Application of CRISPR technology in PD-1

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Abstract. Cancer has been a problem that has plagued human beings for many years. The emergence of immunotherapy that antagonizes antibodies against programmed cell death protein 1 (PD-1) has opened up new ideas for the treatment of different cancers, and the application of CRISPR technology on PD-1 provides new ideas for cancer treatment. However, its potential therapeutic functions and methods have not been fully explored. In this paper, the application of CRISPR technology in PD-1 and more derivative applications will be explored through experiments that study CRISPR-related applications in PD-1. In the application of CRISPR technology on NV, modification of T cells, destruction of PD-1 and HPV combined therapy, verification of the safety of CRISPR technology and other experiments, it has been found that new means can be used to deliver corresponding cells efficiently and accurately, and it can be concluded that CRISPR technology can effectively improve PD-1 therapy. This can be used to improve treatment effectiveness, feasibility, and safety.

Keywords: CRISPR, PD-1, cancer, antibody immunotherapy.

1. Introduction

In today's society, cancer has become an important medical problem affecting human beings. Up to now, there have been about 35 million confirmed cancer cases and nearly 10 million people die of cancer in the world, which shows that cancer has seriously affected human life and health. The main treatment methods for cancer include targeted therapy, surgery, radiation therapy, chemotherapy, traditional Chinese medicine therapy and so on. Although these methods have achieved good therapeutic effects in cancer treatment, there are still some shortcomings in these treatment methods. For example, these treatments are expensive, harmful to patients, long and ineffective. To this end, finding accurate and efficient ways to treat cancer has become a hot topic.

Since the discovery of programmed cell death protein 1 (PD-1) in mice, and this substance promoted the development of cancer treatment methods. So far, the appearance of antibody immunotherapy against PD-1 has opened up new ideas for the treatment of a diverse of various cancers. The appearance of this method has improved the harmful effects of chemotherapy before. However, the existing treatment methods for PD-1 still have some defects, such as side effects, technical difficulty. These shortcomings have led to the lack of widespread application of treatment methods. CRISPR technology is today's hot "gene scissors", since the 1990s, this technology has been applied in many aspects, to promote the development of many medical means, but also to help solve many medical problems.

CRISPR applications on PD-1 antibody immunotherapy to improve the function of PD-1 protein and improve the therapeutic effect has become a new direction for the research and treatment of cancer. In the present study, by using CRISPR technology to test whether Cas9 RNP-mediated destruction of endogenous Pdcd1 site in primary human CAR T cells can enhance the anti-tumor effect, CRISPR/ Cas9-mediated destruction of PD-1 can enhance the anti-tumor effect of human chimeric antigen receptor T cells [1]. In this study, PD-L1 expression was found to reduce the function of CAR T cell leading to impaired tumor clearance in subcutaneous xenograft models. To avoid the inhibited antitumor response, a combination of Cas9 ribonucleoprotein (Cas9 RNP)-mediated gene editing and lentiviral transduction was developed to generate PD-1-deficient anti-CD19 CAR T cells. This is a practical case for using CRISPR technology to improve PD-1 technology. At the same time, CRISPR technology can be seen to ameliorate the anti-tumor efficacy of PD-1 and effectively genetically manipulate human primary T cells, while opening up new avenues for future research and

improved immunotherapies [1]. Meanwhile, studies of long-lived plasma cells that express alpha-PD-1 antibodies were conducted by using an IDLV delivery system to deliver Cas9/sgRNA and the corresponding donor template to human primary B cells, in which an HMEJ donor was constructed [2]. It contains guide RNA target sites on both sides of HA. To facilitate the evaluation of knock-in efficiency, the T2A-CD90 reporter gene downstream of the α-PD-1 gene can be analyzed, resulting in the co-expression of CD90 on the cell surface. The co-delivery of donor and Cas9/sgRNA-IDLV led to efficient and specific expression of the transgene after integration into the GAPDH locus. It improves the effectiveness of treatment and reduces the inefficiency of conducting B cells due to technology, and improves the microenvironment of a variety of immune cells and tumors [2]. There are also some clinical trials that prove the safety and feasibility of this technology, which provides the basic preparation for subsequent corresponding research and application. These findings provide new ideas for studying PD-1 regulatory mechanisms, screening targeted molecules, and identifying potentially sensitive patients. This paper will analyze the application development and improvement of CRIPSR technology in PD-1 protein by analyzing the current status and progress of CRISPR technology to transform key substances in different cancer treatment methods. This research can provide a new design approach for the development of new gene editing techniques in cancer treatment.

