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Review paper

Application of CRISPR/Cas9 in Breast Cancer Treatment: A Mini Review

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Abstract
Breast cancer is the commonest cause of cancer death in women worldwide. Although impressive gains in breast cancer research and treatment have been made over the past decades, breast cancer treatment still remains a significant global challenge. Recently, application of gene editing tools, such as the CRISPR/Cas9 system, has shown a clinical potential to discover novel targets for cancer therapy. CRISPR is a family of DNA sequences found in the genomes of prokaryotic organisms. Cas9 is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence. Cas9 enzymes together with CRISPR sequences form the basis of a technology known as CRISPR/Cas9 that is a genome editing tool used to edit parts of the genome. CRISPR/Cas9 could be a major step forward to cancer management by providing patients with an effective method for dealing with cancers by dissecting the carcinogenesis pathways, identifying new biologic targets, and perhaps arming cancer cells with drugs. Moreover, CRISPR/Cas9 can be employed to rapidly engineer immune cells for cancer immunotherapeutic applications. The CRISPR/Cas9 system has been reported to play an important role in preventing drug resistance in breast cancer. It has also been used to develop early breast cancer diagnostic tools and treatments. Despite the potential of CRISPR/Cas9 in breast cancer treatment, some challenges remain to be solved for clinical application of this system in breast cancer treatment. This review aims to present the application of CRISPR/Cas9 in breast cancer treatment.

Keywords: *Human acellular amniotic membrane, Wound healing, Diabetic rat*

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Introduction

Breast Cancer

Breast cancer is the most common cancer and the leading cause of cancer-related death in women worldwide. There is great variation in breast cancer survival rates around the world, with 5-year survival estimated to be 80% in developed countries and less than 40% in developing countries [1]. Developing countries face resource and infrastructure limitations that challenge the outcomes of breast cancer detection, treatment, recovery, and management [2]. According to the World Health Organization (WHO), improving breast cancer outcome and survival with early detection is the foundation of breast cancer regulation. Various drugs are prescribed to treat breast cancer. In people who are at risk of developing breast cancer, drug treatment with antiestrogens such as raloxifene or tamoxifen may prevent them from developing [3]. Surgery of both breasts is an additional preventive measure in increasing the risk of cancer in women. In patients diagnosed with breast cancer, various management strategies such as targeted therapy, hormone therapy, radiation therapy, surgery, and chemotherapy are used [4].

Epidemiology and Mortality

1.67 million new cases of breast cancer were diagnosed in 2012, 25% of all cancers among women. Studies show that 883,000 cases of breast cancer occur in less developed countries and 794,000 cases in most developed countries [5]. According to reports, 145.2 women in Belgium and 66.3 in Poland among 100,000 have breast cancer [6]. Breast cancer affects one woman out of every eight women in America and one woman out of every 35 women in Asia, and it is still increasing [7], [8].

The fifth cause of death from cancer is breast cancer. The mortality and prevalence of breast cancer in the United States is higher than in the world. In Poland, 17% of diseases are cancer and 14% of deaths are caused by cancer. In 2004, the global death from breast cancer was reported to be 519,000 [9]. In the United States alone, approximately 1,208,000 cases of cancer are reported annually and approximately 538,000 people die from this cancer, representing about one-fifth of all annual deaths from any cause [10].

CRISPR/Cas9 System

A major advance in the field of genomics is the development of CRISPR/Cas9 technology, which has revolutionized gene editing in the 21st century. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), first identified in Escherichia coli in 1987, consists of a group of 29-nucleotide repeat fragments separated by 32-nucleotide fragments of uniquely different sequences [11]. It has been shown to play a role in numerous cellular processes including thermo-acclimation [12], DNA repair [13] and chromosomal rearrangements [14]. In addition, a short palindromic repeat sequence of 24 to 40 nucleotides with an interval of 20 to 58 different nucleotide sequences was identified in several species of bacteria and archaea, such as Streptococcus pyogenes (S. pyogenes), Mycobacterium tuberculosis (Hacobacterium tuberculosis) [15], [16].

