Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/60/20241430

Utilizing the T cell immune response to treat non-small cell lung cancer with CRISPR/Cas9 technology

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Abstract. Non-small cell lung cancer (NSCLC) has the greatest incidence rate and fatality, among all the lung cancer types. Its treatment has been widely concerned by the research community. Currently, many inhibitors used in clinical practice face drawbacks such as increased drug resistance in cancer cells and failure, as well as relatively high cytotoxicity. They not only fail to have a long-term effect on the lesion may also cause certain damage to normal cells. Therefore, finding a treatment method that cancer cells cannot or are difficult to develop drug resistance, can have long-lasting effects, and has low cytotoxicity is currently the research direction of scholars. CRISPR/Cas9 technology, a widely used and convenient gene editing method, can modify the microenvironment of T cells or cancer cells as a therapy of NSCLC. This article aims to review the current mechanisms of using CRISPR/Cas9 technology to treat NSCLC looking forward to more treatment sites and methods in the future.

Keywords: CRISPR/Cas9, T cell, non-small cell lung cancer.

1. Introduction

When compared to other cancers, lung cancer has the greatest incidence and fatality rate in recent years. In recent years, the incidence rate of lung cancer continues to rise, with a high proportion of malignant tumors being the most common form. Lung cancer mainly consists of non-small cell lung cancer (NSCLC), which was more than half of the incidence rate of it, and small cell lung cancer (SCLC). To be more precise, NSCLC is made up of large cell carcinoma, adenocarcinoma (bronchioloalveolar carcinoma), and squamous cell carcinoma (SCC), also known as epidermoid carcinoma [1]. This article will review the therapy of NSCLC for it is a relatively representative type of lung cancer.

A series of DNA sequences known as clustered regularly spaced short palindromic repeat sequences, or CRISPR, is extensively found in bacteria and archaea. Cas sequences are located near the CRISPR sequence, which can encode CRISPR associated protein 9 (Cas9). Using CRISPR sequences, specific DNA sites in the genome of organisms can be identified and cleaved, making it a widely used gene editing technique [2]. The CRISPR/Cas9 system is generally constituted by two parts: Cas9 protein and guide RNA (gRNA), where gRNA is generally a simple nucleotide sequence that can recognize and complement nucleotides on DNA. When gRNA binds to DNA, the endonuclease in Cas9 protein also binds to DNA, causing double stranded DNA to unwind and three nucleotides to be cleaved at the binding site, which generally leads to DNA sequence deletion or insertion mutation. If it occurs in the intron region of a gene, it may cause the gene to fail to express or re express the gene that cannot be expressed. At present, scholars are proficient in using CRISPR/Cas9 technology to perform gene editing

DOI: 10.54254/2753-8818/60/20241430

on different organisms by independently designing gRNA sequences. Compared with transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs), common gene editing technologies, CRISPR/Cas9 has higher editing accuracy and design flexibility, low cost, and a wider range of applications. It is currently a widely used and promising gene editing technology [3].

At present, targeted therapy for NSCLC mainly involves inhibiting oncogenic driving factors and increasing T cell immune response. However, immunotherapy for NSCLC is currently one of the promising treatment methods as it does not cause damage to normal cells due to circulating phosphorus compared to cytotoxic therapy. Immunotherapy mainly suppresses the immune checkpoint of T cells, enhances their immune response, and enables them to more sensitively sense and act on tumor cells. Currently, many inhibitors are engaged in clinical treatment programmed, for example a inhibitor preventing association between cell death programmed protein 1 (PD-1) and PD-1 ligand 1 (PD-L1) [4]. But after a period of action, these inhibitors will cause cells to develop a certain level of resistance, and some patients will show excessive immune response, with T cells showing excessive aggression. The CRISPR/Cas9 technology was considered as a possibility about curing NSCLC by scholars, due to its emergence.

This review aims to summarize the current methods of CRISPR/Cas9 approaches for treating NSCLC, and to describe two ways to modify the microenvironment of the tumor site and the intrinsic T cells, explaining the ways to enhance the sensibility to the cancer cells.

