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# From Gene Editing to Immunotherapy: The Role of CRISPR-Cas9 in Breast Cancer

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### Abstract

Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide with therapeutic resistance and tumour heterogeneity posing major challenges to conventional treatment strategies. Clinical evidence indicates that drug resistance is a great obstacle in breast cancer therapy. Multiple mechanisms contribute to the development of drug resistance, but the underlying cause is usually a shift in the genetic composition of tumour cells. Constant effort is being made to introduce novel therapies with minimal toxicity. The advent of CRISPR-Cas9 genome editing has revolutionized cancer biology by enabling precise and efficient manipulation of genetic material. In breast cancer research, CRISPR-Cas9 has been employed to knockout oncogenes such as HER2 and MYC, repair or model BRCA1/2 mutations, and investigate mechanisms of drug resistance. Furthermore, CRISPR-based approaches are being explored in diagnostics, such as the rapid detection of BRCA mutations, and in immunotherapy, where gene editing enhances the efficacy of immune checkpoint blockade and adoptive cell transfer therapies. Recent studies have also demonstrated the potential of CRISPR-Cas9 delivered via nanoparticles to reprogram tumour microenvironments and improve anti-tumour immunity. However, significant challenges remain, including off-target effects, safe and targeted delivery to tumour tissues, and ethical considerations, particularly concerning germline editing. Despite these limitations, CRISPR-Cas9 holds immense promise in advancing personalized and precision medicine for breast cancer, offering new avenues for effective therapies and early detection. This review current applications, emerging strategies, and future directions of CRISPR-Cas9 in breast cancer research and therapy.

**Keywords:** Breast Cancer, CRISPR-Cas9, Diagnostics, Gene Editing, Immunotherapy, Personalized Medicine.

## Introduction

Breast cancer is a condition where abnormal breast cells grow uncontrollably, forming tumors that can spread to other parts of the body. Symptoms include a new breast lump or swelling, skin dimpling, or nipple changes.

Sharma *et al* (2010) Worldwide, breast cancer comprises 10.4% of all cancer incidences among women, making it the second most common type of non-skin cancer (after lung cancer) and the fifth most common cause of cancer death. In

2004, breast cancer caused 519,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths). Breast cancer is about 100 times more common in women than in men, although males tend to have poorer outcomes due to delays in diagnosis.

Elmore JG *et al* (2005) studies comparing full-field digital mammography to screen film have not shown statistically significant differences in cancer detection while the impact on recall rates (percentage of screening mammograms considered to have positive results) was unclear. One study suggested that computer-aided detection increases cancer detection rates and recall rates while a second larger study did not find any significant differences. Screening clinical breast examination detects some cancers missed by mammography, but the sensitivity reported in the community is lower (28% to 36%) than in randomized trials (about 54%). Breast self-examination has not been shown to be effective in reducing breast cancer mortality, but it does increase the number of breast biopsies performed because of false-positives. Magnetic resonance imaging and ultrasound are being studied for screening women at high risk for breast cancer but are not recommended for screening the general population.

Li, T., Li, S., Kang, Y. *et al* (2024) Derived from a microbial immune defense mechanism, CRISPR/Cas9, characterized as precision, maneuverability and universality in gene editing, has been harnessed as a versatile tool for precisely manipulating DNA in mammals. In the process of applying it to practice, the consecutive exploitation of novel orthologs and variants never ceases.

Cardoso F *et al* (2019) The treatments of breast cancer include surgery, chemotherapy, radiotherapy (RT), endocrine therapy, targeted therapy, and immunotherapy, and the therapeutic schedules require the cooperation of multiple subspecialties. For non-metastatic breast cancer, surgery-based treatment is the standard management, and chemotherapy-based preoperative systemic therapy can reduce tumor volume of the breast, making breast conservation possible, and decreasing the need for axillary lymph node dissection (ALND).

## Mechanism of CRISPR-Cas9

Barrangou R *et al* (2007) performed infection experiments with *Streptococcus thermophilus* and revealed that the CRISPR-Cas9 system provides resistance to phages, providing the first experimental evidence for its adaptive immunity role. Generally, CRISPR-Cas-mediated adaptive immunity occurs over three steps: acquisition, transcription, and interference.

