**CRISPR/Cas9 Technology: Making Possibilities against Breast Cancer**

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**ABSTRACT**

Breast cancer initiation and progression underlies by mutation process which initiates in lobules and promotes metastasis. Genomes are the exact determination to explain cancer etiology. Survey says about nearly 92% of females in the U.S.A. are affected by deadly breast tumors during 2012-2016. The ability to correct such mutations holds huge promise for battling cancer. Recently, because of its great capabilities and accuracy, the CRISPR-Cas9 leading genome editing technique has been extensively used in the therapeutic examination of cancers. A novel tool appearance paved a way for scientists to carry out extensive treatments. CRISPR/Cas editing and developing techniques were first discovered in 1987 by Yoshizum Ishino and his colleagues. These techniques are used for drug discovery and development for formidable diseases. Being a big revolutionary technique, this is used widely to treat dangerous malignant breast cancer. We have discussed the role of CRISPR to fight against oncology infections, described the clear view of breast cancer diagnosis and treatment. A potential therapeutic CRISPR/Cas role in cancer therapy was proposed.

**Keywords:** Breast cancer, CRISPR/Cas9 technology, BRCA1, Mechanism, Application.

**INTRODUCTION**

Cancer is the leading cause of death worldwide. According to a survey by the World Health Organization (WHO), in 2030 23.6 million new cases will arise. Since it is a frightening disease, there is no particular vaccine or treatment to cure completely. Knowledge of somatic alternatives in breast cancer can be gained through studies on the genetic sequence.

Breast cancer is the most threatening disease in both sexes. However, the majority of females are coming in with breast cancer while in men is quite less. Nulliparous women have a severe impact when compared to parous women. Studies show that they help prevent cancer by stopping the growth of free radicals in our environment. In 2018, 266,120 new breast cancer cases were reported; of these, 40,920 cases were fatal. Breast cancer is classified according to the over-expression of the cancer cell membrane, which includes hormone receptor Progesterone (PR), Estrogens (ER), and Human Epithelial growth factor Receptor (HER2). Based on these, breast cancer became divided into triple-positive breast cancer and triple-negative breast cancer. The patients with PR-, ER-, HER2- positive receptors are called triple-positive breast cancer, and the patients with negative receptors are called triple-negative receptors.

The common and foregoing methods is to treat cancer are Surgery (mastectomy or lumpectomy, depending on the virility of the tumor cells), Chemotherapy (the use of drugs to kill the tumor cells), and Radiotherapy (introduction of high radio waves to kill the tumor cells). Chemotherapy is one of the most common methods used to treat tumor cells, but the studies proved that this method causes an unbearable side effect on the human being.

Imagine being able to cure any genetic disease, prevent bacteria without antibiotics, and replace mosquitoes so that they can successfully spread malaria or cancer or transplant animal organs without rejection. The molecular machines to achieve these goals are not in the sci-fi novel set in the distant future. These are the goals achieved by the family of DNA sequences called CRISPR. As a gift of nature, in 1987, a Clustered Regularly Interspaced Short Palindromic Repeats, now commonly known as CRISPR were found in the immune system of *Escherichia coli*, a recurrence of genes 29 nucleotide series along with 32 nucleotides variable sequence fraction.

In familiar usage, CRISPR (Pronounced “crispr”) is shorthand for CRISPR/Cas9 system. CRISPR are specialized stretches of DNA. The protein Cas9 (or “CRISPR associated”) is an enzyme that acts as molecular scissors, successfully slicing the strands of DNA. Based on the structure and sequence of nuclear proteins, the CRISPR/Cas9 bacterial adaptive immunity system became divided into three different types. There are:

1. CRISPR/Cas type I
2. CRISPR/Cas type II, and
3. CRISPR/Cas type III.

Of these, CRISPR/Cas type II is beneficial and works out but type I and type III was unfit for genome editing because of their complex structures. The CRISPR/Cas II system helps to identify and inhibits the viral DNA in the bacterial system. Three main molecules play an effective role in viral DNA modification is listed as
1. Cas9 protein
2. CRISPR RNA
3. Transactivating RNA.

Type II CRISPR helps to eliminate the viral sequence in three stages, phase I is the acquisition of the CRISPR/Cas system, during which it initially detects the viral sequence of DNA. The second stage is CrRNA biogenesis, in which the targeted viral compounds bind to the CRISPR complex. Finally, the CRISPR complex attacks DNA and degrades the occupational space with a single incision. Recently, CRISPR/Cas system is most widely used in cancer research to diagnose the position of cancer and to correct it.

Here, we introduce the CRISPR self mechanism, detail about breast cancer diagnosis and treatment, timeline on CRISPR/Cas9 technology, the role of CRISPR in breast cancer, and its applications and advantages.

**Investigation of Breast Cancer**

The ancient Egyptian book Edwin Smith reported 8 cases of papyrus breast cancer or breast ulcers. In the early 1880s, mastectomy was used to treat patients with breast cancer. Lumpectomy was first introduced in the 1980s, later developed, and in 1985 the advanced lumpectomy was developed; used before systematic adaptive therapy. Chemotherapy is a well-known method of treating cancer, and many therapies have been started since then. Breast cancer is caused by genetic variations such as mutations, recombination, genetic migration, environmental influences, and physical changes.

