Review Article

Research into overcoming drug resistance in lung cancer treatment using CRISPR-Cas9 technology: a narrative review

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Background and Objective: Lung cancer remains a leading cause of cancer-related mortality globally, with drug resistance posing a significant challenge to effective treatment. The advent of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR-Cas9) technology offers a novel and precise gene-editing technology for targeting and negating drug resistance mechanisms in lung cancer. This review summarizes the research progress in the use of CRISPR-Cas9 technology for investigating and managing drug resistance in lung cancer treatment.

Methods: A literature search was conducted using the Web of Science and PubMed databases, with the following keywords: [CRISPR-Cas9], [lung cancer], [drug resistance], [gene editing], and [gene therapy]. The search was limited to articles published in English from 2002 to September 2023. From the search results, studies that utilized CRISPR-Cas9 technology in the context of lung cancer drug resistance were selected for further analysis and summarize.

Key Content and Findings: CRISPR-Cas9 technology enables precise DNA-sequence editing, allowing for the targeted addition, deletion, or modification of genes. It has been applied to investigate drug resistance in lung cancer by focusing on key genes such as epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), tumor protein 53 (*TP53*), and B-cell lymphoma/leukemia-2 (*BCL2*), among others. The technology has shown potential in inhibiting tumor growth, repairing mutations, and enhancing the sensitivity of cancer cells to chemotherapy. Additionally, CRISPR-Cas9 has been used to identify novel key genes and molecular mechanisms contributing to drug resistance, offering new avenues for therapeutic intervention. The review also highlights the use of CRISPR-Cas9 in targeting immune escape mechanisms and the development of strategies to improve drug sensitivity.

Conclusions: The CRISPR-Cas9 technology holds great promise for advancing lung cancer treatment, particularly in addressing drug resistance. The ability to precisely target and edit genes involved in resistance pathways offers a powerful tool for developing more effective and personalized therapies. While challenges remain in terms of delivery, safety, and ethical considerations, ongoing research and technological refinements are expected to further enhance the role of CRISPR-Cas9 in improving patient outcomes in lung cancer treatment.

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Keywords: Clustered regularly interspaced short palindromic repeats and clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9); lung cancer; drug resistance; gene editing; gene therapy

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Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR-Cas9) is a gene-editing technique that enables the precise cutting of DNA sequences, allowing for the addition, deletion, or modification of genes. Essentially, it employs a DNA sequence known as CRISPR-Cas9 to interact with target genes, forming an accurate gene-editing tool (1). CRISPR sequences are DNA sequences found in bacteria and archaea, which protect bacteria from viral infections by recognizing and cutting the DNA of invading viruses. Cas9 is an auxiliary protein of the CRISPR sequence, responsible for identifying and cutting DNA sequences that match the CRISPR sequence (2). By combining the Cas9 protein with the CRISPR sequence, precise cuts can be made to specific DNA sequences (3). CRISPR-Cas9 technology is advancing swiftly and has achieved significant milestones in various domains. For instance, a 2022 clinical study employed CRISPR-Cas9 to genetically alter donor T cells, aiming to treat pediatric patients with drug-resistant leukemia who had no remaining therapeutic options. This phase I clinical trial marked the inaugural use of "universal" CRISPR-engineered T cells in humans, signifying a pivotal evolution in deploying gene-edited cells against cancer. The researchers involved in this trial innovated and employed a novel class of precision universal genome-edited T cells (4). Furthermore, CRISPR-Cas9's potential has been rigorously explored in the realm of chimeric antigen receptor (CAR) T-cell (CAR-T) therapy. A notable study introduced an enhanced gene-editing system capable of augmenting the efficiency of CAR-T therapies and pinpointed a low-toxicity, high-efficiency, virus-independent CAR-T manufacturing approach. The groundbreaking gene-editing technique involves tethering a modified Cas9 enzyme to a single-stranded DNA template by integrating a brief overhanging segment of double-stranded DNA (dsDNA) on both extremities. This not only acts as a blueprint for homology-directed repair but also houses the less hazardous Cas9 target sequence. In comparison with the Cas9 target sequence of dsDNA, the study using a hybrid ssDNA observed an average augmentation in knock-in efficiency and a two- to three-time greater yield. Impressively, with the application of the new Cas9-guided single-stranded templates, approximately half of the T cells adopted the new genes and were thus transformed into CAR-Ts (5). While continual enhancements in CRISPR-Cas9 technology are of paramount importance, its application in addressing existing clinical challenges holds equal significance. For instance, CRISPR-Cas9 can be employed to modulate gene expression, enabling insights into gene roles in disease progression. Alternatively, it can be used to rectify gene mutations, potentially altering the trajectory of mutation-induced diseases. In lung cancer research, CRISPR-Cas9 plays an instrumental role, with drug resistance studies being a focal area of investigation.