Doudna JA *et al* (2014) It can be simply summarized as acquiring foreign DNA to integrate into the host CRISPR locus, producing mature crRNA (CRISPR-derived RNA) and Cas protein cleaving the target sequence under the guidance of RNA. In conclusion, The system includes a Cas9 nuclease and a guide RNA (gRNA). Generally, gRNA consists of crRNA and transactivating crRNA (tracrRNA) that forms base pairs with DNA target sequences, allowing Cas9 to introduce a site-specific DSB into DNA. TracrRNA:crRNA is usually designed as a single RNA complex (sgRNA). The two-component structure of sgRNA and Cas9 is easy to operate. Multiple editing of the target locus can be achieved by designing multiple sgRNAs. Collectively, the 5' end recognizes a specific DNA target sequence by Watson-Crick base pairing, while the Cas9 nuclease binds to the 3' end of the sgRNA, causing a target DSB at about 3 bp upstream of the protospacer adjacent motifs (PAMs). A guide RNA (gRNA) is the "addressing system" for the Cas9 protein, a bacterial enzyme that acts like molecular scissors to cut DNA. The gRNA, made of two parts ([crRNA](#) and [tracrRNA](#)),

directs Cas9 to a specific DNA sequence by base-pairing with the target DNA, allowing for precision genome editing. Scientists use this system to make double-strand breaks in DNA, which cells then repair, leading to genetic modifications.

Qi LS *et al* (2013) A pivotal feature of Cas9 is that it can bind to specific DNA sites via guide RNA and PAM. Thus, a kind of catalytic death Cas9 (dCas9) lacking endonuclease activity can be fused with transcription activator and repressor to regulate gene expression in the whole genome. Transcription inhibition based on dCas9 is called CRISPR interference (CRISPRi), and when dCas9 is co-expressed with sgRNA, it can prevent formation of the transcription initiation complex and transcription extension. CRISPRi not only can effectively inhibit expression of multiple target genes in *Escherichia coli* simultaneously, but its effect also is reversible and does not appear to extend off-target.

## Oncogenes and tumour suppressor genes in breast cancer

Downward J. (2003) Targeting Oncogenes refer to genes whose activation can contribute to the development of cancer. An example of such a mode of oncogene activation is that of *HER-2*, which is seen in about 20% of primary breast cancer cases. Activating point mutations in *ras* codons 12, 13, and 61 prevent the interaction of p21<sup>ras</sup> with GTPase-activating protein (GAP), maintaining p21<sup>ras</sup> in an activated and GTP-bound form and enhancing downstream signalling events such as cell cycle entry/activation.

Nahta R *et al* (2003) Other mechanisms of oncogene activation include chromosomal translocation, whereby a new fusion gene is transcribed into a protein, with enhanced function. In breast cancer, there has been much attention focused on oncogenic components of the cell signalling system, an example of which is the *HER-2/Neu* cascade.

Yarden Y, *et al* (2003) The *HER-2* (human epithelial receptor 2, also known as *HER-2/neu* or *erbB-2*) gene is located on chromosome 17q and encodes a 185-kDa transmembrane tyrosine kinase growth factor receptor

Stachler *et al* (2016) Activating mutations in *PIK3CA* can co-occur with other driver mutations.

Ramjaun, *et al* (2007) An example of this is the presence of both mutant *PIK3CA* and mutant *KRAS* (GTPase-encoding oncogene, first identified in the Kirsten rat-sarcoma virus). Activation of either protein alone results in increased signalling through both the PI3K and mitogen-activated protein kinase (MAPK) pathways

Razavi, Gao, Cerami *et al* (2007) Although *KRAS* mutations are not frequent in breast cancer, they are significantly associated with *PIK3CA* mutations.

Hines WC *et al* (2005) In breast tumours, expressing high levels of *TERT* mRNA is correlated with increased levels of *MYC* mRNA. *MYC* gene amplification and overexpression in breast cancer were first reported in 1986.

Aulmann S *et al* (2002) *MYC* amplification appears to play a role in breast cancer progression because it has been observed in the more aggressive phenotype of ductal carcinoma in situ or in only the invasive component.

Timms *et al* (2014) Sequencing of matched tumour and normal DNA samples determined that 75% of *BRCA1/2* mutant tumours carried a germline *BRCA1/2* mutation. an apparently sporadic breast tumor was seen in one individual carrying a *BRCA2* somatic deleterious mutation. *BRCA1/2* deficiency, including deleterious mutations and *BRCA1* promoter methylation, ranged from ~10 to ~37% across the four subtypes. the highest rates of *BRCA1/2* mutations were observed in triple-negative tumors (15.9%). In one TNBC patient, the only *BRCA1* mutation was somatic; the other six individuals with a single deleterious mutation all carried germline mutations. Normal tissue DNA was not available for one TNBC patient carrying a deletion of one exon in *BRCA1* in their tumor sample. Consequently, it was not possible to determine whether this deleterious mutation was a germline mutation or a somatic mutation. Two additional TNBC tumors carried both germline and somatic mutations. *BRCA1/2* mutations were detected in all three of the other breast cancer subtypes with frequencies ranging from 7.8 to 11.1%.

