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Review Article

Molecular Mechanisms of Breast Cancer Drug Resistance and CRISPR/Cas9 Strategies to Overcome

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Abstract

Breast cancer (BC) is considered as the most frequent cancer among women and a significant contributor to mortality. The CRISPR/Cas9 gene-editing tool has promising applications for BC drug resistance. It is a unique and creative approach that has lately attracted attention and can be used to fix gene alterations related to multidrug resistance. Recent research has effectively investigated and targeted particular genes linked to BC treatment resistance using CRISPR/Cas9 gene editing, including those linked to hormone receptor signaling, drug efflux transporters, and DNA repair pathways. The CRISPR/Cas9 technology's selective disruption or mutation of these genes provides valuable information about their role in resistance and paves the path for cutting-edge treatment options. CRISPR/Cas9 gene editing can overcome BC treatment resistance by identifying crucial genetic variables and revealing new therapeutic targets Despite the advantages, there are limitations in the study on CRISPR/Cas9-based gene editing for BC treatment resistance, for example, off-target effects and the improvement of delivery techniques are still major issues. Successful clinical translation depends on methods to improve the specificity and effectiveness of CRISPR/Cas9 editing and to solve these constraints. This review aims to explore the possibility of CRISPR/Cas9 gene editing as an innovative method of combating BC medication resistance.

Keywords: Breast Cancer, CRISPR/Cas9, Drug Resistance.

INTRODUCTION

Millions of women around the world continue to face breast cancer (BC) as a serious health issue [1]. Although advances in targeted therapy have improved patient outcomes, medication resistance continues to be a significant barrier to successfully treating such life-threatening disease [2]. Changes in some gene sequences and signaling pathways frequently result in resistance to BC medications, making treatment ineffective [3-5]. To increase patient survival rates and achieve long-term remission, medication resistance must be overcome [6]. A revolutionary technique called Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) gene editing has recently emerged as a viable approach to address this challenge [7].

In bacteria and archaea, the system of CRISPR/Cas9 naturally developed as an immune tool for fighting phage invasion and plasmid transmission [8]. Once penetrated by an exogenous

phage or plasmid, bacteria or archaea obtain a portion of their genome to be inserted into the CRISPR spacer regions. When the bacteria get homologous DNA, transcription of the CRISPR region will begin. A single guide RNA (sgRNA) is produced by a sequence of maturation and processing steps. The sgRNA directs Cas9 to cut the DNA strand that breaks the homologous DNA spacer section. The short, guanine-enriched sequence known as protospacer-adjacent motifs, or PAMs, is necessary for the recognition of the sgRNA [8] (Figure 1).

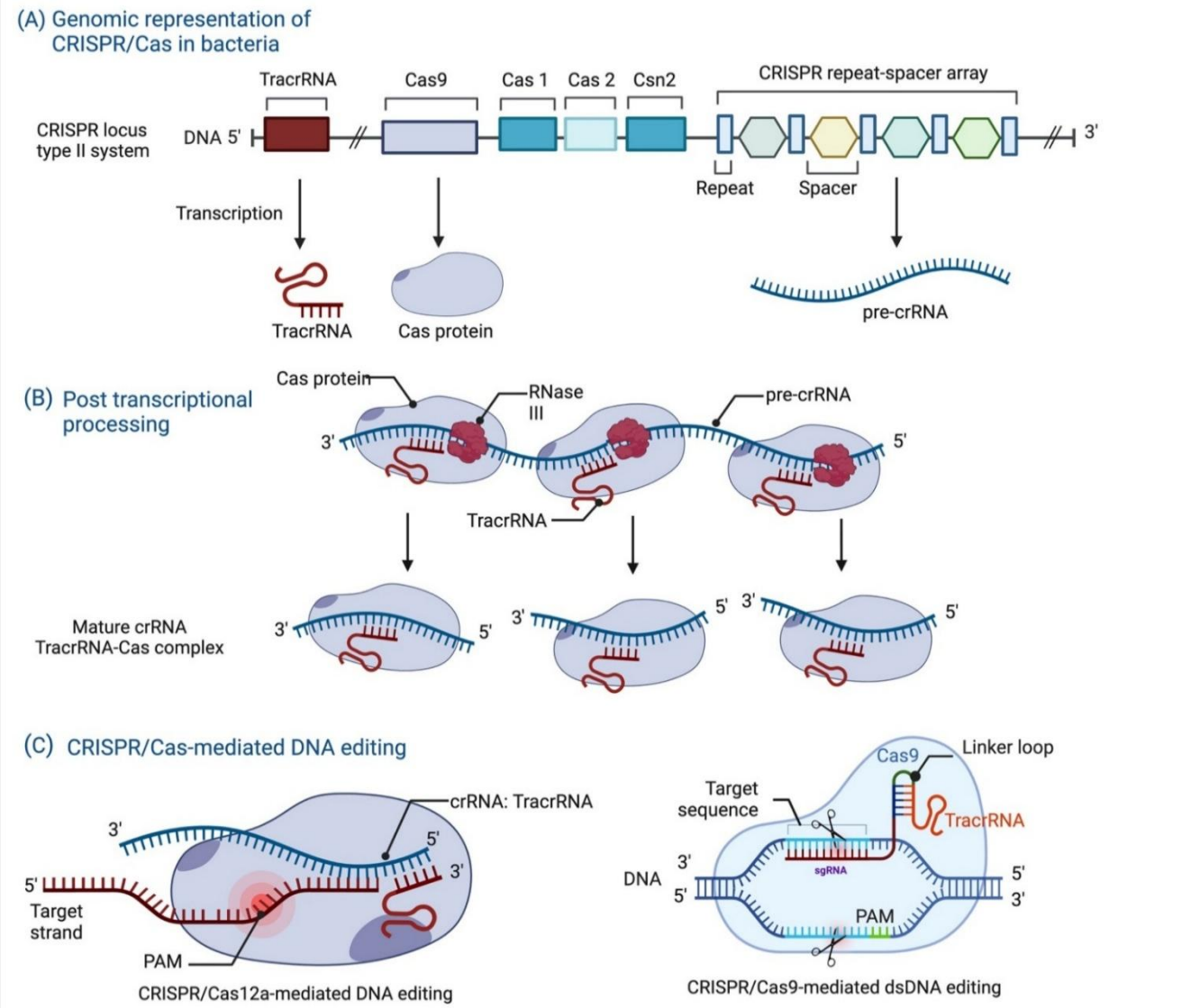

Figure 1. CRISPR/Cas system type II biology. (A) A depiction of CRISPR/genome Cas9's together with pertinent transcription- and translation-related products. (B) Site-specific gene editing using engineered CRISPR/Cas9. (C) ssRNA and dsDNA editing using the CRISPR/Cas system.

CRISPR/Cas9 is a precise and effective gene editing technology that enables researchers to change particular genes within cell DNA [9]. The Cas9 enzyme is guided by a guide RNA molecule to a specified genomic region, where it directs precise alterations, such as gene deletion, knockdown, or activation [10]. It comprises two essential parts: the clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated protein 9 (Cas9) [11]. The CRISPR sequence acts as a guide, targeting specific regions of the genome, whereas the Cas9 protein cuts the DNA precisely at the desired region, acting as a molecular scissors [12]. This gives researchers the opportunity to either disrupt particular genes linked to medication resistance or introduce advantageous alterations to make cancer cells more susceptible to therapy.

Researchers can deliberately edit particular genes linked to drug resistance processes by using CRISPR/Cas9 to precisely and effectively manipulate the genetic material within cells [13]. By

facilitating the creation of novel therapeutic strategies, this ground-breaking technology has the potential to change the treatment approaches for BC.

