The Therapeutic Potential of CRISPR-Cas9 in Drug Resistance During Cancer Treatment

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Abstract. With population aging on the rise, cancer's burden as the main cause of death has dramatically increased globally Although multiple treatment methods are available, the death rate is high. Drug resistance is still the main factor contributing to cancer deaths and recurrence at this time. The implementation of the CRISPR-Cas 9 gene editing approach plays a promising role in reducing drug resistance during cancer therapy because the main mechanism of drug resistance is genetic composition shifting. CRISPR-Cas9 has already served as a potent approach in several industries, particularly in those of agriculture and health. Nevertheless, given the limitations of CRISPR-Cas9 application, more study is required to lessen the unfavorable side effects. The mechanisms of the CRISPR-Cas9 technique and the mechanisms of drug resistance in chemotherapy have been reviewed in this article, along with the history of CRISPR-development, Cas9's current application fields, and potential application opportunities and CRISPR-limitations Cas9's in overcoming drug resistance.

Keywords: Drug resistance, Cancer, CRISPR-Cas9, Gene Editing (GE).

1. Introduction

The 2nd largest cause of death worldwide is cancer [1]. Despite the development of numerous cancer medicines, such as chemotherapy, surgery, radiation therapy, immunotherapy, target therapy, and endocrine therapy, approximately 1 in 6 individuals worldwide still succumb to the disease [1-3]. The emergence of treatment resistance is a major factor resulting in the death of cancer patients (90%) [3]. Through the application of CRISPR-Cas9, some genes associated with drug resistance have recently been discovered. These genes could one day be targeted to help cancer cells overcome their drug resistance [3,4].

1.1. Mechanism of drug resistance

When it comes to cancer treatments, particularly chemotherapy, targeted therapy, and immunotherapy, drug resistance and multiple drug resistance are frequent and significant difficulties [3,5]. Relapses were made more likely by the emergence of treatment resistance in cancer cells [2]. For instance, after chemotherapy and surgery, 50% to 70% of ovarian adenocarcinoma cases recurred within a year [2]. Drug resistance can either be intrinsic, meaning it was in the cancer cells before therapy, or acquired, meaning it appeared in the cancer cells after treatment [2,3,6]. Increased heterogeneity, anticancer agent efflux, senescence escape, drug target alteration, improved ability to repair DNA damage, changes to epigenetics, tumor microenvironment effect (pH level), intracellular and extracellular ATP level, and epithelial-mesenchymal transition factors are just a few of the many drug-resistant mechanisms tumor cells can develop [2,3,6]. As was already indicated, although numerous mechanisms may cause drug resistance in cancer cells, the fundamental one is changes in the genetic makeup of these cells, which may be brought about by heterogeneity or therapeutic interventions [4,7]. Editing the genes causing or linked to drug resistance is therefore required to reduce drug resistance effects and increase the potency of anticancer medicines.

1.2. Genome editing techniques

Numerous techniques for targeted GE have been developed with recent rapid advancements in gene editing technology [8]. Table 1 lists the benefits and drawbacks of the three genome editing

tools: CRISPR, transcription activator-like effector nuclease (TALEN), and zinc finger nuclease (ZFN) [8]. In general, CRISPR-Cas9 outperformed TALEN and ZFN, becoming the most effective gene editing method of the 20th century based on its high efficiency, simplicity, and low cost [8].

Table 1. Differences between TALEN, ZFN, and CRISPR-Cas GE techniques [8].

Feature	TALEN	ZFN	CRISPR-Cas
Cost	High	Low	Low
Ease of design	A little complex	Moderate	Simple
Specifity	Intermediate	Low	High
Pros	Highly effective and specific	Highly effective and specific	Modifies multiple sites in tandem
Cons	Time consuming	Time consuming	protospacer-adjacent motif (PAM) required next to target sequence

2. CRISPR-Cas9

2.1. Mechanism of CRISPR-Cas9

As a powerful GE tool, CRISPR-Cas9 technology comes from a prokaryotic cell's immune system [3,4]. A Cas9 protein and combined with a sgRNA makes up a typical CRISPR-Cas9 system [9]. Trans-activating crRNA (tracrRNA) and CRISPR RNA (crRNA) make up sgRNA [9]. The goal of sgRNA is to instruct Cas9 by recognizing the target sequence through the 5'end and transferring it into the cell [9]. After PAM recognition, the Ruvc domain and MHN domain of Cas9 protein produce double-stranded breaks (DSBs) [9]. Then, the DSBs will then be repaired via either the homology-directed repair pathways or non-homologous end joining pathway [9].

2.2. Development history of CRISPR-Cas9

There are four major components of the CRISPR-Cas9 system, which were discovered at different times respectively [10]. The short DNA repeats CRISPR was first discovered from the DNA sequence of Escherichia coli bacteria by Ishino et al. in 1987 and the Cas9 protein was first mentioned in 2005 [10]. The two RNA molecules crRNA and tracrRNA were found in 2007 and 2011, respectively [10]. The most significant discovery in the history of biology was made by Doudna and Emmanuelle Charpentier in 2012 when they initially proposed genome programmable editing using CRISPR-Cas9. 2013 saw the implementation of CRISPR-Cas9 in eukaryotic cells and germline cells [10]. At the same time, the CRISPR-Cas9 system's development opened up greater possibilities in numerous biomedical sectors.

