Application of CRISPR-Cas 9 system in cancer therapy

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Abstract. There are several gene editing technologies under the background of that time, such as ZFN and TALEN which are hard to use and have high cost. On the contrary, the efficiency and accuracy of CRISPR-Cas system has attracted the attention of scientists. CRISPR is a Short palindromic repeat sequence, and it is widespread in many prokaryotes. The first CRISPR was cloned in E. coli by scientists in 1987, and several other sequences were subsequently cloned. The Cas is a nuclease, then two of them cooperate to become the CRISPR-Cas system. It is not only an acquired immune defense mechanism of prokaryotes against virus, but also a tool for gene editing. As an emerging gene editing technology in recent years, CRISPR-Cas9 technology has been widely applied to the treatment of a variety of diseases. This review focused on the application of this technology in cancer. This review systematically introduced the working principle and immune mechanism of CRISPR-Cas9 and compared CRISPR-Cas9 technology with two previous generations of gene-editing technology, current application of CRISPR-Cas9 in cancer treatment, elaborating it with relevant examples, and finally pointed out the challenges of CRISPR-Cas9 in cancer treatment, looking forward to the development of this technology in the future. The purpose of the paper is to introduce and popularize CRISPR-Cas9 technology, and to provide a direction for future cancer treatment.

Keywords: CRISPR-Cas9; cancer; gene therapy; immunotherapy; CAR T-cell therapy.

1. Introduction

Cancer is a genetic disease caused by epigenetic aberrations; it has a high incidence. Cancer is the most common cause of death worldwide. The data from the Global Cancer Observatory illustrates that cancer kills millions of people around the world every year. On the basis of the World Health Organization, 19.3 million people have cancer in 2020, and nearly 10 million deaths are caused by cancer. The pathogenesis of cancer is mainly caused by genetic mutation, combined with some induced factors, which will lead to the massive spreading and metastasis of these originally few cancer cells [1], in order to the growth of the tumor, the cancer cells in the body will continue to take those healthy cells under their control. Regarding the factors that induce cancer cells, the first is the external environmental factors, such as some radioactive substances, special toxic substances, chemical pollution, viruses, etc. The second is the genetic factors of the family, as well as the self-factors [2]. Cancer is characterized by high mortality, low early diagnosis rate, low curative rate, etc., and conventional treatments, such as surgery, radiotherapy and chemotherapy, may cause radiation damage, drug toxicity, and other adverse effects, even leading to death, which is a major challenge for modern medicine. CRISPR-Cas9 as a burgeoning technology has offered another direction for cancer treatment, and its ability to locate genes efficiently and accurately has contributed to the exploration of cancer causative mechanisms and the study of therapeutic methods.

2. Mechanism of action of CRISPR-Cas9

It is common to see CRISPR-Cas9 system in archaea and bacterial genomes, and it is used to defend bacteria against foreign viruses and plasmids. CRISPR-Cas9 genome editing system consists of Cas9 endonuclease and gRNA, and Cas9 nuclease cleaves the conformational domains with HNH and RuvC activity in the double-stranded DNA sequence to break it. gRNAs are chimeric RNAs formed by the binding of tracrRNAs to crRNAs, the shorter of which is known as sgRNA, and are

able to recognize the PAM in the sequence, which is the motif adjacent to the specific binding sequence. Cas endonuclease cuts the double-stranded DNA three bases upstream of the PAM motif, and, after it has produced a nick, the after the gap is created, repairing it is typically done by NHEJ or HDR. The extraneous DNA fragments are consolidated into the CRISPR repeating spacer array in the host chromosome through the mechanism of action at first, followed by the production of crRNA at the 5' end and CRISPR repeating sequences at the 3' end, which binds to the tracrRNA to form a short RNA, sgRNA, which recognizes the PAM sequence, and Cas9 endonuclease cuts the double-stranded DNA to form a break gap [3, 4].

3. CRISPR-Cas immune mechanism

Some bacteria and archaea have the function of the complex adaptive immune system [5]. CRISPR and the relevant Cas genes work together and form adaptive immune to against the phage infection. The CRISPR-Cas system has about 40% genomes related to bacteria and archaea [6]. The CRISPR-Cas immune mechanism includes three stages, and they are adaption, maturation and interference. The first stage aims at getting the foreign DNA. When the bacteriophage invades the cell, its DNA will be exposed, and at the same time, the CRISPR-Cas system will transcribe and translate the Cas1-Cas2 protein complex, which will recognize the PAM and its proto-spacer as the spacer sequence. Then cut the spacer sequence and insert it into CRISPR sequence .The second stage will synthesize a mature complex which has an ability to cleave nucleic acid. Under the control of CRISPR's leader region, the pre-crRNA, the tracrRNA and Cas9 protein are produced. They become a complex. Formed into small crRNA under NaseIII's processing. The last step is targeting interference, a process that the complex will strike the invading DNA precisely. The complex will scan the exogenous DNA sequence, the pre-spacer sequence that complements crRNA will be recognized during this process [5]. The PAM is located and the DNA double strand is unwound, afterward the crRNA will hybridize with one of the free DNA chains. In the end, the Cas9 protein will cut the target location.

