Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/17/20240655

Application of CRISPR/Cas9 technology in tumor targeted therapy

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Abstract. Cancer has always been one of the major diseases affecting human health, which is characterized by genomic instability, high individual heterogeneity and inhibitory tumor microenvironment. Epidemiological studies have found that the standardized rates of various new malignant tumors have increased significantly in recent years, while the traditional therapeutic methods are easy to cause serious tissue damages, high recurrence rate and other postoperative complications. CRISPR/Cas9 system is a defense mechanism of archaea. It can recognize and integrate invasive DNA into its own genome through three steps: acquisition, expression and interference. When invaded by the same antigen, it can quickly recognize foreign DNA and specifically cut it, thus playing an immune defense role. This biological mechanism was originally mainly used for gene function identification. With the rapid development and interdisciplinary integration of immunology, genomics and clinical medicine, more and more studies have found that CRISPR/Cas9 system combined with targeted therapy can assist immunotherapy, gene therapy and target screening at the genetic and molecular levels, and has high editing efficiency, which has great prospects in the field of tumor treatment. Therefore, the objective of this review is to explore the application of CRISPR/Cas9 technology in adoptive immune cell therapy, tumor gene therapy and targeted gene screening, so as to provide reference for its efficient application in tumor targeted therapy.

Keywords: CRISPR/Cas9, Tumor, Targeted Therapy.

1. Introduction

Tumors are formed by the abnormal proliferation of local tissue cells due to the action of various carcinogenic factors, which can be classified as benign and malignant ones [1]. Genomic instability, point mutation accumulation and structural changes are the characteristics of tumors as genomic diseases [2]. The epidemiological analysis of clinical cases of malignant tumors in tertiary hospitals in China showed that from 2014 to 2021, the standardized rates of colorectal cancer and breast cancer increased significantly, and the increase rates of lung cancer, colorectal cancer, breast cancer and leukemia were all greater than 10%. In addition, the incidence of malignant tumors showed obvious gender and age preference, with the majority of malignant tumors in the elderly. The incidence of male was significantly higher than that of female (58.93%) [3,4]. Around 19.3 million new diagnoses of cancer and 10 million cancer-related mortality were reported worldwide in 2020, which was about 14.73 and 7 million more than that in China [5]. Therefore, the search for more reasonable and efficient tumor treatment methods has been a major problem to be overcome in the fields of public health and medicine.

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Cancer treatment has been discovered in a variety of ways at the moment. Traditional cancer treatments consists of surgery, radiotherapy and chemotherapy. Although they can inhibit the onset and progression of tumors to a certain extent, the recurrence rate remains high. Radiotherapy and chemotherapy are also easy to cause tissue damage and have many toxic side effects, which have many limitations in practical application [6-9]. Targeted therapy is a new type of tumor treatment after traditional chemotherapy. Through computer molecular screening and chemical synthesis, highly effective drug molecules are designed to act on specific targets of tumor cells or immune cells, and then induce tumor cell apoptosis and improve the killing of immune cells. Compared with traditional treatment methods, targeted therapy has high efficiency, high specificity and low toxic side effects [10]. However, due to individual heterogeneity, targeted therapy drugs cannot produce therapeutic effects on patients with target deletion or produce many adverse reactions after medication, which limits their large-scale application in the course of treating solid tumors [11].

With some further researches undergoing, more researchers have discovered that CRISPR/Cas9 is widely favored in various disease treatments due to its low cost, simple operation and high efficiency [5]. It is a defense mechanism found in bacteria and archaea which has a significant impact on adaptive immunity by eliminating foreign phage and plasmid DNA through three stages: acquisition, expression and interference. Based on their composition and action mechanism, Type I, II, III are the three types of this system [12]. The type II CRISPR/Cas9, in contrast to the other forms, uses one specific endogenous Cas9 protein as its trigger and only requires the synthesis of fresh RNA in order to carry out gene editing. Since it is simple to design and highly specific, this technology has currently been the most commonly utilized one in gene editing, including Cas9, crRNA and tracrRNA [13]. When invaded by a phage or a foreign gene, the Cas protein of the bacterial genome recognizes and cleaves the protospacer sequence of the invader through the PAM sequence, and insets it between its own leader sequence and the adjacent repeat sequence to form a new spacer sequence, so when the bacteria are invaded again by the same foreigner, the CRISPR/Cas9 system can specifically cut off the genome, make it become linear and unable to replicate and express, and then degrade it by enzymes in the bacterial body, so as to achieve immune defense.