The CRISPR/Cas9 method has demonstrated a promised perspective in cancer treatment in cellular and animal models. The efficacy of CRISPR/Cas9 technology in cancer treatment is currently being assessed in a number of ongoing clinical trials [14,15]. Nevertheless, research has indicated that this method can also be utilized to increase the potency of targeted therapy and chemotherapy. Drug resistance is a major concern in cancer treatment since it is believed to be the cause of 90% of cancer patients' deaths [16]. While much remains unknown about how cancer cells develop drug resistance, recent research has pointed to a large variety of genes involved in drug efflux, DNA repair, apoptosis, and several cellular signaling pathways as potential causes [17]. Promising achievements in reducing drug resistance and boosting the efficiency of anticancer treatments have been achieved by targeting some of these genes using CRISPR/Cas9 technology. This study first provides a brief history of drug resistance and its key mechanisms in cancer, then discusses several strategies for treating drug resistance in BC. Subsequently, it explores the main ways of CRISPR/Cas9 technology's potential to eradicate drug resistance in breast cancer. Furthermore, it highlights the main limitations of the use of CRISPR/Cas9 system and the main strategies to overcome them. However, to overcome the challenges of employing CRISPR/Cas9 technology in BC resistance to therapies, more research is needed.

CRISPR/CAS9-BASED GENE EDITING ADVANCEMENTS

A single mutation may result in a number of different diseases, such as sickle cell anemia. As a consequence, it is critical to create sensitive, efficient, and concentrated approaches for repairing mutated genes. Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were utilized alongside other targeted genome editing techniques to suppress certain DNA sequences [18,19] and CRISPR-associated protein 9 (Cas9) is the third-generation gene-editing technique that is most often used [20]. Based on the composition and function of Cas-proteins, the relatively new CRISPR/Cas genome editing technology is categorized into two classes: Class I and Class II; while class I systems have several Cas-protein complexes, class II systems contain just one Cas-protein complex. Although Type II CRISPR/Cas-9 is a very basic structure, it has been extensively explored and used in genetic engineering [21].

Cas9 should be combined with guide RNA in order to recognize and cleave DNA at specified sites. Thus, two components form the CRISPR/Cas9 system: a Cas9 endonuclease and a single-stranded guide RNA (sgRNA) [22]. When sgRNA attaches to the target sequence, Cas9 correctly cleaves the DNA to create a DSB (Figure 2). Upon the occurrence of a DNA-DSB, DNA-DSB repair mechanisms initiate genome repair. This method provides various advantages, including high stability, rapid action, and low antigenicity response induction [8,23].

The CRISPR/Cas9 system enables precise targeting of nearly any genomic locus for the correction of disease-causing mutations or silencing of genes linked to disease development by modifying the nucleotide sequence of a short section of guide RNA [24] (Figure 3).

In recent years, the CRISPR/Cas9 genome editing system has proven to be a successful method for targeting and changing genes, including gene replacements, gene insertions/deletions, and single base pair conversions [25]. This approach is more advantageous and beneficial than ZFNs and TALENs [26]. More recently, *Streptococcus pyogenes* type II CRISPR-Cas9 has emerged as a critical tool for targeted genome editing in a broad variety of species, including bacteria, yeast, Drosophila, zebrafish, mosquitoes, mice, plants, and mammals [27]. The CRISPR-Cas9 technique has gained traction in biomedical and therapeutic applications, as well as in the development of animal model systems [28]. After claiming that CRISPR gene editing could be a "possible functional cure for chronic HIV," Excision BioTherapeutics was granted FDA

approval on September 15, 2021, to conduct the first human research using CRISPR gene editing as a treatment for HIV. Genome editing has never before been tried in humans, but that all changes now.

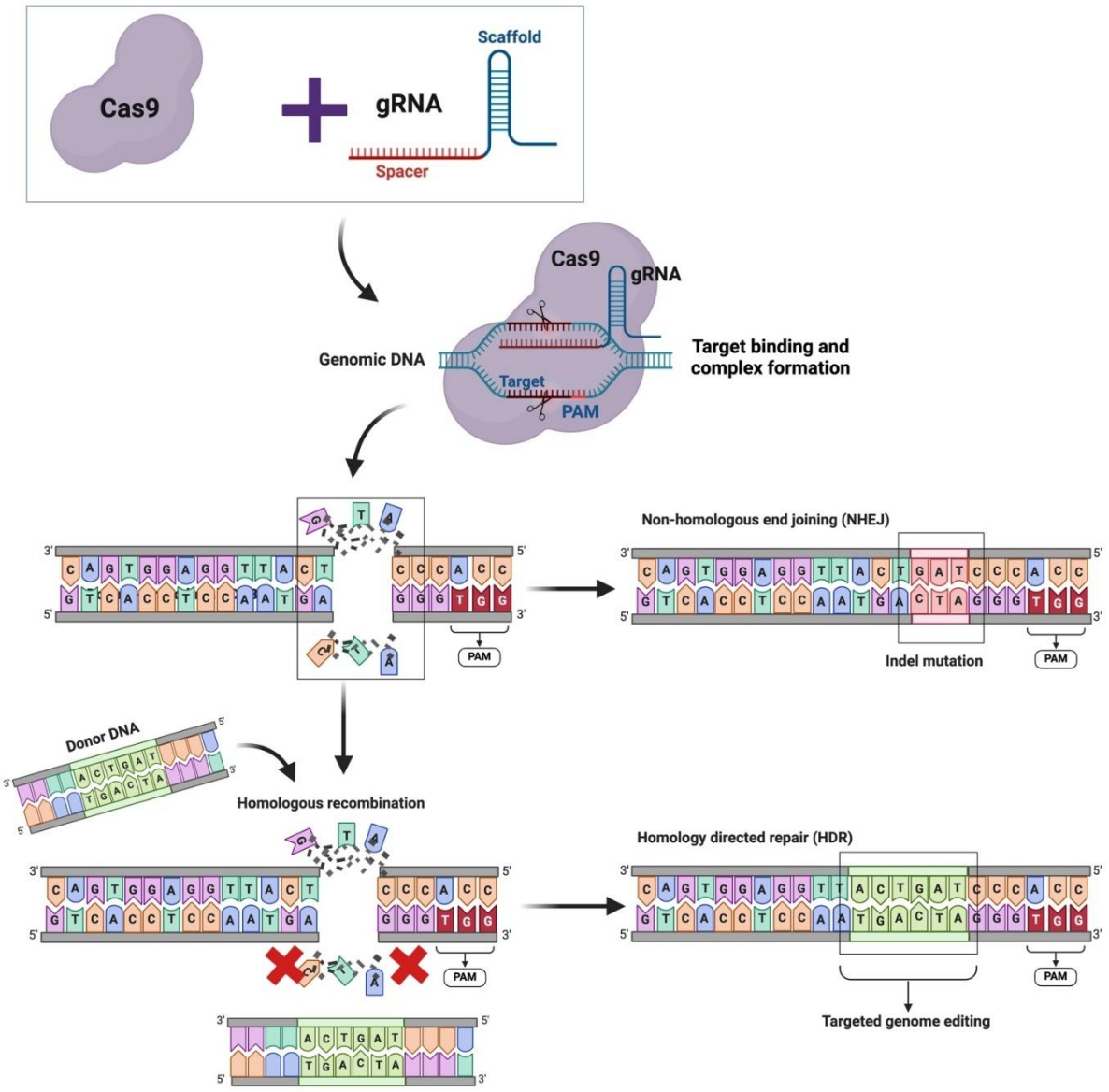

Figure 2. The illustration shows the mechanism of gene editing by CRISPR/Cas9 through homologous recombination and nonhomologous end-joining.

BREAST CANCER and THERAPEUTIC RESISTANCE

According to global cancer statistics, BC has exceeded lung cancer as the most prevalent cancer globally, accounting for 11.7% of all cases and 6.9% of deaths [29]. Breast cancer treatment choices are many and often include a combination of modalities based on the tumor's stage and biology, as well as the patient's acceptance and tolerance. Surgical procedures, radiation therapy, and systemic therapy (chemotherapy, endocrine therapy, and biologic and targeted therapy) are examples of such interventions [30]. Chemotherapy is often indicated for people who are at high risk of developing problems. Patients with BC who have tumors that express *HER2* are treated with *HER2*-targeted treatment in conjunction with chemotherapy. The majority of individuals with illnesses that are HR-positive should get endocrine hormone treatment [31].

