CRISPR/Cas9-Driven Precision Oncology for Lung Cancer: Current Situation and Challenges

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Abstract: Different types of lung cancer are treated in different ways. How to choose accurate treatment plan and reverse drug resistance after treatment will become the key to further improve the prognosis of lung cancer in time to come. CRISPR/Cas9 gene editing instrument. It capitalizes on the natural immune defense system of prokaryotes to accurately modify the target gene, compared to other, the design is simpler and lower cost, only the synthesis of a specific gRNA can target any gene sequence, a wider range of applications. This technology has displayed the great promise in the field of lung cancer study and the treatment, but it still needs to face bottlenecks such as off-target effects and delivery systems, and its actual clinical effectiveness needs to be rigorously verified by more in-depth studies and large-scale clinical trials.

1 INTRODUCTION

Lung cancer ranks as the most common and lethal malignancy globally, accounting for the highest burden and mortality worldwide, which has emerged as a global health crisis, topping the charts as the most common cancer type and casting a long shadow over human health.

Although the current diagnosis and treatment technology has been improved, there is still a lack of early clinical diagnosis of lung cancer. Most patients suffering from this malignant disease have no obvious clinical symptoms, and once diagnosed, most of the patients have poor survival prognosis due to late staging, missing the best time for treatment, resulting in no significant improvement in the 5-year survival rate.

Conventional therapies to treat lung cancer include surgical excision, chemotherapy, and radiotherapy etc. Surgical excision, curative for early-stage disease, is often infeasible for advanced lung cancer patients due to the low early diagnosis rate (only 15%) and advanced-stage presentation in 75% of patients at diagnosis (Memi F,2018). Even if the patient meets the surgical indications, irreversible lung function impairment or inevitable postoperative complications may result.

Chemotherapy, which serves as the cornerstone of treatment for advanced - stage patients who are ineligible for surgical resection, frequently runs into the roadblock of drug resistance, which not only undermines the effectiveness of chemotherapy but also leads to subpar treatment outcomes (Ren F,2024).

For Radiotherapy, despite its role in reducing thoracic recurrence. Its survival benefit is modest (5% improvement at three years) and age-dependent, showing no efficacy in patients over 70 and potential harm in older subgroups. Trial heterogeneity—including variations in radiation doses, timing, and combination strategies—further complicates its clinical utility. It does not respond to distant metastases and may promote the spread of tumor cells due to the "distal effect". Additionally, radiotherapy's nonlethal toxicity profiles remain incompletely characterized, and long-term follow-up data beyond three years are limited, leaving uncertainty about its durability. All of these challenges underscore the need for innovative approaches to enhance treatment precision and overcome the resistance mechanisms.

The gene - editing approach founded on CRISPR and Cas systems has ushered in a novel era for lung cancer research. The first-in-human Phase I trial results demonstrated that CRISPR/Cas9-edited PD-1-targeted T cell therapy in lung cancer patients is generally safe and feasible. This landmark study establishes a critical translational foundation for advancing CRISPR-based gene editing technologies into subsequent clinical investigations (Lu Y,2020).

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Distinguished from earlier gene-editing tools like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), the CRISPR system has established itself as the preeminent gene-editing platform with the highest clinical translatability. This technological advantage stems from its dual attributes: simplified operational process and unprecedented targeting precision. By enabling site-specific genomic modifications with high efficiency, CRISPR technology has not only revolutionized oncogenesis research but also unlocked innovative strategies for personalized cancer treatment. Its capacity to precisely manipulate disease-causing genes positions it as a cornerstone technology in the advancement of precision medicine (Wang SW,2022; Li J,2013).

This paper will explore CRISPR/Cas9's role in lung cancer therapy by examining its technical principles, preclinical breakthroughs in oncogene editing and drug resistance reversal, and translational challenges including delivery efficiency, off-target risks, and ethical considerations, to assess its potential as a transformative therapeutic approach.

2 TECHNICAL BASIS OF CRISPR/Cas9

CRISPR is a sequence consisting of a number of repeated sequence regions and intervals arranged alternately. Where, the sequence of the repeating sequence area is arranged in palindromic manner. The sequence of the spacer region is random, it is derived from the foreign viral DNA sequence and used to recognize this sequence when the foreign virus re-invades, and the Cas protein will interrupt the viral sequence at this time, thereby providing acquired immunity to bacteria and archaea. CRISPR/Cas9 uses a specific RNA to guide endonucrenase to a target, enabling DNA editing (Nussenzweig PM,2020).

