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Advances of CRISPR/Cas9 in cancer research and treatment

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Abstract. Cancer is a disease that is very difficult to cure and a lot of people in the world die because of cancer. In the past, the treatments of cancer could only depend on radiotherapy, chemotherapy and surgery of removing tumors. But these ways have some weaknesses such as harming the health of the body and relapse of cancer. Whereas recently, completely curing cancer has become possible through the advances of gene-editing technologies. Due to its great effectiveness, simplicity of usage, and other advantages, the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 (CRISPR/Cas9) system is a widely used gene editing technique today. But there are still many challenges that are applied to the body. For example, the CRISPR/Cas9 system has an off-target effect and there are no suitable carriers to deliver it to the area of the body. The resolution of these problems may be made possible by future developments in the CRISPR/Cas9 system. The use, restrictions, and potential uses of the CRISPR/Cas9 system in the treatment of cancer are examined in this research. In addition, this paper compares other treatments of cancer to summarize the advantages and disadvantages of different methods.

Keywords: cancer, tumor, CRISPR/Cas9 system, off-target effect, gene-editing technology.

1. Introduction

Nowadays cancer is the leading cause of death in the world, and treating it has long been a medical challenge. The World New statistics on the global burden of cancer in 2020 have been released by the International Agency for Research on Cancer (IARC) of the World Health Organization. According to statistics, there will be 9.96 million cancer deaths and 19.29 million new cancer cases worldwide in 2020. Therefore, cancer treatment has become increasingly important.

Cancer is an illness where some cells can grow out of control. The cells proliferate in the place of the body everywhere and then form tumors. Tumors can be malignant or benign. Malignant tumors have extremely negative effects on the body, but most benign tumors do not affect people's health except benign tumors in the brain. Cancerous tumors can migrate to distant parts of the body to produce new tumors and spread to surrounding tissues. Removing tumors is a method to treat cancer, but sometimes cancerous tumors can grow again. So a treatment to inhabit tumors growth fundamentally is needed. Tumor immunotherapy is an effective way to treat cancer now. It refers to

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enhancing the resistance of specific immune cells to the tumors, while reducing the protection of other regulatory immune cells to the tumors [1]. And this method become more and more perfect with advances in gene editing technology. In recent years, according to Feng Zhang and others, mammals can employ the CRISPR/Cas9 system [2]. This research made it possible to use the CRISPR/Cas9 system in tumor immunotherapy to cure cancer. Currently, CRISPR/Cas9 technology is extensively applied in the field of cancer and offers new and more effective ways to treat cancer [1, 2].

CRISPR/Cas9 is a technology of manipulating DNA. It is an immune response system that evolved by bacteria fighting viruses for a long time [2]. The CRISPR/Cas9 system evolves from the type II CRISPR-Cas system [3]. By establishing base pairs with DNA target sequences, the crRNA (CRISPR RNA) and tracrRNA (trans-activating crRNA) components of the CRISPR/Cas9 system can disrupt the duplex structure of DNA [3]. Double-strand breaks(DSBs) are formed after Cas9 protein cut out the DNA double-strand of the target gene. Then, HDR (homology-directed repair) and NHEJ (nonhomologous end joining) are activated (two self-repair mechanisms of a cell). Thus, using the CRISPR/Cas9 system can realize gene editing such as insertion, knockout and modification of target genes [4]. It improves the function of CAR-T cells by knocking out the T cell inhibitory receptor gene [4]. This method can effectively inhibit the fertility of tumor cells.

The CRISPR/Cas9 technology is used to treat cancer, but it is also utilized to generate medications, increase agricultural output, and do other things because of its simplicity, precision, and ease of modification [2, 4]. Applying CRISPR/Cas9 system to cancer is a big milestone in the history of treating cancer. The CRISPR/Cas9 method has so far improved the way that breast, lung, and prostate cancer are treated. The first report of CRISPR clustered repeats was published by Y Ishino and others in 1987. And then, it was detected in all kinds of bacteria and archaeas in the next decade [5,6]. CRISPR/Cas was first used in biotechnology in 2007 and mature CRISPR RNA was proved to be able to resist foreign viruses or plasmids in the next year [5]. It was applied for a trial which treated a disease in 2013. Nowadays, a lot of clinical data on CRISPR/Cas9 treating diseases was published [6].

