Applying CRISPR-Cas9 technologies for lung cancer treatment

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Abstract. A significant cancerous growth with a high global incidence and death rate, cancer of the lungs—particularly non-small cell lung cancer (NSCLC), which makes up 80% to 85% of cases—poses a serious threat to public health. Drug resistance, damage to cells that are healthy, and limited effectiveness in advanced stages represent a few of the drawbacks of conventional treatments such radiation and targeted therapy. CRISPR-Cas9 gene editing technology, consisting of key elements like Cas9 protein and guide RNA (sgRNA), enables precise DNA cleavage and editing, offering new prospects for NSCLC treatment with advantages such as strong targeting and high efficiency. The procedures for employing CRISPR-Cas9 for curing NSCLC are explained in detail in this publication, including the use of delivery vectors optimized to overcome lung physiological barriers, treatment strategies like oncogene knockout using Cas9 nickases and adjuvant CAR-T therapy through PD-1 gene knockout, and its application in detection and diagnosis for early screening. Additionally, it analyzes the technical advantages of CRISPR-Cas9 compared to traditional therapies and discusses the challenges and solutions in clinical transformation, with the intention of offering a theoretical guide for the accurate management of NSCLC.

Keywords: Non-small cell lung cancer, CRISPR-Cas9, Gene editing, PD-1 gene.

1. Introduction

One of the malignant tumors with the greatest prevalence worldwide, Lung cancer is now a major public health concern that puts lives at risk. The World Health Organization predicts that globally, lung cancer will claim the lives of 1.8 million individuals in 2020, while 2.2 million new cases will be identified. Clinical treatment focuses on NSCLC. It's responsible for 80–85% individuals with cancer of the lungs.

Currently, the main treatment methods for NSCLC include surgical resection, radiotherapy, chemotherapy, and targeted therapy. Traditional radiotherapy and chemotherapy function by non-specifically killing tumor cells, but in the process, they cause severe damage to normal proliferating cells such as bone marrow hematopoietic cells and gastrointestinal mucosal cells. This leads to a range of complications, including bone marrow suppression, gastrointestinal reactions, and reduced immune function, which not only affect the patient's quality of life but also may hinder the continuation of treatment. Although targeted drugs such as EGFR-TKI have significantly improved the prognosis of patients with driver gene mutations, when they receive their first diagnosis, a significant portion of patients already have metastatic tumors that are distant, and most patients develop drug resistance after several months of treatment. These limitations have been driving researchers to continuously explore more accurate and safe treatment strategies.

The CRISPR-Cas9 technology's birth for modifying genes has raised hopes for the treatment of NSCLC. Through sequence-specific recognition mediated by sgRNA and the site-specific cleavage function of Cas9 nuclease, this technology can achieve precise editing of tumor-related mutant genes or modify immune cells to enhance their anti-tumor activity. Compared with traditional therapies, it boasts several outstanding advantages. Firstly, it has strong targeting, enabling it to specifically act on tumor cells or mutant genes, thereby minimizing damage to normal tissues. Secondly, it has high editing efficiency, allowing for stable genetic modification at the genome level. Thirdly, it is versatile—it can not only directly repair carcinogenic mutations but also modify CAR-T cells to overcome tumor immune escape, providing multiple approaches for tumor treatment.

The implementation of the mechanism of CRISPR-Cas9 technology for the therapy of NSCLC, which is a type of lung cancer, will be methodically explained in this article. Its technical advantages over conventional therapies will also be thoroughly examined, and the difficulties in achieving clinical transformation and possible solutions will be covered. It is envisaged that this would offer useful theoretical references for the accurate management of NSCLC.

