited to preclinical models and lacks assessment of potential off-target effects and delivery safety, which remain key barriers to clinical translation.

In a 2024 study, researchers constructed a functionalized nanoplatfrom FCPCV to deliver the CRISPR/Cas9 system and knock out the CCL5 (C-C motif chemokine ligand 5) gene in BC [8]. CCL5 can promote the immunosuppressive recruitment of Treg. In vitro and in vivo experiments showed that this strategy significantly enhanced CD8⁺ T cell infiltration and immune factor (IFN- γ , TNF- α , GZMB) secretion, thereby inhibiting the progression of TNBC [8]. This highlights the potential of the Cas9 tool not only in direct gene knockout, but also in reshaping the tumor microenvironment to enhance existing immunotherapy. However, before application in human studies, challenges in nanoparticle delivery specificity and biosafety still need to be further optimized.

4.3. Challenges and future aspects of gene repair and deletion mechanisms

Overall, the Gene repair and deletion mechanisms offer a novel strategy for precision BC treatment, altering pathogenic gene expression at the source. They are particularly suitable for personalized interventions targeting specific mutation-driven tumors. These mechanisms, through direct editing at the DNA level, have the potential to achieve more thorough therapeutic effects, theoretically surpassing traditional expression control methods.

Current research focuses primarily on in vitro models and animal studies, and has initially demonstrated their targeting and editing efficiency. In particular, nanoparticle- or virus-based delivery systems demonstrate excellent tissue penetration and gene delivery specificity. However, in vivo application of these mechanisms remains limited by common challenges such as delivery stability and long-term safety. Furthermore, as a therapeutic approach relying on intracellular repair responses, their therapeutic efficacy may be affected by biological differences, such as deviations from the repair pathway, leading to some heterogeneity in response.

Future research will further optimize the coordinated design of editing systems and delivery platforms to enhance their ability to control expression in complex in vivo environments. Furthermore, bioinformatics prediction and personalized screening techniques will be combined to improve the controllability and specificity of gene repair pathways. In addition, the gene deletion

mechanism can also serve as a basic module for other treatment strategies, and can be used in combination with expression compensation, immune activation and other mechanisms to construct a higher-dimensional comprehensive treatment system, thereby expanding its practical application prospects in the precision treatment of BC.

5. Conclusion

Current research on genetic engineering therapies for BC is increasingly diverse, but it often focuses on targets or technologies, exhibiting a "single breakthrough, fragmented pathways," and lacking systematic integration at the mechanistic level. This paper attempts to categorize genetic engineering strategies in BC into three main categories, based on "therapeutic mechanism": RNAi, gene compensatory expression, and gene repair and deletion mechanisms. Within this framework, the paper systematically organizes various research cases and their key technical tools. This perspective not only helps clarify the core logic and functional differences between different strategies but also provides a clear reference framework for subsequent research, contributing to the construction of a more systematic and comparable knowledge system based on fragmented research.

Although the three strategies differ in their mechanisms and pathways of action, they all face common challenges, such as delivery system optimization, biosafety assessment, and individual variability in response. Currently, most research remains in the laboratory or animal model stage and remains far from clinical application. Future efforts should continue to optimize expression efficiency, enhance targeting, and control side effects. Furthermore, combined strategies (such as interference + compensation, repair + immune activation) should be explored to develop more systematic and personalized BC treatments.

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