2. CRISPR/Cas9 technology

2.1. Principle

When the virus first invades bacteria, the double-stranded DNA of the virus is injected into the cell. Cas9 protein cuts out a sequence of the exogenetic DNA as an identification of recognizing the invasive virus based on the protospacer-adjacent motif (PAM, a short sequence). NGG serves as PAM's recognition site in the CRISPR/Cas9 system (GGG, CGG, AGG, TGG) [5,6]. Cutting function of Cas9 protein depends on the HNH nuclease domain and RuvC-like nuclease domain. The HNH domain cuts the targeted DNA strand (A DNA stand of pairing with the base of sgRNA) while the RuvC domain cuts the non-target DNA strand [6]. Upstream of the PAM sequence, between the third and fourth bases, is the cutting site [6]. The cutting sequence is incorporated into the bacteria's CRISPR sequence as a new interspaced sequence. Then, when this virus invades the bacteria which has the new CRISPR sequence carrying virus information, CRISPR/Cas9 system can identify the virus and break the duplex structure of virus DNA. Pre-crRNA is produced by the CRISPR sequence under the controlling leader sequence. The tracrRNA is transcribed at the same time. The pre-crRNA becomes mature by RNase III catalyzing, and then combines with tracrRNA to form sgRNA (small guide RNA). The sgRNA combines with Cas9 protein to form a compound. It can identify the interspaced sequence that pairs with the base of sgRNA. The double strand of exogenetic DNA is disengaged. One strand is paired with the base of sgRNA, and the other one is free. At last, the DNA of the virus is broken by the function of Cas9 protein.

2.2. Application

The CRISPR-Cas9 system is naturally present in bacteria and can be used to target and destroy cancer cells, whose task is to recognize and cleave the DNA of the virus or phage, so that the virus or phage can kill [7]. The CRISPR-Cas9 system can treat cancer at the gene level [8]. For instance, ovarian

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cancer, one of the prevalent gynecological malignancies, is dangerous to patients' lives and health because it has a high death rate. Its treatment is mainly surgical surgery, with chemoradiotherapy as an adjuvant treatment for ovarian cancer. Advanced ovarian cancer is particularly prone to distant metastases, so the clinical therapy of ovarian cancer also focuses on the diagnosis and management of ovarian cancer metastases. The CRISPR-Cas9 system mediated by genome editing technology as an efficient and practical emerging gene editing technology can obtain gene knockout mutants more rapidly than conventional techniques. The development of precision medicine approaches for cancer depends on the identification of gene mutations that fuel cancer development, and CRISPR gene editing has the speed and efficiency to produce gene knockouts that control endogenous gene expression and reproduce genomic alterations related to cancer. Making gene knockout mice models is now common practice because of the CRISPR technology's ease of use and great efficiency. Additionally, tissue-specific cancer models can be created by selectively introducing every element of the CRISPR gene-editing system into particular somatic cells. Acute myeloid leukemia, for instance, can be brought on by using CRISPR to alter the Tet 2, Runx 1, Dnmt3a, Nf 1, and Smc 3 genes in hematopoietic progenitor cells [8]. The quick creation of cancer models with a complicated phenotype is made possible by administering CRISPR to the liver, pancreas, or lung. Monitoring the lineage alterations of cancer cells is a crucial CRISPR use in cancer research. Cancer's heterogeneity, in which cancer cells continuously acquire genetic variations, produces cell clones with various properties, is one of the disease's key hallmarks. Understanding internal tumor heterogeneity and tracking the generation and evolution of new clones gave scientists a more comprehensive understanding of [9].

2.3. Limitations

Despite CRISPR extensive use in cancer treatment settings, the use of this technology still has some limitations and concerns that need to be addressed [9]. Induction of DSBs in the nuclease approach may cause large unplanned deletions and in some cases drive chromosome division, which may cause loss of tumor suppressors as well as compromise other normal cellular functions [10,11]. The p53 pathway may be activated as a result of DNA damage's secondary effects, which may then cause cell death or the selection of cells with diminished p53 pathway activity [12]. These limitations all raise concerns that CRISPR therapy may enrich for triggering cancer cells, but also note that there is no clinical evidence to support the idea that CRISPR would cause or promote the growth of cancer [8].

2.4. Comparison

The three common ways to treat cancer are chemotherapy, surgery and radiotherapy. Chemotherapy is the abbreviation of chemical drug therapy, which is the use of chemical drugs to prevent the increase of cancer cell infiltration, and metastasis, until finally kills cancer cells. Patients can choose to take oral or inject drugs, which can partly damage all the cells in the body, but work best when faced with rapidly dividing cells. When DNA is self-replicated, the drug disrupts the double helix of DNA, thereby killing the cells. Cancer cells reproduce rapidly. When a large number of chemotherapy drugs enter cancer cells, DNA is rapidly destroyed during the process of exposure, and it is difficult to repair itself, so cancer cells die at a much higher rate than other cells. Chemotherapy can also destroy tumor cells, but sometimes it can injure innocent normal cells, such as immune cells. At the same time, the toxic and side effects of chemotherapy are relatively many. Many clinical patients are not willing to choose chemotherapy because they cannot bear the toxicity of chemotherapy, such as loss of appetite, weight loss and hair loss after chemotherapy, etc., which leads to the decline of the quality of life due to the toxicity of chemotherapy. While CRISPR-Cas9 gene editing may permanently impair tumor survival genes, which could circumvent the recurrent administration restriction of conventional cancer medicines, increase therapeutic efficacy, and necessitate fewer therapeutic interventions [13,14]. This is not chemotherapy, with no side effects, and the cancer cells treated with this method will never be active again. In order to permanently stop cancer cells from duplicating, the molecular scissors of Cas9 will chop off the DNA of cancer cells [15]. The method is undoubtedly a promising option for treating human diseases that can fundamentally cure the disease. Immune cells have immune surveillance,

