Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/8/20240426

Advancements in the research of combining MRI and CRISPR technology for the treatment of breast cancer

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Abstract. Cancer remains a significant contributor to global mortality, posing a considerable public health challenge. Among the various types of cancer, breast cancer stands out as one of the most prevalent among women worldwide. Ongoing efforts are dedicated to the development of innovative treatments with minimal side effects. A groundbreaking genome technique for editing, CRISPR-associated protein-9 (Cas9), also known as clustered regularly interspaced short palindromic repeat (CRISPR), has become an effective instrument for genetic modification. This technology has revolutionized genome editing across diverse biological fields, including its applications in genome therapy and tumor research. With ongoing advancements in sensitivity, specificity, and diagnostic accuracy, magnetic resonance imaging (MRI) has gained increasing recognition for the detection of breast cancer. MRI's capability to produce high-resolution images plays a pivotal role in aiding healthcare professionals in precise localization of breast masses, tumors, and other abnormalities. Additionally, it facilitates the assessment of parameters like tumor size, extent, and lymph node involvement, contributing to the formulation of more accurate treatment strategies. Here is a review summary of the combined utilization of MRI and CRISPR technology in breast cancer treatment. It explores the synergy between these two technologies, discussing their advantages and disadvantages and their potential to enhance diagnostic and therapeutic outcomes.

Keywords: CRISPR, MRI, Breast Cancer.

1. Introduction

Cancer is a complex and well-known disease, posing a significant threat to modern human health. Breast cancer ranks first among female malignancies, and the incidence of cancer is often genetic. The incidence rate of women who is after menopause is much higher. As a member of the most common malignant tumors that typically occur in breast glandular epithelial tissue, Breast cancer seriously affecting women's health, and even endangering their lives. Many scientists have contributed to the study of breast cancer. In 2022, the emergence of 3D-printed breast cancer tumor models marked a significant advancement in breast cancer research [1]. Although the global mortality rate of breast cancer

DOI: 10.54254/2753-8818/8/20240426

has gradually declined, but its situation in remote areas remains to be precarious—new treatments strategies and methods cannot be popularized to those places so quickly.

The Magnetic Resonance Imaging (MRI) technique offers high spatial anatomical detail as well as cellular density and perfusion data, making it sensitive in cancer detection. It also provides the highest soft tissue contrast of any imaging modality. MRI has been effective in visualizing the anatomical structure and function of tumors at high resolution. Nonetheless, concerns about the specificity and false positives of MRI persist. CRISPR—famous for its cheapness, precision and versatility, is the brilliant technology which can edit the specific gene with high precision. Deriving from ancient bacterial, this technology has been used all around the world.

Recently, scientists have made new progress in cancer research and treatment in cancer research and treatment. However, it is still a hard task to locate the residual cancer cells in the breasts as following treatment remains. The configurations of cancer cells will be changed drastically. It is why although MRI is the most accurate technique, using MRI to detect the specific position of cancer cells is still difficult.

This paper summarizes some of the proven methods about MRI and CRISPR for detecting breast cancer, and also the combinations of both. The paper focuses on the detailed principles, limitations, advantages and disadvantages of all the methods listed below. The clinical application of some methods was mentioned with its data.

2. Current status of breast cancer detection methods

2.1. Biopsy test

The methods of breast cancer biopsy include puncture biopsy and surgical biopsy. A needle biopsy is a biopsy of a breast mass or lymph node to collect breast cancer tissue. Fine needle puncture (FNA) and core needle puncture (CNB) are two methods of needle biopsy. In FNA, a fine needle is inserted into the mass and cells are extracted under negative pressure and then analyzed by staining or immunohistochemical evaluation. CNB is made by puncturing the mass with a larger needle, obtaining a tissue sample, and then confirming the diagnosis through pathological examination. A surgical biopsy is a surgical removal of a lump or lymph node to collect breast cancer tissue. Biopsies are inexpensive and allow for a more detailed pathological evaluation. But biopsies can cause trauma and other complications.

