International Journal Of Public Mental Health And Neurosciences
ISSN: 2394-4668
(An Official publication of Sarvasumana Association)

CRISPR-Based Approaches for Targeting Breast Cancer: A Comprehensive Genomic Investigation

Tayyaba Ahmad[1], Nethra S[1] and Preenon Bagchi[2]
[1]Padmashree Institute of Management and Sciences, Bengaluru, India
[2]Institute of Biosciences and Technology, MGM University, Aurangabad, India
Corresponding author mail Id: 23tayyabaahmed@gmail.com

ABSTRACT

Breast cancer can occur in women and rarely in men. It includes lumps in breast and change the texture of breast. Breast cancer follows autosomal dominant inheritance pattern. About 5% to 10% of breast cancer follows hereditary pattern. Breast cancer is generally caused by germinal mutation in certain genes but mainly in BRCA1 and BRCA2gene which are present on chromosome number(17q21-31) and chromosome number (13q12-13) respectively. These genes are human tumorsuppressor genes which encodes for BRCA1 and BRCA2 proteins. Breast cancer can be halted by CRISPR gene editing with no sign of toxicity in mouse (done by researchers in Boston Children's Hospital).

Keywords: Breast Cancer, Autosomal Dominant, Suppressor Gene, CRISPR

INTRODUCTION

Breast cancer is the most prevalent cancer entity in women and the second leading cause of death from neoplasia after lung cancer, according to cancer statistics. Researchers all across the world are attempting to enhance the prevention, detection, and treatment of breast cancer, as well as the quality of life for patients and survivors. Breast cancer is classified into four types: ductal carcinoma in situ, invasive ductal carcinoma, inflammatory breast cancer, and metastatic breast cancer. Breast cancer can affect both men and women, although it affects women more frequently. Breast cancer is most commonly caused by cells in the milk production duct (invasive ductal carcinoma). Breast cancer can potentially start in lobular glandular tissues (invasive lobular carcinoma). Breast cancer is caused by gene mutations in the breast, such as BRCA1 (BReast CAncer gene) and BRCA2 genes [1]. Breast cancer accounts for 10.4% of all cancer cases in women, making it the second most frequent non-skin cancer and the fifth most common cause of cancer mortality. Cancer cells share DNA and RNA with cells from the species from which they arose. This is why they are frequently missed by the immune system. As immunotherapy becomes more popular in cancer treatment, CRISPRcas9 can guide immune cells toward cancer cells and boost antitumor immune responses [2].

INTRODUCTION TO CRISPR CAS9

Researchers identified CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, as a prokaryotic immune system in bacteria and archaea. It is a genomic tool that assists researchers in altering, removing, or editing genomic sequences. This is not only a technique for advanced science but also a boon to modern science, as the basic principle is now easily understood by scientists; it is considered a simple and easy to implement method, causing a drastic positive change in the medical field as well. CRISPR was discovered in the E.Coli genome in 1987 as a series of repetitive fragments of 29 nucleotides in length interspaced with variable sequence pieces of 32 nucleotides. The identification of comparable short repeat palindromic sequences of 24-40 nucleotides in different species of bacteria and archaea resulted from interest in the CRISPR system and its related casgenes. The repetition sequences are separated by varied lengths of 20-58 nucleotides [3-5].

Although CRISPR was previously thought to work as an RNA interference mechanism, it was discovered that it serves as a genetic memory of invading pathogens (Fig. 1). Cas proteins, which act as guided endo-nucleases, employ this memory to check for invading DNA and inhibit it by causing double stranded breaks.

CRISPR systems are categorized into six varieties, which are further divided into two classes: type I and type III. Both types of CRISPR systems use a set of Cas-proteins. A multi-protein CRISPR RNA complex known as cascade recognizes the target DNA in type I systems, which is subsequently cleaved by Cas3. Cas10 forms a cascade like complex that recognizes and cleaves the target in type III systems. To scan bind and cleave the target DNA sequence, type II CRISPR systems require only one protein, Cas9 [4].

CRISPR-Cas9 is being investigated as a potential cure for a variety of disorders, including cancer.

Researchers at Brigham and Women's Hospital are using CRISPR to modify cancer cells, transforming them into killer cells capable of delivering medicines directly to tumors. Several genome-editing methods with diverse uses have been developed during the last 20 years. CRISPR/Cas9 is a technology inspired by the bacterial immune system that allows for the repair, insertion, or deletion of genetic material in both in vitro and in vivo settings. The discovery of this enthralling bacterial immune defence mechanism has resulted in a hitherto unprecedented revolutionary breakthrough in medical sciences and biotechnology (Fig. 2) [1-5].