Great technology has been developed to diagnose breast tumors, some of which have been discussed: Mammography is the most effective way to detect the presence of tumor cells in the human body, but fail (or not) to detect when the tumor cells are dense or mutated. Only an expert can handle this. Careless handling of it by specialists can lead to unnecessary surgery. Fluorescence In Situ Hybridization (FISH) helps to map specific cells in the breast, to determine if cells have mirror copies of the HER2 gene, and to distinguish between individuals with positive and negative cases. Experts tested the enzyme-linked immunosuppressant (ELISA) using two antigen sandwich ELISAS to identify tumor-affected areas. Here are some common ways to diagnose breast cancer.8, 9

Dennis et al. concluded that, along with transtuzmub, have shown that chemotherapy is effective in treating HER/positive metastatic cancer and reducing mortality.10

Joseph et al. concluded and showed the great result in chemotherapy with concentrations of cyclophosphamide, methotrexate, and fluorouracil.11 Gunter et al., studies have shown the impact of Pathological Complete Response (PCR) after neoadjuvant therapy for breast cancer. Anthra cyclins and taxanes were used for selected patients with intrinsic breast cancer. This therapy showed a positive result in patients with HER2 positive (non-luminal), luminal B/HER2- negative tumors. It has shown alternative results for luminal and non-luminal tumours.12

D.L. Hershman et al., Conducted experiments based on consensual and persistent hormone therapy. Hormone therapy includes Tamoxifer and Aromatase inhibitor (AI). Of the 8,769 women, only 49% of patients underwent full-time hormone therapy. Their studies concluded that there was an increase in mortality from continuous and non-compliant hormone therapy.13

**TIMELINE ON CRISPR/Cas9 TECHNOLOGY**

1987 - Repetitive elements in bacterial adaptive immune system was discovered.  
2002 – Named as CRISPR.  
2005 – CRISPR with viral Cas gene was identified (PAM).  
2007 – Role of CRISPR in bacterial immune system was reported.  
2012 – Development of CRISPR as gene editing tool.  
2013 – To control specific sequence of gene expression by CRISPR Interference (CRISPRi).  
2015 – Targeted transcription was achieved in bacteria using CRISPRi.  
2017 – Successful implementation of CRISPR/Cas9 in human embryos by modifying beta-globin gene mutations.  
2018 – CRISPR/Cas9 editing helped in first line chemotherapies in lung cancer.  
2019 – ‘Prime editing’ technique was published as report.  
2020 – Nobel Prize in chemistry awarded to Emmanuelle Charpentier, and Jennifer Doudnafo development of genome editing by CRISPR/Cas9.14

**Self-mechanism of CRISPR/Cas9 Technology**

CRISPR/Cas editing and developing techniques were first discovered in 1987 by Yoshizum Ishino and his colleagues. The genome of the different organism consists of a series of messages and instructions within their sequence of DNA. Genomic editing is involved to make changes the series of sequence to get changes in the instructions. It is performed by adding or deleting the DNA sequences and tricking the cells natural DNA repair mechanism into introducing the changes one wants. CRISPR/Cas9 provides the same.

The CRISPR Cas9 system, which is found in the adaptive immune system of bacteria, can regulate the genetic materials through three processes, called adaptation, expression, and interaction. The adoption phase is...
CRISPR Cas9 plays an interesting role in gene therapy, in both cell lines and a mammal, shorting out monogenic disease; induce protective mutations, helps in CRISPR-mediated cancer gene therapy. CRISPR toolbox plays an exclusive role in gene silencing, with the use of CRISPR STOP a cluster of genes and transcripts were targeted. This CRISPR toolbox of genetic management helps in mutation. For example, *Oriza sativa* GBSSI used as a target for mutation using the CRISPR\Cas9. This is effectively used in transcriptome analysis, which enables targeted nucleotide alternations.

CRISPR, a natural tool, plays a broader role. Many ideological discussions, laws, and ethics are being developed because of CRISPR. It has played a key role in the treatment of various diseases and recent years the demolition of the gene, although interesting things about the future of CRISPR continue to bombard us in the years to come. Thus, CRISPR plays a major role in various fields as a developing tool in genetics, which has had a huge impact and revolutionized the biosphere.

**Role of CRISPR in Breast Carcinoma**

The CRISPR / Cas9 gene creates a favourable environment for genetic modification; Creates plasmid vectors and validates genetic algorithms in breast cancer treatment. Studies show that it is the best output technology for treating breast carcinoma.

Studies with TS genes are discussed. Human organoids from human epithelial subsets are classified based on the expression of two proteins, CD49F and APCM. The most commonly used mutated TS genes are p53, PTEN, and RB1. The sgRNA presented by the Cas9 system targets three TS genes. P53 is subject to nutlin-3A, while PTEN and RB1 perform immunoblot analyses. As P53 did not degrade, genetic modification occurred. To find out the capabilities of CRISPR / Cas9 editing, the next-generation sequencing was followed by transduction. CRISPR/Cas9 is simultaneous or sequentially knocks out the TS function in breast tumorigenesis.