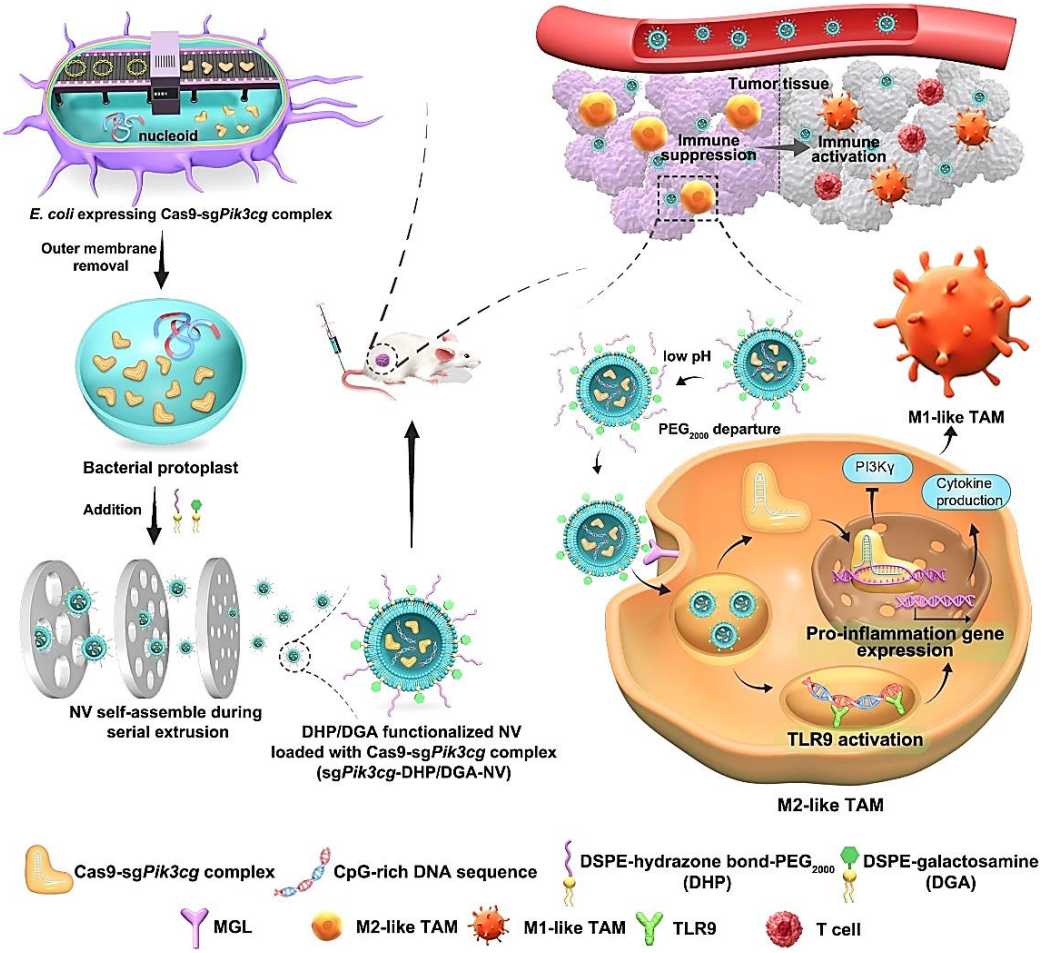

Figure 1. Schematic diagram of the prepared nanovesicles for improving the anti-tumor efficacy [3].

2. CRISPR-related applications in PD-1

Zhao et al. used nanovesicles (NVS) derived from bacterial protoplasts to achieve TAM targeting [3]. In the study, a gene-edited delivery system was developed to efficiently encapsulate Cas9-sgRNA RNP with functionalized NVS derived from *E. coli* protoplasts, as shown in Fig. 1. This approach

was able to reprogram tumor-associated macrophages (Tams) and demonstrated the efficacy of tumor therapy. Targeted NVS edited by CRISPR technology are loaded with two key components: Targeting Pik3cg's Cas9-sgRNA ribonucleoprotein and CPG-rich bacterial DNA fragment, which allowed it to reduce cancer cell elasticity and proliferation and improve apoptotic immunity of cancer cells in cancer therapy. This technology demonstrates CRISPR technology's application in the treatment of cancer at the NV level, eliminating the immunosuppressive and fibrotic barriers in the tumor microenvironment, and enhancing the efficacy of different therapeutic methods. It provides a new idea for the future combination therapy of oncology and is expected to become an effective method for the cure of cancer. Meanwhile, it also offers a new idea for the application of CRISPR technology in PD-1: using active nanovesicles to effectively encapsulate Cas9-sgRNA RNP so that it can be successfully delivered and activated corresponding cells to improve the function of PD-1. The disadvantage of this technique is the re-editing of relevant sites such as TAM reprogramming therapy surface based on PI3Kγ blocking.