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recognition and killing ability for malignant tumors, using CRISPR -Cas9 technology to produce genetically engineered immune cells, enabling them to destroy cancer cells more efficiently, which has gradually developed into a hot field in modern cancer therapy.

3. The latest application of CRISPR/Cas9 technology in cancer treatment

CRISPR/Cas9-based CAR-T cell therapy uses gene editing to enhance the ability of CAR-T cells to kill tumor cells. The specific process involves culturing CAR-T cells in cell culture dishes, preparing CRISPR/Cas9 tools, importing the required DNA sequences into Cas9, introducing these tools into target CAR-T cells, and check gene editing efficiency. By PCR or sequencing test. Subsequently, the efficiency of tumor cell killing by CRISPR/Cas9-edited CAR-T cells is tested. So far, the challenge in treating tumor cells has mainly consisted of two aspects. Tumor heterogeneity and drug resistance. Tumor heterogeneity refers to the diversity and complexity of tumor cells in morphology, genetics, and function. Compared to normal cells, tumor cells have a more complex and diverse composition, and different tumor cell types respond differently to therapeutic agents. Therefore, tumor heterogeneity makes it difficult to establish a universal therapeutic regimen for the treatment of tumor cells, requiring individualized treatment to be performed according to specific circumstances. Another obstacle is drug resistance. With the application and development of drugs, an increasing number of tumor cells are developing resistance to certain drugs, which means that treatments are becoming less and less effective. Many mechanisms of drug resistance have been discovered, including dysfunction of the cell's apoptotic machinery, presence of tumor stem cells, and tumor cell invasion and metastasis. These mechanisms not only make tumors more difficult to treat, but often lead to treatment failure. Many new therapies such as targeted therapy, immunotherapy and gene therapy have emerged in this field, but they also have many shortcomings and imperfections. As a new gene-editing technique, CRISPR/Cas9 has some clear advantages over conventional therapeutics. First, CRISPR/Cas9 technology can achieve efficient gene editing. Conventional treatments often use chemotherapy drugs or radiation therapy to kill cancer cells.

However, these methods inevitably cause harm to human health during the treatment process. In contrast, specific genes in cancer cells can be directly edited by the CRISPR/Cas9 technique, avoiding damage to normal cells and achieving therapeutic goals by targeting problems present in tumor cells. increase. Secondly, with CRISPR/Cas9 technology, highly individualized treatment plans can also be achieved. Due to the high heterogeneity of tumor cells from individual to individual, conventional drug treatments are often ineffective. However, CRISPR/Cas9 technology can be customized to each individual's specific circumstances to effectively improve treatment outcomes. Furthermore, CRISPR/Cas9 technology exhibits a certain level of specificity. When treating tumor cells, it is necessary to target different subpopulations individually because tumor cells are heterogeneous. With CRISPR/Cas9 technology, more precise primers can be designed to edit specific genes and sequences, enabling more efficient and precise treatments. Finally, CRISPR/Cas9 technology has plenty of potential applications. CRISPR/Cas9 technology, which has a very broad variety of uses and market potential, can be used to treat genetic illnesses, generate new drugs, and improve precision medicine in addition to cancer therapy. In summary, CRISPR/Cas9 technology offers significant advantages in terms of accuracy, individualization, specificity and application prospects compared to conventional therapeutics. Continued advances in CRISPR/Cas9 technology could also solve the human cancer problem.