Drug resistance is the most frequent reason for treatment failure in the complex field of BC therapy. Many BC tumors that respond initially to chemotherapy recur and acquire resistance to numerous anticancer drugs with varied structures and mechanisms of action, a condition referred to as “multidrug resistance” (MDR). This is mostly

caused by genetic changes that impact the efficiency and unfavorable effects of chemotherapy and other adjuvants [32]. Numerous processes, including increased drug efflux, genetic factors, growth factors, enhanced DNA repair ability, and increased xenobiotic metabolism, may contribute to resistance [33]. When the efflux of chemotherapy medications from cancer cells is increased, drug accumulation in tumor cells is diminished. Because of this, chemotherapeutic drug resistance develops at a much higher rate than previously thought [34]. The classic method for preventing the growth of acquired drug resistance is to combine agents with distinct targets.

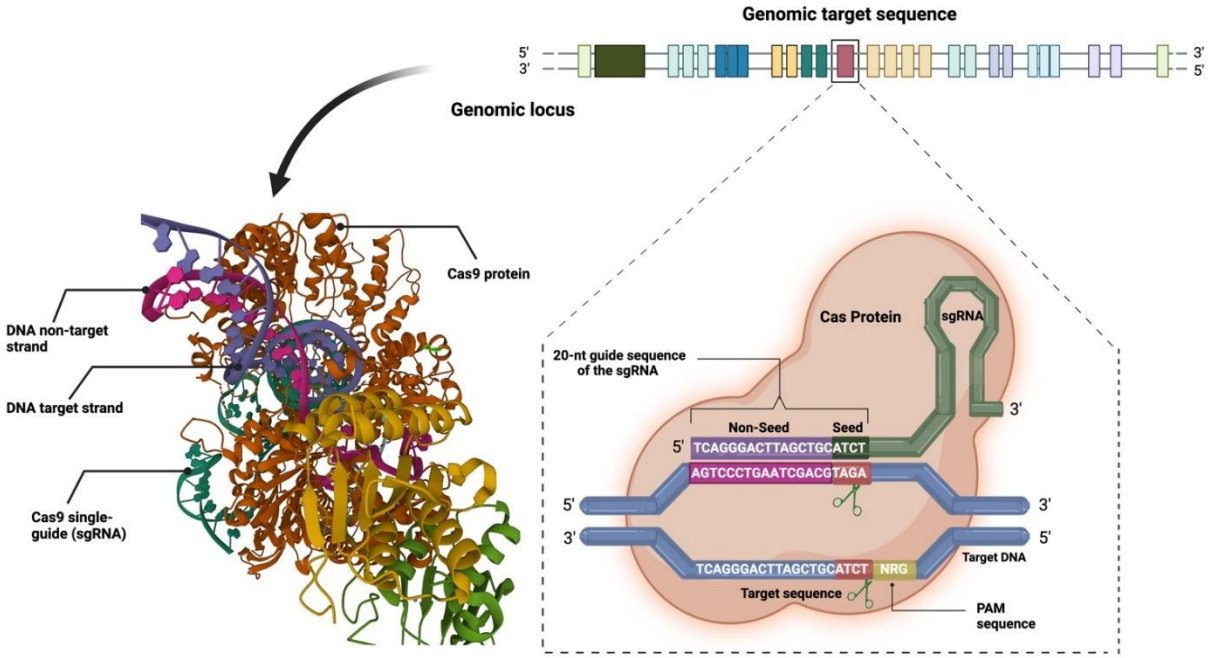

Figure 3. A schematic representation of the CRISPR/Cas9 genome editing system. The CRISPR/Cas9 technology allows for precision targeting of certain genomic loci to fix mutations that cause cancer or silence genes. By changing the nucleotide sequence of a short guide RNA, Cas9 protein can be guided to the appropriate genomic spot, allowing for precise DNA sequence editing."

Nonetheless, since each drug's mechanism of action is complex and interdependent, its impact is difficult to predict. Another strategy in MDR instances is to increase the anticancer drug's specificity to decrease the likelihood of developing resistance [35]. If resistance factors are eliminated using gene editing techniques such as the CRISPR/Cas9 system, which may be better compared to other gene editing technologies, anticancer drugs can be reused [36].

CRISPR/ CAS9'S POTENTIAL ROLE in BREAST CANCER DRUG RESISTANCE

CRISPR/Cas9 has demonstrated considerable promise in a number of biotechnology and medical fields. Even though it hasn't been thoroughly investigated, it shows promise as a viable tactic to deal with the problem of drug resistance. Several applications of CRISPR/Cas9 in the context of drug resistance are listed below:

Drug Efflux

Estimates indicate that the 48 ABC genes in the genetic code are divided into seven subfamilies, each of which serves a distinct function (ABCA-ABCG) [37]. They include ABCB1, ABCC1, and ABCG2. These transporters significantly enhance the development of MDR for cancer chemotherapy [38]. The *MDR1* (P-glycoprotein, ABCB1) ABC transporter P-glycoprotein, or ABCB1, was discovered to be highly expressed in BC cell lines with MDR for the first time [39]. This gene is expressed in the gastrointestinal tract, pancreatic ductulus, liver, and kidney, as well as the endothelial cells that comprise the blood-brain barrier (BBB). When ABCB1 is active, it may dramatically impair the net absorption and penetration of a wide variety of therapeutic

substances into healthy cells in the gut, kidneys, and liver. The expression of ABCB1 in breast cancer varies significantly across people [40]. Additionally, BC cells overexpress a protein (MRPI) that enhances the resistance to anticancer drugs [41].

Multidrug resistance is frequently caused by ATP-binding cassette (ABC) transporters, which remove drugs from tumor cells before the development of therapeutically active concentrations. ABC transporters regulate the distribution, absorption, and excretion of numerous chemical substances [42]. ABCB1 expression may be used as a biomarker to predict the efficacy of anticancer medication, according to a study done in women with breast cancer [43]. These efflux pumps may be inhibited through knockout techniques, restoring the efficacy of already available medications and obviating the need for novel treatments.

Resistance to one anticancer drug increases cancer cells' resistance to other anticancer drugs. In most cases, chemotherapy fails because of multidrug resistance [44]. Thus, reversing ABC-mediated multidrug resistance utilizing the CRISPR/Cas9 system is possible and has been successfully employed, and this technology may assist in re-sensitization to current medicines *in vitro* or *in vivo*. MDR1 has been effectively disrupted utilizing the CRISPR/Cas9 system in doxorubicin (DOX) resistant MCF-7/ADR cells, and sensitivity has been restored [45]. Plasmid therapy decreased MDR1 protein expression by 25%, but increasing the plasmid dosage did not result in a substantial drop in MDR1 protein expression [46] (Figure 4). This finding implies that drug resistance in BC cells may be defeated by utilizing the CRISPR/Cas9 system as a gene editing tool to remove a drug resistance-related gene.

According to the data, cells transfected with sgRNAs become more vulnerable to DOX in an ovarian cell line because the CRISPR/Cas9 system reduces the expression of P-gp, a glycoprotein encoded by the ABCB1 gene in humans. As a consequence, the target gene was efficiently downregulated, and the cell line A2780/ADR was edited using this method to restore its nonmalignant phenotype [47]. Chemotherapeutic compounds that target ABCB1 substrates are more sensitive in MDR cancer cells, and knocking ABCB1 down boosts doxorubicin and other anticancer drugs' intracellular accumulation.

CRISPR/Cas9 might be a significant tool in the study and eradication of MDR cancers, and this work sheds insight on and provides vital clues about its prospective uses [48]. In MDCK cells, after the successful knockout of endogenous Mdr1 (Abcb1) by CRISPR-Cas9, a cell line with no cMdr1 background was established. Therefore, the use of CRISPR-Cas9 technology in the study of drug transport mechanisms is strongly encouraged [49].