2. Lung Cancer

The most prevalent and dangerous aggressive tumor in the world is lung cancer. According to recent estimates by the International Cancer Research Agency, it is cancer that has the highest number of deaths. By 2022, nearly 2.5 million people were diagnosed and more than 1.8 million will die.The death toll is more than twice that of colorectal cancer, and it ranks first in the world in cancer mortality for ten consecutive years [1]. In addition, China continues to have the highest incidence and mortality rates of lung cancer worldwide, with China continuing to have the highest rates of both cases and deaths. The incidence after the age of 40 increased with age, with more males than females, but females have increased in recent years. The high-risk groups include smokers and passive smokers, occupational exposed persons with long-term exposure to asbestos, air pollution exposed persons, and family history of lung cancer or basic lung diseases [2]. Therefore, treatment scheme exploration and technology innovation are very important.

2.1. Non small Cell Lung Cancer

Non small cell lung cancer (NSCLC) constitutes 80–85% of cancer of the lung's occurrences. [3]. Its etiology is related to smoking , including passive smoking , asbestos and other occupational and environmental exposure, genetic susceptibility, and chronic lung disease; It has a variety of driving gene mutations, such as EGFR mutation, which is common in Asian non-smoking adenocarcinoma patients, ALK fusion, which is common in young non-smoking patients, and so on [4]. These mutations are important targets for targeted therapy.

2.2. Small Cell Lung Cancer

Small cell lung cancer (SCLC), which has a high degree of aggressiveness, accounts for 15% to 20% of lung malignancies. Its etiology is highly correlated with smoking and the risk of smokers is much higher than that of non-smokers, and passive smoking may also increase the risk, occasionally associated with environmental or occupational exposure. In terms of gene mutation, the inactivation of tumor suppressor gene is the main feature. The mutation rate of TP53 and RB1 genes is very high [5]. The primary cause and progression of SCLC is the inactivation of both genes. Moreover, it may involve gene changes such as myc family amplification, but there is no clear high-frequency driving mutation target like NSCLC.

2.3. Conventional Lung Cancer Treatment Approaches

In the traditional treatment of lung cancer, radiotherapy kills cancer cells locally by radiation, which is effective for local tumors but easy to damage the surrounding normal tissues , such as radiation pneumonia. Chemotherapy with cytotoxic drugs for systemic treatment can deal with metastatic lesions, but the selectivity is poor, which often leads to adverse consequences like nausea and inhibition of bone marrow. The challenges and dilemmas of treatment are: the curative effect on advanced or metastatic lung cancer is limited, drug resistance is prone to lead to treatment failure, and the individual reactions of patients are different, lack of accuracy, toxic and side effects also seriously affect the quality of life and treatment compliance.

3. CRISPR-Cas9 Technology

Crispr-Cas is an adaptive immune system that can fight the invasion of viruses and plasmids by bacteria and antiques. Firstly, the exogenous DNA fragment was integrated into CRISPR array, transcribed to generate pre crrna, and processed into mature crrna. Finally, crrna guided Cas protein to cut complementary exogenous DNA. The Crispr-cas9 technology was originally suggested in 2012 by American scientist Jennifer Doudna and French scientist Emmanuelle Charpentier. It was the first time to prove that cas9 protein can perform double fracture of the thread of target DNA under the guidance of tracrrna and crrna [6].

3.1. Key Elements of CRISPR-Cas9

3.1.1. Cas9 Protein

Cas9 protein is an endonuclease ,which is DNA cleaving enzyme, belonging to CRISPR related protein family. It has two catalytic domains: The HNH structure domain performs the function of cutting the target chain complementing the sgRNA, while the RuvC structure domain performs the function of cutting the non-target chain [7]. Figure 1 shows the specific structure of cas9 protein, including HNH and ruvc domains.