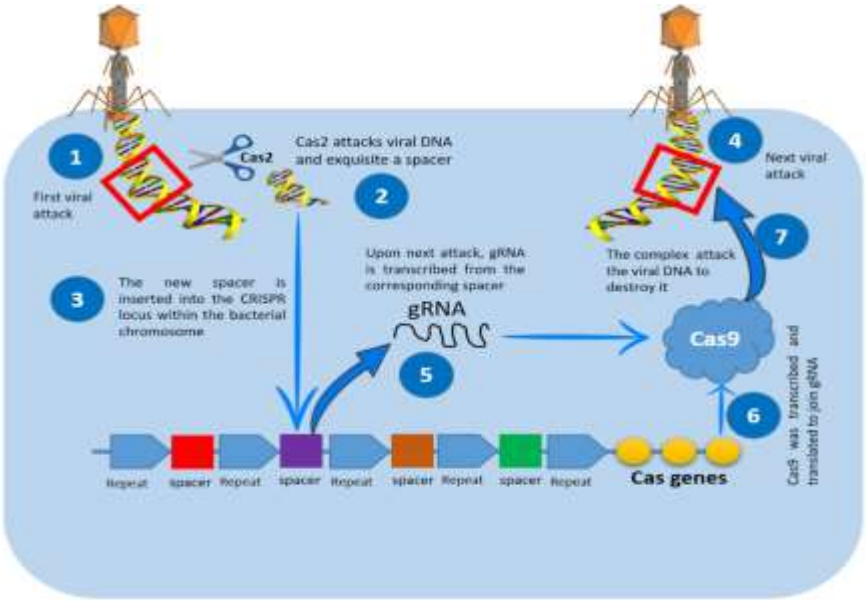

Figure 1 depicts how CRISPR/Cas9 functions as a bacterial immune system. When an intruder (plasmid or virus) penetrates bacteria [1,] it instructs a nuclease called Cas2 to cut a short region of the viral genome (spacer) [2, 3] and insert it between two repetitions in the CRISPR locus. When this sort of invader returns [4], the bacteria transcribe its spacer to form crRNA [5, which is then developed by tracrRNA]. After identifying the invader genome (by crRNA), both forms of RNA linked with Cas9 [6] will be directed to it to break it (via Cas9). [adapted from (6)]

Medullary carcinoma, mutinous carcinoma, and tubular carcinoma are less prevalent forms of breast cancer.

Medullary carcinoma is an invasive breast cancer that forms a clear barrier between tumor tissue and normal tissue. They are responsible for only 5% of all breast cancers [7].

Mucous-producing cancer cells generate mutinous carcinoma. They are also known as colloid carcinoma [7, 8].

Tubular carcinoma accounts for 2% of breast cancer diagnoses. Breast cancer can be caused by a variety of factors, including genetics, hormones, nutrition and lifestyle, and exposure to UV and X-rays [7, 8, 9].

Breast cancer can be cured using gene therapy and CRISPR. The goal of gene therapy is to provide a nucleic acid-based medication that either corrects or destroys the cells harboring genetic abnormalities [10].

Figure 2: CRISPR/Cas9 research and therapy areas in breast cancer [reproduced from (6)]

TRIPLE NEGATIVE BREAST CANCER

It is clinically inactive for the expression of estrogen and progesterone receptors (ER/PR) as well as the HER2 protein. The majority of breast tumors are hormone receptor positive, which is defined as Luminal A, which is hormone receptor positive (HR+) and human epidermal growth factor receptor (HER2-) with a low level of (ki67), and Luminal B, which is (HR+,HER2+, and a high level of ki67) [11-12].

Crisprcas9 therapeutic application A study of normal mammary gland parenchyma cells, including their immune-phenotype, is required to completely comprehend the molecular and pathologic aspects usually associated with the triple negative phenotype. The more central luminal cells are known to express low molecular weight cytokeratins such as CK7, CK8, CK18, and CK19, as well as MUC1alpha6integrin, BCL1, ER, PR, and GATA3 [13]. Approximately 56% of the genes for triple negative breast cancer and basal-like breast cancer overlap. The overlap ratio between triple negative breast cancer and basal-like breast cancer can be as high as 60%-90%. According to epidemiological data, triple negative breast cancer is most common in premenopausal young women under 40 years old, accounting for around 15-20% of all breast cancer patients. Triple-negative breast cancer is highly aggressive, with 46% of triple-negative breast cancer patients having distant metastasis [14].When compared to other breast cancer subtypes, triple negative breast cancer has a worse overall prognosis, as evidenced by a greater risk of early recurrence and distant metastatis to the brain and lungs.