The efficacy of breast cancer treatment drugs is influenced by the ATP-binding cassette efflux transporter (ABC) [50]. Numerous biological variables influence the function or production of BCRP in BC, including the pro-inflammatory cytokines IL-1, TNF-α, IL-6, and INF [51]. In drug-resistant MCF-7 BC cells, extracellular vesicles containing ABCB1, ABCG2, and ABCC2 sequester anticancer drugs, preventing them from reaching their intracellular targets [52]. Lymphatic metastasis, tumor size, and poor pCR have been linked to high BCRP/ABCG2 expression [53]. Different anticancer agents, such as anthracyclines, mitoxantrone, flavopiridol, and antifolates, are all known to be resistant to BCRP, which is found in a wide range of tumors [54]. For this reason, CRISPR/Cas9 could be utilized to identify drug-resistant genes in breast cancer, which could lead to a new approach to treatment [55]. Also, if resistance factors can be eliminated, anticancer agents can be more effective. Deletion of Pik3ca and Pik3cb led to the downregulation of P-gp/ABCD1 and BCRP/ABCG2 transporters, which allowed human epidermoid carcinoma and non-small cell lung cancer MDR cells to become drug-responsive again after CRISPR-Cas9 deletion of these genes. As a consequence, gene knockdown using CRISPR/Cas9 is very precise and advantageous, making it ideal for studying BCRP-mediated therapy resistance in BC [56].

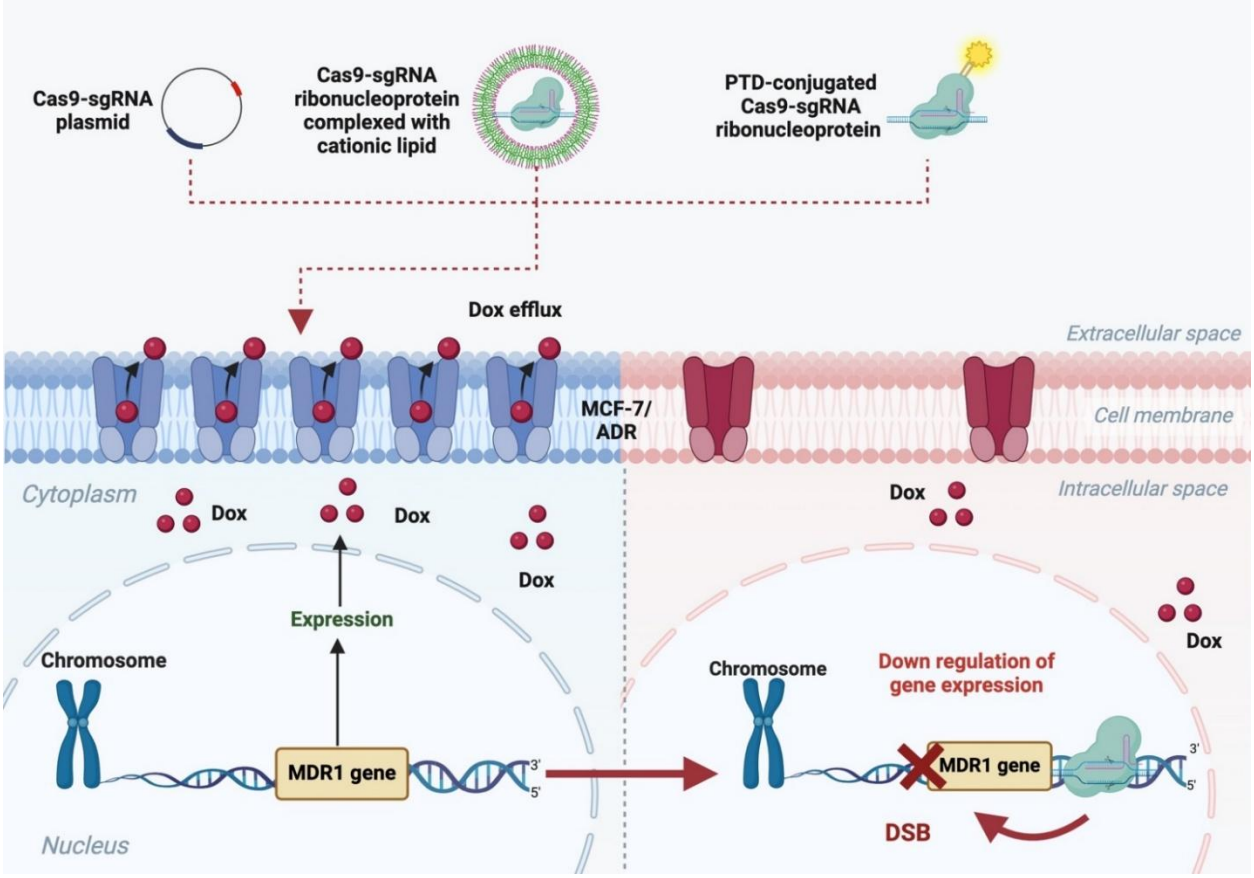

Figure 4. Multidrug-resistant cells (MCF-7/ADR) were transfected with a plasmid for Cas9-sgRNA expression, and the Cas9-sgRNA ribonucleoprotein complex was delivered intracellularly using either a PTD peptide or Lipofectamine.

Apoptosis Inhibition

Normal breast development requires a balance of proliferation and cell death, and tumor growth is most likely the consequence of both excessive proliferation and decreased cell death. The activation of various intrinsic and extrinsic signaling pathways is necessary for the initiation of cell death, and these pathways are often dysregulated in cancer [57].It is critical to understand how proliferation and apoptosis interact in order to comprehend how chemotherapy, radiation, and hormone therapies impact tumor development or regression. Apoptosis can be induced by any or all of these methods. Inhibition of apoptosis is a key mechanism for increasing chemotherapy resistance in cancer cells. Apoptosis is mediated by proteins like members of the Bcl-2 family and the tumor suppressor p53, which are typically dysregulated in MDR malignancies, while IAPs, which are involved in caspase in activation, are typically overexpressed in malignant tumors [58]. In addition, PI3K/AKT survival pathways, which are closely linked to the development of resistance, are often involved [59,60]. Increased Bcl-2 expression has been correlated with drug resistance in BC [61], either antagonists or inhibitors of Bcl-2 proteins may be able to overcome this resistance [62].

A critical function for PI3K/AKT as a critical link in modifying breast cancer multidrug resistance. When activated, P-glycoprotein (P-gp, ABCB1), multidrug resistance-associated protein 1 (MRP1, ABCC1), and BC resistance protein (ABCG2) were found to be effective ABC transporters that effectively expressed the PI3K/AKT pathway in the biological basis of cancer, inhibiting chemotherapy response and facilitating drug excretion. The PI3K/AKT pathway regulates apoptosis, which influences MDR by inhibiting this process, and aberrant PI3K/AKT signaling activation also leads to an increase in Bcl-2 expression, which results apoptosis inhibition and MDR [63]**.** Endocrine resistance is related to PI3K activation in BC, discovering PI3K inhibitors an interesting method for reversing endocrine resistance. Through amplification of the PI3K/AKT pathway, extranuclear ER signaling contributes considerably to endocrine therapy resistance [64].