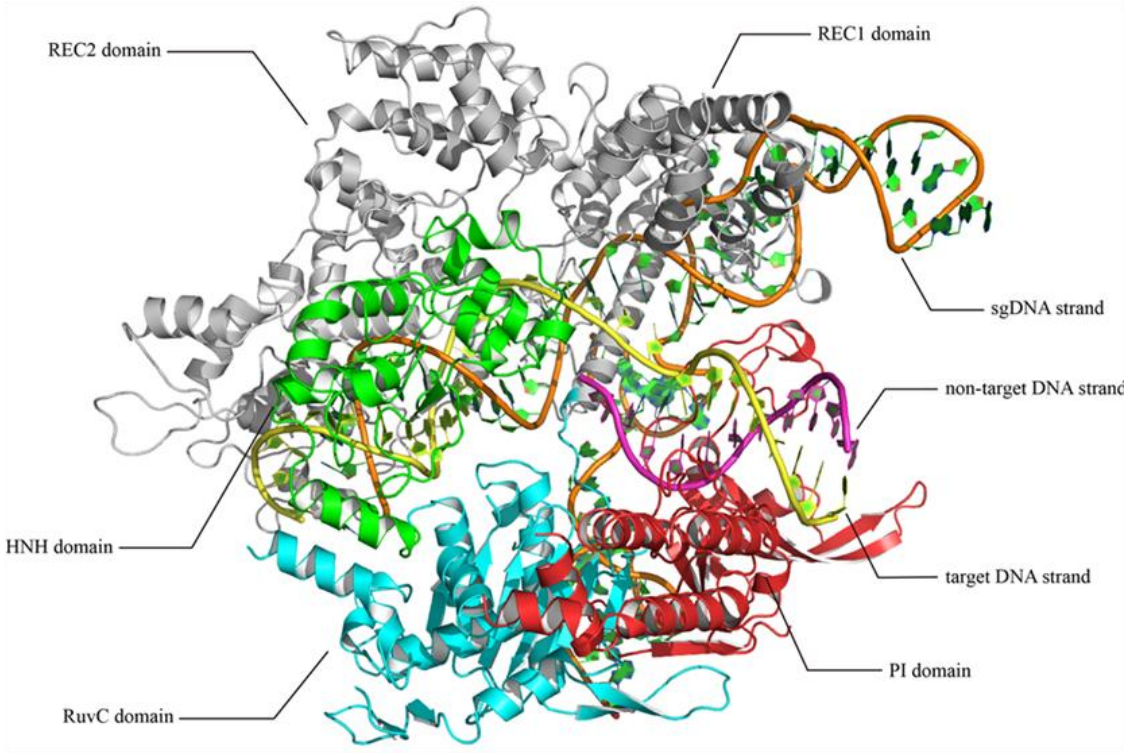

Figure 1. Structure of Cas9 protein [8]

3.1.2. Guide RNA (sgRNA)

CRISPR RNA (crrna) and trans activated crrna (tracrRNA) constituents of sgRNA. A 20 nt targeted segment is present in Crrna , which can complement and pair with the target DNA. Tracrrna is responsible for binding Cas9 protein to form Cas9 sgRNA complex [9].

3.2. Detailed Steps of DNA Cutting

DNA cleavage has detailed steps first Cas9 protein scans DNA to identify protopacer adjacent motif (PAM) sequence which is protopacer adjacent motif usually NGG this is located downstream of the target sequence which is the 3' end and is the necessary site for Cas9 binding and cleavage then Cas9 untied the DNA double strand near the PAM site to form an R-loop structure containing RNA-DNA hybrid strand which is single stranded RNA and DNA complementary pairing replaced free single stranded DNA which is ssDNA and possibly unpaired DNA loop at the same time the 20nt

target sequence of sgRNA was paired with the target DNA's homologous segment then HNH domain cleaved the target chain complementary to sgRNA and ruvc domain cleaved the non target chain and finally formed double strand break which is DSB finally the cells directly connect the broken ends through non homologous end joining which is NHEJ this is easy to introduce insertion or deletion mutations leading to gene knockout or complete self repair through homologous recombination repair which is HDR precise insertion or replacement of genes this is for gene knockin when there is a repair template. Figure 2 shows the process of cas9 protein scanning and fixing DNA double strand, RNA double strand uncoupling DNA double strand and knocking out gene fragments.