## Role of CRISPR-Cas9 in understanding drug resistance

Drug resistance remains a critical obstacle in breast cancer therapy. Using CRISPR screens researchers can:

- Identify genes involved in multidrug resistance
- Study mechanisms of hormonal and targeted therapy resistance.
- Develop drug-resistant cell lines to test new interventions. This helps design more effective therapeutic combinations.

Vaghari-Tabari *et al* (2022) Chemotherapy is a common approach in the treatment of cancers. However, cancer cells often have the ability to resist the effects of chemotherapeutic drugs, which can lead to chemotherapy failure. An important feature of drug resistance is that resistance to one drug may lead to resistance to other drugs, which is called multidrug resistance (MDR). Curcumin is a natural compound that has been extensively studied to overcome MDR in cancers because it appears to be nontoxic even in high concentrations. The effect of metformin, an antidiabetic drug, on the drug resistance of cancer cells has also been considered in recent years. It seems that metformin can attenuate p.gp activity in doxorubicin-resistant cancer cells in a dose-dependent manner and potentiate the apoptotic effects of doxorubicin.

Shafiei-Irannejad *et al* (2018) The design of nanoparticles for effective delivery of anticancer compounds and overcoming drug resistance in cancers has been one of the most interesting areas of research in recent years, and the results reported so far have been very interesting. For example, codelivery of metformin and doxorubicin in the form of PLGA–TPGS nanoparticles can enhance drug cellular uptake and attenuate drug efflux in DOX-resistant breast cancer cells, leading to enhanced cytotoxicity and apoptosis and attenuated drug in breast cancer cells.

Yang Y *et al* (2016) In bacteria, the CRISPR/Cas9 system acts like an RNA-based immune system. This system can be used to edit genes in eukaryotic cells, including MDR-related genes. For this purpose, single guide RNA (sgRNA) can be designed to complement the desired sequence and enter the target cell along with Cas9, an endonuclease. sgRNA can guide Cas9 to the target sequence, and Cas9 can cause a double-strand break in the target sequence. In this way, it is possible to delete or insert the desired sequence in to genes.

Ha JS *et al* (2016) In a study of DOX-resistant breast cancer cells, the *MDR1* gene was targeted and downregulated using the CRISPR/Cas9 system. The results of this study showed that the cytotoxicity of DOX is significantly increased in resistant breast cancer cells treated with Cas9-sgRNA plasmid. Flow cytometric analysis performed in this study showed that targeting MDR1 using CRISPR/Cas9 system in DOX-resistant breast cancer cells increased drug accumulation within the cell and quadrupled drug uptake compared with untreated cells.

### **CRISPR-Cas9 in functional genomics and breast cancer modelling**

CRISPR-Cas9 enables the creation of precise in-vitro and in-vivo breast cancer models by editing specific oncogenes (HER2, MYC) and tumour suppressor genes (BRAC1/2, TP53). Through genome-wide knockout or activation screens, researchers can identify critical genes involved in tumour progression, metastasis and drug sensitivity. This approach accelerates the discovery of novel therapeutic targets and helps understand the molecular mechanisms underlying tumour heterogeneity.

## **CRISPR - based diagnostics and early detection**

Vaghari-Tabari *et al* (2022) In general, using the genome-wide CRISPR knockout screens technique, it is possible to examine a large number of genes in an experiment. A significant number of studies in recent years that have focused on the identification of genes involved in drug resistance have used this technique and reported interesting results. For example, some preclinical studies using the CRISPR screen technique have elucidated the mechanism of action of PBRM1 and SGOL1 in cancer drug resistance. PBRM1 is a SWI/SNF complex subunit. This complex is involved in altering the nucleosome position and controlling chromatin availability and appears to have tumour-suppressive properties.

Beyond therapeutics, CRISPR systems such as Cas12 and Cas13 have been adapted for rapid and sensitive detection of genetic mutations associated with breast cancer, including BRCA1/2 and PIK3CA. These CRISPR based biosensors offer point of care diagnostic potential, allowing fast and accurate detection from minimal samples. Integration with fluorescence or microfluidic platforms enhances their clinical applicability for early diagnosis and genetic screening.