GENES RESPONSIBLE FOR BREAST CANCER

Every human being possesses both the BRCA1 and BRCA2 genes. A limited number of persons have mutant BRCA1 and BRCA2 genes.

BRCA1 GENE: The gene is found on chromosome 17. It has 22 exons that cover approximately 110kb of DNA. This gene codes for a protein that functions as a tumor suppressor. Tumor suppressor proteins inhibit cells from developing and dividing too quickly or too fast. The BRCA1 protein aids in the repair of damaged DNA in the nucleus of many different types of normal cells. To repair DNA breaks, BRCA1 interacts with multiple other proteins. BRCA1 mutation increases your risk by 65% [15].

BRCA2GENE: The gene was discovered on chromosome 13 in humans. The BRCA2 gene includes several copies of a 70 amino acid pattern known as the BRC motif, and these motifs mediate binding to the RAD51recombinase, which is involved in DNA repair. BRCA2 is thought to be a tumor suppressor gene. In eukaryotes, the BRCA2 protein plays a crucial role in homologous recombinational repair. BRCA2 mutations increase the risk of breast cancer by 45% [15-17].

PROTEINS RESPONSIBLE FOR TUMOR SUPPRESSION IN BREAST CANCER

MASPIN is a protein that is encoded by the SERPINB5 gene in humans. SERPINB5 was discovered to operate as a tumor suppressor gene in epithelial cells, inhibiting cancer cells' capacity to infiltrate and metastasis to other organs [10-17].

The CCN6 gene encodes a protein that appears to be involved in bone formation and the preservation of cartilage, which covers and protects the ends of bones.

BECLIN 1 is a tumor suppressor protein that is found in both mouse and human breast and ovarian malignancies. This protein enhances the plasma membrane localization of ECadherin, a breast tumor suppressor molecule that prevents tumor development and metastasis when only present on the surface of cells [10-17].

Lipocalin 2 gene is thought to be a significant cause in triple-negative breast cancer, an aggressive type of the disease with few effective treatments[10-17].

Genome editing is the process of modifying DNA by inserting, removing, or replacing sequences. Current strategies involve incorporating a double-stranded base repair mechanism into the DNA.A tumor-specific CRISPR gene editing system encapsulated in a nanogel may be able to prevent the progression of triple negative breast cancer [10-17].

METHODS TO REDUCE BREAST CANCER BY CRISPR

1. A polymer system was created by a research team at the Harvey Perkins Institute of Medical Research. In the study, a synthetic polymer system delivered CRISPR to breast cancer cells, which successfully restored the expression of two genes named MASPIN and CCN6. Typically, these genes are not active in breast cancer, therefore the team was able to "turn on" these genes in a mouse model of breast cancer, which resulted in decreased tumor development. More research is needed before applying this technique to people, but the team members have demonstrated for the first time that a wholly synthetic (and targeted) CRISPR delivery approach may be employed in a mouse model of breast cancer and there was no observable toxicity [10-17].

2. A tumor-targeted CRISPR gene editing technology (encapsulated in a nanogel and injected into the body) capable of successfully and safely halting the progression of triple-negative breast cancer. A proof-of-principle study done in human tumor cells and mice by researchers at Boston Children's Hospital indicates a potential genetic therapy for triple negative breast cancer. The investigation demonstrated that the CRISPR editing technique may target breast tumors and knock out the breast cancer-promoting gene, lipocalin2. The method reduced tumor development by 77% in a mouse model while causing minimal harm in normal tissues [10-17]. Actually, hormone treatment, such as HER2drugs, cannot be used to treat triple negative breast cancer. Because chemotherapy is not an option, a novel technology such as CRISPR is employed [10-17].

3. A germline mutation in the BRCA1 gene results in a defective BRCA, which increases the chance of developing breast cancer. Breast cancer is caused by a BRCA1 mutation in exon 11. The BRCA1 protein is involved in the repair of double stranded breaks (DSB) in DNA via homologous recombination. As poly [ADP-ribose] polymerase inhibitors (PARP) and platinum compounds produce DSB, the HR

(homologous recombination) pathway is required for repair. Many individuals with genetic BRCA1 and BRCA2 mutations do not respond to PARP therapy [10-17].