Due to the rapid development of genomics and the progress of medical treatment, our understanding of tumors gradually deepened from the cellular level to the molecular level. After CRISPR/Cas9 system was applied to tumor treatment, studies found that the participation of this specific immune defense mechanism could not only optimize immunotherapy, but also be used for tumor target screening or inducing tumor cell apoptosis at the genomic level, which has paved the way for significant prospects tumor therapeutics [12].

2. Immunotherapy

2.1. Adoptive immune cell therapy

Tumor immunotherapy is defined as the treatment of tumors by artificially enhancing or inhibiting the body's immune function when it is not functioning properly (immunodeficiency or hyperimmunity). It mainly includes adoptive immune cell therapy, immune checkpoint monoclonal antibody therapy, tumor vaccine therapy, and non-specific immune stimulation therapy (cytokine therapy). Adoptive Cell Therapy (ACT) is considered to be the most potential therapy in tumor therapy. By harvesting immune cells (often T cells) from the patient, expanding and identifying them functionally in vitro, followed by reintroducing them into the patient's body, it primarily achieves the goal of attacking tumors or provoking the immune system to fight back cancerous cells [14-16]. ACT, which includes T cell receptors T (TCR-T) and chimeric antigen receptor T (CAR-T) cell treatment, is now the most developed and popular immune cell therapy. It exhibits good targeting features both in vivo and in vitro [17]. In order to enhance the tumor killing ability of lymphocytes, according to studies, the combination of CRISPR/Cas9 with cell therapy can dramatically enhance the T lymphocytes' capacity for tumor cell detection and killing in patients, leading to effective tumor treatment [18].

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2.2. CRISPR/cas9 Utilized in CAR-T immunotherapy

CAR-T immunotherapy combines single-chain antibodies (scFV) that perceive tumor-related antigens as well as T lymphocyte mobilization motifs and transducts gene into T cells to endow them with tumor targeting, enhanced killing activity and long-lasting killing through the bonding of the high affinity of antibodies for tumor antigens with the killing mechanism of T lymphocytes. The therapeutic effect of highly efficient cancer cell recognition and killing is achieved by transfusing these cells back to the patient, which has played an unprecedented role in the management of hematological malignancies [19]. Nevertheless, the large-scale application of CAR-T therapy is limited by the time-consuming and expensive T cell personalized manufacturing methods, as well as the difficulty in producing enough high-quality T cells in patients with lymphopenia and individual heterogeneity. Therefore, the generation and functional enhancement of universal CAR-T cells have become the focus of research. With the progress of gene editing technology, CRISPR/Cas9 technology has been widely used in allogeneic CAR-T cell therapy due to its well flexibility and high efficiency.

Many studies have reported that CRISPR/Cas9 can simultaneously knock out the endogenous TCR and HLA molecules, so as to generate allogeneic cells for CAR-T therapy. Ran used CRISPR/Cas9 technology to target knockout of endogenous TCR, HLA-1, and PD-1 CAR-T cells, which showed significant anti-tumor activity in vitro and in animal models [20]. Xuhua Zhang used the high-fidelity Cas9 mutant eSpCas9 to target CD3 and HLA-1 at the same time, and successfully generated a universal CD3 and HLA-1 double negative CAR-T cell population, without finding off-target mutations [21].