## **CRISPR-Cas9 in immunotherapy and tumour microenvironment modulation**

Liu *et al.* (2023) For oncology patients, the immune system tends to present poor co-stimulation and high co-suppression in TME. Moreover, T cells generally develop phenotypic and functional states that are difficult to exert antitumor effects, such as poor proliferation and severe apoptosis. Relevant molecules can be knocked in via CRISPR-Cas9, so CAR-T can “arm” itself to improve the effect of immunotherapy. TME harbours a variety of immunosuppressive cells, such as MDSCs, tumour-associated macrophages (TAMs), and regulatory T cells (Tregs). Tumour cells up-regulate ligands (e.g., PD-L1) and produce other common inhibitory signals, generating a “brake” effect. In this condition, CAR-T cell therapy can be developed by knocking out these molecules using CRISPR-Cas9.

Wei L *et al* (2019) Tumour-infiltrating lymphocytes (TILs) have been proved to be efficient in treating metastatic melanoma. To further improve the curative effect of TIL therapy, CRISPR-Cas9 can be designed to reverse the repressed state of T cells. For instance, the metabolism-related factor Regnase-I is a negative regulator of antitumor response. Hence, knocking down Regnase-I allows much better infiltration and persistence of T cells in TME. Another research confirmed that the zinc finger transcription factor Gata-3 is non-negligible, which leads to the dysfunction of T cell.

Singer *et al* (2016) transduced sgRNAs targeting Gata-3 along with lentiviruses CRISPR-Cas9 into CD8<sup>+</sup> T cells, demonstrating that the antitumor function of TILs can be progressed with increased frequency of IFN $\gamma$ <sup>+</sup> and IL-2<sup>+</sup> cells on account of the disruption of Gata-3.

CRISPR-Cas9-based unbiased genome-wide T cell screening has been employed to identify genes in mammalian cells that significantly affect cancer cell survival, proliferation, migration, and drug resistance, based on which we can explore new targets for cancer therapy. Additionally, targeting immunosuppressive factors like TGF- $\beta$  and VEGF helps reprogram the tumour microenvironment, enhancing anti-tumour immunity and response to checkpoint inhibitors.

## **Nanoparticle-mediated delivery and clinical translation**

Effective delivery of CRISPR components remains a key challenge. Advanced nanoparticle and viral-vector systems

are being developed to deliver Cas9 and guide RNA specifically to tumour cells. Lipid nanoparticles (LNPs) offer improved stability, reduced immune response and precise targeting to breast tumour tissues. These systems increase editing efficiency while minimizing off-target effects, making a major step toward safe clinical application of CRISPR based therapies.

## Challenges and Ethical considerations

Despite its transformative potential, the application of CRISPR-Cas9 in breast cancer therapy faces several challenges. Off-target mutations remain a major concern, as unintended DNA cleavage can disrupt essential genes or activate oncogenic pathways. Achieving efficient, tissue-specific delivery of CRISPR components to tumour sites is another obstacle, particularly in solid cancers like breast carcinoma. Immunogenicity and degradation of Cas9 proteins also limit therapeutic success.

From an ethical standpoint, germline editing raises serious concerns regarding heritable genetic alterations and long-term consequences for future generations. Strict ethical regulations, risk assessment, and clinical validation are essential before CRISPR-based therapies can be safely implemented in humans. Balancing innovation with responsibility is key to the technology's acceptance in clinical oncology.<sup>1</sup>

## Future prospects

The integration of CRISPR-Cas9 with precision medicine, artificial intelligence, and advanced delivery systems is likely to revolutionize breast cancer management. Future directions include combining CRISPR-based gene editing with immunotherapy, chemotherapy, and targeted molecular treatments to overcome drug resistance and improve therapeutic response. Continuous improvements in Cas9 variants, base editors, and prime editing tools will further enhance accuracy and safety.

## Conclusion

In conclusion, CRISPR-Cas9 represents a powerful platform linking gene editing, diagnostics, and immunotherapy in breast cancer research. Although technical and ethical challenges remain, ongoing advancements promise to make CRISPR-guided approaches an integral part of personalized and precision oncology, offering new hope for effective and less toxic breast cancer therapies.

