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CRISPR-Cas9 Gene-Editing Technology for the Screening of Drug Resistance Genes in Tumor Cells

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Abstract. Chemotherapy is the most widely used and effective treatment method in today's cancer treatment. However, the rise of cancer drug resistance has limited chemotherapy effectiveness. The molecular mechanisms and related genes by which many cancer cells acquire resistance to chemotherapy drugs remain to be identified. Recent advancements of CRISPR-Cas9 genome-editing techniques have drawn rigorous exploration of precision oncology applications. CRISPR-Cas9 technology can knock out, inhibit or activate the expression of specific genes. By observing the phenotype of gene-edited cells, drug-resistant genes in carcinoma cells can be identified. This paper will decipher the molecular basis of CRISPR-Cas9 technology in targeted gene modification and give examples to illustrate its application in the screening of drug resistance genes in three types of human cancers. Additionally, the application limitations and emerging horizons of CRISPR-Cas9 gene modification technique in strategies for overcoming anticancer drug resistance are discussed. The continuous development and optimization of this technology are expected to provide strong support for breaking through the bottlenecks in tumor treatment and pushing precision cancer medicine to new heights.

Keywords: CRISPR-Cas9, drug-resistance cancer, gene screening, drug resistance mechanism

1. Introduction

Cancer treatment remains highly challenging and results in high mortality, although medical institutions currently use methods such as surgery, chemotherapy, targeted therapy, and radiation therapy to treat cancer. Despite advancements in these modalities, many patients experience disease recurrence or progression, highlighting the urgent need for more effective strategies. Therefore, it is necessary to explore new cancer treatment methods and improve existing cancer therapies. The CRISPR-Cas9 system was first discovered in prokaryotes and is an effective tool for prokaryotes (e.g., bacteria) to defend against foreign viral invasion. Over the past decade, this technology has evolved rapidly, transitioning from a microbial defense mechanism to a versatile gene-editing platform with broad applications in biomedical research. CRISPR-Cas9 enables gene editing, which makes it a tool for a variety of cell types and organisms. A critical hurdle in modern oncology lies in tumor cells' resistance to therapeutic agents. This resistance often arises from genetic mutations,

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epigenetic modifications, or adaptive changes in tumor cell populations, rendering standard treatments ineffective. While introducing the principle of CRISPR-Cas9 technology, this essay examines the evolving role of CRISPR-Cas9 technology in characterizing chemoresistance pathways across multiple cancer lineages, while objectively assessing its experimental validity and technical boundaries within pharmacological discovery contexts.

2. CRISPR-Cas9 for transcriptional regulation, genome-wide screening, and drug resistance research

In prokaryotes, the CRISPR-Cas9 complex operates as a molecular surveillance system, identifying foreign viral DNA (e.g., phages) for targeted degradation. Its locus includes spacer regions, containing DNA sequences from exogenous viruses and short repetitive DNA sequences encoding CRISPR-RNAs (crRNAs). Additionally, there are four genes encoding CRISPR-associated proteins (Cas) lie near the CRISPR locus [1]. The tracrRNA gene located upstream of the CRISPR locus is transcribed and base-pairs with crRNA. Subsequently, the Cas9 protein assembles with the crRNA-tracrRNA duplex to generate a functionally active ribonucleoprotein (RNP) complex [1]. Foreign DNA recognition by the CRISPR-Cas9 system is mediated by its RNP assembly, where Cas9 executes site-directed nucleolytic cleavage through coordinated HNH and RuvC domain activities. Currently, CRISPR-Cas9 libraries was designed for key gene screening or target discovery related to specific phenotypes or cellular functions. These libraries typically employ virus-packaged sgRNA collections for high-throughput delivery. In CRISPR knockout (CRISPRko) systems, negative selection enables the identification of single-guide RNA (sgRNA) depletion or reduction within a cell population. CRISPRko is widely used to detect impaired cellular fitness, including reduced viability, diminished drug sensitivity, decreased proliferative capacity, and loss of migratory ability. Notably, sgRNA target sites can be designed across virtually all genomic regions-not just protein-coding genes but also non-coding regulatory elements for direct functional modulation [2]. In contrast, CRISPRa (CRISPR activation) employs a positive selection approach for analysis, where the most highly enriched target genes serve as the primary research focus. By designing a sgRNA library targeting sites near promoter regions, endogenous transcriptional activation can be achieved. The Synergistic Activation Mediator (SAM) system works in concert with the dCas9-VP64 fusion protein to enhance transcriptional activity, which leads to overexpression of the target gene [2].

3. Mechanism of generation of drug-resistant tumor cells

Two hypotheses exist regarding the formation of drug-tolerant persister cells (DTPs) [3]. The first hypothesis suggests untreated tumors have already contain a subpopulation of cancer cells with DTP characteristics, which survive through a clonal selection mechanism after drug treatment. The second hypothesis posits that pharmacologic intervention may cause a phenotype transformation in carcinoma cells, which convert into DTPs. Analytical findings reveal the persistence of a quiescent cellular subset exhibiting constitutively elevated levels of JARID1B (H3K4-specific demethylase) among treatment-experienced, rapidly proliferating malignant cell populations. This subpopulation demonstrates significant enrichment in residual tumor cells following chemotherapeutic treatment [4]. Emerging experimental evidence supports a paradigm wherein drug-tolerant persisters (DTPs) are primarily driven by stochastic reprogramming processes, instead of being predominantly governed by pre-existing clonal selection mechanisms [5]. By employing CRISPR gene knockout/activation technology combined with bioinformatics approaches, researchers can precisely and efficiently identify key genes associated with cancer drug resistance.