Studies on triple-negative cancer with PARP1 inhibition have been described. Cell lines with BRCA1 wild type and BRCAm, inhibitory poly (ADP) ribose polymerase (PARP1) are selected from triple-negative cancer patients. The CRISPR device was introduced to inhibit the single gene (PARP1) that produces modified cell lines. The CHOPCHOP algorithm was selected to initiate sgRNA and target PARP1. The cluster of the three gRNA is synchronized based on the capabilities and off-targeting effects. Although gRNA2 and gRNA3 reached 82% efficiency in PARP1 failure, gRNA1 efficiency reached 23%. Therefore, gRNA1 was cloned using some protocols followed by the researchers. This method of targeting BARC wild type and BARCm type using inhibitor PARP1 with CRISPR device potential has shown a positive effect on breast cancer treatment.

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**Figure 1:** Action of CRISPR\Cas9 system

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Significance of CRISPR/Cas9 Technology

The CRISPR / Cas9 practice system plays an important role in cancer treatment in many ways. It is used to model mice for tumor disease. Cas9 genetic modification technology helps modify T-cells and gathers patients immunity to work against cancerous carcinoma29. CRISPR device helps CRISPR-based screening techniques on the widescreen help identify the required genes and drug targets. The myeloid leukemia tumor suppressor ASXL1 was converted to a mouse model using the Cas9 devices. This helped to increase the lifespan of the rat30. The main failure of chemotherapy treatment is the ability of tumor cells to fight chemotherapy drugs.

CRISPR Cas9 paves the way for the inactivation of drug-resistant genes in tumor cells, increasing the potential of genes in tumor cells, and enhancing the effectiveness of chemotherapy31. CRISPR / Cas9 sometimes cause off-target mutations, leading to a risk factor. The CRISPR / Cas9 system should be carefully designed to avoid this risk. Guide RNA could not understand some molecular mechanisms. Online tools can help you find off-targets and capabilities. Cech and his colleagues introduced the "pop-in/pop-up" approach to reducing the efficiency of CRISPR / Cas932. Compared to other engineering nucleases such as ZFNs and TALENs, CRISPR / Cas9 have higher target efficiency, lower cost, and easier testing.

CONCLUSION

CRISPR/Cas9 an engineering technique is playing a major role in the biological field, providing new alternations in an easy way for various disease treatments; any organism’s genome can be easily modified. As a part, the CRISPR toolbox in breast cancer research has a great potential to treat tumor cells efficiently. The researchers say genome-wide specifications should be improved in the CRISPR technique. Being a developing and most preferable tool, it takes a major place in research field to diagnose and treat diseases, in gene-editing and gene therapy. This method of gene-editing technique is a simple process that can be carried out easily. The development of the CRISPR/Cas9 system from the basic level of the clinical method shows a powerful way in treating drug-resistant. With the potential of the CRISPR/Cas9 system, it can easily overcome the complexity of cancer and gives a suitable cancer drug-resistant. The future with CRISPR has a great impact in the world.

From the knowledge of the above review, we conclude that even numerous old and present strategies are being used to treat breast cancer; there is reducing in mortality rate. By introducing the CRISPR gene editing method, demonstrated preferable execution over different treatments and medicines. It can prevent more mortality rate than other treatments.

Subsequently, we sum up yet there is a particular antitumor drug for complete cure from breast cancer. From the experimental results, the CRISPR technique can act effectively than different methods.
REFERENCES


13. Dawn L. Hershman, Theresa Shao, Lawrence H. Kushi, Donna Buono , Wei Yann Tsai, Louis Fehrenbacher, Marilyn Kwan, Scarlett Lin Gomez, Alfred I. Neugut, Early discontinuation and non-adherence to adjuvant hormonal therapy are associated with increased mortality in women with breast cancer, Breast Cancer Research and Treatment, 126, 2011, 529–537. https://doi.org/10.1007/s10549-010-1132-4


24. Rachel L. Mintz, Yeh-Hsing Lao, Chun-Wei Chi, Siyu He, Mingqiang Li, Chai Hoon Quek, Dan Shao, Boyuan Chen, Jing Han, Sihong Wang, Kam W. Leong, CRISPR/Cas9-mediated mutagenesis to validate the synergy between PARP1 inhibition and chemotherapy in BRCA1-mutated breast cancer cells, Bioengineering and Translational Medicine, 5, 2020, e10152. https://doi.org/10.1002/btm.2.10152

25. Xueli Tian, Tingxuan Gu, Satyananda Patel, Ann M. Bode, Mee-Hyun Lee, and Zigang Dong, CRISPR/Cas9 – An evolving biological tool kit for cancer biology and oncology, npj Precision Oncology & Published in partnership with The Hormel Institute, University of Minnesota, 8, 2019.


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