Over the years, research has shown that CRISPR has evolved over time as an adaptive immune system and protects bacteria and archaea against foreign DNA invaders such as viruses and plasmids [17].

CRISPR/Cas systems are divided into 2 classes, 6 types, and 33 subtypes, characterized by the involvement of different Cas proteins in the CRISPR framework that target DNA, RNA, or both [18], [19]. This classification is given in Table 1 [20].

Table 1. Classification of the CRISPR/Cas Systems [20]

CRIPSR/Cas Systems						
Class	1			2		
Protein type	Multiplex			Single		
Type	I	III	IV	II	V	VI
Corresponding Cas protein	Cas 3	Cas 10	Cas 8	Cas 9	Cas 12a, Cas 12c, Cas 13a	Cas 13b, Cas 13c

History

CRISPR was first reported in 1987, after which the technology evolved step by step [11]. Francisco Mojica was the first to identify sequence patterns as CRISPR in the early 1990s. After studying these repeats for the next ten years, Mojica made the key finding in 2003 that the repeating DNA corresponedwith segments of DNA that matched the viruses attacking the bacteria [21]. The immune role of CRISPR in bacteria was first discovered by Barrangou et al. in 2007 [22]. In 2012, the molecular process of CRISPR technology was characterized by Jennifer Doudna, Emmanuel Charpentier and their teams. In fact, CRISPR was discovered as a genome engineering tool [23].

In 2013 Feng Zhang demonstrated the application of CRISPR in eukaryotic cells, how CRISPR can be used to genetically modify eukaryotic cells [24].

In 2014, Kevin Esvel traised the concept of CRISPR -CRISPR gene drives-genetic components that "cheat evolution." Also eradicating malaria and other vector-borne diseases with the help of CRISPR-based gene drivers [25].

In 2015, Guangzhou University used CRISPR to modify human embryos [26].

In 2018, the first human trials for CRISPR were approved [27], [28].

A paper on the development and use of prime editing was published in Nature in October 2019 by Andrew Anzalone, a postdoc researcher from Dr. David Liu's team [29].

In 2020, Victoria Gray was the first patient who received the sickle cell disease treatment, and her encouraging outcomes quickly gained attention [30]. Also, less than six months later, published data revealed that ten patients who had undergone the CTX001 therapy had achieved considerable advancements. And seven-were treated for beta-thalassemia and three for sickle cell anemia [31].

CRISPR and treatment of diseases

Sicle Cell Disease (SCD)

Sickle cell disease (SCD) is an inherited monogenic disorder that causes significant mortality and morbidity worldwide. There is no definitive cure for SCD. Rapid and significant progress in genome editing approaches is valuable as a therapeutic because it is possible to correct mutations in patient-derived hematopoietic stem/progenitor cells (HSPCs), inducing fetal hemoglobin expression to bypass sickling of red blood cells. (RBCs) are present. or creating modified induced pluripotent stem cells (iPSCs). The recent discovery of CRISPR/Cas9, which has revolutionized genome engineering, has made it possible to translate these concepts into a clinical reality [32].

Beta Thalassemia

Production of β-thalassemia patient-specific induced pluripotent stem cells (iPSCs), gene modification based on homologous recombination following pathogenic mutations or deletions in the β-globin gene (HBB), and hematopoiesis derived from them, is an excellent therapeutic way to treat beta-thalassemia. During the studies, it was found that the hematopoietic differentiation efficiency of β-Thal iPSCs was greatly improved after modification by the CRISPR/Cas9 system [33].

Myotonic Dystrophy Type 1 (DM1)

Myotonic dystrophy type 1 (DM1) is a severe neuromuscular disorder with variable multisystem

features for which no cure has yet been found. To ameliorate the symptoms of DM1 disease and restore cellular homeostasis, CRISPR/Cas approaches directed towards mutations in DNA and RNA are of great interest [34].

Huntington's Disease (HD)

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease caused by the expansion of three CAG nucleotides in exon 1 of the Huntingtin (HTT) gene. CRISPR-based genome editing tools that are simple to use and highly efficient open new ways to treat this disease [35].

