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CRISPR/Cas9 in the treatment of β-thalassemia

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Abstract. Based on clinical evidence, medication resistance poses a significant challenge to the treatment of cancer. It causes the disease to become uncontrollable and raises death rates. Drug resistance arises from a variety of causes, but a change in the inherited makeup of tumor cells is typically the root reason. The ability to modify the genome is growing with the recent discovery of clustered regularly interspaced short palindromic repeats (CRISPR)/associated (Cas)9 technology, which may be helpful in reducing drug resistance. Owing to its exceptional accuracy and efficiency, the CRISPR/Cas 9 system has been used to investigate the relevant roles of cancer-causing genes, create animal models of tumors, and identify potential therapeutic targets. As a result, it has emerged as the go-to technique for therapeutic gene editing. Utilizing CRISPR/Cas 9 technologies in the treatment of different diseases is growing. Because oncogene regulation differs from normal gene regulation, the CRISPR/Cas 9 system offers efficient methods for oncogene elimination, interference with expression, and modification of activity, all of which can effectively impede the growth of tumors. This article discusses the potential of the CRISPR/Cas9 system to identify resistance targets in drug-resistant breast cancer and reverse resistance gene alterations. Furthermore, the difficulties that prevent this technology from being clinically applicable and emphasize the CRISPR/Cas9 systems are discussed. The CRISPR/Cas9 system will be a crucial component of personalized medicine and is anticipated to have a significant impact on reducing drug resistance in cancer therapy.

Keywords: CRISPR/Cas9, medical applications, cancer.

1. Introduction

The illness known as cancer arises from an imbalance in normal regulation and an overabundance of cell division in the body. Cancer cells are defined as hyperproliferating cells that frequently infect nearby tissues and even spread to other parts of the body through the lymphatic and/or circulatory systems (cancer metastasis). Notably, malignant tumors include cancers and sarcomas. A cancer is a malignant tumor, not a cancer. There are numerous varieties of cancer, and the location, malignancy, and metastasis of the cancer all affect how severe the disease is. Once diagnosed, it is often treated by combining surgery, chemotherapy and radiotherapy. If the cancer is untreated, the final result usually results in death. With the ubiquity of bad lifestyles, cancer is becoming more and more frequent. According to the International Center for Cancer Research (International Agency for Research on Cancer, IARC) A Cancer Journal for Clinicians Global Cancer Statistics Report 2018: Estimates of the incidence and Mortality of 36 cancers in 185 countries worldwide ① shows that there were 1,8078,957 new cancer cases worldwide in 2018, The incidence rate is 236.9/10 ten thousand; 9 555 027 cancer deaths, Mortality rate is 125.2/10 ten

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thousand. Among them, new cancer cases and deaths in China accounted for 23.7% and 30% of ②, respectively. That means that there were 4,285,033 new cancers in China in 2018, of which 2,8,65,174 cancer patients died [1].

The International Institute of Cancer has released its 2020 Global Cancer Data, which shows that breast cancer is now the most common cancer worldwide in women, surpassing lung cancer in terms of new cases. In China, the growth rate of breast cancer incidence of cancer is twice that of the global growth rate, ranking the top in the incidence of malignant tumors in the female population. This leads to an increasing number of patients and medical stress. As the most widely distributed cancer among Chinese women, breast cancer is characterized by high survival rate and long treatment time. This leads to an increasing number of patients and medical stress [2]. Patients were aged 26 - 73 years, The mean age was 51.91 ± 9.47 years, Most of the patients were aged 45 - 59 years, For middle-aged women, 91.98% of the patients were married with a living spouse; More than half of the patients were not highly educated, Junior high school (37.79%) and primary school and below (34.73%): retired patients (28.63%) and unemployed patients (27.48%) Nearly employed patients could not work during chemotherapy (33.59%): nearly half of them had a monthly income of 1000 - 3000 (43.51%): most of the patients had urban medical insurance (68.32%) [3].

There are three phases to the CRISPR/Cas system's operation. The acquisition of additional spacers is the first step, CRISPR locus expression, including transcriptional and post-transcriptional processing, is the second, and disruption of the CRISPR/Cas system is the third [4]. Recent research on gene function, virus-host interactions, and recombinant vaccines has benefited greatly from the use of CRISPR/Cas 9. Its high efficiency, simple design and wide application provide strong support for these studies. In many cases of viral genome editing, CRISPR/Cas 9 has shown higher editing efficiency by simultaneously editing multiple sites in the viral genome to produce a multivalent recombinant vaccine against highly infectious strains. The development of recombinant vectors / plasmids with multiple antigens will be important for vaccine development, especially in the context of outbreaks of coronavirus and its varieties, and the importance of future vaccine development is more prominent. Therefore, the development process of multivalent vaccines should focus on a broad host range and rapid development [5].

2. CRISPR technology

The CRISPR system was initially identified in 1987. But it hasn't been widely applied until 2013 that CRISPR technologies began to be widely used. Over the last five years, CRISPR has revolutionized not only the domains of chemical biology and medicine, but also biotechnology and biotechnology. Following the initial successful use of these technologies in the genetic alteration of mammals, experts from all over the world contributed favorably to the advancement of these technologies using their specialized knowledge.