4. Comparison of three generations of gene editing technologies

The main advantages of ZFNs are strong targeting and easy in vitro delivery. However, it is relatively difficult to assemble structures with high affinity of zinc finger structural domains to long nucleotides, with limited target selection and large off-target effects. Compared with ZFNs, TALENs are easier to design and construct, can be made in large quantities in a short period of time, and TALE repeat sequences can even bind with high affinity to the desired DNA sequence. TALE repeat arrays can be easily extended to any length required, often binding longer sequences than ZFNS. At the same time, there are fewer restrictions on the selection of target sites. But there are also some disadvantages, such as off-target effects, and TALENs volume is larger, can affect the efficiency of transfer and expression in cells, therapeutic utility ratio has also fallen. The high repeatability of TALENs also affects the efficiency of their transport by vectors and has a certain degree of cytotoxicity. Compared with ZFNs and TALENs, CRISPR-Cas9 does not require large DNA fragments for each new target site. The technology is simpler and more operable. Secondly, CRISPR-Cas9 is more flexible in the selection of target sites than ZFNs and TALENs, and the efficiency of gene editing is higher. However, there are also some defects, such as the size of Cas9 protein, which is much larger than TALENs, greatly affecting the transport of vectors to it, low cell adaptability, and off-target effects [7].

5. CRISPR-Cas9 in Cancer Therapy

Cancer therapy has been heavily influenced by the study of CRISPR-Cas9 in recent years, and also has many applications in mediating drug targets, solving drug resistance problems and constructing cancer models [8]. The primary focus of this paper is on the use of CRISPR-Cas9 in cancer therapy,

and it categorizes treatment methods into gene therapy and immunotherapy, and explains them with specific research examples.

5.1. Gene therapy

Gene therapy refers to the process of curing cancer by editing or knocking out specific genes to inhibit tumor growth. Oncogenes, also known as tumor suppressor genes, have important roles in normal cells to regulate cell growth and inhibit tumorigenesis. Silencing, deletion and mutation of such genes often lead to cancer development and contribute to cancer progression [9], and restoration of oncogene activity or corrective repair of such genes is expected to be therapeutic for cancer. Overexpression of Myc genes has been associated with several types of lymphomas, and studies have shown that it has been possible to successfully edit Trp53, an oncogene in Arf $^{-/-}$ μ-Myc lymphomas, for treatment of the associated lymphomas [10]. PTEN is another oncogene, and Moses et al. effectively enhanced PTEN expression in melanoma and triple-negative breast cancer cell lines by using CRISPR/dCas9 in combination with the transactivating factor VPR in cancer cells with low expression of PTEN to inhibit its oncogenic effects [11]. Valletta's report noted that CRISPR-Cas9-mediated ASXL1 mutation-corrected leukemia cell xenograft mice showed a significant prolongation of survival compared to uncorrected xenograft mice [12]. In addition to inhibiting cancer development through the manipulation of oncogenes, knockdown or editing of certain specific genes can also serve as an antitumor.YES1 is utilized to regulate cell growth, survival, and apoptosis, and is elevated in lung cancer. Non-small-cell lung cancer resistance to dasatinib can be effectively reduced by knockdown of YES1 by CRISPR-Cas9 [13]. Furthermore, removal of the epidermal growth factor receptor allele by CRISPR-Cas9 significantly inhibited the growth and proliferation of lung cancer cell lines H1975, A549 and H1650 [14]. The results of the study have been able to demonstrate that targeting HPV E6 and E7 ontogenesis using CRISPR-Cas9 can enhance the effectiveness of radiation therapy for the treatment of cancer [15].

5.2. Immunotherapy

Immunotherapy mainly refers to the enhancement of the body's immune system to attack tumor cells in order to inhibit the development of cancer. T-cells have a significant role in recognizing and killing tumor cells. CAR is a modular fusion protein. Chimeric antigen receptor (CAR) T-cell therapy is a treatment that transfers CAR genes into specific T cells that are isolated and enriched from the patient's body, and then activates CAR-T cells to destroy tumor cells by genetically modifying T cells to express specific receptors that recognize tumor antigens [16]. CAR-T cells, the development has been the five dynasties. CRISPR-Cas9 is used in the fifth generation of CAR-T cells, which Miss TRAC (TCR) [17]. Insertion of CAR into the TRAC promoter can stably drives CAR expression, and simultaneous knockdown of TCR eliminates the effects of GvHD [18]. T-cells modified by CAR can recognize CAR-targeting antigens to promote their proliferation, cytokine production, and toxicity against tumor cells [17]. The MSKCC research group has constructed CAR-T cells that precisely kill cancer cells for a long period of time [19], this innovation for T-cell improvement is a breakthrough for removing cancer cells.In addition to enhancing the effectiveness of T cells, lifting the inhibition of T cells is another way to facilitate the immune system to attack cancer cells. PD-L1 is an immunosuppressive molecule, which prevents autoimmune diseases by appropriately suppressing the immune system under normal conditions. While the activity of T-cells are inhibited by interaction between PD-L1 expressed in tumor cells and PD-1 on T-cells, promoting their apoptosis to weaken and evade the attack of the immune system. The results of using CRISPR-Cas 9 editing PD-1 of T cells in human clinical experiments in patients with advanced non-small cell carcinoma reported by lu et al. proved its safety and feasibility [20].