2.2. Endoscopic breast examination

The internal tissue of nipple overflow in breast cancer patients, which can be directly and clearly observed by endoscope. The test can be used to detect breast overflow as early as possible in patients with disease, especially in patients with intraductal breast cancer has shown great advantages. But endoscopy can’t detect all breast tissue, only some areas. Therefore, there may be misdiagnosis or missed diagnosis, which will inevitably produce certain diagnostic errors.

2.3. Supersonic inspection

Ultrasonography is used to show images of suspicious substances in breast cancer patients without damage. Under normal circumstances, for breast diseases, B-ultrasound can be used as the preferred inspection means, which is not only simple, fast, but also can be used repeatedly. But ultrasound can’t provide a more accurate image than an X-ray or MRI, and can be affected by factors such as breast shape and size, breast density, and so on. Moreover, ultrasound is less effective for detecting deep breast tissue and calcified tumors, which may lead to misdiagnosis.

2.4. X-ray examination

The gold standard method for early detection of breast cancer is mammography, which is the basis for mammography programs that are being expanded worldwide to detect the early stages of small malignancies [2, 3] Although evidence supports that mammography reduces the risk of death, there is

DOI: 10.54254/2753-8818/8/20240426

still debate about the overall effectiveness of the test in breast screening and diagnosis [4]. However, the X-ray display of breast tissue is not clear enough, can only preliminarily determine whether there is a lesion, but can't determine the degree of malignancy of tumor cells, for some cases, the early detection of cancer is poor.

2.5. MR examination

Today, MRI is the best imaging method for breast cancer lesions. First, MRI can provide high-resolution and multi-dimensional images, and small changes in breast tissue can be observed from multiple angles, making it easier to find abnormal areas. In addition to T1-weighted and T2-weighted imaging, the routine breast MR Protocol includes dynamic enhanced MRI (DCE MRI) [5]. Due to the long MRI scanning time and low specificity, the indication of MRI is usually limited to the screening of designated patients with clinical indications and women at high risk of breast cancer.

3. Clinical applications of MRI

Kuhl et al. presented a simplified breast MRI protocol consisting of one pre-contrast picture and one post-contrast image that can be obtained in as little as three minutes to reduce MRI scan duration and increase diagnostic accuracy [6]. In using this short MRI acquisition, using only the maximum intensity projection image, the sensitivity can reach 91%. When reading the DCE AP breast MRI image, the radiologist will use the MIP image to quickly see if there is abnormal enhancement in the detected breast, and then compare the subtraction image with the T1-weighted comparison image to observe the lesion. The absence of other additional pulse sequences and the absence of time-dynamic signatures did not have a significant effect on the diagnosis of breast cancer. This work represents an early effort to simplify MRI screening for breast cancer.

Not only that, a recent study investigated the diagnostic effectiveness of simplified breast MRI protocols. The protocol consisted of a comparison of pre-T1-weighted sequences and an initial comparison of post-T1-weighted sequences, both of which were strongly associated with fat saturation in 100 cancers. The reader interprets the first post-comparison T1-weighted image, the maximum intensity projection image, and the first post-comparison image subtracted after image post-processing [7]. Each sequence in the study protocol achieved an average sensitivity of 93-96%, with an average interpretation time of 44 seconds. In previous studies, the total scanning time of the simplified schemes studied was 3-10 minutes. The authors conclude that simplifying MRI not only reduces costs, but also makes breast tissue MRI more accessible and improves clinical diagnostic efficiency. Therefore, greatly shortening MRI for breast cancer is feasible and will be widely used in clinical practice as soon as possible after continuous improvement. However, due to limitations in the number of patients surveyed, more studies are needed to evaluate the value of simplified MRI for breast cancer screening.