Widespread in bacteria and archaea, CRISPR systems are adaptable immune systems. Dr. Nakata's team identified a unique repeat sequence in Escherichia coli (E. coli) in 1987 [6]. Two decades later, in 2002, the sequence was dubbed CRISPR, or arranged in a regular, spaced-out short palindromic repeat. Simultaneously, the genes next to the repetitive sequences were discovered to be CRISPR-associated (Cas) genes. The fundamental elements required for the process of adaptive immunity are the CRISPR array and Cas proteins. Since 2007, more light has been shed on the following unraveling of the molecular intricacies of CRISPR-Cas systems. In vitro cleavage of target DNA by pure Cas9 under the supervision of crRNA/tracrRNA was demonstrated in 2012. Later in 2013, mammalian cells were able to change their genomes using the type II CRISPR Cas9 technology. Subsequently, several CRISPR-Cas single effector enzymes have been discovered and altered for use in a variety of species.

CRISPR-Cas systems are split into two classes, Class 1 and Class 2, based on notable differences in the Cas proteins involved in binding guidance and target cleavage. These classes are further grouped into six groups based on the "signature genes." Based on their multi-subunit effector complexes, Type I, III, and IV systems are classified as Class 1 systems; on the other hand, Type II, V, and VI systems' single, massive, multi-domain effectors are categorized as Class 2. The researchers are still looking into different CRISPR systems, therefore the classification work is not yet complete. Furthermore, the

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classification has to be updated as new information based on structural, biochemical investigations, and comparative genomic research of CRISPR components becomes available. The review by Koonin et al. provided an overview of the most recent categorization of CRISPR-Cas systems. The Type II system, which had Cas9 from Streptococcus pyogenes, and the Type V system, which contained Cas12a (formerly known as Cpf1) from Acidaminococcus sp (AsCas12a), the Lachnospiraceae bacterium (LbCas12a), and Francisella novicida (FnCas12a), were the most commonly utilized systems (figure 1) [7, 8].

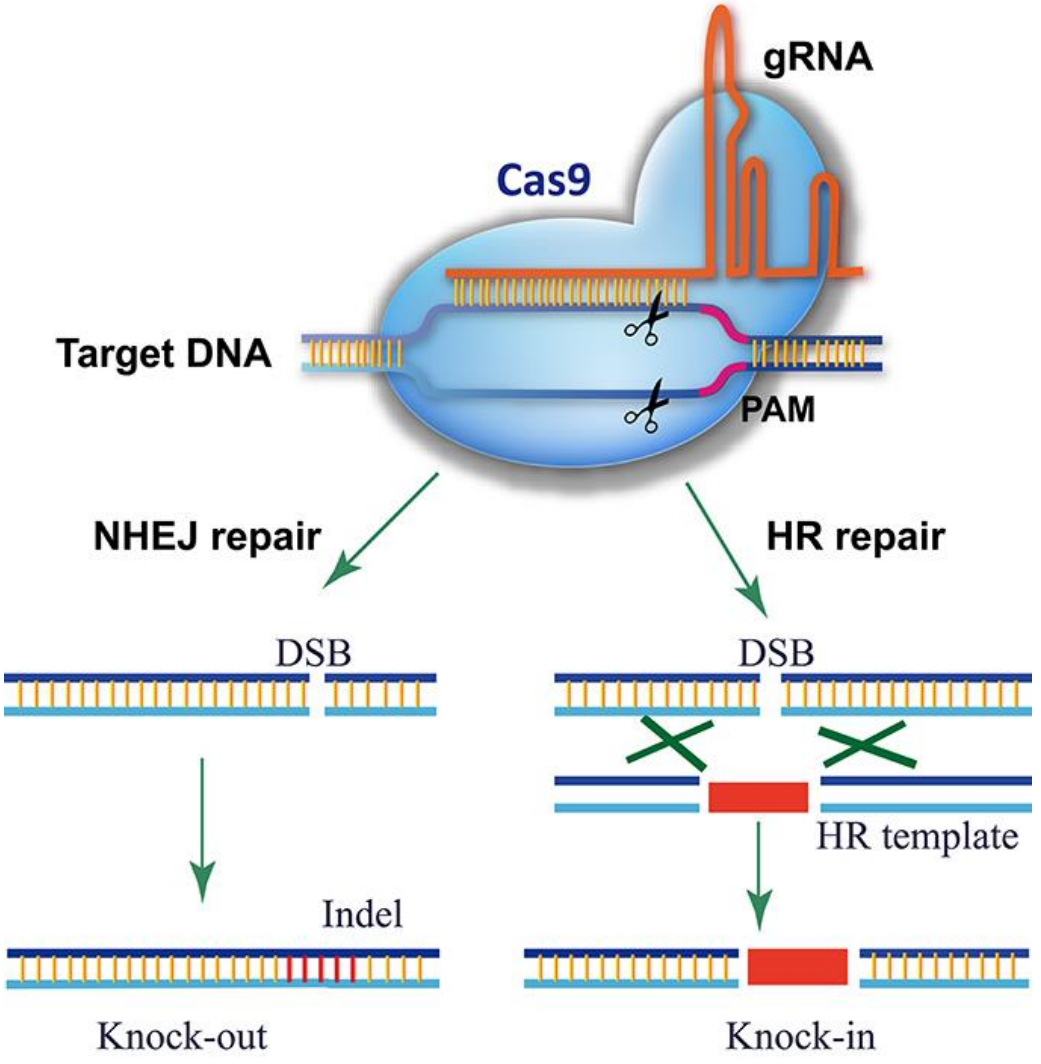

Figure 1. Schematic of Cas9/gRNA genome editing [9].