It is taxonomically categorized into Class 1 and Class 2, with the latter distinguished by simpler functional protein architecture compared to multi-protein complexes in Class 1 systems. Among Class 2, Type II Cas9 and Type V Cpf1 represent single-component nuclease effectors (Memi F,2018). In general, the core of the class 1 is a complex composed of proteins such as the helicases Cas3 and Cas8 and the polymerase Cas10. The second key type of CRISPR-Cas is distinguished by its unique functional proteins, namely single CrRNA-binding proteins with multiple domains. This binding protein internally integrates all the components needed to perform nucleic acid cleavage. It can do with only one group the work that the first class of systems requires multiple groups to do together

Adaptive immunity mediated by CRISPR/Cas systems progresses through three conserved phases: 1) foreign DNA derived from invading phages or plasmids is captured and integrated as spacer arrays flanked by palindromic repeats into host CRISPR loci, establishing sequence-specific immunity; 2) transcription of CRISPR loci generates pre-crRNA, which undergoes maturation via RNase III cleavage directed by trans-activating tracrRNA transcribed from upstream regions, forming a functional tracrRNA: crRNA duplex; 3) this ribonucleoprotein complex guides Cas9 nuclease to introduce double-strand breaks (DSB) at complementary protospacer adjacent motif (PAM)-containing genomic sites, synergistically disrupting invading nucleic acids.

Compared to other gene editing technologies, this system has the highest targeting efficiency (50% to 80%) through preferential binding to open chromatin regions and utilization of truncated sgRNA (2-3nt shortening). The core advantage of CRISPR/Cas9 over other genome editing tools lies in its unique RNA-guided targeting mechanism: target DNA recognition is achieved via 20-nucleotide sgRNA base pairing, avoiding the technical bottlenecks of ZFNs and TALENs that rely on complex DNA-binding protein design, significantly reducing design complexity and implementation costs. This system supports highly efficient multiplexed genome editing, enabling simultaneous multi-site editing in mammalian cells and other systems through co-delivery of multiple gRNAs, with efficiency far surpassing traditional tools. Additionally, CRISPR/Cas9 achieves 1500-fold improved DNA specificity compared to wild-type Cas9 through optimized PAM sequences (e.g., CGGH consensus) combined with Cas9 nickase strategies (e.g., D10A mutants). Targeting efficiency is further enhanced permanent gene modification at the DNA level independent of cell cycle phase; and enhanced precision with off-target effects reduced to <0.1% through optimized sgRNA design and homology-directed repair (HDR) integration (Karimian A,2019). These technical advancements have propelled CRISPR-Cas9 to the forefront of translational applications, particularly in precision oncology for diseases such as lung cancer, where its modularity and efficiency enable targeted therapeutic interventions (Memi F,2018).

3 CURRENT PROGRESS

3.1 Genome Ablation

In a study, researchers exploited the mutation-generated protospacer-adjacent motif (PAM, 5'-CGG-3') of the oncogenic EGFR L858R mutation (CTG→CGG) in NSCLC. The researchers designed a specific sgRNA and delivered CRISPR/Cas9 via adenoviral vectors (Ad/sgEGFR + Ad/Cas9). This landmark research, which was published in Nucleic Acids Research in 2017, first established the in - vivo proof - of - concept for allele - specific CRISPR/Cas9 - mediated oncogene ablation. Findings indicated that CRISPR/Cas9-mediated ablation of the L858R mutation in EGFR-upregulated lung cancer cells suppressed tumor cell proliferation. This study addressed precision oncology challenges and provided a template for personalized therapies targeting PAM - generating mutations (Karimian A,2019).

Findings from a human Phase I trial (NCT02793856) evaluated CRISPR-Cas9-engineered T cells with PD-1 gene modulation in individuals with advanced NSCLC. Researchers introduced plasmids carrying Cas9 and sgRNA into patients' T cells. In order to achieve accurate knockout of the PD-1 gene, analysis of results revealed that the condition of 8 patients showed a notable stable trend (Lu Y,2020).

3.2 Genome Cleavage

A study advanced allele-specific CRISPR/Cas9 for Non-Small Cell Lung Cancer (NSCLC) by targeting Epidermal Growth Factor Receptor (EGFR) L858R mutations (T→G transversion). The strategy exploited the mutation-generated PAM (5'-CGG-3') to design a specific sgRNA, delivered via lentiviral vectors. In vitro, this system achieved 37.9% target specificity in mutant cells (NCI-H1975), confirmed by digital PCR and T7 endonuclease assay. Functional studies showed selective EGFR downregulation, reduced proliferation (MTT/colony formation, $p<0.0005$), and smaller tumor growth in vivo ($p=0.015$). The approach targets between 15% and 35% of cancer mutations characterized by C>G, A>G, and T>G nucleotide substitutions. And highlights PAM-dependent specificity as a key precision oncology tool (Koo T,2017).