6. Conclusion

According to research, this review focuses on the use of the CRISPR-Cas9 in cancer therapy, expanding the description of gene therapy and immunotherapy. Despite the edges and potential of the CRISPR-Cas9, it still faces certain challenges. In clinical application, its safety and whether its effect is significant still needs to be solved such as its efficiency, the methods of transportation to the target location and the edit cell's adaption. The cancer is fundamentally a genetic level disease. And from this review, the use of immunotherapy may achieve better treatment results. As CRISPR technology advances, the number of living examples of CRISPR-Cas9 used in cancer will increase, and the technology will become more efficient and safer.

Authors contributions

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

References

[1] Hausman, Daniel M. "What Is Cancer?" Perspectives in Biology and Medicine, vol. 62 no. 4, 2019, p. 778-784.

[2] Roy, P S, and B J Saikia. "Cancer and cure: A critical analysis." Indian journal of cancer vol. 53,3(2016): 441-442.

[3] Ma, Yuanwu et al. "Genome modification by CRISPR/Cas9." The FEBS journal vol.281,23(2014):5186-93.

[4] Jiang, Fuguo, and Jennifer A Doudna. "CRISPR-Cas9 Structures and Mechanisms." Annual review of biophysics vol. 46 (2017): 505-529.

[5] Hryhorowicz, Magdalena et al. "CRISPR/Cas9 Immune System as a Tool for Genome Engineering."Archivum immunologiae et therapiae experimentlis vol. 65,3 (2017): 233-240.

[6] Amitai, Gil, and Rotem Sorek. "CRISPR-Cas adaptation: insights into the mechanism of action." Nature reviews. Microbiology vol. 14, 2 (2016): 67-76.

[7] Gupta, Rajat M, and Kiran Musunuru. "Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9." The Journal of clinical investigation vol. 124,10 (2014): 4154-61.

[8] Bhat, Ajaz A et al. "Integration of CRISPR/Cas9 with artificial intelligence for improved cancer therapeutics." Journal of translational medicine vol. 20,1 534. 18 Nov. 2022,

[9] Morris, Luc G T, and Timothy A Chan. "Therapeutic targeting of tumor suppressor genes." Cancer vol. 121,9 (2015): 1357-68.

[10] Malina, Abba et al. "Repurposing CRISPR/Cas9 for in situ functional assays." Genes & development vol. 27,23 (2013): 2602-14.

[11] Moses, Colette et al. "Activating PTEN Tumor Suppressor Expression with the CRISPR/dCas9 System." Molecular therapy. Nucleic acids vol. 14 (2019): 287-300.

[12] Valletta, Simona et al. "ASXL1 mutation correction by CRISPR/Cas9 restores gene function in leukemia cells and increases survival in mouse xenografts." Oncotarget vol. 6,42 (2015): 44061-71.

[13] Garmendia, Irati et al. "YES1 Drives Lung Cancer Growth and Progression and Predicts Sensitivity to Dasatinib." American journal of respiratory and critical care medicine vol. 200,7 (2019): 888-899.

[14] Koo, Taeyoung et al. "Selective disruption of an oncogenic mutant allele by CRISPR/Cas9 induces efficient tumor regression." Nucleic acids research vol. 45,13 (2017): 7897-7908.

[15] Zhen, Shuai, and Xu Li. "Oncogenic Human Papillomavirus: Application of CRISPR/Cas9 Therapeutic Strategies for Cervical Cancer." Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology vol. 44,6 (2017): 2455-2466.

[16] Dastjerd, Niloufar Tavakoli et al. "Gene therapy: A promising approach for breast cancer treatment." Cell biochemistry and function vol. 40,1 (2022): 28-48.

[17] Razeghian, Ehsan et al. “A deep insight into CRISPR/Cas9 application in CAR-T cell-based tumor immunotherapies.” Stem cell research & therapy vol. 12,1 (2021)428. 28.

[18] Dimitri, Alexander et al. “Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing.” Molecular cancer vol. 21, 1(2022): 78. 18.

[19] Eyquem, Justin et al. “Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection.” Nature vol. 543,7643 (2017): 113-117.

[20] Lu, You et al. “Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer.” Nature medicine vol. 26,5 (2020): 732-740.