By concentrating on the BCL-2 family's role in a number of human malignancies, researchers were

able to develop inhibitors that specifically target certain BFL-1 and BCL-w proteins in a study experiment to assess BH3 mimetic sensitivity. Additionally, a CRISPR/Cas9 screen revealed that BFL-1 and BCL-w enhance resistance to all tested BCL-2, BCL-XL, and MCL-1 inhibitors [65]. MCL-1 inhibitor S63845 was reprogrammed in breast cancer cell lines utilizing S63845-resistant cells when combined with CRISPR/Cas9 technology. As a whole, these studies support the clinical testing of MCL-1 inhibitors in BC [66]. hnRNPA2/B1 has been demonstrated to stimulate tumor development *in vitro* and *in vivo* by participating in a number of signaling pathways. CRISPR-CAS9 knockout of this gene in MCF-7 cells has been applied; included are the PI3KAKT and STAT3 pathways. It is possible to treat BC by targeting the hnRNPA2/B1 or STAT3 pathways [67]. The CRISPR-Cas9-based genome editing technique completely knocked out ARID1A in BT474 cells, which activated the expression of ANXA1 and increased the trastuzumab resistance. According to the findings of Berns et al., ANXA1 may be a potential target for the therapy of HER2-targeting antibody resistance (Figure 5) [68].

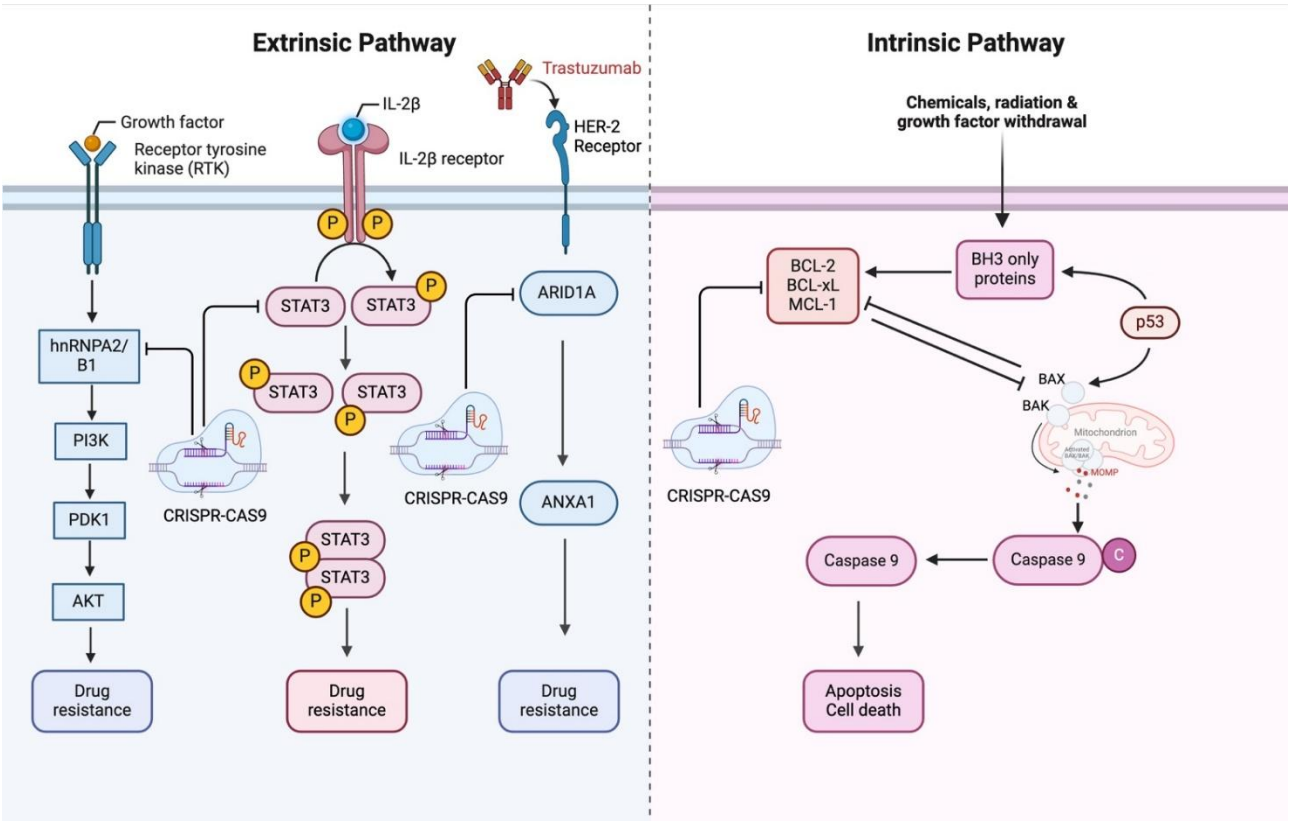

Figure 5. Shows how breast cancer treatment resistance develops in both intrinsic and extrinsic mechanisms and how a genome editing technology based on CRISPR/Cas can eliminate this obstacle.

Drug Target Alteration with Epigenetic Modifications in Breast Cancer

A therapy's efficacy is determined by its molecular target and any alterations to that target, such as mutations or changes in expression levels, which can lead to drug resistance in cancer. Human epidermal growth factor receptor 2 (HER2), an EGFR family receptor tyrosine kinase, is highly expressed in BC patients, and resistance to inhibitors targeting this kinase may develop with prolonged use. Resistance to chemotherapy and molecularly targeted therapies is currently a significant limitation to cancer research progress. In addition to altering specific pharmacological targets, drug resistance can also be induced by modifying the signal transduction system responsible for drug activation. Anticancer medications often exploit interactions with intracellular target proteins to induce lethal effects on cancer cells. Disrupting the normal function and/or expression of these enzymes leads to reduced medication effectiveness [60,69]. Tamoxifen's effect on breast cancer patients is reduced by changes in estrogen and progesterone receptor expression levels. Stopping cancer cell proliferation by targeting particular proteins

involved in tumor formation is possible with targeted therapy. If the medications' targets are altered during targeted treatment, this might lead to drug resistance. Drug targets may be altered by secondary mutations or epigenetic changes in the expression levels of the target protein. The use of estrogen receptor suppressors in BC therapy is another example of how altering the medication target can produce resistance. Resistance to hormone treatment is commonly attributed to epigenetic alterations [69,70].

Existing medicines are unable to destroy cancer stem cells (CSCs), also known as cancer starting cells, because of increased resistance to treatment regimens. The triple-negative subtype of breast cancer is more prone to recurrence and metastasis, and it is a poor prognosis subtype of heterogeneous breast cancer. The TNBC is characterized by the lack of progesterone and estrogen receptors (PR and ER), as well as the absence of HER2 overexpression [71]. Only a minority of the overall number of cancer cells in a tumor mass is assumed to be CSCs; however, because of their resistance to therapy, they can potentially self-renew and differentiate [72]. There is evidence that WAVE3 contributes to the growth and metastasis of BC, promotes the self-renewal of CSCs, and regulates CSC-specific gene transcription in triple-negative breast cancer (TNBC) by interacting with the transcription factor Y-box-binding protein-1 (YB1), a CSC maintenance gene. CRISPR/Cas9 knockdown of WAVE3 reveals the WAVE3/YB1 signaling axis as a regulator of CSC-mediated resistance to therapy and provides a potential therapeutic window for TNBCs. Additionally, by precisely targeting BCSCs, targeting WAVE3 may be employed as a potential therapeutic method for treating metastasis in patients with TNBC malignancies [73,74].

Breast cancer, which is ER-positive, has been discovered to have endocrine resistance, which has been connected to treatment resistance in the illness and includes ER receptors. The transcription of the estrogen receptor 1 (ESR1) gene and the proteasome-dependent degradation of the ER protein control the expression of ER. Hormone treatment resistance in breast cancer is caused by mutations in the Esr1 gene. About 50% of all endocrine resistance instances are caused by a mutation in ESR1 [75,76], as a possible mechanism for tamoxifen resistance, lower ESR1 mRNA expression levels have been suggested [77]. ESR1 mutations in HR-positive BC occur almost exclusively following the administration of aromatase inhibitors in a metastatic setting [76]. Therefore, ER inhibition may reduce relapse and prolong the patient's life. By "knocking in" ESR1 gene mutations and expressing them under the estrogen receptor's endogenous promoter, a new model of ESR1 mutations has been developed for endocrine therapy resistance, notably to aromatase inhibitors [78]. On MCF7 breast cancer cells, CRISPR/Cas9 genome editing was employed to demonstrate the significance of ER mutations and the use of knock-in mutational models for investigating new treatment methods for endocrine-resistant BC caused by ER mutations [79]. Furthermore, CDK8 inhibition by CRISPR/CAS9 knockout suppresses estrogen-dependent transcription and the growth of estrogen receptor-positive BC [80]. In PIK3CA, mutated-ER+ B inhibitors BC of PI3K are active, and through the use of CRISPR/Cas9-based sgRNA knockout screens, various negative regulators of mTORC1 have been identified, the loss of which confers resistance to PI3K inhibition. This shows the function of restored mTOR signaling in conferring resistance to PI3K inhibition and proposes therapeutic strategies to inhibit or reverse this resistance [81].