Lung cancer is a highly malignant tumor that ranks among the leading causes of global mortality, with a 5-year survival rate that is often less than 20% for advanced-stage disease (6,7). It includes non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). The conventional treatment approach involves surgery, which should be the first line of action when possible (8,9). Other treatment conducted with drugs including chemotherapy, targeted therapy, and immunotherapy, are usually administered in patients with inoperable lung cancer or after surgery. Novel adjuvant therapies have emerged, which involve the use of corresponding antitumor drugs before surgery (10). These treatments aim to control tumor lesions prior to surgery and eliminate potential residual tumor cells. However, drug resistance remains a significant challenge in chemotherapy, targeted therapy, and immunotherapy, which are the focus of recent research (11-14). The emergence of drug resistance severely limits the clinical efficacy of lung cancer treatments (*Figure 1*) (15). The development of treatment resistance in cancer patients is a complex process driven by multiple reasons such as genetic heterogeneity, tumor microenvironment interactions, cancer stem cells, metabolic reprogramming, epigenetic

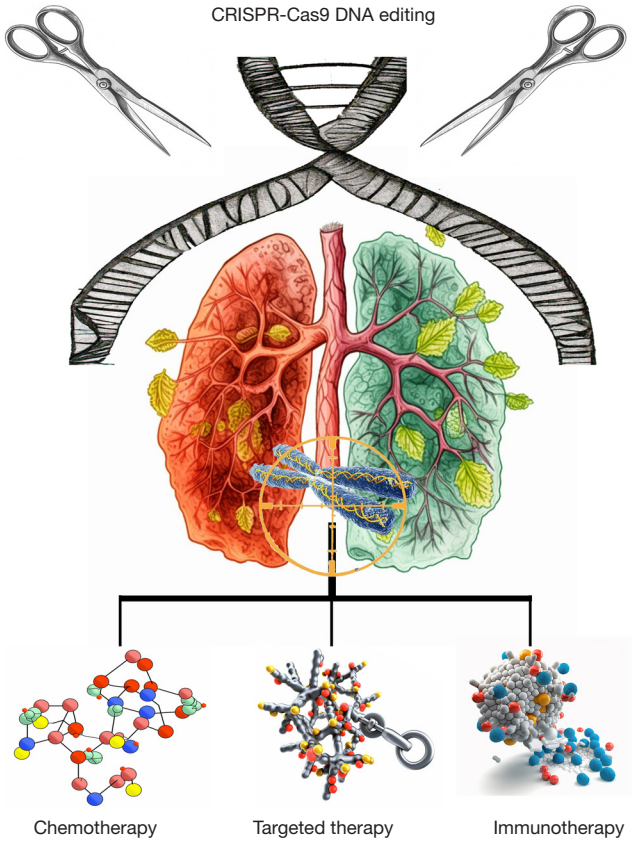

Figure 1 The main treatment methods of lung cancer and the risk of drug resistance. CRISPR-Cas9, CRISPR and CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats.

alterations, and selective pressure from prolonged therapies. These diverse mechanisms enable tumor cells to evade the effects of therapeutic agents, presenting a major challenge in cancer management that requires a deeper understanding to develop more effective strategies. To overcome the challenge of treatment resistance in cancer, various therapeutic strategies have been explored. These include targeting multiple signaling pathways simultaneously, leveraging combination therapies to prevent the emergence of resistant clones, developing novel drug classes that can overcome specific resistance mechanisms, utilizing immunotherapies to harness the body's immune system, and employing liquid biopsies to monitor the evolution of resistance, which all leads to personalized treatment.

Before the advent of CRISPR-Cas9 technology, researchers had been working to improve drug resistance by altering by-pass signaling of the treatment target, which fail to work as usual, but the results were not ideal. CRISPR-Cas9 technology has emerged as a promising approach to address the challenge of cancer drug resistance. By precisely editing the genome of tumor cells, CRISPR-Cas9 can disrupt key resistance-conferring genes or sensitize cancer cells to existing therapies. This technology allows for targeted manipulation of genetic and epigenetic factors driving resistance, enhancing the efficacy of cancer treatments. This technique also allows researchers to study genetic variation and drug resistance mechanisms in lung cancer cells with persistent effects, enabling the development of more precise treatments. In this review, we summarize the progress in CRISPR-Cas9 research related to drug resistance in lung cancer. Our aim is to provide a valuable reference for the further development of CRISPR-Cas9-based gene intervention systems, which may ultimately improve the management of drug resistance in patients with lung cancer. We present this article in accordance with the Narrative Review reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-592/rc).

Table 1 Summary of the search strategy

Items	Specification
Date of search	September 6, 2023
Databases and other sources searched	Web of Science, PubMed
Search terms used	((TS=(CRISPR-Cas9)) OR TS=(gene therapy)) OR TS=(lung cancer) AND TS=(drug resistance) AND TS=(gene editing)
Timeframe	From January 1, 2002, to September 6, 2023
Inclusion criteria	Article or review in English
Selection process	Double-blind search, aggregated together for inclusion in the review

CRISPR-Cas9, CRISPR and CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; TKI, tyrosine kinase inhibitor.

Methods

Literature selection

The word "CRISPR" first appeared as a name for a technology in 2002; therefore, in this review, we only searched for literature published between 2002 and September 2023. The database searched was Web of Science and PubMed, and the language of the literature was specified as English. The types of literature searched were research articles and reviews. Keywords searched include but were not limited to "CRISPR-Cas9", "lung cancer", "drug resistance", "gene editing", and "gene therapy". The references from all the retrieved articles were manually read and are listed in the list of references. The search strategy is summarized in *Table 1*.