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## References

- Aulmann S, Bentz M, Sinn HP. C-myc oncogene amplification in ductal carcinoma in situ of the breast. *Breast Cancer Res Treat* 2002;74:25-31
- Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*. 2007;315:1709–12.
- Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. *Ann Oncol*. 2019;30(8):1194–1220. doi: 10.1093/annonc/mdz173
- Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B.E.; Sumer, S.O.; Aksoy, B.A.; Jacobsen, A.; Byrne, C.J.; Heuer, M.L.; Larsson, E.; et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012, 2, 401–404
- Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014;346(6213):1258096
- Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer*. 2003 Jan;3(1):11-22. doi: 10.1038/nrc969. PMID: 12509763.
- Elmore JG, Armstrong K, Lehman CD, Fletcher SW. Screening for Breast Cancer. *JAMA*. 2005;293(10):1245–1256. doi:10.1001/jama.293.10.1245
- Gao, J.; Aksoy, B.A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S.O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal*. 2013, 6, pl1.
- Ha JS, Byun J, Ahn DR. Overcoming doxorubicin resistance of cancer cells by Cas9-mediated gene disruption. *Sci Rep*. 2016;6:22847.
- Hines WC, Fajardo AM, Joste NE, Bisoffi M, Griffith JK. Quantitative and spatial measurements of telomerase reverse transcriptase expression within normal and malignant human breast tissues. *Mol Cancer Res* 2005;3:503-9.
- Li, T., Li, S., Kang, Y. *et al.* Harnessing the evolving CRISPR/Cas9 for precision oncology. *J Transl Med* **22**, 749 (2024). <https://doi.org/10.1186/s12967-024-05570-4>
- Liu, Z., Shi, M., Ren, Y. *et al.* Recent advances and applications of CRISPR-Cas9 in cancer immunotherapy. *Mol Cancer* **22**, 35 (2023). <https://doi.org/10.1186/s12943-023-01738-6>
- Nahta R, Hortobágyi GN, Esteva FJ. Growth factor receptors in breast cancer: potential for therapeutic intervention. *Oncologist*. 2003;8(1):5-17. doi: 10.1634/theoncologist.8-1-5. PMID: 12604728.
- Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*. 2013;152:1173–83.
- Ramjaun, A.R.; Downward, J. Ras and phosphoinositide 3-kinase: Partners in development and tumorigenesis. *Cell Cycle* **2007**, 6, 2902–2905
- Razavi, P.; Chang, M.T.; Xu, G.; Bandlamudi, C.; Ross, D.S.; Vasan, N.; Cai, Y.; Bielski, C.M.; Donoghue, M.T.A.; Jonsson, P.; et al. The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers. *Cancer Cell* **2018**, 34,

- Shafiei-Irannejad V, Samadi N, Salehi R, Yousefi B, Rahimi M, Akbarzadeh A, Zarghami N. Reversion of multidrug resistance by co-encapsulation of doxorubicin and metformin in poly(lactide-co-glycolide)-d- $\alpha$ -tocopheryl polyethylene glycol 1000 succinate nanoparticles. *Pharm Res.* 2018;35:119.
- Sharma, Ganesh N.1,\*; Dave, Rahul1; Sanadya, Jyotsana2; Sharma, Piush2; Sharma, K. K3. VARIOUS TYPES AND MANAGEMENT OF BREAST CANCER: AN OVERVIEW. *Journal of Advanced Pharmaceutical Technology & Research* 1(2):p 109-126, Apr–Jun 2010.
- Singer M, Wang C, Cong L, Marjanovic ND, Kowalczyk MS, Zhang H, et al. A distinct gene module for dysfunction uncoupled from activation in tumor-infiltrating T cells. *Cell.* 2016;166(6):1500–11.
- Stachler, M.D.; Rinehart, E.M.; Garcia, E.; Lindeman, N.I. PIK3CA Mutations are Common in Many Tumor Types and are Often Associated with Other Driver Mutations. *Appl. Immunohistochem. Mol. Morphol.* **2016**, *24*, 313–319
- Timms, K.M., Abkevich, V., Hughes, E. *et al.* Association of *BRCA1/2* defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Res* **16**, 475 (2014). <https://doi.org/10.1186/s13058-014-0475-x>
- Vaghari-Tabari, M., Hassanpour, P., Sadeghsoltani, F. *et al.* CRISPR/Cas9 gene editing: a new approach for overcoming drug resistance in cancer. *Cell Mol Biol Lett* **27**, 49 (2022). <https://doi.org/10.1186/s11658-022-00348-2>
- Wei L, Lee D, Law CT, Zhang MS, Shen J, Chin DW, et al. Genome-wide CRISPR/Cas9 library screening identified PHGDH as a critical driver for Sorafenib resistance in HCC. *Nat Commun.* 2019;10(1):4681.
- Yang Y, Qiu JG, Li Y, Di JM, Zhang WJ, Jiang QW, Zheng DW, Chen Y, Wei MN, Huang JR, et al. Targeting ABCB1-mediated tumor multidrug resistance by CRISPR/Cas9-based genome editing. *Am J Transl Res.* 2016;8:3986–94.
- Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol.* 2003 Feb;2(2):127-37. doi: 10.1038/35052073. PMID: 11252954.