2. The remedial mechanisms of CRISPR/Cas9 for NSCLC

The sensibility of T cells of tumor cells can be affected by many factors, including the intrinsic and microenvironment of the tumor. In clinical trials, determining the distinguishing characteristics of T cells from other cells as the therapeutic site for cancer is extremely important [5]. Therefore, this article reviews two aspects: gene editing of T cells and gene editing of cancer cells to affect the microenvironment.

2.1. CRISPR/Cas9 application on editing of T cells

2.1.1. PD-1 gene

The immunosuppressive receptor PD-1 is frequently expressed in tumor locations, bone marrow cells, and T cells. Its main function is to downregulate immune responses, which can inhibit tissue damage and autoimmune reactions caused by excessive immune cell responses. However, tumor cells also evade immune responses through this mechanism. Transmembrane sections and extracellular IgV-like domains make up PD-1. A tyrosine-based switching motif (ITSM) and an immune receptor tyrosine-based inhibitory motif sequence make up the intracellular tail region. When PD-1 binds to PD-L1, phosphatase is recruited to ITSM and causes dephosphorylation of signaling kinase, leading to inhibition of signaling kinase activity and inhibition of T cell expansion[6].

Yang et al. [7] transfected the CRISPR/Cas9 system into T cells through electroporation, amplified it, and injected it into mice with malignant melanoma. The tumor size was measured using a caliper. Experiments have shown that tumor growth in mice injected with treated T cells is significantly inhibited, and due to malignant melanoma, the amount of T cells in mice decreases. The injected T cells can proliferate steadily in mice and have a longer survival time. Knocking out of the PD-1 gene of T cells can effectively enhance cell activity and have better therapeutic effects on tumor cells. Meanwhile, NSCLC is similar to malignant melanoma, both of which are solid tumors, and this method mainly targets T cells for modification, with strong universality. This article speculates that it can be analogized to the treatment of NSCLC.

Lu et al. [8] first identified a pair of exons targeting a single directional RNA (sgRNA) gene, and then transfected plasmids carrying Cas9 and sgRNA into T cells through electroporation. In T cells, after confirming through flow cytometry that the PD-1 gene has been disrupted and the expression of PD-1 has significantly decreased, ex vivo cultured T cells are then infused into the patient's body. Through experimental data, it can be concluded that T cells edited by CRISPR/Cas9 technology exhibit stronger

DOI: 10.54254/2753-8818/60/20241430

cell activity and expansion ability compared to unedited T cells. The results showed that 11 out of 12 patients had mild treatment adverse reactions, but none of them had treatment related adverse events ⩾ grade 3. Meanwhile, resulting from next-generation sequencing technology (NGS) detection, among all off-target locations, most of the mutation sites were located between genes or within introns, with little impact on gene transcription. The results indicate that this method has good biosafety and there are very few off target events of injected T cells. However, due to the lack of an effective control group and a small sample size in this experiment, further research is needed on the effectiveness of this method and the optimal injection amount of edited T cells.

2.1.2. T cell immunoglobulin mucin 3 (TIM-3 gene)

TIM-3 is an immunosuppressive factor easy detected from individuals with NSCLC. When TIM-3 interacts with its ligand galactose agglutinin-9, it can mediate effector T cell apoptosis and enhance regulatory T cell-mediated immune response, thereby achieving anti-tumor immune effects and ultimately promoting tumor cell growth and secretion of inflammatory factors. Through the research of scholars, it has been found that TIM-3 has a high expression rate in $CD8^+$tumor infiltrating lymphocytes ($CD8^+$TIL) and can serve as one of the therapeutic sites for CRISPR/Cas9 technology in treating tumor cells[9]. In recent years, inhibiting TIM-3 expression has become another approach with high anti-cancer research value, following the PD-1 gene and CTLA-4 gene.