Furthermore, it has been shown that HER2 mutations in human BC cells result in multidrug resistance. Chemoresistance in MCF7 has been related to Akt1 activation by HER2/PI-3K, indicating that this protein might serve as a novel biological target for therapy to enhance the prognosis of women with BC [82]. Trastuzumab was found to be ineffective against P95HER2-positive breast tumors [83]. It is possible to use the CRISPR/Cas9 system to target the mutated form of HER2 in order to disrupt or inactivate it. Trastuzumab in breast cancer sensitivity was shown to be reduced when HER2 or non-HER receptors were activated [84]. In a breast cancer cell line, the

CRISPR/Cas9 system directly targeted and inhibited the HER2 mutation and exerted its effect through a dominant negative mutation, which may show the anti-cancer effects of HER2-targeting by CRISPR/Cas9 [85]. By reactivating or sensitizing tumors to anti-HER2 TKI therapy, CRISPR/Cas9 deletion of HER2 and CDK12 may considerably improve the prognosis of patients with HER2-positive BC [86].

Drug Interaction and Activation

Drug-drug interactions (DDIs) have the potential to compromise clinical treatment by inducing an adverse event or modifying the clinical response adversely. Treatment may also be paused if the dose of a medication has to be altered in order to prevent or reverse an undesirable DDI or if either drug needs to be discontinued. The CDK4/6 inhibitors are utilized in patients with hormone receptor-positive BC. Mechanisms for drug inactivation can reduce the amount of free drug that can bind to its intracellular target [87]. It has been demonstrated that cancer drugs are inactivated by CYP450 class I metabolizing enzymes [88]. Inactivation of the pRB and p53 pathways in breast cancer predicts resistance to anthracyclines and mitomycin in vivo [89].

It has been demonstrated that combinatorial CRISPR-Cas9 genetic interaction mapping effectively identifies a large number of therapeutically significant genetic interactions in cancer. Observed differences in genetic interaction between cell lines via systematic CRISPR could be reproduced to a large extent as drug-drug interactions [90,91]. Knowledge of the causes of DDI, in combination with a comprehensive understanding of patient/drug-specific features, may therefore, serve as the basis for determining the optimal treatment options for BC patients. The CRISPR-Cas9 technology, which is extensively utilized to generate animal models of absorption, distribution, metabolism, and excretion (ADME), may also be used to examine the mechanism of drug resistance associated with DDIs in humans, as well as drug inactivation [92]. It has been proven that an erlotinib-docetaxel interaction is possible and that docetaxel acts on erlotinib in rats through the CYP3A enzyme [92,93]. Additionally, when used in combination with quizartinib to treat malignant lesions, pharmacological ABCB1/ABCG2 inhibitors decrease quizartinib's brain accumulation in mice [94].

Epithelial-Mesenchymal Transition (EMT)

Cancer cells may become more mobile as a consequence of the stimulation of EMT-transcription factors (EMT-TFs), enhancing their potential to disseminate and form tumor clusters that can then migrate collectively within a tumor bed [95]. During the EMT, epithelial cells revert to a front-back polarity, lose their epithelial differentiation, gene expression profile, and morphology, release their lateral cell junctions and connections to the basal substrate, and elongate in order to become motile and invasive. Tumor cell tractability EMT is regulated by Snail family members, which are transcription factors. Additionally, it has been associated with cancer-related characteristics such as an increase in apoptosis resistance and immunosurveillance evasion, as well as the potential to grow endlessly [96,97]. Chemoresistance has been linked to the expression of Snail family proteins in BC [98]. Potential therapeutic targets for EMT genes could be identified through the use of CRISPR/Cas9 gene editing.

CRISPR/Cas9 gene editing allows the identification of genes involved in EMT, which can lead to the development of therapeutic interventions. When the EMT genes in human ovarian cancer (RMG-1) cells were altered using CRISPR/Cas9, it was shown that Snail1 was important [99]. CRISPR/Cas9-mediated CPEB2 knockdown in the MCF10A cell line causes an increase in EMT and increased tumor sphere formation [100]. Reductions in EMT, migration, and invasion were observed in *in vitro*, and tumor metastasis was inhibited when Kindlin-2 was knocked down in CRISPR/Cas9-derived MDA-MB-231 cells and 4T1 cell lines in a mouse xenograft model [101]. Genetic editing of AGTR-1 using CRISPR/Cas9 was shown to inhibit DNA repair and regulate EMT in triple-negative cell lines. Losartan treatment reduced the protein

expression of EMT markers. In addition, the study noted that there is no published data demonstrating the effectiveness of losartan in this tumor type [102]. Further, Table 1 showed different CRISPR/Cas9 applications that targeted several genes to overcome drug resistance.

Table 1. Several applications of CRISPR/Cas9 in the context of drug resistance.