3.3 Reduction of Drug Resistance

Gefitinib is used as a linear epidermal growth receptor - tyrosinase suppressant (EGFR-TKIs) in the treatment of lung cancer patients after a period of acquired resistance. The main mechanism is the secondary mutation of T790M at the second site of EGFR, which increases the affinity between ATP and EGFR-TKIS junction domain, resulting in EGFR-TkIs cannot effectively block the signal pathway and produce drug resistance.

Guernet and colleagues combined single guide RNA with single-stranded DNA fragments carrying unique genetic tags, can precisely track thousands of tumor clones at the single-cell resolution. It has successfully constructed resistance models driven by EGFR T790M/KRAS G12D mutations and those related to EML4-ALK rearrangements, confirming that drug resistance can be dynamically formed through multiple mechanisms such as gene amplification and bypass signaling activation, which have made significant breakthroughs in NSCLC drug resistance research. By simulating the clonal interactions in the tumor microenvironment, this system provides innovative tools for optimizing personalized treatment strategies, effectively evaluating combination therapies, and deciphering the special drug resistance mechanisms in patients with EGFR/ALK co-mutations (Cheung AH,2018; Marino FZ,2017).

3.4 Economic Efficiency

The continuous innovation of CRISPR-Cas9 technology has brought new hope for alleviating the economic burden on lung cancer patients. A team led by Irene Lara-Sáez developed a cationic polymer delivery system for CRISPR/Cas9 ribonucleoprotein (RNP), enabling simpler and cost-effective construction of lung mutation models using chemical vectors and off-the-shelf synthetic reagents. This virus-free approach significantly reduces the technical barriers and production costs of gene editing. Its precision in inducing genetic modifications not only allows efficient screening of oncogene combinations but also expands therapeutic possibilities beyond traditional gene inactivation. As this technology becomes widely adopted in cancer modeling, it will drive rapid iterations of personalized treatment protocols, ultimately mitigating long-term economic burdens on lung cancer patients through dual pathways of improved treatment efficiency and shortened R&D cycles (Guernet A,2016).

4 CURRENT LIMITATIONS

4.1 Off Target Effects

The mismatch between the designed specific sgRNA and the non-target DNA sequence leads to unexpected mutations in the gene locus, which is called off-target effect. Off-target will not only reduce the efficiency of genome editing, but also lead to the rearrangement of chromosomes, and even destroy genes that are not perfectly matched, and may also lead to the inactivity of functional genes.

Within CRISPR/Cas9-based genome editing for lung cancer, the origination of off-target genomic alterations is governed by multi-dimensional regulatory networks. The mismatching between sgRNA and target sequence is the key factor that causes the off-target phenomenon.The effective concentration of Cas9/sgRNA complexes exhibits a concentration-dependent relationship with cleavage specificity - when complex concentrations exceed physiological thresholds, Cas9 cleavage specificity decreases significantly. This concentration-dependent risk is particularly prominent in lung cancer therapy, as high-dose delivery strategies are often employed to overcome the low transfection efficiency of tumor cells. PAM sequence, length and adjacent seed region can affect the missed target to some extent. In addition, the structure, shear activity and plasmid transfection concentration of Cas9 affected the off-target to some extent. Notably, the type of transfected cell is also a key factor affecting off-target.

From the sgRNA point,it was confirmed that the accuracy and specificity of sgRNA gene editing were proportional to GC content in sgRNA seed region, and when GC content was 40% ~ 60%, off-target was not easy to occur (Ren X,2014). When two G bases are added to the 5 'terminal of the sgRNA, the incidence of off-target events can be reduced. In addition, the commonly used sgRNA guidance sequence composed of 20 nucleotides was shortened by 2 to 3 nucleotides, which could not only ensure the efficiency of targeting binding sites, while also reduce the occurrence of off-target. From the cas9 point, the fusion protein d Cas9-Fok I monomer has the cutting ability only when it is combined with two Sgrnas, thus forming a correct double-stranded incision and reducing off-target (Lara-Sáez,2024; Pattanayak V,2013).