Flowing the study by Kuhl, Mango, and colleagues, multiple investigations discovered that DCE AP offers higher than average patient sensitivity and specificity for lifetime risk of breast cancer when compared to a full DCE MRI screening regimen. Recently, studies have been conducted to assess the detection and characterization of breast lesions using ultra-fast breast DCE MRI. The high time-resolution picture sequences obtained during the very early contrast enhancement phase are used to generate the maximum slope of contrast enhancement and time curve in MIP images, thereby describing breast lesions. [8, 9]. Mann et al. conducted a retrospective analysis covering 160 patients with different pathologic types, including benign and malignant. They tested the efficiency of ultra-fast dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) in the diagnosis of breast disease. [9]. The study used an MRI protocol consisting of 20 time-resolution angiographic sequences (called TWIST sequences) that were collected before and after the injection of contrast agent [9]. The researchers generated images by selecting the most enhanced 3x3x1 voxel regions within the lesion area and analyzed them. The time resolution of each TWIST sequence was 4.3 seconds, and the overall acquisition time for the full ultra-fast MRI protocol was 102 seconds.

Van Zelst et al. evaluated the diagnostic performance of ultra-fast dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) in 201 patients with breast cancer [8]. They applied the ultra-

DOI: 10.54254/2753-8818/8/20240426

fast breast dynamic contrast enhanced MRI protocol described in Mann et al. 's study, which uses TWIST sequences to generate images. In addition, they used breast cancer screening protocols that included diffusion-weighted imaging (DWI), T1-weighted volumetric interpolation fast Dynamic contrast enhanced MRI (VIBE DCE MRI), and T2-weighted turbo Spin Echo (TSE) MRI. The acquisition time of the complete protocol was 13 minutes, while the acquisition time of the ultra-fast DCE MRI protocol was 1 minute and 42 seconds.

Abe and his team conducted a retrospective study involving 60 patients who underwent breast dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) examinations and had histopathological follow-ups. [10]. The study used an ultra-rapid DCE MRI protocol with 5 T1-weighted sequences collected pre-operatively and 8 T1-weighted sequences collected postoperatively. The scheme also uses a fat saturation and Enhanced Sensitivity Coding (SENSE) accelerator to capture images at a time resolution of every 7 seconds [10]. The above findings indicate that ultra-fast DCE MRI improves specificity while maintaining a high diagnostic sensitivity [10]. Not only that, the Ultrafast DCE MRI also reduces the acquisition time and reading time table 1, which is conducive to reducing the time and cost of DCE MRI in breast cancer screening.

Table 1. List of studies evaluating Ultrafast MRI screening protocols for breast cancer.

	Study (year)	Mann	Van Zelst	Abe
Ultrafast protocol	Acquisition time	120s	120s	156s
	Interpretation time	*	69.2s	*
	Sensitivity	90%	84%	85%
	Specificity	67%	82%	79%
Breast cancer risk		*	High	*
Full protocol	Acquisition time	*	180s	416s
	Interpretation time	*	89.7s	*
	Sensitivity	*	86%	*
	Specificity	*	72%	*

4. CRISPR detection technology for Breast Cancer

CRISPR technology has found extensive applications in both fundamental and translational analysis within the biological field of cancer. This adaptable method may be used to focus on oncogenes and tumor suppressor genes (TSGs), thereby through a variety of methods, reducing the development of cancer. These targets could be reached by through knockout, gene suppression, epigenetic changes, and gene editing, as summarized in Table 2. The nonhomologous end joining (NHEJ) or homology directed repair (HDR) pathways are used in the CRISPR process, illustrated in Figure 1. Most cell types predominantly use the NHEJ pathway, resulting in double-strand breaks (DSBs), which are characterized by the arbitrary deletion or insertion of nucleotide bases at the site of cleavage. This process is prone to mistakes, resulting in the premature or dysfunctional synthesis of polypeptides and frame-shift mutations. Conversely, the HDR pathway is no error, DNA damage can be repaired by utilizing a homologous section from the donor DNA template [11]. CRISPR has proven effective in deleting viral oncogenes and cellular in different types of cancer.

4.1. Immunotherapy based on CRISPR

Tumor formation is significantly influenced by compromised immune systems. Numerous methods are used by cancer cells to avoid detection by the immune system, including altering immune cell performance and creating an immune-compromised tumor microenvironment. Consequently, the importance of strengthening the immune system has been acknowledged to target cancer cells, as outlined in Table 3. From a variety of angles, CRISPR-based genetic changes have been investigated as

DOI: 10.54254/2753-8818/8/20240426

way to treat a number of disorders connected to immune dysfunction [12]. The use of CRISPR technology has improved resistance against tumors in BC through the mechanisms depicted in Figure 2.