WHY CRISPR IS REQUIRED TO DETECT BREAST CANCER

Several technologies are now used to detect breast cancer, including mammography, MRI (magnetic resonance imaging), and ultrasound. Although they are the favored approaches, they cannot be regarded the definitive answer to breast cancer. The preceding approaches are simpler to use than CRISPR, although they are not as advanced. As with these procedures, numerous obstacles must be overcome before obtaining the final answer to the disease (breast cancer). So, in mammography, X-rays are used to inspect each breast horizontally and vertically to check for tumor growth as well as clusters of pus in the breast to avoid cancer, but this technique can only identify the early stage of breast cancer or the normal stage, not the metastatic stage. MRI is a procedure in which magnetic resonance is used instead of rays to identify tumors and cysts in the breast that might progress to breast cancer, however it is not regarded the best option since it can provide false positive findings and is expensive. According to studies, if a woman is suspected of having a known genetic mutation, an MRI will have a better sensitivity for cancer diagnosis than mammography. Ultrasound is also employed in the screening of malignancies such as breast cancer, where ultrasonic waves are used to detect cysts, which can be solid or fluid-filled, and ultrasounds can also be used to find tiny tumors that are not easily apparent by mammography. The usage of other invasive procedures like as screening, biopsy, and many more might be minimized on a large scale with the use of CRISPR/Cas since biopsy (tissuesampling) findings are not always reliable and may vary, therefore CRISPR CANCER is an option in that scenario as well (Fig. 3) [10-17].

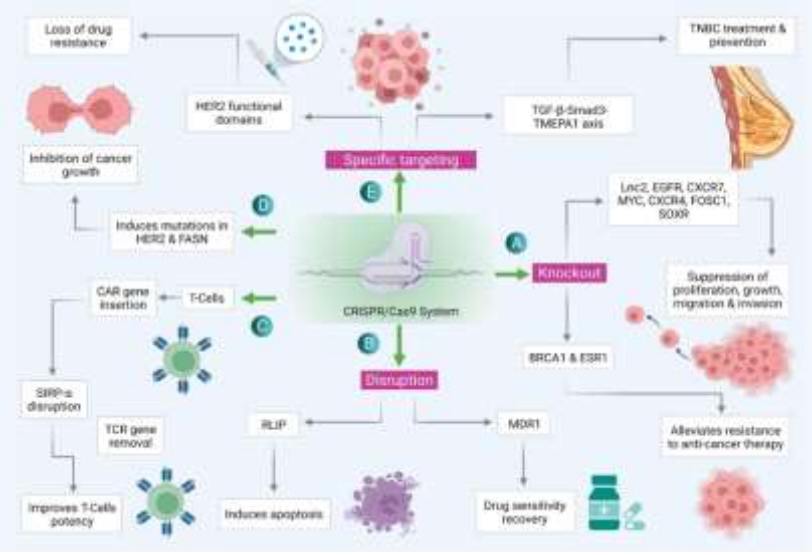

Figure 3: Cancer therapy using the CRISPR/Cas9 system: A knock-out of numerous oncogenes, the overexpression or dysregulation of which leads to either therapeutic resistance or cancer growth. B Drug resistance genes RLIP and MDR1 in BC are disrupted using CRISPR/Cas for drug sensitivity restoration. C T-cells are utilized for immunotherapy in BC, and CRISPR/Cas has been employed in T-cells for CAR gene insertion, TCR gene elimination, and SIRP- disruption, increasing their potency. D Mutations in HER2 (human epidermal growth factor receptor 2) and FASN (Fatty acid synthase) caused by CRISPR/Cas9 decrease cancer cell development. The TGF-Smad3-TMEPAI axis plays a role in cancer cells by allowing them to escape TGF-mediated growth inhibition, and the functional domains of HER2 are required for carcinogenic activity, so targeting them with CRISPR/Cas results in TNBC treatment and drug resistance loss [reproduced from (7)].

(***CRISPR TECHNOLOGY FOR BREAST DIAGNOSTIC MODELLING THERAPY***)

ADVANCE ARTIFICIAL INTELLIGENCE IN DETECTING BREAST CANCER

Nowadays, radiologists have developed an artificial intelligence system to detect breast cancer in women all over the world, which is more advanced than mammography because mammography sometimes requires double screening or examination, so this new technology has been introduced to the modern era [18].