Blaeschke et al. [10] transfected plasmids into T cells through electroporation, knocked out their TIM-3, and activated and proliferated them in contact with leukemia target cells. According to the experimental findings, children with high TIM-3 expression have a recurrence rate of pediatric acute lymphoblastic leukemia is much higher than lower patients. Simultaneously, when T cells without TIM-3 expression are specifically co-cultured with leukemia target cells, their activity is higher than that of untreated T cells. This suggests that TIM-3 gene knockout can strengthen T cell immune responses, which is precisely what is required for the treatment of NSCLC. Therefore, the TIM-3 gene can serve as one of the sites for treating NSCLC through CRISPR/Cas9 technology.

Ciraolo et al. [11] first designed the gRNA sequence for binding to specific exons of target genes. The CRISPR/Cas9 system was transfected into mouse T lymphocytes to disrupt the genes expressing PD-1, lymphocyte activation gene-3 (LAG-3), and TIM-3 in the cells. After that, the expression of these was examined and identified using protein blot analysis, and it was shown that the rate of protein synthesis had dropped by almost 30%. Next, scholars used similar methods to transfect the CRISPR/Cas9 system into mouse $CD8^+$T cells and amplify these T cells in vitro. The experimental results showed that the expression of PD-1, LAG-3, and TIM-3 in cells transfected with sgRNA was significantly reduced. After antigen-specific stimulation, compared to the control group there was no significant change in the levels of inflammatory factors produced by the experimental group. The function of $CD8^+$T cells in the experimental group was not significantly affected, indicating that TIM-3 knockout would not affect the normal function of T cells. After injecting T cells into the mouse body again, the tumor growth rate reduced, and the survival rate increased. This study demonstrated through fluorescence labeling that $CD8^+$T cells in the experimental group were significantly enriched in tumors and enhanced their ability to persist at the tumor site, demonstrating a good tumor cell clearance effect. However, the inhibitory effect on tumor growth in the experimental results was relatively mild, and no relief cases were found. Therefore, further research and experiments are needed to more effectively utilize the TIM-3 action site for cancer treatment.

2.2. CRISPR/Cas9 application on editing of cancer cells

2.2.1. Indoleamine 2,3-dioxygenase 1(IDO1) gene

Heme enzyme TIDO1 is extensively expressed in a wide range of tissues and cells and is intimately linked to numerous biological processes, including aging, immunity, and the development of numerous illnesses. IDO1 catalyzes the metabolism of tryptophan in dogs by combining molecular oxygen with a pyrrole ring that cleaves substrates. In addition, IDO1 is a rate-limiting enzyme in this pathway. When

DOI: 10.54254/2753-8818/60/20241430

IDO1 is in an active state, its heme iron is in a divalent ferrous state. In theory, IDO1, as a rate limiting enzyme, does not undergo any changes in heme iron during the catalytic process. However, due to the reducibility of ferrous ions, IDO1 is prone to self-oxidation, requiring the presence of reducing agents to maintain enzyme activity. During the catalytic process, the consumption of tryptophan and byproducts produced during metabolism can produce immunosuppressive effects in the immune response process, allowing cancer cells to achieve immune escape[12]. Therefore, inhibiting the expression of IDO1 can slow down the inhibitory effect on immune response in the microenvironment.

Ye et al. [13] first cultured human adipose derived mesenchymal stem cells (hADSCs), and then co cultured them with the CRISPR/Cas9 system carrying IDO1 sgRNA encoding. They used Western blotting to determine the knockout efficiency of their IDO1 gene, and finally injected the cultured hADSCs into rats with colitis. The research results indicate that the intestinal mucosa of rats injected with processed hADSC is normal, with only mild congestion and a lower disease activity index. The infiltration of inflammatory cells can be effectively reduced with high IDO1 expression, resulting in inhibiting the inflammatory response of damaged colon tissue. This indicates that upregulation of IDO1 expression can suppress the immune response effect, i.e. alleviate the inflammatory response. On the contrary, in the treatment process of tumor cells, experiments should achieve enhanced immune response by inhibiting the expression of IDO1 gene.