2.3. Application fields of CRISPR-Cas9

In addition to its use in the study of cancer treatment resistance, CRISPR-Cas9 has made a substantial contribution to a number of other industries, such as biotechnology, medicine, and agriculture [11]. Cystic fibrosis, -thalassemia, sickle cell anemia, and other conditions are among the 22 CRISPR-Cas9-associated gene treatments that have been authorized to treat human illnesses [11]. Additionally, CRISPR-Cas9 is used in HIV treatment and CAR-T cell therapy, both of which are crucial therapeutic applications of the gene editing technology [11]. The use of CRISPR-Cas9 in agriculture can strengthen a crop's resistance to disease and drought, extend its shelf life and boost its nutritional value, and raise the muscle mass of cattle animals used in animal husbandry [11].

3. Present progress of CRISPR-Cas9 application in cancer cell drug resistance

The study found that CRISPR-Cas9 may be a helpful technique to overcome medication resistance during many cancer treatments [3]. For instance, by employing CRISPR-CAS9 to target the ABCB1

gene, osteosarcoma cells' drug resistance to DOX was reduced [3]. Additionally, employing CRISPR-Cas9 to delete the ACBC1 gene can make breast cancer cells more chemo-sensitive and accumulate more doxorubicin (DOX) [3].

The positive and negative selection helped to expose the medication resistance mechanisms, and the CRISPR-Cas9 library screens were crucial to identify target genes associated with drug resistance [12]. In addition, CRSIPR-Cas9 screening is more successful than RNAi screening because it is more sensitive to the screening process and has a lower off-target effect [12]. superior to RNAi screening in effectiveness Currently, CRISPR-Cas9 technology has discovered more than 20 genes linked to cancer medication resistance (figure 1) [3].