Alzheimer's Disease (AD)

Alzheimer's disease (AD) is an irreversible and progressive condition that manifests as cognitive impairment and amyloid beta (Aβ) plaques and neurofibrillary tangles. In the management of AD, CRISPR/Cas9 genome editing is promising. Which is an extensive task that requires the translation of this technological approach into therapeutic applications. So far, viral and non-viral methods are available for delivery of CRISPR/Cas9 systems [36].

Cardiovascular Disorders (CVDS)

Cardiovascular disease (CVD) is one of the leading causes of death in the world. CVD is widely classified as hereditary and non-hereditary diseases. The therapeutic potential of CRISPR/Cas9 for CVD is a challenge for several reasons, particularly the postmitotic nature of cardiomyocytes. The main cellular targets for using this technique are cardiac fibroblasts, endothelial cells, and smooth muscle cells [37].

Metabolic Disorders

Metabolic liver disease (MLD) is caused by a defect in a transporter protein that leads to abnormal metabolism of carbohydrates, protein, and fat. Studies conducted in gene editing-based therapy suggest the use of CRISPR-Cas9 to treat metabolic disorders. However, CRISPR-Cas9 technology against metabolic disorders requires more effort [38].

Cystic Fibrosis (CF)

Mutations in the gene encoding the chloride ion channel CFTR lead to cystic fibrosis (CF), which is the most common autosomal recessive genetic disease. The disease presents several symptoms but most often involves the bronchi and lungs, which are affected by recurrent bronchitis and bronchopneumonia caused by certain bacteria such as Pseudomonas aeruginosa and Staphylococcus aureus. In the treatment of CF, encouraging results have been observed in the potential application of the CRISPR technique. Because CF is caused by a series of mutations, the CRISPR strategy may be an important tool for treating CF in the foreseeable future [39].

Eye-Related Disorders

Retinitis pigmentosa (RP) is an inherited pigmentary dystrophy of the retina that may cause vision loss. Several mutations lead to this disease. CRISPR-Cas9 gene editing technique can improve visual function by stopping retinal degeneration in rat, and this achievement shows the possibility of in vivo gene correction using CRISPR-Cas9 technology.

Several mutations have also been reported to cause cataracts. Several studies using the CRISPR-Cas9 system have recently been developed to study congenital cataracts in humans [38].

Viral Infection

HIV is a virus that attacks the human immune system and causes acquired immunodeficiency syndrome (AIDS). Although AIDS is now manageable with antiretroviral drug therapy (HAART), there is no lifelong cure for AIDS. It has been observed that CRISPR-Cas9 can lead to its mutation and degradation by affecting the HIV-1 provirus DNA. It has been found that CRISPR-Cas9-mediated gene editing can inhibit several steps of HIV-1 infection.

Hepatitis B Virus (HBV) causes chronic hepatitis, which is an infectious disease in the world. It has been observed that CRISPR-Cas9 can reduce HBV by inhibiting viral replication. CRISPR-Cas9 has also been shown to cleave viral DNA and suppress HBV. Therefore, disrupting the HBV genome through CRISPR-Cas9 technology can be a promising anti-HBV therapy in the future.

Hepatitis C Virus (HCV) is a single-stranded RNA virus that causes hepatitis C, an inflammatory liver disease. In previous studies, the CRISPR-FnCas9 system was used to inhibit HCV in eukaryotic cells.

Human Papillomavirus (HPV), a double-stranded DNA virus that infects mucosal cells, causes about 11 percent of cancers in women. By targeting CRISPR-Cas9 technology, the therapeutic potential of gene editing against genital warts can be realized [38].

The outbreak of severe acute respiratory syndrome (SARS)-CoV-2 began in December 2019 and spread rapidly to become a global pandemic. The CRISPR-based DETECTR assay will be a faster and more accurate alternative in the prevention of further outbreaks of SARS-CoV-2 [40].