In addition, targeted editing of immunosuppressive genes in tumor cells or immunosuppressive factors in the tumor microenvironment by CRISPR/Cas9 can effectively enhance the anti-tumor ability of immunocytes or inhibit the apoptosis of them. Programmed death 1(PD-1) is an important inhibitory immune checkpoint in the body, which can inhibit the immune activity of T cells after binding to PD-1 receptor (PD-L1), followed by immune escape of tumor cells. Hu et al. used CRISPR/Cas9 to knock out the PD-1 receptor and successfully block the interaction between PD-1 and PD-L1. The results showed that this method could significantly enhance the cytokine production of CAR-T lymphocytes and the cytotoxicity of them to malignant cells, with stronger tumor control ability and lower recurrence rate in vivo, providing a fresh approach to treating solid tumors [22]. Zhang Linlin used PiggyBac-transposase system and CRISPR/Cas9 system to stably express EGFRVIIICAR gene in human primary T cells, and specifically knocked out PD-1. They proved that PD-1 knockout could significantly improve the cytolytic activity of CAR-T lymphocytes towards glioma. In addition, after the targeted knockout of SHP-1 and CD133 CAR genes by CRISPR system, the tumor killing activity of CAR-T cells was significantly improved [23]. Similarly, CRISPR/Cas9 technology can also be used for targeted editing of immunosuppressive factors in the tumor microenvironment. Adenosine is a key immunosuppressive factor that accumulates in the tumor microenvironment under hypoxic conditions, which can inhibit immune responses after binding to A2AR receptor. Giuffrida deleted the A2AR gene locus with CRISPR/Cas9, which effectively blocked the adenosine-A2AR immunosuppressive pathway and enhanced the immunotherapy effect of CAR-T cells [24].

2.3. CRISPR/Cas9 utilized in TCR-T immunotherapy

Despite the importance of CAR-T immunotherapy in tumor treatments, its application in solid tumor treatments is limited due to the loss of cancer targets, tumor ag inhibition and the inhibitory action in tumor microenvironment. In contrast, TCR-T therapy is more generalized and versatile in tumor antigen recognition, and has shown greater potentiality in tumor remedy [25]. TCR-T therapy is achieved by screening and distinguishing TCR successions that can explicitly tie to the objective antigen, transferring them into fringe blood-inferred T lymphocytes (or heterologous T lymphocytes) by variation, and then transfusing them back to the patient to explicitly perceive and kill the tumor cells expressing the antigen, so as to achieve the purpose of treating tumors. However, due to the existence of exogenous and endogenous TCR molecules, it is easy to pair endogenous α chains with exogenous β chains or endogenous β chains with exogenous α chains in the treatment, which is responsible for the development of autoimmune diseases. In addition, endogenous α chains can also competitively bind to CD3 molecules,

DOI: 10.54254/2753-8818/17/20240655

which limits the formation of transgenic TCR/CD3 complexes and blocks its binding to MHC molecules on tumor cell surface, furthermore inhibiting the immune response of T-lymphocytes. Therefore, the formation and optimization of TCR molecules has always been a key issue to be solved in TCR-T cell therapy [23].

As CRISPR/Cas9 gene editing technology advances, more studies have revealed that it can not only specifically knock out the endogenous TCR gene, but also introduce synthetic tumor-specific TCR sequences, thereby significantly improving the targeted killing ability of T cells. Feng Juan et al. used CRISPR-Cas9 technology to knock out the TCR gene in CD8+T cells and Jurkat cells, and expressed the TCR gene in two kinds of cells by recombinant lentivirus. The study indicated that the endogenous TCR knockout can effectively enhance the expression of transgenic TCR. Moreover, the targeted killing ability of the two cell lines against HPV16 positive SiHa cells was enhanced [26]. Mastaglio et al. created a method to knock out the endogenous TCRαchain to achieve single TCR editing (SE), and demonstrated that the TCR expression of edited T cells was significantly up-regulated, which could effectively recognize and bind to NY-ESO-1(pos) on the surface of tumor cells and mediate tumor rejection without causing xenograft versus host disease [27].