When artificial intelligence (AI) capabilities are used with this technology, there is greater accuracy for changing gene mutations, molecular cloning, and produces alterations in the tumor genome. AI is one of the emerging methods to cancer immunotherapy and vaccine creation, and it is quickly becoming a potent tool for gene prediction. As a result, this new approach to genome editing and cancer treatment is associated with the generation of a large amount of information, which is actually very expensive to perform laboratory trial and error for gene editing, and artificial intelligence analyzes gene editing more accurately by analyzing data and developing a knowledge model. Artificial intelligence approaches can also help to speed up cancer therapy by detecting knowledge patterns in gene editing [18]. Knowledge-based methods, machine learning methodologies, and agent-based models are all part of this artificial intelligence strategy. The purpose of feature selection of canceromics data, biology, and pathological entities is determined by knowledge-based approaches. Using machine learning computational techniques, we can simply enter the name of the gene to be edited, and the cloud-based search engine will return a list of guides that we may filter by projected on-target or off-target effects. Machine learning and agent-based models can be used to analyze epitope prediction and immunological prediction [18].

ROLE OF CXCL12 IN TRIPLE NEGATIVE BREAST CANCER

The knockout and co-knockout approach for specific genes such as CXCR4 or CXCR7, which are responsible for triple negative breast cancer, was used in this technique. The solo knockout of CXCR4 and CXCR7 genes resulted in a decrease in growth, cell proliferation, migration, and invasion, which was followed by the co knockout of CXCR4 and CXCR7 genes in a triple negative breast cancer cell line. To record the experimental alterations, PCR sequencing and western blotting are performed. CXCL12 generated distinct alterations in migration and invasion in single knockout and co-knockout cells. This results in higher migration and invasion in CXCL12-added cells compared to non-added CXCL12-added cells [10-17].

ETHICAL ISSUES

CRISPR/Cas gene editing methods are proving to be effective research tools. [6-7]. Though it raises ongoing ethical difficulties, it has been chosen as the preferred technology for genome editing due to its high degree of simplicity and the demand of very little work. As a result, these characteristics make this technique appealing to any biotechnology or bioinformatics lab [6-7].

The disadvantages of this technique to date include the off-target effects, which may have a variety of pathogenic repercussions, but the on-targets may also result in a variety of deletions and genomic rearrangements [6-7].

FUTURE PERSPECTIVES

CRISPR/Cas9 is a potent technology for genome editing in laboratories. After establishing its safety characteristics, it can also be used as a therapeutic alternative. As a result, before using CRISPR/Cas9 directly to human cells, animal cancer models and bioinformatics models should be employed as a preclinical platform to construct models to fully uncover the causal genes of such illness. Again, these models should be explored in conjunction with cancer patients, making it possible to quickly uncover alternative resistance mechanisms and develop novel treatment techniques. Doudna and Charpentier have claimed that this approach, when combined with an RNA-guided genome editing tool, can serve as an excellent platform for customized treatment.where it gives unique potential to modify human genome readily and straightforwardly in specified approach. This approach is a game-changing tool that is being used to treat a variety of ailments. However, its inclusion in the system poses a number of social and ethical problems, not just for

humans but also for other animals and the environment. Furthermore, despite a few technical challenges in targeting cancer genes, the possibilities of gene therapy using CRISPR/Cas9 remain promising. This therapy will hopefully rely on carefully designed sgRNA, monitoring of potential off-target effects, and efficient delivery. Furthermore, from basic research to clinical application, this approach is opening up new avenues for the treatment of chemotherapeutic drug resistance [6-7].

CONCLUSION

This ground-breaking technique has the potential to heal a variety of human ailments. In this review, we highlight the most advanced CRISPR/Cas9-based approaches for addressing the challenges associated with many types of cancer, including breast cancer, which is caused not only by genetic mutations but also by epigenetic mutations, making it an ideal tool for dealing with the mutations that underpin this disease. The use of CRISPR/Cas9 in somatic cells is morally acceptable due to the low risk relative to the advantages, but germ-line applications for human embryos pose substantial dangers compared to possible benefits, with unknown detrimental impacts on future progeny [6-7].