Use of CRISPR-Cas9 in ameliorating drug resistance in lung cancer

This section discusses four main topics: CRISPR-Cas9 technology's role in targeting gene expression or mutations related to lung cancer drug resistance, its application in addressing lung cancer's drug-resistant signaling pathways, its interaction with immune-associated mechanisms in lung cancer drug resistance, and its potential for identifying the key genes that contribute to drug resistance in lung cancer.

Investigating drug resistance in lung cancer with CRISPR-Cas9-targeted genes

CRISPR-Cas9 can accurately target the critical genes of lung cancer cells, such as tumor-suppressor genes and proliferation-related genes, to inhibit tumor growth and spread (16,17). Several crucial genes involved in regulating drug resistance in lung cancer have been identified, among which (I) epidermal growth factor receptor (*EGFR*); (II) Kirsten rat sarcoma viral oncogene homolog (*KRAS*); (III) tumor protein 53 (*TP53*); (IV) B-cell lymphoma/leukemia-2 (*BCL2*); (V) phosphoinositol 3-kinase α (*PIK3CA*); (VI) anaplastic lymphoma kinase (*ALK*); and (VII) mesenchymal-epithelial transition (*MET*), are discussed in detail below.

(I) *EGFR* is one of the most common mutated genes in lung cancer, with mutations leading to *EGFR* overactivation, thus promoting tumor growth and metastasis (18). *EGFR* mutations are closely associated with sensitivity to targeted therapy, but resistance can also develop during treatment (14,19). *EGFR* can guide the use of multiple targeted drugs, and CRISPR-Cas9 technology has been used to achieve *EGFR* gene expression inhibition and mutation repair. Researchers have designed specific RNA sequences using CRISPR-Cas9 technology to precisely cut the DNA sequence of the *EGFR* gene, thereby inhibiting its expression (20). This method has been demonstrated in both *in vitro* and *in vivo* experiments and has been shown to inhibit the growth of lung cancer cells (21). Additionally, CRISPR-Cas9 technology can be used to design specific RNA sequences that target Cas9 at the *EGFR* gene's mutation site, after which a DNA repair template can be used to repair the mutation (22). The repair template, which can be single-stranded DNA or dsDNA, contains the normal *EGFR* gene sequence. The repair template works alongside Cas9 to restore the mutant site to the normal *EGFR* gene sequence, thus reinstating its normal function (23,24).

(II) *KRAS* is another frequently mutated gene, with

mutations causing excessive *KRAS* activation that promotes tumor growth and metastasis. *KRAS* mutations are found in approximately 25% of those with NSCLC, which is a common type of lung cancer (25). Oncogenic mutations in *KRAS* typically occur at hotspots in the protein (e.g., codons 12, 13, and 61), inducing protumorigenic signaling via downstream effector pathways by raising steady-state levels of *KRAS* proteins in the guanosine-5'-triphosphate (GTP)-bound state. A few examples of these pathways include mitogen-activated protein kinase (MTK) and phosphatidylinositol 3-kinase (PI3K) (26,27). It has been reported that CRISPR can be used to perform a positive-selection deep mutational scanning screen for mutations in *KRAS G12C* that confer resistance to *KRAS G12C* inhibition in Ba/F3 cells cultured for 7 days in the absence of interleukin-3 and in the presence of one of two *KRAS G12C* inhibitors, *MRTX1257*, or sotorasib. CRISPR-Cas9 may have the same effect as *KRAS G12C* inhibitors in countering drug resistance (28-30).

(III) *TP53* is a tumor-suppressor gene. Mutations result in the loss of *TP53* function, promoting tumor growth and metastasis (31). Several studies have investigated the use of CRISPR-Cas9 to knock down *TP53* in lung cancer cells (32-34). One study demonstrated the existence of an autophagic switch that occurs in response to the antitumoral drug cisplatin, which can affect the sensitivity of cancer cells to this treatment (35). CRISPR-Cas9 was also used to generate a set of isogenic NSCLC cell lines expressing wild-type p53 (H460wt) and a knockout of p53 (H460crp53) in order to determine how cisplatin sensitivity and autophagic function are affected by p53 status (36).

(IV) *BCL2* has been identified as a contributing factor to the development of resistance against EGFR-tyrosine kinase inhibitors (TKIs) in NSCLC (37). A study reports low levels of *BCL2* expression detected in NSCLC samples collected prior to resistance development and, conversely, report highly levels detected subsequent to the emergence of resistance (38). This suggests that upregulation of *BCL2* may be a latent driver for resistance exploitation and that targeting *BCL2* may be a useful strategy in treating NSCLC with acquired resistance (39). There have been no studies that have employed CRISPR to knock out *BCL2* in lung cancer. However, for other cancer types, such as prostate or liver cancer, CRISPR-Cas9 has been used to successfully interfere with *BCL2* expression *in vitro* and *in vivo* and to block the resistance caused by *BCL2* expression (40,41).