4.2 Delivery Challenges

At present, in vitro delivery technology has been developed to some extent, while there are still some limitations, likephysical methods (e.g., electroporation, liposome transfection) are simple and efficient in vitro, but hydrodynamic injection causes liver damage and cardiovascular dysfunction; viral vectors (e.g., adenoviral, AAV, lentiviral) are among the earliest developed delivery technologies, emerging in the 1990s. Their delivery efficiency is high, but there are potential risks of immunogenicity, insertion mutations, and high off-target effects, which limit their further application. Nonviral vectors (e.g., lipid-based/polymer-based nanocarriers) may effectively solve the problems of potential toxicity and capacity limitation caused by viral vectors. And has the advantages of simple and easy to obtain, low cost, safety and so on. Among them, the strategy based on liposomal delivery of Cas9 mRNA/sgRNA has entered the clinical experimental stage,further propels the implementation of CRISPR/Cas9 genome editing vectors in creating innovative gene therapy modalities.The novel biocompatible, non-immunogenic and biodegradable non-viral nanomaterials are ideal vectors to reduce the risk of organ toxicity and local inflammation. It will provide a new impetus for the targeted delivery and clinical conversion of CRISPR/Cas9 systems in the future (Wu X,2014).

4.3 Ethical Concerns

CRISPR/Cas9 raises complex ethical considerations, with core controversies centered on technical risks, clinical trial design, stakeholder influences, and patient autonomy.

The technology’s potential risks in somatic gene therapy—including off-target effects, mosaicism formation, and uncertainties regarding long-term safety—stand in stark contrast to the reversibility of traditional drug trials. For example, it may induce new cancers or genetic defects.And its irreversibility and "all-or-nothing" nature pose unique challenges for clinical trial design, necessitating phased monitoring mechanisms and reliance on extrapolation of primate experimental data, yet existing regulatory frameworks remain ill-adapted to such novel therapies (Li L,2015).

Debates also revolve around the ethical boundary between gene therapy and enhancement. While lung cancer treatment falls within the scope of disease correction, the technology’s efficiency may blur the

line between therapeutic and optimizing interventions. Historical precedents (e.g., IVF and mitochondrial transfer) demonstrate shifting public acceptance with accumulating evidence of medical benefits, but CRISPR/Cas9's non-therapeutic applications in animal models—such as antiviral resistance and muscle enhancement—hint at potential "slippery slope" risks (Baumann M,2016).

Stakeholder dynamics are critical: pharmaceutical companies may emphasize benefits under economic pressures, while public sensitivity to issues like "designer babies" could lead to either exaggerated fears or underestimation of risks in the oncology context. Additionally, the high costs of genetic screening and insurer payment restrictions may force patients to assume treatment risks with limited information, exacerbating tensions between patient autonomy and medical decision-making. Therefore, it is necessary to establish a rigorous ethical review mechanism for clinical trials to ensure that the risk-benefit ratio is reasonable and adopt a dynamic informed consent model to allow patients to continue to participate in decision-making during treatment.

5 CONCLUSION

CRISPR/Cas9 has established itself as a revolutionary tool in precision oncology, particularly in lung cancer treatment, by enabling unprecedented gene-editing capabilities. Current progress highlights its utility in oncogene ablation (e.g., EGFR mutations), overcoming drug resistance through clonal profiling, and generating genetically engineered preclinical models. However, technical bottlenecks remain significant hurdles. Ethical considerations, such as the risk of therapeutic creep toward enhancement and regulatory gaps, further complicate its implementation.

Looking ahead, advancements in CRISPR/Cas9 for lung cancer will likely focus on three key areas. First, improving targeting precision through computational sgRNA design tools and high-fidelity Cas9 variants could mitigate off-target risks. Second, developing novel delivery systems—such as cell-penetrating peptides, organ-specific nanocarriers, or viral vectors with reduced immunogenicity—will be critical for achieving efficient and safe in vivo genome editing. Third, integrating CRISPR/Cas9 with emerging therapies (e.g., immunotherapy, targeted kinase inhibitors) may synergistically enhance therapeutic outcomes while addressing tumor heterogeneity.

To address these challenges and opportunities, broad cooperation and joint efforts from all sectors of the world have become essential. It is necessary to strengthen international cooperation to develop more detailed and comprehensive legal regulations to ensure the healthy development of this technology under the double framework of moral and legal law. At the same time, it is also necessary to ensure the security and controllability of the technology. In addition, public participation is also indispensable, through enhancing social ethical and moral awareness and scientific literacy, jointly promote the rational application of gene editing technology.

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