Cancer Research Using Crispr/Cas9 Gene Editing Technology

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Abstract. Gene editing technology has completely transformed the life sciences and medical treatment, because it makes precise and stable genetic alterations to genomic sequences possible. Among gene editing technologies, CRISPR system is now the most employed one. CRISPR technology is currently often used in the medical field including cancer study as well as genome editing in a number of animals. Besides, the CRISPR system has been widely used in agriculture, industry and other fields, such as crop breeding, industrial microbial design, viral nucleic acid detection, etc. In the framework of people's knowledge of cancer research and genome editing technology, this paper focuses on the adhibition of CRISPR/Cas9 in cancer modeling, high throughput genetic analysis of tumor cell metastasis-related genes, as well as cancer treatment. It indicates that CRISPR/Cas9 techniques can be a promising tool in cancer diagnostics and therapeutics. Although CRISPR / Cas9 technology has limitations such as being off-target, this technology undoubtedly has great potential in cancer or other diseases in the future.

Keywords: CRISPR/Cas9, Cancer research, Modeling, Cancer treatment.

1. Introduction

One of the leading causes of disease-related death is cancer, and the prevalence of cancer is currently rising globally. Thus, cancer-related research has always been a hot topic. Meanwhile, scientists have made great progress and breakthroughs in the prevention and treatment of cancer, and some methods can even lead to prolonged survival or even cure. At present, cancer treatment mainly includes surgery, radiotherapy, chemotherapy and targeted drug therapy. Conventional surgery is to treat tumors by removing cancer-infiltrating tissue or expanding the scope of resection, but it is very likely that there are still residual tumor cells that have not been removed. Therefore, postoperative recurrence is prone to occur. Some tumor cells are sensitive to radiotherapy or chemotherapy, but the process of radiotherapy and chemotherapy also causes damage to normal cells. Although the adverse reactions of drugs can be reduced by targeted drug therapy, the drug resistance of tumors is easy to produce which will lead to cancer recurrence. As molecular biology improves by leaps and bounds, cancer immunotherapy has become a new way of tumor treatment in recent years. In the 1970s and 1980s, with the development of DNA recombination technology and gene cloning technology, gene therapy has shown a unique edge in therapeutic various major diseases. After decades of research, gene therapy has expanded from the initial treatment of single-gene genetic diseases to the treatment of infectious diseases, cardiovascular diseases, autoimmune diseases, and metabolic diseases, as well as malignant tumors. The emergence and improvement of gene editing have promoted the research progress of gene therapy greatly.

Up to now, gene editing technology has developed three generations over time. Zinc finger nuclease (ZFN) technology is the first generation; The second generation is comprised of transcription activator-like effector nucleases (TALENs), while the third generation includes the CRISPR/Cas system. In addition, the emergence of single-base gene editing technology (Base Editor, BE) in 2016 and the advent of Prime Editor (PE) in 2019 has pushed gene editing technology to a new climax. ZFN acts as a synthetic restriction endonuclease. The primary functional areas of ZFN are the DNA cleavage domain and zinc finger DNA binding domain. Researchers targeted different DNA

sequences by modifying the DNA binding domain, and then specifically cleaved by the DNA cleavage domain. In addition, ZFN technology is able to interact with the mechanism of intracellular DNA repair, allowing researchers to freely edit the genome. The advantage of ZFN technology is that it has high specificity, thus avoiding the immune response, but the high specificity has brought another disadvantage, that is, genetically engineered zinc finger DNA binding protein has become a problem. In addition, ZNF technology is easy to off-target, leading to cell death or additional mutations. The TAL effector (TALE) can combine with the target sequence specifically by connecting a segment of artificial TALE with FokI nuclease to form TALEN, a useful tool for specific genome editing. Because they can bind to DNA target sites with high specificity, the off-target effect is very weak. In addition, compared with ZFN technology, its design is not so complicated, and it is not as susceptible to the binding of the surrounding connection environment as ZFN. However, the assembly of TALEN coding plasmids is a lengthy and high-intensity work process.