3. CRISPR/Cas9 Is Better Than Other Methods for Overcoming Drug Resistance in Breast Cancer.

Traditionally, medicines with distinct targets have been combined to reduce acquired drug resistance. But because one drug's methods of action interact with the others, it is difficult to predict the outcome. Enhancing the anticancer agent's specificity is a further way to reduce the likelihood of acquired drug resistance, particularly in MDR situations. Furthermore, it would be possible to reuse currently available anticancer medications by inhibiting or reversing resistance mechanisms [10].

A sequence of genetic events leads to the multistep and complex process of cancer development, including breast cancer. Numerous point mutations, copy number changes, and chromosome

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rearrangements have been found in the genome sequencing of several cancer types. Oncogenes and drug-resistant genes have been validated using a variety of in vivo and in vitro methods. These techniques can be divided into two categories: gain-of-function (cDNA-based over-expression) and loss-of-function (RNAi, for example) of the target gene. In the past few decades, these methods have played a crucial role in many important discoveries in cancer biology, but they also have some serious drawbacks. CDNA based expression systems may elevate gene expression to a supraphysiological level, which may have adverse effects on biological processes within cells.Furthermore, RNA interference (RNAi) only partially knocks down a gene's mRNA levels; residual mRNAs may still be functional. This may make it difficult to identify targets that need whole mRNA inactivation. Although genome engineering in mouse or human cell models is more comprehensive, conventional methods have found it to be time-consuming and technically difficult. The predominant technique for simulating various cancer forms in mice has been the Cre/LoxP system. The intricate genetic processes involved in cancer has made it difficult to use functionally analyze the importance of individual mutations or the combinatorial impact on genes related to carcinogenesis.[11].

The CRISPR/Cas9 technology provides a quick mechanism for specific endogenous locus alteration, circumventing the drawbacks of the previously stated techniques. This approach could be applied, for instance, to somatic genome engineering, genetic screening in vivo or in vitro, CRISPR-based effector regulation, and genetic variant modeling. Recent studies have shown that CRISPR/Cas9-mediated NHEJ or HDR can effectively disrupt or modify genes in different types of cancer cells. It has been effectively applied to patient-derived xenografts and established cell lines to modify, reorganize, or introduce new mutations at several locations in the mammalian genome. Since CRISPR/Cas9-based genome editing has advanced, many of the difficulties in producing.

4. Conclusions

The area of molecular biology has undergone a dramatic shift in the past few years due to the astounding advancements made in the development of numerous CRISPR-based technologies, especially in human cells and mammalian model systems. In addition to the widely utilized Cas9 system, other CRISPR-related nuclease variations are still being identified and explored, offering possible enhancements in accuracy, effectiveness, and transport. Meanwhile, there are countless opportunities for diverse applications with additional improvement of the well-characterized CRISPR systems that are already available. However, there are still certain technical obstacles to overcome. CRISPR-based technologies have enormous Possibility of therapeutic use as a generally applicable method. In June 2016, the United States approved the first CRISPR clinical trial. At the West China Hospital in Chengdu, the first patient received the modified CAR-T cells later in October of that same year. Following the successful correction in 2017 of a detrimental mutation in genes in human embryos, more CRISPR-related clinical trials are being looked at.

But as the CRISPR revolution gains momentum, worries about their application on a social and ethical level are also growing. Greater thought should go into the therapeutic uses of these effective instruments. In May 2018, the CRISPR gene therapy experiment was put on hold by the US Food and Drug Administration (FDA) without providing any information. Undoubtedly, CRISPR-based treatment is still in its infancy and has a number of issues that need to be resolved. The off-target effect is one of the main worries. Various Cas nucleases have been designed to increase the accuracy of targeting. The delivery efficiency of CRISPR-based gene therapy is another significant barrier. In addition to technological obstacles, the public is also interested in social and ethical issues. Although a study suggested that Cas9 might cause humans' adaptive immune reactions, no workable remedy has yet been discovered. In addition, a different investigation noted that Cas9 editing caused unintentional alterations to the human germ line and that it activated a p53-mediated DNA damage response, a characteristic shared by cancer cells. There is still more work to be done in developing CRISPR-based therapy because the reasons for these issues are still unclear. All things considered, we think that when CRISPR is used more and more, amazing advancements and uses may become possible.

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Author contributions

All the authors contributed equally and their names were listed in alphabetical order.

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