(V) *PIK3CA* is a signaling pathway gene, whose mutations cause the overactivation of *PIK3CA*, thus promoting tumor growth and metastasis (42). There are currently no studies that have specifically focused on using CRISPR-Cas9 to target *PIK3CA* in lung cancer. However, similar studies have been conducted in breast cancer. CRISPR-Cas9-based single-guided RNA (sgRNA) knockout screens were carried out on MCF7 and T47D estrogen receptor $(ER)^+$ breast cancer cell lines mutated in *PIK3CA* to investigate possible mediators of inhibitor response (43). In one study, the researchers used CRISPR/Cas9 technology to knock out the *PIK3CA* gene in these cell lines and then treated a group with dimethyl sulfoxide and another group with PI3Kα inhibitor (alpelisib or taselisib) for 20 days. The genomic DNA of each group was extracted, and the sgRNA sequences were amplified via polymerase chain reaction and then retrieved via next-generation sequencing. The results of this study suggest that genomic alterations involving the genes that potentially affect mechanistic target of rapamycin (mTOR) activity are present in a significant proportion of primary and metastatic *PIK3CA*-mutant breast tumors and may play a role in limiting the sensitivity to PI3Kα inhibition. However, this study did not specifically arrive at a conclusion regarding the effects of the CRISPR knockout of *PIK3CA* on the sensitivity to PI3Kα inhibitors (44).

(VI) *ALK* is a mutated gene that causes overactivation of *ALK*, promoting tumor growth and metastasis (45). Oncogenic *ALK* gene rearrangements occur in a small percentage of patients with NSCLC and result in the overexpression and ligand-independent activation of *ALK* (46). The nature of the fusion partner determines the degree of activation (47). Although echinoderm microtubule-associated protein-like 4 (EML4) is the most common fusion partner, multiple other fusion partners have been reported (48,49). Five *ALK* inhibitors have been approved by the United

States Food and Drug Administration (FDA) for use in treating NSCLC (alectinib, brigatinib, ceritinib, lorlatinib, and crizotinib) (50). Although these inhibitors have shown efficacy, resistance mechanisms to them exist and are an active area of research. *ALK* gene rearrangements can lead to drug resistance in NSCLC (51). Resistance mechanisms mediated by *ALK* include *ALK* amplification, copy number gain, and mutations of the *ALK* kinase domain. Depending on the resistance mechanism, high-potency, second-generation ALK-TKIs can be used (to target *ALK*-dependent resistance); otherwise, agents that target multiple pathways in addition to *ALK* (to target *ALK*-independent resistance) can be used (52). In other type of cancer, neuroblastoma, researchers used CRISPR-Cas9-based screening to identify genes associated with *ALK* inhibitor resistance in neuroblastoma cell lines. One study has found that mutations in *ALK* signaling pathways confer resistance to *ALK* inhibitors in neuroblastoma cells, resulting in collateral vulnerability (53).

(VII) *MET* is a signaling pathway gene, with mutations leading to overactivation of *MET*, thud promoting tumor growth and metastasis. *MET* mutations are closely associated with resistance to targeted therapies. CRISPR-Cas9's regulation of these genes primarily involves expression regulation and mutation repair. Some studies have shown that the activation of *MET* negatively affects the effectiveness of TKIs in NSCLC due to crosstalk between *MET* and receptor tyrosine kinase (*RTK*) signaling pathways which starts with the activation of rat sarcoma virus (Ras) protein, a small G protein anchored to the plasma membrane(54,55).

In the evasion of cell death induced by EGFR-TKIs, *MET* amplification promotes downstream signal transduction through bypass activation. Therefore, MET amplification is an important mechanism of EGFR-TKI resistance, with a prevalence of 5–21% observed after first- or second-line EGFR-TKI resistance, ~5% after first-line therapy, and ~19% after later-line osimertinib resistance (56). Another study found that MET alterations are a recurring and actionable resistance mechanism in ALK-positive lung cancer. This suggests that targeting both *ALK* and *MET* pathways may be an effective treatment strategy for patients with this type of cancer. In one report, two patients with *ALK*-positive lung cancer and acquired *MET* alterations achieved rapid clinical and radiographic response to *ALK-MET* combination therapy. Therefore, the study provides rationale for pursuing *ALK-MET* combination therapy in clinic (57). Studies have also demonstrated that CRISPR-Cas9 can effectively interfere with MET both *in vivo* and *in vitro* (58,59). Therefore, it is believed that the CRISPR-Cas9 function of *ALK* gene editing, when combined with CRISPR-Cas9's simultaneous targeting of *MET* and *ALK* expression or mutation, may have a role in regulating drug resistance in NSCLC.