In the genome of Escherichia coli, CRISPR were initially identified in 1987 and also proved to have the ability to modify genes in living cells in 2012 [1]. After a period of time, the CRISPR/Cas system has since been optimized and expanded, among which, CRISPR/Cas9 and its modified versions have been used for genome engineering nowadays widely. CRISPR is an adaptive defense mechanism used by bacteria and archaea to combat external DNA and viruses. For further research, the system can be split into two categories. The first CRISPR / Cas's system exists in bacteria and archaea, and contains effector complexes is more than one Cas protein (usually 4-7 Cas protein subunits); the second CRISPR / Cas's systems are almost entirely confined to bacteria, and their respective multi-structural domain protein effector complexes [2]. CRISPR/Cas9 can modify the genome sequence of cells as well as introduce epigenetic and transcriptional modifications. There is no doubt that CRISPR/Cas9 has great prospects for cancer research.

2. Cancer modeling

2.1. Establish animal cancer modeling

Cancer is generated by the multi-step accumulation of mutations that confer certain typical biological characteristics to the malignant phenotype of transformed cells. One of the main purposes of cancer study is to characterize these mutations and evaluate their effects on carcinogenesis [3]. After analyzing the tumor cell genome sequencing data, numerous gene mutations related to cancer have been found and the establishment of animal cancer models (such as mice) carrying mutant genes is an efficient approach to studying the features of these mutations. However, the conventional ways to establish genetic modification animal model is not only time-consuming but laborious intensive. To destroy PTEN and P53, researchers have injected the CRISPR system into adult mice's tail veins, which are two important tumor suppressor genes. After that, the liver tumor model of adult mice was successfully established [4]. However, the above experiments should be paid attention to about how the plasmid can reach the cells of specific argan to play an effective role. Besides, T-cell acute lymphoblastic leukemia (T-ALL) zebrafish model was established and reported for the first time by placing the mouse gene Myc open reading frame under the control of the zebrafish rag2 promoter [5]. The model established is able to realize specific multi-gene knockout in this study and study the effect of multi-gene interaction on lung cancer phenotype, and more accurately study the mechanism of cancer occurrence.

Tumorigenesis is the result of a combination of mutations in multiple genes. In addition to changes in the above-identified cancer-related genes, one challenge facing cancer research is how to recognize key mutations that lead to cancer. In order to figure out the correlation between the deficiency of gene function and the phenotype of tumor growth and metastasis, measure the sequence of DNA and gene knockout mediated by CRISPR / Cas9 have been combined. In each of these tests, the impact of mutation on the growth of the initial tumor was positively linked with the rate of metastasis. The study shows that screening based on Cas9 is a reliable means for systematically determining the genetic phenotype of cancer evolution in vivo. The relationship between genes and cancer can be

better reflected by this high-throughput sequencing for excavating disease-related genes. The interaction of multiple genes on phenotypes and their corresponding relationships can be revealed as well.

2.2. Modeling chromosomal rearrangements in cancer

Chromosomal rearrangements refer to changes in the position of chromosomal segments, including chromosomal deletions, duplications, inversions, and ectopics. Chromosomal rearrangements have the potential to induce cellular carcinogenesis, which means implies that in cancer cells, the presence of chromosomal rearrangements (e.g., translocations, inversions or deletions) may occur with reorganization of nuclear structure and repositioning of genes may alter their expression, such as the formation of human lymphomas and leukemias due to chromosomal rearrangements [6]. Using traditional methods to produce cancer models of chromosomal rearrangements requires the addition of loxP sequences at two rearrangement sites to produce chromosomal rearrangements by Cre-loxP recombination, which is a site-specific recombinase technology used to perform deletion, insertion, transposition, and inversion operations at specific sites in cellular DNA. The feature is that it allows DNA modification to be performed on specific cell populations or to have the modification process triggered by a specific external stimulus. However, generating chromosomal rearrangements using CRISPR/Cas9 technology is very simple, requiring only targeted cleavage of two rearrangement sites. Therefore, CRISPR/Cas9 technology will greatly reduce the cost and time required compared to traditional methods, which will help researchers to model chromosomal rearrangements. Human chromosome 2 rearrangement leads to the expression of EML4-ALK fusion and triggers non-small cell lung cancer. Some researchers used virus-mediated CRISPR/Cas9 technology to edit rearrangements in adult mouse somatic cells and rapidly established a mouse model for lung cancer caused by the fusion gene EML4-ALK [7]. Such models provide a very effective platform for scientists to study the mechanisms of cancer development in depth and to screen antitumor drugs at the animal level.