Model	Medicine to test	Target/delivery	Intervention	The study's findings	Ref.
MCF7-Cell line	PI3Kα inhibitors	sgRNA screen/ Lentivirus	CRISPR/Cas9 sgRNA knockout screens	Numerous negative regulators of mTORC1 have been found using CRISPR/Cas9-based sgRNA knockout screens, the loss of which provides tolerance to PI3K inhibition. This demonstrates the importance of restoring mTOR signaling in giving resistance to PI3K inhibition and suggests therapeutic options for preventing or reversing this resistance.	81
MCF7-Y537S anti-estrogen tamoxifen and fulvestrant-resistant breast cancer cell lines	Tamoxifen and fulvestrant / CDK7 inhibitor, THZ1	CDK7 in MCF7-Y537S cells	The gene-editing tool CRISPR-Cas9 was utilized to introduce one allele of tyrosine 537 into MCF7 breast cancer cells in the ESR1 gene, and CDK7 inhibitor was used to limit CDK7 phosphorylation and proliferation in Cell line: MCF7-Y537S	New treatment techniques may be examined via the use of knock-in mutational models, and CDK7 inhibition may be used to treat endocrine-resistant breast cancer that is driven by ER mutations. ER mutations play a vital role in endocrine resistance development.	79
In vivo and *in vitro* use of xenografts derived from cells or patients.	Anti-HER2 TKIs (lapatinib)	HER2/ CDK12	CRISPR/Cas9 knockout technique for inhibition of HER2/ CDK12	Inhibiting the HER2/CDK12 axis with CRISPR/Cas9 gene-editing technology, tumors become more susceptible to anti-HER2 TKI therapy.	86
Xenograft mouse model	-	HnRNPA2/B1	CRISPR-CAS9 knockout of hnRNPA2/B1 in MCF-7 cells	*In vitro* and *in vivo*, the STAT3 pathway regulates apoptosis and autophagy in breast cancer MCF7 cells. Therefore, inhibiting the hnRNPA2/B1 or STAT3 pathways may be utilized to treat breast cancer.	67
The ATCC provided the MCF7, BT474, SKBR3, HCC1954, and ZR75.1 breast cancer cell lines cultured in DMEM with 8% FCS and 1% penicillin/streptomycin.	Trastuzumab	ARID1A	knockout of ARID1A in BT474 cells	When ARID1A was entirely knocked out, activating ANXA1 expression in BT474 cells increased trastuzumab resistance. According to the findings of this investigation, ANXA1 may provide a novel target for the therapy of HER2-targeting antibody resistance.	68
Cell lines BT-474, SKBR-3, and MCF-7 from ATCC were used in this study.	Trastuzumab	ERBB2/retro virus	In order to edit the HER2 (ERBB2) gene, CRISPR/Cas9 technology is employed in BC cells that have been amplified with HER2, which is a target for the monoclonal antibody trastuzumab.	CRISPR/Cas9 technology has been used to limit HER2 proliferation and tumorigenicity by directly targeting and suppressing HER2 mutations (proliferation and tumorigenicity).	85
PDX tumor models in vivo and triple-negative and HER2-amplified cell lines *in vitro*.	Both docetaxel and anti-HER2 therapies, as well as MCL-1 inhibitor S63845 alone.	MCL-1	MCL-1 inhibitor S63845 in combination with CRISPR/Cas9 technology in breast cancer cell lines using S63845-resistant cells	MCL-1 has been identified as a target in TNBC and HER2-amplified breast tumors, and S63845 has demonstrated promising activity in PDX models as an MCL-1 inhibitor. These findings provide a solid foundation for future clinical research.	66
RNA-guided endonuclease (RGEN) systems pre-treated resistant MCF-7/ADR cells	Doxorubicin	mdr1 gene/ Cas9-sgRNA plasmid or the Cas9-sgRNA ribonucleoprotein complex	down-regulate mdr1 gene by CRISPR/Cas9	After disrupting MDR1 utilizing the CRISPR/Cas9 technology, drug resistance and sensitivity were restored in resistant MCF-7/ADR cells. As a consequence, Cas9-mediated gene disruption represents a potentially useful technique for overcoming cancer cells' resistance to several medicines.	46
MDR cell lines KBV200 and HCT-8/V overexpressing ABCB1	Vincristine ar doxorubicin	ABCB1-/lentivirus	CRISPR/Cas9-mediated ABCB1 KO	ABCB1 has been knocked out by the CRISPR/Cas9 system in MDR cancer cells, increasing the sensitivity of ABCB1 substrate chemotherapeutic drugs, giving light and offering vital insights on the prospective uses of CRISPR/Cas9 in the research and elimination of MDR cancer.	48
BC cells from human MDA-MB-231	-	WAVE3	WAVE3 was knocked out using CRISPR/Cas9, and a relationship between WAVE3 and Y-box-binding protein-1 (YB1) was established.	WAVE3/YB1 signaling axis is a regulator of CSC-mediated resistance to treatment, and this work suggests WAVE3/YB1 as a novel therapeutic window for TNBCs.	73

sgRNA single guide RNA, *CDK7* cyclin-dependent kinase 7, *HER2* Human epidermal growth factor receptor 2, *CDK12* cyclin-dependent kinase 12, *HnRNPA2/B1* heterogeneous nuclear ribonucleoprotein A2/B1, *ARID1A* AT-rich interactive domain-containing protein 1A, *ERBB2* erb-B2 receptor tyrosine kinase 2, *MCL-1* myeloid cell leukemia-1, *ABCB1* ATP binding cassette subfamily B member 1, *WAVE3* wiskott-aldrich syndrome verprolin-homologous protein 3, *TNBCs* triple negative breast cancer, *BC* breast cancer, *MDR* multidrug resistance, *RGEN* RNA-guided endonuclease.

APPROACHES to CRISPR DELIVERY

The delivery of Cas9 into cells is critical in gene editing. CRISPR-Cas9 gene editing therapies are delivered *in vivo* via delivery vectors or physical methods. Several different kinds of non-viral vectors have been created for *in vivo* CRISPR-Cas9 use (Table 2). There are several different types of nanoparticles in this category: lipids, polymers, lipid nanoparticles, peptides, and inorganic. Electroporation and microinjection are the two most commonly used methods for delivering CRISPR/Cas9 technology to target cells in clinical trial studies [103]. The microinjection approach may be used to inject plasmid DNA with both the Cas9 protein and the sgRNA, as well as inject the Cas9 protein with the sgRNA. For example, cargo can be delivered directly to a target site inside the cell using a microscope and needle [104]. In zebrafish [105,106], mouse [107] RNP has been successfully microinjected into embryos. In addition, other organisms were successfully administered RNP using this technique, including the olive fruit fly [108], for limb regeneration [109]. High-voltage pulses may create nanometer-sized breaches in the cellular membranes of suspended cells by electroporation, enabling components with hydrodynamic diameters of several nanometers to enter the cell and conduct effective gene editing [110,111]. Due to its simplicity and high efficacy, electroporation is currently one of the most popular commercial gene transfection techniques. This technique has been used to successfully introduce the CRISPR system into a variety of cell types with varying degrees of success [112].

Table 2. Delivery systems of CRISPR-Cas9 for target gene editing.

CRISPR-Cas Form	Delivery System	Study Objective	Target	Level of target gene after delivery system	Mechanism of action	Ref.
Plasmid DNA	Non-viral vector	MCF-7 cells, MCF-7/DDP	ERCC1	Knockdown	Drug sensitivity was dramatically increased, and ERCC1 mRNA levels were lowered in the cisplatin-resistant MCF-7 cell line by the dual-targeted polyplexes harboring CRISPR/Cas9 plasmids.	113
CRISPR/Cas9	pCR4-TOPO TA	-MCF7 cell line -Nude mice	miR-23b, miR-27b	Knockdown	MiR-23b and miR-27b are knocked out using CRISPR/Cas9 in MCF7 cells and nude mice, which suppresses the growth of breast tumors.	114
CRISPR/Cas9	-	MCF-HGH, MDA-MB-468 cell lines	LINC00511, BCL2, SURVIVIN	Knockdown	Through the suppression of antiapoptotic genes, LINC00511 knockdown procedures using CRISPR/Cas9 improved the apoptosis of BC cells.	115
CRISPR/Cas9	Using ultrasound and artificial nanobubbles (Gas Vesicles, GVs)	Modified 4T1 cell (4T1-Cas9-hyg stable cell line)	Cdh2	Knockdown	To prevent tumor invasion and metastasis, an ultrasound in conjunction with GVs may be an efficient way to carry out CRISPR/Cas9 gene editing on the Cdh2 gene.	116
CRISPR-Cas9 plasmid	Chitosan-based delivery	-	VEGF	Knockdown	BC invasion potential and breast cancer stem cell viability can both be decreased by using chitosan/VEGF CRISPR/Cas9 plasmid/protamine complexes to lower VEGF expression.	117

ERCC1 excision repair cross-complementing 1, *BCL2* B-cell lymphoma 2, *Cdh2* cadherin-2, *VEGF* vascular endothelial growth factor A, *CRISPR* clustered regularly interspaced short palindromic repeats, *GVs* gas vesicles.

Because of its greater transfection efficiency and wider application to a broader spectrum of cells, viral vector-mediated nucleic acid transduction has emerged as the preferred technique for CRISPR/Cas9 genome editing. Adenovirus (AV), lentivirus (LV), and adeno-associated viruses (AAV) are among the most common viral vectors [118]. AAVs are the most extensively used vectors for CRISPR genome editing due to their low immunogenicity relative to other viruses and minimal toxicity in animal models. They have been authorized for multiple human clinical studies in gene augmentation therapy [119]. CRISPR/Cas9 and AAV vectors have been used in mouse studies for

genome editing in vivo in the brain [120,121], muscle [122], retina [123-126], liver [127-130], heart [131-133], and lung [134,135], using an AAV vector and CRISPR/Cas9 as an antiviral against Orth-poxviruses [136]. Another vector often utilized in clinical trials is the lentivirus, which can infect both proliferating and non-dividing cells. Clinical experiments have shown that when utilized for *ex vivo* transduction, lentivirus outperforms other vectors [137]. Many studies have been undertaken employing the CRISPR-Cas9 system for gene editing in human hematopoietic stem and progenitor cells using lentiviruses [138], delivery of CRISPR/Cas9 to Huh7 tumors through a lentiviral vector with a modified tropism for hepatic tumors [139], and sickle cell disease gene therapy [140]. For genome editing *in vivo* using adenovirus, retrovirus, and herpes simplex virus, there are a number of limitations. Adenoviral vectors (AVs) have a large packing capacity, which allows them to contain all of the components required for genome editing, including the Cas protein and one or more sgRNAs. If the AV genome does not integrate into the host genome, re-expression of Cas9 and sgRNA may decrease off-target effects [118,137].