CRISPR-Cas9 targeting signaling pathways related to drug resistance in lung cancer

The complex process of tumor drug resistance often transcends the function of single genes, making the study of relevant signaling pathways particularly crucial (60). CRISPR-Cas9 may influence the activity of the signaling pathways related to lung cancer drug resistance by regulating key nodes, thereby improving drug sensitivity (*Figure 2*). The currently known signaling pathways related to drug resistance in lung cancer include (I) the PI3K/protein kinase B (Akt)/mTOR signaling pathway; (II) Wnt/β-catenin signaling pathway; (III) Notch signaling pathway; (IV) Hedgehog signaling pathway; (V) nuclear factor-κB (NF-κB) signaling pathway; (VI) adenosine triphosphate (ATP)-binding cassette transporter signaling pathway; (VII) DNA repair signaling pathway; and (VIII) rat sarcoma virus (RAS)/rapidly accelerated fibrosarcoma (RAF)/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway. (I) The PI3K/Akt/mTOR signaling pathway is essential for cell growth and survival and is involved in a variety of cellular processes, including cell proliferation, apoptosis, metabolism, and angiogenesis (61). (II) The Wnt/β-catenin signaling pathway is an important pathway for embryonic development and tissue regeneration. It has been implicated in numerous cellular processes, including cell proliferation, differentiation, and epithelial-mesenchymal transition (EMT) (62). (III) The Notch signaling pathway is a vital cell fate-determining pathway and involved in various cellular processes, including cell proliferation, differentiation, and cancer cell stemness (63). (IV) The Hedgehog signaling pathway is another critical cell fate-determining pathway and participates in numerous cellular processes, including cell proliferation, differentiation, and apoptosis (64). (V) The NF-κB signaling pathway, an essential inflammatory and immune response pathway, is involved in various cellular processes, including cell

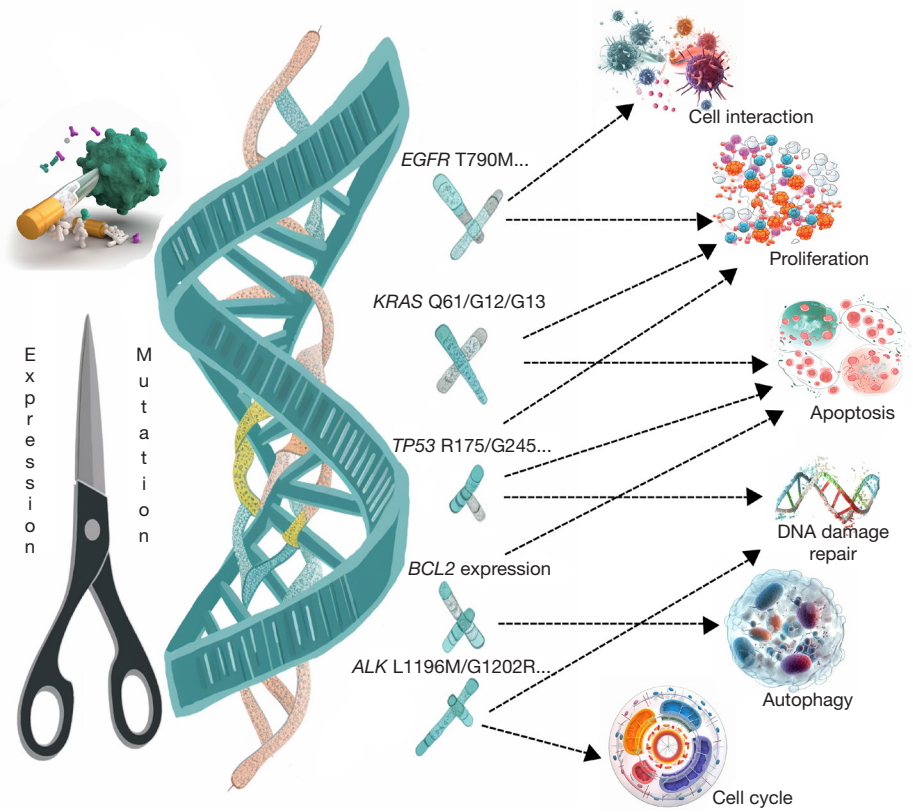

Figure 2 Use of CRISPR-Cas9 technology for the editing of tumor driver genes and its effect on the biological behavior of tumor cells. *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *TP53*, tumor protein 53; *BCL2*, B-cell lymphoma/leukemia-2; *ALK*, anaplastic lymphoma kinase; CRISPR-Cas9, CRISPR and CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats.

proliferation, apoptosis, inflammation, and immune response (65). (VI) The ABC transporter signaling pathway, includes ABC transporters, which are a class of transmembrane proteins that transport chemotherapy drugs from the intracellular to extracellular space, thus reducing drug accumulation in cells and decreasing drug efficacy (66). (VII) The DNA repair signaling pathway is closely linked to DNA repair, which is a vital repair mechanism for cells to ameliorate the damage to DNA caused by chemotherapy drugs, thereby reducing drug efficacy (67). (VIII) The RAS/RAF/MEK/ERK signaling pathway, which is critical to cell growth and survival pathway, is implicated in various cellular processes, including cell proliferation, apoptosis, metabolism, and angiogenesis. Abnormal activation of the RAS/RAF/MEK/ERK signaling pathway is closely related to the development of various tumors and is an essential mechanism of drug resistance in tumor-targeted therapy (68).