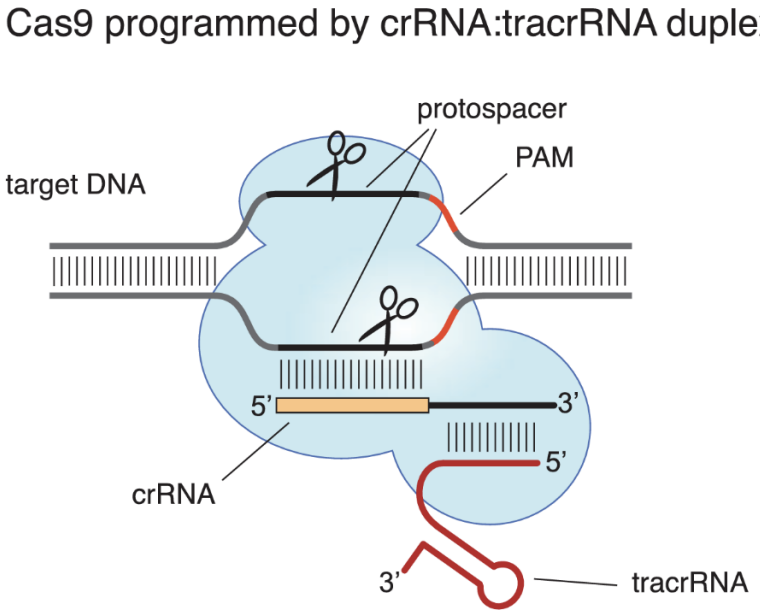

Figure 2. Crispr/Cas9 Knockout mechanism [6]

4. The utilization of crispr-cas9 technology for lung cancer treatment

Because of the high incidence of NSCLC and the long doubling cycle of cancer cells, CRISPR technology is mainly used in the treatment of NSCLC.

4.1. Delivery vector

Gene therapy for lung diseases is progressing slowly due to the special physiological barrier of the lung, such as mucus layer, cilia clearance. Traditional viral vectors ,such as AAV, have some problems, such as immunogenicity, long-term expression of Cas9 and the risk of Miss target. Non-viral vectors like LNPS and lipid nanoparticles , have excellent performance in liver delivery, but the delivery efficiency is low in the lung due to their negative charge and mucus layer repulsion. Researchers use surfactant modification or ligand targeting strategies to overcome these obstacles. These particles are composed of two molecules: a lengthy lipid tail and a strongly charged cap group. Lung gene therapy's transport issue has been solved by the particles' ability to bind with the mRNA which is charged negative and aid the mRNA get away from the cell structure that will phagocytize the atoms thanks to the head group's positive charge [10].

4.2. Treatment strategy

4.2.1. Knockout of oncogenes

CRISPR technology can use Cas9 incision enzyme ,such as cas9d10a, to cut only single strand DNA (SSBs), and SSBs are transformed into lethal double strand breaks in cancer cells with active replication, so as to achieve selective killing. Limitations of traditional CRISPR-Cas9: traditional Cas9 nuclease causes uncontrollable genomic variation ,such as large deletion and chromosome fragmentation, through NHEJ repair, and is also toxic to non dividing cells, which limits its

therapeutic application. Unique advantages of Cas9d10a: it only retains the activity of HNH domain, cuts single stranded DNA, and avoids the direct production of DSBs, thus avoiding genome variation. Single chain incision SSB only breaks one chain, and the other complete chain can be used as a repair template to ensure high fidelity [11].