In a recent study, a group of researchers used CRISPR/Cas9 technology to edit two key genes, PDCD1 and LAG3, in CAR-T cells. These two genes encode immune checkpoint receptors that can suppress the activity of T cells when they bind with their ligands. By editing these genes, the researchers made CAR-T cells more sensitive to tumors and capable of killing more cancer cells. This study provides a more effective tool for CAR-T cell immunotherapy. Moreover, Joshua team have tried to treat liver cancer using CRISPR/Cas9 technology. Colorectal cancer and liver cancer are common malignant tumors caused by the uncontrolled proliferation and malignant transformation of some cells in the human liver tissue. Fortunately, research has already proven that these two disorders

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can be treated using CRISPR/Cas9 technology [16]. The following is the process of scientists successfully treating liver cancer using CRISPR/Cas9 technology: Step 1: Identify liver cancer-related genes. Through a huge number of tests and data analysis, researchers pinpoint the genes most closely associated with the onset and progression of liver cancer, and they prove that these genes are useful therapeutic targets for the disease. Step 2: Design CRISPR/Cas9 target sequence. Using the identified gene sequences linked to liver cancer, create CRISPR/Cas9 target sequences that can accurately cleave the target DNA sequence. Step 3: Synthesize and validate CRISPR/Cas9 tools. they synthesize the designed CRISPR/Cas9 target sequences and verify their effectiveness and specificity in liver cancer cells through in vitro experiments. Step 4: Construct vectors and prepare plasmids to integrate the synthesized CRISPR/Cas9 target sequences into an appropriate vector and prepare the plasmids. Step 5: Import CRISPR/Cas9 plasmids and treat liver cancer import the plasmids into liver cancer cells, use CRISPR/Cas9 technology to cleave the gene DNA sequence related to liver cancer, and observe the therapeutic effect. In summary, the key to their successful treatment of liver cancer using CRISPR/Cas9 technology lies in the precise identification of liver cancer-related genes, designing effective CRISPR/Cas9 target sequences and vectors, and developing an effective treatment plan [17]. By editing genes related to liver cancer, they successfully inhibited tumor growth, demonstrating the high efficiency of CRISPR/Cas9 technology and indicating its potential for developing more effective liver cancer treatments. According to a different study, the technology behind CRISPR/Cas9 can be utilized to cure cancerous tumors like pancreatic cancer. With this technology, we seem to be gradually overcoming various types of cancer.

Similarly, Lihongyi team think that editing the Kras gene can help the human immune system better recognize and attack cancer cells. This research provides a new approach to treating pancreatic cancer. Recently, a multicenter clinical trial called "BEACONCRC" used CRISPR technology to develop personalized treatment plans for colorectal cancer patients. The study aims to use CRISPR/Cas9 technology to edit cancer-related genes and use immunotherapies such as CAR-T cells to achieve more precise and effective colorectal cancer treatment. In addition, Genetic illnesses including hereditary malignancies can be identified and prevented using CRISPR/Cas9 technology. Scientists successfully inhibited the growth of breast and ovarian cancer by editing the BRCA1 gene [16]. The BRCA1 gene encodes a protein that regulates DNA repair, maintains genome stability, and controls cell apoptosis. The BRCA1 gene's malfunction can occasionally result from mutations, which raises the risk of getting breast or ovarian cancer. Researchers modified the BRCA1 gene and restored its function using CRISPR/Cas9 technology to slow the spread of malignant tumors. Through this, they successfully suppressed the development of breast and ovarian cancer. The specific experimental process is as follows: - Obtain cancer cells from the patient for cultivation - Use CRISPR/Cas9 tools to select targets for the BRCA1 gene - Transfect these tools into the target cancer cells - Detect the gene editing effect using PCR or sequencing methods - Test the effects of BRCA1 gene editing on breast and ovarian cancer. This method provides a new direction for the treatment of hereditary cancers.

The use of targeted gene editing and gene expression regulation methods based on CRISPR/Cas9 will help develop more personalized and efficient treatment strategies [17]. Additionally, fields like tumor marker discovery and early cancer diagnosis can benefit from the use of CRISPR/Cas9 technology, and it is anticipated to play a crucial part in cancer treatment in the future.

4. Conclusion

This paper provides a quick overview of the CRISPR/Cas9 system's history and fundamental principles. It looks into the usage of the CRISPR/Cas9 system to treat ovarian and liver cancer. Finally, compare the benefits and drawbacks of using the CRISPR/Cas9 system to treat cancer to those of more traditional cancer therapies, such as chemotherapy.

The prospects for the application of CRISPR/Cas9 technology in cancer treatment are extensive. This technology can efficiently and accurately edit and regulate gene targets, helping to develop more personalized and efficient cancer treatment plans. In addition, the technology of CRISPR/Cas9 is also applicable to other fields, such as the identification of tumor markers, early cancer diagnosis, and

modification of CAR-T cells, bringing more possibilities for future cancer treatment. Thanks to its high efficiency and simplicity, CRISPR/Cas9 has greatly enhanced the development of cancer treatment research. However, off-target effects remain the biggest problem for this system, limiting its clinical application. Currently, studies have shown that by establishing a CRISPR paired nickase system, the specificity of the CRISPR/Cas9 system can be improved, reducing the occurrence of off-target effects. In conclusion, the use of the CRISPR/Cas9 system in the fight against cancer is essential., and it is anticipated that when the CRISPR system develops further in the future, cancer will be better avoided and treated.

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