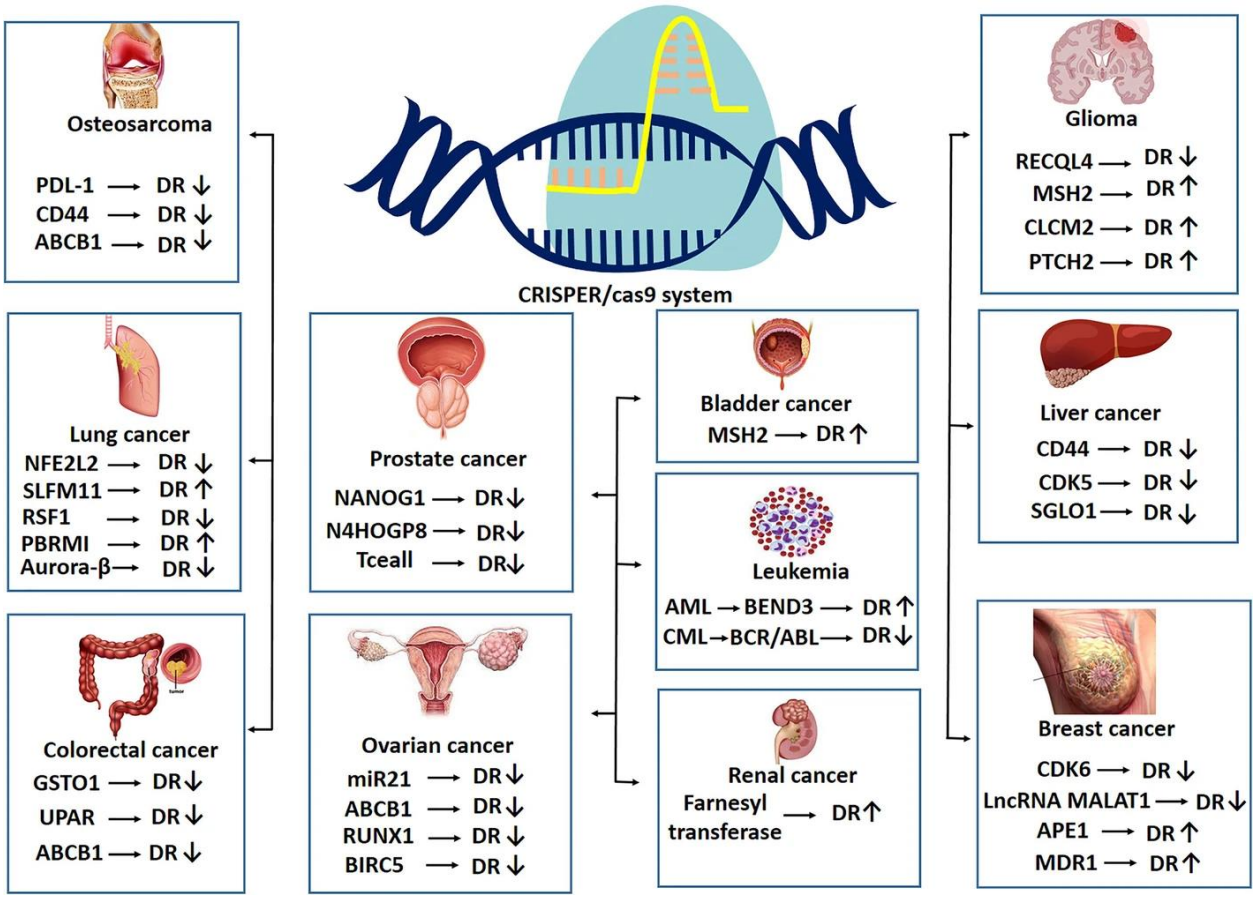

Figure 1. Target genes for medication resistance have been identified, and CRISPR/Cas9 has been shown to reduce drug resistance [3].

DR: drug resistance

At present, the utilization of CRISPR-Cas9 technology shows efficacy by targeting some identified genes in cancer cells, which included increased cancer cells' sensitivity to chemotherapy, increased drug accumulation in cancer cells, increased cytotoxicity of drugs, reduced cancer cell proliferation, and increased chances of apoptosis, etc. [3]. For instance, the identification of gene SLFN11 in lung cancer, gene BIRC5 in ovarian cancer, etc. [3]. These identified genes are the potential targets for overcoming drug resistance 18. For example, the chemosensitivities of cisplatin increased in cancer cells by targeting the RUNX1 gene in ovarian cancer [3]. Using CRISPR-Cas9 targets the gene PARP1 which is involved in DNA repair could decrease the DNA repair ability as well as improve the effectiveness of anti-cancer drugs, such as doxorubicin [3]. Using CRISPR-Cas 9 targets and knockout RECQL4 gene in glioma cells could increase the temozolomide toxicity, so the level of apoptosis markers and DNA damage increased [3]. Some genes were identified that have shown a significant effect in reducing drug resistance after the application of CRISPR-Cas9, but the specific mechanism is still unclear [3]. In addition, most research about overcoming drug resistance by utilizing CRISPR-Cas9 is currently in the clinical trial stages, there are some outcomes of clinical

trials illustrated the effectiveness [3]. Therefore, CRISPR-Cas9 could be an effective and promising approach to overcoming drug resistance in the future.

4. Limitations of CRISPR-Cas9 application

Nevertheless, off-target is a significant limitation which needs further investigations in the CRISPR-Cas9 application, the solutions to minimize the off-target effects include optimization of sgRNA, using high specificity promoters and surface receptors with high expression in cancer cells, or using D10A-mutated Cas9 [3]. In addition, the off-target effects of Cas9 variants eSpCas9 and SpCas9-HF1 are also decreased significantly [3]. Another limitation results from antigen-specific T-cells which are against Cas 9 proteins directly [7]. This limitation can be minimized by modifying Cas9 to escape the immune system or suppress the immune system directly [7]. Besides, since most research about CRISPR-Cas9 is conducted in vitro, hence the effectiveness of the treatment effect is also determined by the delivery methods [13]. At present, the most commonly adopted delivery approach for CRISPR-Cas9 is the AAV vectors, which can trigger the immune reaction or increase off-target mutations, some enzymes also have the ability to degrade vectors [3]. Thus, nanotechnology-based vectors were invented, such as lipid nanoparticles, nanoparticles, inorganic nanoparticles, porous nanoparticles, etc. [13]. This will enhance therapeutic efficacy and suppress side effects of CRISPR-Cas9 gene editing prominently [13]. Moreover, due to the heterogenicity of cancer cells, targeting only one specific gene could kill the sensitive clone but no other sub-clones [4]. Therefore, the application of multiple sgRNA could be considered as a possible solution [4]. The ethical issue of CRISPR-Cas9 gene editing also cannot be ignored, on one hand, the high expenditure of CRISPR-Cas9 due to individualized therapy leads people unable to access it equally [4]. On the other hand, research about CRISPR-Cas9 technology involved in zygotes and embryos also needs to be considered in terms of ethics and law [4]. Finally, the consideration of potential adverse effects on ecological environments and other organisms is also necessary [4].

5. Conclusion

In conclusion, drug resistance is a leading cause of death among cancer cases. Although there are many drug resistance mechanisms, genome mutation of cancer cells is the root cause. Hence, the research and application of CRISPR-Cas9 GE technique are conducive to screening out and editing more genes related to drug resistance in the clinical aspects, which will offer more possibilities to overcome drug resistance and then reduce the mortality of cancer patients. Thus, CRISPR-Cas9-based therapy can be the basis of personalized medicine in cancer treatment in the future. However, because CRISPR - Cas9 is still in the development stage, limitations and side effects during this therapy need to be minimized and solved by more research, and the ethical issues about gene editing are also under debate.

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