4.2. T Cell-based immunotherapy using CRISPR

Using T cells as a cancer therapy target has gained considerable attention due to their ability to distinguish between oneself and the other, because of their wide range of T cell repertoire. In traditional immune therapy approaches, in order to identify tumor-associated antigens (TAAs), patient T cells are genetically modified to express T cell receptors (TCR) or chimeric antigen receptors (CAR T cells). [13]. However, this technique requires a lot of time and heavily reliant on the individual patient's T-cell characteristics [12, 14]. Through the integration of the CAR gene into the T-cell receptor constant (TRAC) locus, TCRs have been replaced with CARs [13]. The ectopic production of CARs in T cells can increase T cell potency, inhibit terminal differentiation and limit lymph node exhaustion, according to research using mouse models, Acute Myeloid Leukemia (AML) [12]. Therefore, CRISPR holds promise for enhancing T-cell effectiveness against tumor growth in breast cancer.

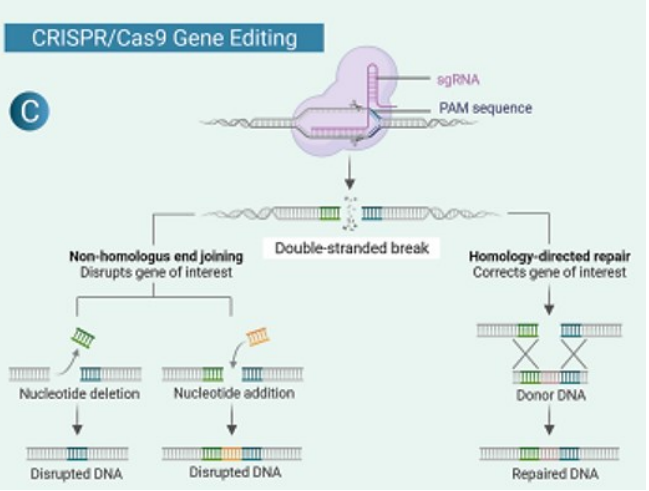

Figure 1. The diagram of CRISPR gene editing.

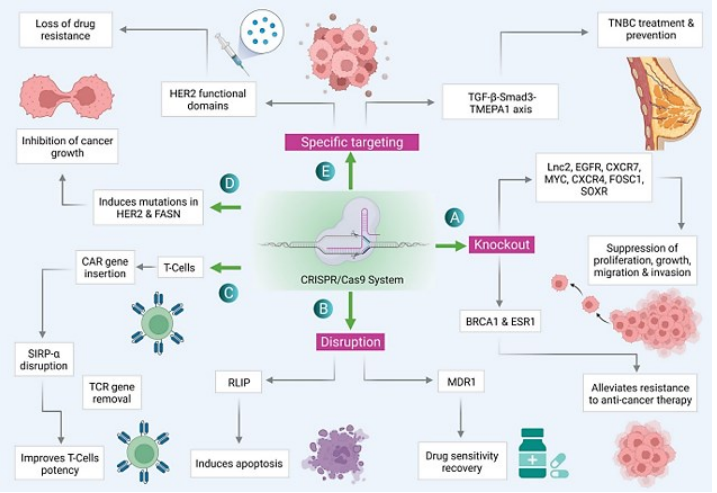

Figure 2. CRISPR system use in the treatment of cancer.

A various oncogene whose overexpression or dysregulation result in either therapeutic resistance or cancer growth can be knocked off. B Using CRISPR, the genes RLlP and MDR1 that are responsible for drug resistance in BEC are shut down to restore drug sensitivity.C In breast cancer, T-cells are

DOI: 10.54254/2753-8818/8/20240426

employed for immunotherapy. CRISPR has been used to T-cells for CAR geneinsertion, TCR gene removal, and SIRP-a disruption, enhancing its potency.D CRISPR causes mutations in fatty acid synthase and human epidermal growth factor receptor 2, which inhibits the proliferation of cancer cells. E TGF-Smad3-TMEFPSince the functional domains of HER2 are necessary for carcinogenic activity and Al axls helps cancer cells avoid TGfF-mediated growth suppression, their targeted targeting with CRSPR results in the therapy of TNBC and the loss of rug resistance, respectively.