CRISPR-Cas9 reshapes biological function by making different adjustments at multiple points within the signaling pathway. For example, in the PI3K/Akt/mTOR signaling pathway, CRISPR-Cas9 can target several genes, such as *PIK3CA*, *AKT1*, and *MTOR*. Knockout of the *PIK3CA* gene can inhibit PI3K signaling pathway activity by altering key gene regulations, thus affecting cell proliferation and growth in breast cancer. Moreover, by knocking out the *AKT1* gene in prostate cancer, the expression level of Akt protein can be reduced, impacting the downstream signaling pathway activity of Akt, another essential molecule in this signaling pathway. Simultaneously, *MTOR* gene knockout can decrease the phosphorylation level of mTOR protein, affecting the activity of the mTOR signaling pathway at the level of protein posttranslational modification (69,70).

CRISPR-Cas9 targeting immune escape in lung cancer resistance

Immune escape refers to the phenomenon in which certain

pathogens or tumor cells evade the host immune system's attack and clearance through various means, resulting in disease persistence or recurrence (71). The targeting of immune escape by CRISPR-Cas9 in lung cancer resistance is primarily based on the regulation of key genes involved in the process (72). The key genes of immune escape can be categorized as follows: (I) immune recognition genes, which includes T-cell receptor genes, B cell receptor genes, MHC molecular genes, and others, all of which play a critical role in antigen recognition and identification within the immune system (73); (II) immune signal transduction genes, which encompasses those related to the JAK-STAT signaling pathway, NF-κB signaling pathway, and PI3K-Akt signaling pathway, among others, and which are essential for signal transduction and immune response regulation within the immune system (74); (III) immune regulatory genes, which include immunosuppressive factor genes and immunostimulatory factor genes, which play a significant role in immune response regulation within the immune system (75); (IV) immune effector genes, comprising cytotoxin genes, chemokine genes, and cytokine genes, which are crucial for pathogen killing, immune cell attraction, and immune response regulation within the immune system (76); and (V) immune escape genes, which include tumor mutation genes, pathogen mutation genes, and immunosuppressive factor genes, which play a vital role in immune system evasion.

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway is discussed as an example here. JAK-STAT signaling involves five key proteins: JAK protein, STAT protein, suppressor of cytokine signaling (SOCS) protein, cytokine receptor, and protein inhibitors of activated STAT (PIAS) protein. The JAK and STAT proteins are involved in the phosphorylation and dephosphorylation processes, while the SOCS and PIAS proteins serve as negative regulatory factors of JAK-STAT (77). The cytokine receptor is responsible for binding with cytokines and recruiting JAK and STAT proteins to regulate immune cell differentiation and function (78). CRISPR-Cas9 technology is capable of a variety of functions, such as knockout, addition, repair, and modification. Previous studies have demonstrated that CRISPR-Cas9 can significantly regulate the activity of the JAK-STAT pathway (79-81). Additionally, researchers have used CRISPR-Cas9 technology to target immune escape mechanisms in lung cancer cells, such as those involving programmed death ligand 1 (PD-L1) and transforming growth factor-β (TGF-β) (82). By cutting these genes and reducing their expression levels, the immune cells' ability to attack lung cancer cells is enhanced. Moreover, novel immune cells have been developed, including CAR-Ts and natural killer cells, which more effectively target lung cancer cells (83).

Application of CRISPR-Cas9 in identifying the key genes and molecular mechanisms of lung cancer drug resistance

In addition to being applied in the knockout of the genes known to be related to resistance, CRISPR-Cas9 has been used as a powerful tool for exploring novel key genes and molecular mechanisms of drug resistance in lung cancer. By using CRISPR-Cas9 to knock out specific genes in lung cancer cells, researchers can identify which genes are integral to drug resistance and which genes can be targeted to overcome drug resistance (84,85). For instance, a study published in the prestigious journal *Cancer Research* in 2017 employed CRISPR-Cas9 to knock out the gene for the myeloid cell leukemia-1 (MCL-1) protein in lung cancer cells (86). The authors of this paper discovered that MCL-1 plays a critical role in mediating resistance to chemotherapy drugs such as cisplatin and etoposide. By targeting MCL-1 using CRISPR-Cas9 or other drugs, they were able to sensitize lung cancer cells to chemotherapy. Another study published in the esteemed journal *Nature Communications* in 2018 used CRISPR-Cas9 to knock out the gene for the Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1 (KEAP1) protein in lung cancer cells (87). KEAP1 is a negative regulator of the nuclear factor erythroid-2-related factor 2 (NRF2) pathway, which is often overexpressed in lung cancer and contributes to drug resistance. It is an E3 ubiquitin ligase, a critical regulator that targets NRF2 for ubiquitylation and subsequent degradation (88). The researchers found that knocking out KEAP1 increased the sensitivity of lung cancer cells to chemotherapy drugs such as cisplatin and paclitaxel. These studies highlight the potential of CRISPR-Cas9 to identify key genes and molecular mechanisms of drug resistance in lung cancer and to aid in the development of new therapeutic strategies to overcome drug resistance. However, further research is required before the intricate molecular mechanisms of drug resistance in lung cancer can be more fully comprehended and to develop safe and effective methods for delivering CRISPR-Cas9 to lung cancer cells in humans.