Cancer

Cancer is a complex disease. Indeed, it is a genomic disease caused by mutations in DNA that activate oncogenes and inactivate tumor suppressors, as well as disrupt the regulation of the epigenome, which coordinates normal gene expression. Although CRISPR is still a young technology, it has affected almost every aspect of cancer biology. For cancer treatment in the coming years, CRISPR will take its first real steps in clinical medicine. By elucidating the role of individual genes in the behavior of cancer cells, CRISPR has been and will continue to enable the next generation of immunotherapy, attribute the functional effect of repetitive coding variants, and reveal the role of non-coding and regulatory elements in tumorigenesis [41].

Breast Cancer and CRISPR/Cas9 Technique

CRISPR/Cas9 is a powerful tool to study and treat various cancers including breast cancer (BC). Genes involved in causing cancer often fall into two groups: proto-oncogenes, which cause cell growth and proliferation, and when mutated or activated, cause tumor growth. And tumor suppressor genes (TSGs) involved in DNA repair and cell growth control, which when mutated or inactivated lead to genomic instability and uncontrolled proliferation. Many mutations, at the nucleotide, transcriptional and epigenetic levels, may lead to aberrant expression or their inhibition. CRISPR/Cas9 can target mutations at any level and thus have great potential in the treatment of BC [42].

Genes encoding growth factors and their receptors, transcription factors (TF), signal transducers and chromatin remodeling proteins have oncogenic potential [43]. CRISPR/Cas9 can be used to directly target these oncogenes, destroy them, and inhibit cancer growth through various mechanisms. This technique has been successfully used to eliminate cellular and viral oncogenes in various cancer models, cervical cancer [44], endometrial cancer [45], prostate cancer [46] and including leukemia [47]. PI3KCA, HER2/ErbB2 and MYC oncogenes are involved in BC [48] and it has been observed that deletion of HER2 led to decreased viability of HER2þ BT-474 and SKBR-3 cells [49].

Also, one of the other targets that can be removed to achieve beneficial results in relation to breast cancer is kinome. In fact, kinomes are protein kinases that play a role in the phosphorylation of proteins and lipids [50]. Their irregularity is a common feature of many cancers, one of which is BC [51]. And the important thing is that some oncogenes that have been discussed and well studied, such as HER2, PI3KCA and FGFR, are members of this family that are prone to knockout through CRISPR/Cas9 [52].

Another technique that can be discussed is immunotherapy. Immunotherapy involves boosting the

immune response to suppress tumor cells. Cancers may evade immune responses by overexpressing immune checkpoint proteins that are normally responsible for preventing autoimmune responses [53]. CRISPR/Cas9 knockout programmed cell death ligand 1 (PD-L1), which binds the programmed cell death receptor 1 (PD-1) on immune cells [54], [55], [56], [57]. May induce an immune response against the tumor [58], [59]. CRISPR knockout of CDK5 has also been shown to reduce PD-L1 expression and inhibit tumor growth [60], [61].

From the research done in the field of breast cancer and the use of CRISPR/Cas9 technique, in a reported research, in cancer models, proteasome inhibitors have been shown to have anticancer properties and increase apoptosis. In addition, it sensitizes tumor cells to extrinsic and intrinsic pro-apoptotic signals. Therefore, the proteasome has become a target for antitumor therapies. Breast cancer proliferation has been shown to be controlled by a site-specific proteasome phosphorylation process [62], and interference and disruption of this process may be beneficial in disease control.

In previous studies, researchers used the CRISPR/Cas9 system and engineered dual-specificity tyrosine-regulated kinase 2 (DYRK2) (enzymes that phosphorylate proteasome components) to disrupt tumorigenesis of proteasome-addicted human breast cancer cells in mice [63].

Conclusion

According to the results of various studies and researches, despite the fact that the CRISPR/Cas9 system has great potential in the treatment and prevention of all types of cancers, especially breast cancer, there are still many challenges and limitations in using this technique clinically. More studies and researches are needed to achieve more useful results in the use of CRISPR/Cas9 technique in the future.

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Conflict of interests

The authors declare that there are no competing interests.

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