4.2.2. Adjuvant CAR-T therapy

After car-t cells recognize tumor antigen, the expression of PD-1 is up-regulated. Although this process can promote self depletion, it can also prevent its over activation from injuring normal tissues. However, the combination of PD-L1 in the tumor microenvironment and PD-1 in car-t cells can activate the protective mechanism and make the tumor realize immune escape; CRISPR-Cas9 can disrupt the immune checkpoint signal, by deleting the gene for PD-1 of car-t cells, increasing T cell persistence and killing capacity [12]. It is safe and has a minimal chance of missing the target, according to the first phase I clinical investigation conducted on humans. The specific process is to isolate T cells from the patient's peripheral blood, transfect cas9 and sgRNA plasmids by electroporation, and target PD-1 gene exon 2. Then the T cells which were edited were reinfused three times ,day 1, 3 and 5, and the doses were 20%, 30% and 50% of the total dose, respectively. Cyclophosphamide was used for lymphocytic clearance three days before reinfusion [13].

4.3. Detection and diagnosis

The PAM sequence of target DNA was recognized by sgRNA to form ribonucleoprotein complex. When sgRNA is complementary to target DNA, cas14a1 is activated to specifically cleave target dsDNA, while retaining the non-specific cleavage ability with cleavage activity of adjacent ssDNA or fluorescent probes, thereby releasing fluorescent signals for visual detection. The study showed that the sensitivity of the system was significantly improved by optimizing the sgRNA design and reaction conditions. This method can detect gene mutation when the number of tumor cells is very small ,such as the mutation rate of 1%, which is suitable for early screening or monitoring of minimal residual lesions [14]; The traditional CT detection usually needs the tumor to reach a certain volume to be found.

5. Conclusion

This study investigates how CRISPR-Cas9 methodology for modification of genes can be accustomed to cure NSCLC, a malignancy with high incidence and mortality that is challenging to traditional therapies. The research shows that CRISPR-Cas9 with its precise targeting and efficient editing capabilities provides innovative solutions to overcome the limitations of conventional treatments such as radiotherapy chemotherapy and targeted therapy. Optimized delivery vectors like surfactant-modified or ligand-targeted nanoparticles address lung physiological barriers for effective gene delivery. Strategies including oncogene knockout using Cas9 nickases such as Cas9D10A achieve selective cancer cell killing while reducing genomic instability. PD-1 gene knockout in CAR-T cells enhances anti-tumor persistence and activity. Moreover CRISPR-Cas14a1-based detection systems show high sensitivity for early NSCLC screening expanding its clinical use. These applications confirm CRISPR-Cas9's potential as a precise and versatile tool in NSCLC diagnosis and treatment. The research is significant for advancing precision oncology in NSCLC. By solving the core limitations of traditional therapies such as off-target toxicity drug resistance and inefficiency in late-stage treatment CRISPR-Cas9 paves the way for personalized therapeutic strategies. Its ability to directly edit cancer-driving genes modify immune cells and enable early detection improves treatment efficacy and enhances patient quality of life taking a critical step toward reducing the global burden of NSCLC. Future research should focus on several directions. Combining CRISPR-Cas9 with other therapies like immune checkpoint inhibitors epigenetic modulators or radiotherapy may synergistically enhance anti-tumor effects and overcome the limitations of single therapies. For example, combining PD-1-edited CAR-T cells with small-molecule inhibitors targeting EGFR or ALK mutations might address multi-drug resistance in advanced NSCLC. Addressing existing

challenges in CRISPR-Cas9 application is crucial. It is crucial to develop more accurate Cas enzymes, which include high-fidelity Cas variants, to decrease off-target effects, optimize delivery mechanisms to improve lung-specific targeting, and investigate the ongoing security of gene-edited cells in vivo. Expanding CRISPR-based strategies to other genetic subtypes of NSCLC including those with rare driver mutations and investigating its potential in small cell lung cancer could broaden its applicability. To confirm the safety and effectiveness of these strategies supporting the integration of preclinical discoveries into routine medical care, extensive clinical trials need to be conducted. Through these efforts CRISPR-Cas9 may become a cornerstone of NSCLC treatment revolutionizing the fight against this devastating disease.

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