As previously mentioned, NRF2, which is encoded by gene *NFE2L2*, is a transcription factor that plays a pivotal role in regulating the antioxidant response in cells. In

lung cancer, NRF2 is frequently overexpressed, leading to increased resistance to chemotherapy and radiation therapy (89-93). The use of CRISPR-Cas9 to knock out NRF2 in lung cancer cells has been shown to increase the sensitivity of these cells to chemotherapy and radiation therapy. Several studies have investigated the efficacy of CRISPR-Cas9 in knocking out NRF2 in lung cancer cells. For instance, according to a study published in 2018, knocking out NRF2 in lung cancer cells increased their sensitivity to chemotherapy (94). In another study published in 2019, CRISPR-Cas9 was used to knock out NRF2 in lung cancer cells and reduce tumor formation in mice (95). Schlafen 11 (*SLFN11*) is a gene involved in DNA repair and replication. Recent studies have shown that *SLFN11* expression is associated with lung cancer sensitivity to chemotherapy and immunotherapy. Knocking out *SLFN11* using CRISPR-Cas9 in lung cancer cells has been shown to reduce their sensitivity to chemotherapy and immunotherapy (96). One study demonstrated that *SLFN11* can sensitize tumor cells to interferon-γ (IFN-γ)-mediated T-cell killing (97), while another study found that *SLFN11* is responsible for platinum treatments activated immune-related pathways in high-grade serous ovarian cancer (98). In SCLC, poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitor was found capable of regulating *SLFN11* expression and demonstrated therapeutic synergy with temozolomide (96). In line with this reasoning, CRISPR-Cas9 may have a similar function in lung cancer.

Aurora B is a protein that plays a crucial role in cell division and is often overexpressed in lung cancer cells. Recent studies have investigated the use of CRISPR-Cas9 in targeting Aurora B in lung cancer cells as a potential therapeutic strategy. This research indicates that using CRISPR-Cas9 to knock out Aurora B in lung cancer cells reduces their ability to form tumors in mice (99). Another study demonstrated that using CRISPR-Cas9 to knock out Aurora B in lung cancer cells increases their sensitivity to chemotherapy drugs such as paclitaxel (100). Polybromo-1 (*PBRM1*) is a gene that plays a crucial role in regulating gene expression and is often mutated in lung cancer. Recent research has investigated the use of CRISPR-Cas9 to knock out *PBRM1* in lung cancer cells as a potential therapeutic strategy. One study reported that knocking out *PBRM1* using CRISPR-Cas9 in lung cancer cells reduced their ability to form tumors in mice (101). Another study revealed that knocking out *PBRM1* using CRISPR-Cas9 in lung cancer cells increased their sensitivity to chemotherapy drugs such as cisplatin and gemcitabine (101).

Glycan-related genes play a pivotal role in the complex mechanisms underlying multidrug resistance (MDR) in cancer. Altered glycosylation patterns, facilitated by aberrant expression or mutations in genes involved in glycan biosynthesis and remodeling, can significantly influence drug transport, cell signaling, and adhesion - all of which contribute to the development of MDR. For instance, the upregulation of glycoprotein drug transporters, such as P-glycoprotein, is a well-characterized mechanism that enables cancer cells to actively efflux chemotherapeutic agents (102). Furthermore, glycans can modulate key signaling cascades linked to MDR, including the PI3K/Akt, MAPK, and Wnt pathways, promoting cell survival and proliferation (103,104). In this context, the emergence of CRISPR-Cas9 technology holds great promise for targeting glycan-related genes to overcome MDR. One study demonstrated that via CRISPR-Cas9 editing, deacetylated glycan sialic acid (Sia) regulated a MDR pathway in colon and lung cancer and disrupt drug efflux and alter glycosylation patterns (105). Additionally, combinatorial CRISPR approaches targeting multiple glycan-associated genes may provide a more comprehensive strategy to address the complexity of MDR mechanisms, thereby resensitizing resistant cancer cells and improving treatment outcomes.

Future research directions in the use of CRISPR-Cas9 technology to counter treatment resistance in lung cancer

Current advancements in CRISPR-Cas9 technology primarily center around enhancing editing efficacy and safety while fostering novel applications. This emphasis on the enhancement of editing efficacy can be categorized into two domains: the inception of new editing instruments and refinement of established research tools. Notably, considerable strides have been made within this research dimension. Comprehensive analyses of viral genomes have unearthed a plethora of potential CRISPR-based genome-editing mechanisms. One groundbreaking study completed a thorough examination of the CRISPR-Cas system in viruses, particularly phages, that plague bacteria and archaea. Astonishingly, the research identified approximately 6,000 phages equipped with CRISPR-Cas systems, spanning every acknowledged variant of the CRISPR-Cas framework. These systems represent promising tools for potential gene-editing applications (106). A case in point is the recently identified CRISPR-Cas12n (107). This system is a derivative of the V-U4-type CRISPR-Cas, closely related to TnpB—the transposition-linked nuclease and the putative

progenitor of V-type CRISPR frameworks, and may be an early evolutionary bridge in TnpB's transition into other V-type Cas nucleases.