REFERENCES

1. BRCA: the breast cancer gene, National breast cancer foundation
2. Yang H, Jaeger M, Walker A, Wei D, Leiker K, Weitao T. Break Breast Cancer Addiction by CRISPR/Cas9 Genome Editing. J Cancer. 2018 Jan 1;9(2):219-231. doi: 10.7150/jca.22554. PMID: 29344267; PMCID: PMC5771328. **[3]**Breast cancer progression can be halted with CRISPR gene editing CRISPR technology for breast cancer :diagnostics, modelling and therapy doi:10.1002/adbi.201800132
3. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 2018 Nov;25(1):1234-1257. doi: 10.1080/10717544.2018.1474964. PMID: 29801422; PMCID: PMC6058482.
4. Rodríguez-Rodríguez DR, Ramírez-Solís R, Garza-Elizondo MA, Garza-Rodríguez MD and Barrera-Saldaña HA: Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases (Review). Int J Mol Med 43: 1559-1574, 2019
5. Sabit, H., Abdel-Ghany, S., Tombuloglu, H. *et al.* New insights on CRISPR/Cas9-based therapy for breast Cancer. *Genes and Environ* **43**, 15 (2021). https://doi.org/10.1186/s41021-021-00188-0
6. Karn, V., Sandhya, S., Hsu, W. et al. CRISPR/Cas9 system in breast cancer therapy: advancement, limitations and future scope. Cancer Cell Int 22, 234 (2022). https://doi.org/10.1186/s12935-022-02654-3
7. Impact of CXCR4 andCXCR7 knockout by CRISPR/Cas9 in breast cancer and delivery potential of gold nanomaterials, JananeePadayachee,Moganavelli Singh doi.org/10.1177/1849543520983196
8. Rodríguez-Ruiz A, Krupinski E, Mordang JJ, Schilling K, Heywang-Köbrunner SH, Sechopoulos I, Mann RM. Detection of Breast Cancer with Mammography: Effect of an Artificial Intelligence Support System. Radiology. 2019 Feb;290(2):305-314. doi: 10.1148/radiol.2018181371. Epub 2018 Nov 20. PMID: 30457482.
9. Chu QD, Peddi PE, Ellis MJ, and Ma C, 2012, Molecular Basis of Triple Negative Breast Cancer and Implications for Therapy, International Journal of Breast Cancer, https://doi.org/10.1155/2012/217185
10. Morrow M, Waters J, Morris E. MRI for breast cancer screening, diagnosis, and treatment. Lancet. 2011 Nov 19; 378(9805):1804-11. doi: 10.1016/S0140-6736(11)61350-0. PMID: 22098853.
11. Sabit H, Abdel-Ghany S, Tombuloglu H, Cevik E, Alqosaibi A, Almulhim F, Al-Muhanaa A. New insights on CRISPR/Cas9-based therapy for breast Cancer. Genes Environ. 2021 Apr 29;43(1):15. doi: 10.1186/s41021-021-00188-0. PMID: 33926574; PMCID: PMC8082964.
12. Padayachee J, Singh M. Therapeutic applications of CRISPR/Cas9 in breast cancer and delivery potential of gold nanomaterials. Nanobiomedicine (Rij). 2020 Dec 24;7:1849543520983196. doi: 10.1177/1849543520983196. PMID: 33488814; PMCID: PMC7768851.
13. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res. 2020 Jun 9;22(1):61. doi: 10.1186/s13058-020-01296-5. PMID: 32517735; PMCID: PMC7285581.
14. Wijshake T, Zou Z, Chen B, Zhong L, Xiao G, Xie Y, Doench JG, Bennett L, Levine B. Tumor-suppressor function of Beclin 1 in breast cancer cells requires E-cadherin. ProcNatlAcadSci U S A. 2021 Feb 2;118(5):e2020478118. doi: 10.1073/pnas.2020478118. PMID: 33495338; PMCID: PMC7865132.

15. Gokhale S. Ultrasound characterization of breast masses. Indian J Radiol Imaging. 2009 Jul-Sep;19(3):242-7. doi: 10.4103/0971-3026.54878. PMID: 19881096; PMCID: PMC2766883.
16. Anders C, Carey LA. Understanding and treating triple-negative breast cancer. Oncology (Williston Park). 2008 Oct;22(11):1233-9; discussion 1239-40, 1243. PMID: 18980022; PMCID: PMC2868264.
17. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma KK. Various types and management of breast cancer: an overview. J Adv Pharm Technol Res. 2010 Apr;1(2):109-26. PMID: 22247839; PMCID: PMC3255438.
18. Bhat, A.A., Nisar, S., Mukherjee, S. *et al*. Integration of CRISPR/Cas9 with artificial intelligence for improved cancer therapeutics. *J Transl Med* **20**, 534 (2022). https://doi.org/10.1186/s12967-022-03765-1