3. Application to tumor cells

3.1. Target screening

Originally used mainly for gene function research, CRISPR technology has become a useful tool in the field of cancer therapy, which can assist researchers to systematically identify potential therapeutic targets through genome and library screening [28]. Matthew et al. performed CRISPR gene screening on CD8+ T cells andre-identified traditional immunotherapy targets like PD-1, among which infiltration and degranulation screening found RNA helicase Dhx37, and their expressions in CD8+ T cells were detected by immunofluorescence. A mouse model was established to demonstrate that CD8+ T cells from Dhx37 knockout mouse had observably enhanced antigen specificity for triple negative breast carcinoma, confirming the potential of Dhx37 as a function adjustment factor in CD8+ T lymphocytes [29]. Steinhart et al. used CRISPR/Cas9 technology to perform a genome-wide screening of RNF43-mutant pancreatic ductal adenocarcinoma cells and found that Frizzled-5 receptors can effectively promote the proliferation of pancreatic tumor. The specific antibody to Frizzled-5 receptor can significantly inhibit the proliferation of pancreatic ductal adenocarcinoma cells with RNF43 mutation in mice and humans, suggesting that Frizzled-5 receptor can be used as a new target for immunotherapy in the future [30].

3.2. Gene Therapy

Due to the outbreak of genome editing and various disease targets, gene editing technology has penetrated into the field of targeted gene editing. Compared with other gene editing technologies, CRISPR/Cas9 has more efficient editing efficiency, good safety and simple operation. At present, it has become a common technology for tumor target editing, which directly or indirectly targets cancer mutated genes or key metabolic genes to kill cancer cells. Zhao Chuanqi et al. constructed a nano-delivery platform. Through CRISPR/Cas9 technology and the metalions released by the platform, they down-regulate the methionine transporter and activate the cGAS/STING signaling pathway, respectively, to alleviate the methionine competitive pressure of T lymphocytes, which results in enhancing the killing activity of lymphocytes against tumor [31]. The synergistic nano-CRISPR scaffold constructed by Gong Chang Yang and other researchers can initiate pyroptosis by self-supplying bioactive proteins within the tumor, thereby inducing immunogenic death and self-adjuvant effect, reversing the tumor immunosuppressive microenvironment, triggering as well as magnifying the adaptive immune cascade for tumor counteraction [32]. Cheong's team used CRISPR/Cas9 to target and edit the immunoglobulin heavy chain constant region genes of IgM+ mouse B cells, hybridoma cells, and human B cells to induce double-strand breaks, so that IgM antibodies can complete the class conversion to IgA, and thus obtain tumor-specific antibodies [33].

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4. Conclusions

In summary, CRISPR/Cas9 technology has important effects in targeted tumor therapy, immunotherapy, gene therapy and target screening with its high accuracy and few side effects of gene editing. However, the practical application of CRISPR/Cas9 technology also faces many challenges, mainly including off-target and low delivery efficiency. Although Erwei Zuo et al. developed GOTI to detect off-target mutations with the CRISPR single-base editor, cytosine-based editing still induced single-nucleotide substitutions more than 20 times, which is more frequently than CRISPR-Cas9 or adenine-based ones, ultimately leading to potentially off-target genetic changes [34]. Therefore, the question of how to ensure the fidelity of CRISPR technology remains to be solved. At the same time, long-term delivery of Cas protein using CRISPR system in the form of DNA is also easy to induce off-target effects, and since Cas protein is easy to induce the body's own immune response, long-term use will also lead to an increase in the body's immune response [35]. So, an effective delivery system for Cas protein, which can directly introduce Cas protein and specific sgRNA, is urgently needed to ensure the efficient delivery of the CRISPR/Cas9 system.

Although there are still many challenges and bottlenecks in CRISPR/Cas9 application, with the deepening of research, the discovery and application framework of CRISPR/Cas9 must be significantly improved. Nowadays, CRISPR/Cas9 technology has been applied to clinical practice and one day, CRISPR/Cas9 will provide a great impetus for the progress of cancer medical treatment in the future.

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