Concerning specific diseases, specialized gene-editing tools promise enhanced outcomes. In a seminal study, researchers dissected DNA sequences of the fetal hemoglobin gene posttherapeutic editing using both CRISPR-Cas9 and base editors. Their findings indicated that Cas9 editing frequently yielded multifaceted, unsupervised editing across varied DNA sequences. Consequently, they innovated base editing which, as the results suggested, augmented fetal hemoglobin expression more effectively than did CRISPR-Cas9. Furthermore, the study demonstrated that the diversity of insertion and deletions instigated by CRISPR-Cas9 could spawn unanticipated phenotypic fluctuations. Such challenges might be circumvented through base editing, offering a potentially safer and more efficient therapeutic strategy for ailments such as sickle cell disease and β-thalassemia (108). Concurrently, the pioneering team behind the CRISPR-Cas9 technology has persisted in refining this gene editing approach. Their recent revelation is Fanzor, a novel RNA-steered DNA-cleaving enzyme observed in eukaryotes. Notably, this nascent CRISPR-like system is reconfigurable, allowing it to edit the human genome. Furthermore, the compact nature of the Fanzor system renders it more amenable to cell and tissue delivery as compared to the traditional CRISPR-Cas mechanisms. Additionally, lacking paracrine cleavage activity, Fanzor allows for greater precision in genome editing. The implications of this seminal work suggest that RNA-induced DNA cleavage might be ubiquitous across organisms (109).

Drawing from these investigations, we can speculate that deploying CRISPR-Cas9 in lung cancer drug resistance research may lead innovations in the following domains: (I) improvement of sgRNA design; (II) refinement of the Cas9 structure, active center, and specificity; (III) enhancement of vectors and delivery modalities, including the harnessing of complementary techniques such as extracellular vesicle delivery; and (IV) the customization of technology to align with specific tumor attributes or those of drug-resistant tumor cells.

As CRISPR-Cas9 technology continues to advance at a rapid pace, an increasing volume of research is being published on the addressing safety concerns pertaining to its use in humans. A recent study noted that CRISPR/Cas9 gene editing can induce cytotoxicity and genomic instability depending on the target sequence sites within the human genome. This undesirable effect operates via the pivotal tumor suppressor protein p53 and is influenced by various epigenetic factors in the DNA sequence proximate to the edited site and its surrounding area. Through computational methods, the authors of this study scrutinized the widely used CRISPR libraries tailored for human cells and identified 3,300 target sequence sites exhibiting pronounced toxic effects. They further revealed that nearly 15% of human genes involve at least one editing site associated with toxicity (110).

Other studies underscored the risks intrinsic to CRISPR therapies, emphasizing the potential for damage to the genome (111,112). Despite CRISPR genome editing demonstrating remarkable efficacy, its safety profile is not uniformly positive. At times, fragmented chromosomes fail to restore their functionality, undermining genome stability, which may lead to adverse oncogenic effects over time. CRISPR therapy has been broadly applied to address an array of conditions, from cancers to genetic syndromes; however, evaluations of its impact on immune system white blood cells indicates that up to 10% of treated cells exhibit a significant loss of cytogenetic material. This can in turn induce genomic instability, elevating cancer risks. The inaugural CRISPR clinical trial, conducted by the University of Pennsylvania in 2020, employed CRISPR technology on T cells from a donor. The T cells were engineered to express a receptor targeting cancer cells and to concurrently disrupt genes encoding the native receptor to avert autoimmunity (113). Another study discussed whether the potential advantages of CRISPR therapy might be overshadowed by the inherent risks of genomic cleavage (114). This speculation was predicated on the notion that damaged DNA may not invariably undergo successful repair, leading to the loss of significant chromosomal segments or even entire chromosomes. Such chromosomal aberrations can render the genome unstable and in a state reminiscent to that observed in cancer cells. In extreme scenarios, CRISPR therapy might inadvertently catalyze cellular carcinogenesis (111).

Nonetheless, in the specific context of lung cancer drug resistance research, our stance is that the immediate priority may lean toward extending patient survival and elevating the quality of treatment. Decisions on genomic implications may be deferred pending a more comprehensive understanding provided by ongoing research.

Conclusions

CRISPR-Cas9 was first reported to have the ability to

reprogram DNA in 2012 (115). Over the past decade, CRISPR-Cas9 technology has achieved several milestones: it is the subject of a Nobel Prize for chemistry, and the first drug developed based on CRISPR/Cas9 technology will be approved this year for the treatment of sickle cell and other diseases (116). This review outlines the progress in the research on the treatment of drug resistance in lung cancer using CRISPR-Cas9 technology. The basic principle of CRISPR-Cas9 technology and its application in lung cancer treatment are discussed, specifically the work to overcome drug resistance in lung cancer via CRISPR-Cas9-targeted genes. The purpose of this review is to serve as a valuable reference for the further development of CRISPR-Cas9-based gene intervention systems, which may ultimately improve the management of drug resistance in patients with lung cancer.

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Footnote

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