CRISPR/CAS9 LIMITATIONS and CHALLENGES

While CRISPR/Cas9 technology is incredibly promising, it is now impossible to employ efficiently in medicine because of technological hurdles.

Off Targets

Apart from the fact that Cas9 binds to genomic sites that are not intended for cleavage, posing a significant obstacle to CRISPR/Cas9 genome editing, sgRNA's ability to recognize genomic sites with a few nucleotide mismatches frequently results in on-target and off-target mutations during DSB repair. Off-target effects may result in a deadly genetic mutation if they are severe enough. Off-target effects may be classified into two types: those induced by sequence similarity between target loci and those caused by off-target sites located elsewhere in the genome [141-144]. While eradicating off-target effects is not feasible, it is conceivable to minimize off-target effects while maintaining high effectiveness and minimal off-targets in crucial locations by choosing only on-target sgRNAs that are highly efficient and have few or no off-targets [145].

A number of approaches have been taken to reduce the number of off-target mutations; it may be possible to avoid or minimize off-target mutations by selecting specific target regions and optimizing Cas9 and guide RNA [146]. It has been suggested that approaches for genome-wide off-target detection, such as CRISPR amplification and off-target CRISPR amplification, would soon enable the accurate and sensitive detection of editor-induced off-target changes [147]. To minimize off-target effects while increasing on-target mutagenesis, a synthetic switch was constructed that controls Cas9 expression in both transcription and translation [148].

Homology Efficiency

This is the second most significant challenge facing the widespread use of CRISPR/Cas9 gene editing in clinical settings. The adoption of homology-directed recombination (HDR) or non-homologous end-joining (NHEJ)-induced indel creation repair procedures has a major impact on the final results of gene editing [141,149]. Precise genome editing may be achieved by enhancing HDR efficiency by suppressing the NHEJ pathway [149]. Precision gene editing using CRISPR-Cas9 and HDR was accomplished by demethylating histone H2A-K15 [150]. Histone deacetylase inhibitors may enhance homology-directed DNA repair mediated by CRISPR/Cas9 [151]. An anti-CRISPR protein-based CRISPR-Cas9 activation technique has been shown to improve the accuracy of genome editing in situations where HDR is prominent. Cell cycle-dependent activatable systems may be used in a variety of additional CRISPR-Cas systems and anti-CRISPR combinations [152-154]. A new approach suggests that Cas9 RNPs encapsulated in poly-L-glutamic acid (PGA) nanoparticles might boost the efficacy of CRISPR/Cas9 gene editing [155].

DNA Damage and Toxicity

There is a chance that CRISPR/Cas9 may induce DNA damage, making it dangerous. CRISPR-Cas9 genome editing, for instance, induces a p53-mediated response to DNA damage.

DNA damage induces cell cycle arrest, which can then lead to apoptosis [156]. The elimination of DNA damage necessitates the development of safer CRISPR/Cas9 applications. CRISPR safety has been addressed in a number of ways; transiently blocking p53-linked genes with non-redundant functions in CRISPR-induced DDR has been hypothesized as a feasible technique; these genes could serve as new pharmacological targets for modifying the CRISPR-p53 response. Additionally, cells with mutations in these genes may be more susceptible to DNA damage caused by CRISPR [157].

Immunogenic Toxicity

Apart from technological issues, CRISPR/Cas9 gene therapy, like other kinds of gene therapy, raises concerns about immunogenic toxicity. Anti-Cas9 antibodies reveal an immune response to intracellular bacterial proteins. In patients with preexisting anti-Cas9 CD8+ cytotoxic T lymphocytes, it may be more difficult to prevent immunological destruction of CRISPR-Cas9-corrected cells, thereby reducing the likelihood of anti-Cas9 formation during CRISPR-Cas9 gene therapy. Charlesworth et al. found that human volunteers acquired anti-Cas9 antibodies against the two most often employed bacterial orthologs, *Staphylococcus aureus* (SaCas9) and *Streptococcus pyogenes* (SpCas9), prior to participating in their research. Additionally, Cas9-based medicines have been linked to an increased incidence of Staphylococcus infections in hospitals [158]. Anti-SaCas9 and anti-SpCas9 antibodies were found in 10% and 2.5% of 200 human serum samples in the USA population respectively [159].

Delivery System

There are various limits or downsides to the delivery of CRISPR-Cas9 gene editing. Adenovirus (AV), lentivirus (LV), and adeno-associated viruses (AAV) are among the most common viral vectors that have been utilized to deliver gene editing tools to mice despite the fact that they sometimes induce immunological toxicities [118]. To address this, adenoviral vector safety and efficacy may be greatly improved by reducing the host immune response, therefore providing new opportunities for the creation of more effective viral vectors. Immunogenicity may be reduced in vivo by using poly (lactic-glycolic) acid copolymer as an encapsulant for recombinant adenovirus [160]. The delivery of CRISPR/Cas9 technology is restricted by AAVs' low cargo capacity, which is a significant constraint; as a consequence, devising a method for using AAV vectors to accomplish cell type-specific CRISPR activity is crucial [161], which implies that when AAVs are utilized for delivery, they can be encoded on distinct vectors in order to overcome this issue [162,163]. The immune response of the host influences the delivery effectiveness of AVV vectors. Therefore, techniques that minimize the host immune response to viral vectors enhance delivery [163,164]. As an alternative to viral delivery, lipid nanoparticles (LNPs) are easily accessible, affordable to manufacture, and highly compatible. While LV and AdV vectors have a greater package capacity for transport than AAV vectors, an immunological response or inflammation in the host may potentially provide substantial hurdles for AdVs and LVs [161,165,166].

Although electroporation may be utilized in vivo for certain target tissues, it is most often performed ex vivo. Electroporation uses a high-voltage shock to permeabilize cell membranes, which is toxic and may result in the permanent permeabilization of cells treated with electroporation. Damage to cells and a high degree of technical proficiency and physical dexterity are two additional drawbacks of microinjection [167,168]. One method of cell engineering uses a technique called microfluidic squeezing, which relies on a temporary mechanical membrane breach to transport chemicals to cells; alternatively, it may be applicable [169].

CONCLUSIONS and PERSPECTIVES

The development of CRISPR/Cas9 gene editing technology has created new opportunities for combating BC medication resistance. The potential of CRISPR/Cas9 as a formidable tool for understanding the genetic and molecular mechanisms behind drug resistance and discovering important genes and pathways implicated in BC resistance has been highlighted in this review. CRISPR/Cas9 offers a platform for the

creation of novel treatment approaches by precisely modifying gene expression and function, allowing researchers to understand the mechanisms underlying resistance.

The studies covered in this review have shown the successful use of CRISPR/Cas9 gene editing in identifying and focusing on particular genes linked to BC treatment resistance, including those involved in DNA repair pathways, hormone receptor signaling, and drug efflux transporters. These discoveries have improved our comprehension of the mechanisms underlying drug resistance while also laying the framework for the creation of personalized therapeutic strategies that can go around resistance and enhance patient outcomes.

The use of CRISPR/Cas9 gene editing as a fresh approach to combating BC medication resistance is incredibly promising. CRISPR/Cas9-based techniques have the potential to completely change how BC is treated by elucidating the molecular causes of resistance and locating new therapeutic targets. However, further research and advancements are required to optimize these aspects and improve the clinical translation of CRISPR/Cas9 technology.

Conflict of Interest

The authors declared that they have no conflict of interests.

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