3. High throughput genetic analysis of tumor cell metastasis-related genes

The search for mutations that cause cancer is a difficult task in cancer research, because tumorigenesis is the result of several genetic abnormalities acting together. In this case, high throughput screening (HTS) can be of great help in finding the key mutations, which is based on experimental methods at the molecular and cellular levels and is characterized by small amounts, rapidity, sensitivity and accuracy. In short, it means that a large amount of information can be obtained through a single experiment, and then the researcher can find valuable information from it. Feng Zhang's lab collaborated with Sharp's team to perform a high-throughput random knockout of a gene in a non-metastatic lung cancer cell line using a CRISPR/Cas9 library. These cells were then transplanted into immunodeficient mice, where some cells left their original location and migrated with blood vessels to form highly metastatic tumors. The sgRNA sequences of metastatic tumors were examined for numerous genes involved in cancer development and spread as well as microRNA. Thus, CRISPR/Cas9 library technology provides a novel approach to search for important genes that cause cancer cell migration.

4. Cancer treatment

4.1. CRISPR for gene therapy

CRISPR / Cas9 has two ways to treat cancer. One is to directly attack key genes in cancer cells, and the other is to edit immune cells and then attack cancer cells through immune cells. Targeting MCL-1 kills Burkitt lymphoma cells driven by the transcriptional regulator c-MYC, so a large number of lymphoma cells can be found to die after knocking out the MCL-1 gene in mice by CRISPR / Cas9. Some viruses can cause cancer, such as Epstein-Barr virus (EBV) infection can lead to Burkitt

lymphoma. Knockout of EBV by CRISPR/Cas9 can significantly inhibit tumor proliferatio [8]. The experiments have shown that oncogene knockout can be used as a potential cancer treatment.

One application of CRISPR / Cas9 in cancer treatment is to edit CAR-T cells, which can attack tumor cells [9]. Among the enrolled patients with non-small cell lung cancer, more than 50 % of the patients were infused with PD-1-edited T cell and found that it had a certain effect and no one had serious adverse reactions related to treatment. This shows that it is safe and feasible in general. All these indicate that CRISPR has unlimited potential in the field of cancer treatment.

4.2. Screening for cancer drug targets using CRISPR /Cas9 technology

Under continuous phage selection pressure, bacteria developed the CRISPR system as a powerful acquired immune defense against exogenous DNA invasion. CRISPR together with Cas's genes form a highly conserved system in which a single-stranded guide gene (single guide RNA, sgRNA) and a Cas9 protein with nucleic acid endonuclease activity make up the CRISPR/Cas9 system, which recognizes specific DNA sequences carried on the sgRNA by the target sequences carried on sgRNAs recognize specific DNA sequences and can repair mutated sites or achieve functional deletion of important genes [10]. The identification of novel therapeutic targets is currently one of the primary objectives of cancer research. Genome sequencing and CRISPR genome editing are being used in conjunction to find mutant targets. However, if the mutation sites are in a non-coding region or at the 5' end of an exon, it is possible that even if a mutation occurs and will not have an impact on the cancer phenotype, even though the function of the gene may be altered [11]. Shi et al. (2015) enhanced this approachby using the CRISPR system to screen for cancer-related drug targets [12]. His team created CRISPR target sequences that corresponded to exonic regions of genes encoding functional structural domains of proteins, ensuring that mutations occurred in sequences encoding key structural domains. As mutations occurred in regions encoding critical structural domains, proteins lost these structures. This approach was used to screen 192 chromatin-regulated regions of mouse acute myeloid leukemia cells and identified 6 recognized and 19 potential therapeutic targets. This strategy is critical because it is a powerful tool for selecting and analyzing drug targets to help develop cancer therapies.

5. Conclusion

This article describes the adhibition of CRISPR / Cas9 in cancer animal modeling, high-throughput analysis of tumor cell metastasis-related genes, and cancer gene therapy and cancer drug screening. It is clear that the two key factors of cancer gene therapy are gene specificity and safety. Although gene therapy drugs and technologies are entering the clinical stage, there are still aspects of the molecular mechanisms of cancer that are not yet understood, and the existing gene therapy methods still need to be further investigated in terms of expression stability, targeting and long-term safety. Future research will most likely focus on potential off-target issues, as the specificity of the CRISPR system is determined by base pairing. However, it is believed that with the development and improvement of various gene editing technologies, researchers will have more effective tools to carry out basic life science and human disease research. It is also hoped that in the near future, gene editing technologies will bring revolutionary changes in the treatment of hereditary illnesses and cancer.

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