The current treatment of PD-1 is achieved by blocking the mutual effect between PD-1 and PD-L1 [4]. First, 10 clinical trials of NSCLC and 9 clinical trials of other types of tumors were analyzed. The response rate to anti-PD-1 /PD-L1 therapy was evaluated and subsequently demonstrated by the application of CRISPR technology that PD-L1 K162 methylation limits the interaction of PD-L1 and PD-1 and inhibits their immunosuppressive function. It was also verified that the methylation of PD-L1 K162 catalyzed by SETD7 was eliminated by abnormal acceptance of IL-6. Subsequently, a series of experiments demonstrated that SETD7-mediated anti-tumor immunity requires the methylation of PD-L1 K162. This experiment identified SETD7-induced PD-L1 K162 methylation as a key modification of PD-1 and PD-L1 interactions and that PD-L1 K162 hypermethylated tumors are resistant to PD-L1 and anti-PD-1 therapy, finding that PD-L1 is a non-histone substrate of SETD7. And PD-L1 K162 hypomethylation contributes to cancer immune surveillance [4]. This study shows that abnormal IL-6 stimulation significantly inhibits SETD7 expression and PD-L1 K162 methylation, and SETD7-mediated PD-L1 K162 methylation is not only a key step in PD-1/PD-L1 interactions, and represents a negative predictive marker for anti-PD-L1 and anti-PD-1 therapy. This study explores the immunopathogenesis and treatment resistance mechanisms associated with PD-L1, providing more possibilities for long-term survival in advanced patients and providing treatment evidence in patients at an early stage, as well as providing new means of treatment and new options for cancer screening. The shortcomings of this trial are the lack of a reliable anti-PD-1 treatment response biomarker, which leads to significant side effects and economic costs, the clinical benefit of most patients treated is not satisfactory, such a biomarker to predict patient response or harm is not fully predictive, the detection efficiency is low, and the therapeutic effect is not ideal.

The first human Phase 1 clinical trial can be used to test the safety and feasibility of multiple CRISPR-Cas9 gene editing on T cells of patients with advanced, refractory cancer [5]. By hypothesizing that removal of endogenous T cell receptor (TCR) and immune checkpoint molecule PD-1 would increase the function and persistence of engineered T cells. And removing PD-1 holds the potential to increase safety and decrease autoimmune-induced toxicity. This study demonstrated the safety and feasibility of using CRISPR-Cas9 technology. This is a typical clinical experiment that applies CRISPR technology to PD-1, which shows that T cells modified by CRISPR-Cas9 technology can implant at a high level and persist for a long time, and continue to transport to the tumor at a level close to that in the blood compartment, no chromosome translocation is detected in the experiment, and the occurrence of off-target editing is reduced. It promoted the evolution of NYCE cells. Improving the efficiency of treatment, have significantly improved the symptoms of patients, but also reduce the symptoms of adverse reactions. It is worth noting that one patient in this clinical trial had a tumor regression after treatment, which shows that this technology is expected to become a new way to cure cancer. However, this technology also exposed some of the shortcomings of this technology: CRISPR technology has a certain toxicity, the cells under this technology still need to prolong the life cycle, and more patients are still needed to verify the safety and feasibility of this technology.

The synergistic anti-tumor effect of PD-1 blocking and the sensible combination of CRISPR-Cas9-mediated HPV knockout on cervical cancer was studied, and the combination of immunotherapy and targeted therapy was attempted to explore a new way to treat cancer [6]. Targeting the application between HPV and PD-1 and CRSIPR technology, we disrupted immune checkpoint PD-1 and HPV by using CRISPR/Cas9 genome editing tools in a human SCID mouse model of SiHa cell xenotransplantation [6]. After a series of experiments, the corresponding signs and material data of mice were monitored, and data analysis showed that: CRISPR technology can mediate CC cells to efficiently knock out HPV while HPV 16E6/E7 can up-regulate PD-expression. In addition, in the experimental subjects of recombinant HU-PPL-SCID, mixed anti-HPV and anti-PD-1 CRISPR/Cas9 therapy can synergistically induce long-lasting anti-tumor effects. But in this experiment, it caused lymphocyte dysfunction, triggering immune escape of tumor cells and enhancing the disease.

3. Conclusion

Cancer has been a problem that has plagued human beings for many years. The emergence of immunotherapy that antagonizes antibodies against PD-1 has opened up new ideas for cancer cure, and the application of CRISPR technology on PD-1 provides new ideas for cancer treatment. However, its potential therapeutic functions and methods have not been fully explored. In this paper, the application of CRISPR technology in PD-1 and more derivative applications has been explored through experiments that study CRISPR related applications in PD-1. In the application of CRISPR technology on NV, modification of T cells, destruction of PD-1 and HPV combined therapy, verification of the safety of CRISPR technology and other experiments, it has been found that new means can be used to deliver corresponding cells efficiently and accurately, and it can be concluded that CRISPR technology can effectively improve PD-1 therapy and further to improve the therapeutic effect, feasibility and safety.

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