Table 2. Latest research on using CRISPR/Cas9 for treating (BC) highlights various genetic modifications.

Target Gene	Cell line	CRISPR approach	Effects
MYC Oncogene	-	CRISPR mediated mutagenesis	Reduced MYC expression leads to a decrease in cell proliferation.
CXCR7 and CXCR4	$MDA-MB-213$	Cas 9 knockout	Decreased tumor cell proliferation, tumor development and invasion
PTEN	$SUM159$	CRISPR activation	Lowers cancer aggressiveness
miRNA23b and miR27b	$MCF-7$	Cas 9 knockout	Decreased tumor growth
MASTL	$tumor\ cell\ lines$	CRISPR	Restricts cell proliferation
FASN	$MCF-7$	CRISPR	Hinders DNA replication, migration, cell adhesion, cell growth, survival, and proliferation of cells.
CDK7	$TNBC\ cell\ lines$	CRISPR	inhibits tumor development and cell proliferation
CXCR7 and CXCR4	$MDA-MB-213$	CRISPR	Decreased tumor cell proliferation, tumor development and invasion

Table 3. Drug sensitization of several genes is one of the uses of CRISPR in targeted immune treatment.

Target Gene	Cell line	Effects
Replacement of TCR with CAR	T-cells	Increases T cell potency while decreasing lymph node depletion and terminal differentiation.
SIRP-α silencing	Macrophages	'Do not eat me' signal is absent, which causes the cancer cells to be destroyed.
P38	Mouse models of established tumors	Improve T cell anti-tumor performance for Adoptive Cell Therapy.
Cdk5 knockout	TNBC	Inhibiting tumor development in murine melanoma and reducing lung metastasis in triple-negative breast cancer are both achieved by downregulating PD-L1 expression.
PI3K	-	Overcomes chemotherapy resistance.
APLNR deletion	Animal model	Reduces the sensitivity and effectiveness of checkpoint blocking.
MALAT1 promoter deletion	BT-549 TNBC model	Increases paclitaxel and doxorubicin sensitivity.

5. Limitations and future development

Off-target effects are a limitation of CRISPR/Cas9 technology and are considered a significant potential risk for gene therapy in vivo. While some computer programs have been used to optimize the design of Sgrnas, because off-target effects are a limitation of CRISPR/Cas9 technology, their specificity cannot be 100 percent guaranteed and are considered an important potential risk for in vivo gene therapy [15]. In addition to the design of the sgRNA, the Cas9 protein itself also plays a crucial role. For example, optimization of the Cas9 protein, such as protein engineering, can significantly reduce off-target effects.

DOI: 10.54254/2753-8818/8/20240426

One way to reduce off-target effects is to use an optimized Cas9 protein, such as SpCas9-HF1 [16]. It should be noted that in vivo, the problem of off-target effects has still not been fully solved, and is closely related to the way gene editing technology is delivered.

MRI also produces some artifacts in breast cancer examination, which poses certain challenges to the diagnosis and treatment of breast cancer. DCE MRI showed poor anatomical details of breast tissue. Because breast tissue is similar to muscle and fat tissue, this similarity can create artifacts in the image. The appearance of such artifacts can lead to unclear boundaries of breast cancer, difficulty in accurately assessing the magnetic field strength of its size and extent of spread, and optimized imaging sequences to improve visualization and discrimination of breast cancer.

The combined application of CRISPR gene editing technology and MRI brings great potential for the treatment of breast cancer. In terms of improving treatment accuracy, we need to further study the genetic and molecular characteristics of breast cancer, improve the delivery system of CRISPR technology, enhance the accuracy of MRI image processing and interpretation, strengthen multidisciplinary collaboration, and build large sample databases. The combined application of these measures will help improve the accuracy of CRISPR gene editing technology and MRI in breast cancer treatment, and bring better treatment results for breast cancer patients.