Yu et al. [14] used zeolite imidazoline framework (ZIF-8) as a metal organic carrier for CRISPR/Cas9 delivery. The carrier also simultaneously delivered the sound sensitizer hematoporphyrin monomethyl ether (H) and compounded it with Lactobacillus GG (LGG) to maintain LGG activity and MHS therapeutic properties. Finally, through ultrasound control, it was delivered to the hypoxic tumor core, knocking out IDO1 related genes, improving T cell activity, triggering a stronger immune response, and achieving the goal of eliminating cancer cells.

The experimental results indicate that sgRNA remains stable and highly active within 12 hours. The CRISPR/Cas9 nano system exhibited negligible cytotoxicity when incubated with cancer cell for 24 hours, with an average lethal rate of 91% for tumor cells, indicating its good targeting effect on tumor cells. At the same time, scholars detected the experimental results through immunofluorescence staining and Western blotting, proving that the introduction of CRISPR/Cas9 achieved the degradation of IDO1 in cancer cells, indicating that the CRISPR/Cas9 system can be effectively delivered by ZIF-8. In the same time, and the target gene sites can be precisely knock down. It also shows good hypoxia targeting and tumor cell targeting, which can enhance the immune response to tumor cells. However, in this experiment, the main concentration was on primary tumor cells, and the immune activation effect after tumor metastasis was average.

2.2.2. CD9 gene

The lungs contain interleukin-16 (IL-16), a cytokine that promotes inflammation and is released by cells. Additionally, it can cause inflammation in places like the lungs, which can result in pneumonia, and it acts as a chemotactic factor for eosinophils and $CD4^+$cells. Generally speaking, IL-16 often binds to CD4 receptors, but scholars have found that IL-16 can display its activity in areas where CD4 is not expressed and have found that CD9 receptors may serve as alternative receptors for CD4, co-mediating tumor cell migration with IL-16. As a member of the transmembrane protein superfamily, the CD9 receptor is involved in the regulation of several physiological processes, such as adhesion, metastasis, apoptosis, and cell motility. It is primarily heavily expressed on the surface of T cells that have been activated [15]. Jian C. Qi et al. [15] found through experiments that anti CD9 mAb has a certain inhibitory effect on IL-16 mediated cell migration, and it is in a dose-dependent mode, with a maximum inhibitory migration rate of about 50%. It has been proven that CD9 can serve as a substitute receptor for CD4 and mediate cell migration with IL-16. Therefore, inhibiting the expression of CD9 may also achieve the effect of inhibiting tumor cell migration and treating cancer.

Blake et al. [16] cultivated human lung epithelial cells, or A549 cells, with fetal bovine serum (FBS) and antibiotics to create the wild-type A549 cells. In a separate group of A549 cells, the CD9 gene was deleted using CRISPR/Cas9 transfection to create the knockout type A549 cells. The presence of

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knockout type A549 and a cell surface expression rate of CD9 below 3% suggest that the system effectively deletes the CD9 gene. The experimental results showed that the migration rates of knockout type A549 is quite different from wild-type A549 is significant. The response migration rate of knockout type A549 was significantly reduced from 29% of wild-type A549 to 15%, and in the presence of IL-16, the migration rate of knockout type A549 was only 0.2%. This experiment not only once again proves that CD9 can play a role as a substitute receptor in cell migration, but also demonstrates that this method can effectively reduce the migration rate of cells. In the future, this method can be used to reduce lung cancer cell migration and increase the immune response rate of T cells.

3. Conclusion

Throughout the world, lung cancer is the leading cause of cancer-related incidence and death, thus finding efficient therapies and survival plans is crucial. This review demonstrates the promise of CRISPR/Cas9 technology to treat NSCLC by focusing on intrinsic T cells as well as the tumor microenvironment. The modification of the tumor site and the enhancement of the immune response within the tumor area present promising avenues for improving therapeutic outcomes.

While remarkable progress has been made in understanding and utilizing CRISPR/Cas9 for cancer treatment, much of the research is still in preclinical stages. Many target sites remain untested experimentally and are based on theoretical predictions, not enough experimental data to support some of the results, necessitating further empirical studies to validate these approaches. Continued research and clinical trials are essential to fully realize the potential capacity of CRISPR/Cas9 technology in combating NSCLC and improving patient prognosis.

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