6. Conclusion

The application of CRISPR in imaging increases in past few years and MRI a major approach in medical imaging. Having reviewed some wide-spread breast cancer detection methods, we find that the unsatisfactory result in early diagnosis and the shortage on sensitivity and specificity still remains to be a vital problem. Several current breast cancer detection techniques are outlined. MRI offers spatial detail and the image of tissue in a high resolution. But MRI have to overcome obstacles which is the lack of specificity and existence of false positives. CRISPR and Cas proteins are found in a bacterial immune system. CPISPR has become effective tool for genome edition. For further development, we need to further study the genetic and molecular mechanism of breast cancer, strengthen multidisciplinary collaboration, and build large sample databases. The method brings a new pathway in breast cancer therapy and makes the result more dependable for patients.

References

[1] Zhao S Y, Wang H, Tang L, et al. 2021 3D bioprinted breast cancer tumor model and doxorubicin susceptibility test. *Chinese Journal of Health Inspection* 31 776–779.

[2] Secretan B L, Scoccianti C, Loomis D 2015 Breast-cancer screening — viewpoint of the IARC working group. *N. Engl. J Med*. 372 2353–8.

[3] Duffy S W, Vulkan D, Cuckle H, Parmar D, Sheikh S, Smith R A, et al. 2020 Effect of mammographic screening from age 40 years on breast cancer mortality (UK Age trial): final results of a randomised, controlled trial. *Lancet Oncol.* 21 1165–72.

[4] Esserman L J 2017 The Wisdom study: breaking the deadlock in the breast cancer screening debate. *Breast Cancer* 3 1–7.

[5] Morris E A, Comstock C E, Lee C H 2013 ACR BI-RADS® magnetic resonance imaging American college of radiology, *Reston*.

[6] Kuhl C K, Schrading S, Strobel K, Schild H H, Hilgers R D 2014 Abbreviated breast magnetic resonance imaging: first postcontrast subtracted images and maximum-intensity projection-a novel approach to breast cancer screening with MRI. *J. Clin. Oncol.* 32 2304–2310.

[7] Mango V L, Morris E A, et al. 2015 Abbreviated protocol for breast MRI: are multiple sequences needed for cancer detection? *Eur. J. Radiol.* 84 65–70.

[8] Zelst J, Vreemann S, Witt H J, et al. 2018 Multireader study on the diagnostic accuracy of ultrafast breast magnetic resonance imaging for breast cancer screening. *Invest Radiol.* 53 579–586.

[9] Mann R M, Mus R D, et al. 2014 Platel novel approach to contrast-enhanced breast magnetic resonance imaging for screening. *Invest Radiol.* 49 579–585.

DOI: 10.54254/2753-8818/8/20240426

[10] Abe H, Mori N, Tsuchiya K, et al. 2016 Kinetic analysis of benign and malignant breast lesions with ultrafast dynamic contrast-enhanced MRI: comparison with standard kinetic assessment. *Am. J. Roentgenol.* 207 1159–1166.
[11] Cong L, Zhang F 2015 Genome engineering using CRISPR-Cas9 system. *Methods Mol. Biol.* 1239 197–217.
[12] Azangou-Khyavy M, Ghasemi M, Khanali J, Boroomand-Saboor M, Jamalkhah M, Soleimani M, et al. 2020 CRISPR/Cas: from tumor gene editing to T cell-based immunotherapy of cancer. *Front. Immunol.* 11 2062.
[13] Grenier J M, Yeung S T, Khanna K M 2018 Combination immunotherapy: taking cancer vaccines to the next level. *Front. Immunol.* 9 610.
[14] Eyquem J, Mansilla-Soto J, Giavridis T, Hamieh M, Cunanan K M, et al. 2017 Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature* 543 113–7.
[15] Khoshandam M, Soltaninejad H, Mousazadeh M, Hamidieh A A, Hosseinkhani S 2023 Clinical applications of the Cas9 genome editing system: Delivery options and challenges in precision medicine. *Gene transmission* 1 282–10.
[16] Kleinstiver B P, Pattanayak V, Prew M S, et al. 2016 The high-fidelity CRISPR-Cas9 nuclease had no detectable genome-wide off-target effects. *The natural world* 529 490–495.