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4. CRISPR-Cas9-driven discovery of drug-resistant gene screening across cancer subtypes

4.1. Hepatocellular carcinoma

Despite conferring survival advantages in advanced hepatocellular carcinoma (HCC), sorafenib's clinical utility remains constrained by both innate and adaptive resistance mechanisms within tumor cell populations [6]. A genome-scale CRISPRa screen leveraging the Synergistic Activation Mediator (SAM) platform was implemented in Huh7 and MHCC-97H hepatocellular carcinoma models to systematically map genetic drivers of sorafenib refractoriness [7]. The study found that overexpression of LRP8 protein significantly inhibited sorafenib-induced apoptosis, whereas knocking down LRP8 expression using a lentiviral CRISPR system markedly enhanced apoptosis in both cell lines [6]. These results suggested that LRP8 may promote the development of drug-resistant tumors by suppressing sorafenib-induced apoptosis.

4.2. Pancreatic cancer

Pancreatic malignancies persist as a critical oncological burden, demonstrating high mortality indices among solid tumors globally. The clinically silent progression characteristic of this malignancy establishes chemotherapy as a cornerstone therapeutic modality. Gemcitabine remains the first-line chemotherapeutic standard for pancreatic adenocarcinoma management. Genome-wide CRISPRko screening conducted in the TB32047 murine pancreatic carcinoma cell model, coupled with pharmacological profiling under gemcitabine pressure, mechanistically validated DCK and CCNL1 as core molecular determinants mediating intrinsic gemcitabine resistance [7].

DCK is pivotal for gemcitabine’s therapeutic efficacy, which phosphorylates gemcitabine into mono-, di-, and tri-phosphate metabolites, exerting cytotoxicity via DNA chain termination [8]. Consequently, DCK deficiency abrogates gemcitabine activation, rendering the drug ineffective. Genetic ablation of DCK induces profound intrinsic gemcitabine resistance across pancreatic carcinoma model systems, establishing this metabolic enzyme as a determinant of drug sensitivity.

The study revealed that CCNL1-deficient cells exhibited significantly stronger resistance to gemcitabine compared to WT and NC cells, though this resistance was less pronounced than in DCK-knockout cells. Knockout of CCNL1 aberrantly activated pro-survival genes within several signaling pathways. The study revealed that knockout of CCNL1 in TB32047 cells elevated phosphorylation levels of Erk, Akt, and STAT3, which demonstrated that CCNL1 deficiency causes gemcitabine resistance through activation of the ERK/AKT/STAT3 survival signaling axis [7].

4.3. Lung cancer

Emerging evidence demonstrates that a diverse array of genetic modulators, spanning transcriptional regulators, epigenetic modifiers, and metabolic enzymes – collectively orchestrate the multifactorial chemoresistance observed in lung carcinoma cells (LCC), revealing an intricate genomic landscape of therapeutic refractoriness. CRISPR-Cas9-mediated genetic ablation of MCL-1 profoundly sensitizes lung carcinoma cells (LCC) to conventional chemotherapeutics. CRISPR-based technique is also applied in disruption of KEAP1 expression. KEAP1 serves as a central suppressor of NRF2 signaling, while NRF2 functions as a master transcriptional regulator coordinating cellular antioxidant defense mechanisms in malignant cells. In lung malignancies, constitutive activation of the NRF2 signaling axis drives chemoresistance through pleiotropic cytoprotective mechanisms. The KEAP1 knockout mutants have a higher sensitivity to cisplatin and paclitaxel. Moreover, the

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knockout of the NRF2 gene in LCC by CRISPR-Cas9 techniques can also augment their sensitivity to chemotherapy and radiotherapy [9].

5. Conclusion

The principal obstacle in contemporary oncology stems from tumor cell pharmacological refractoriness, which severely attenuates therapeutic efficacy across multiple treatment modalities while its intricate molecular pathogenesis remains incompletely mapped. CRISPR-Cas9 functional genomics empowers systematic interrogation of chemoresistance mechanisms through precision editing modalitie (including gene disruption, transcriptional modulation, and epigenetic remodeling)—enabling causal identification of genetic determinants across diverse malignancies through combinatorial genotype-phenotype mapping. In hepatocellular carcinoma, genome-scale activation or knockout screens have identified key genes associated with resistance to sorafenib and lenvatinib, whose expression is jointly regulated by genetic alterations and epigenetic mechanisms. In pancreatic cancer, genome-wide knockout screens have uncovered genes linked to gemcitabine resistance, which mediate resistance by affecting drug activation or activating survival signaling pathways. In lung cancer, the technology has helped identify genes regulating chemotherapy sensitivity, which influence drug-resistant phenotypes through involvement in anti-apoptotic and antioxidant pathways. This technological paradigm unmasks the molecular choreography of oncological refractoriness, simultaneously decoding the pathobiological circuitry driving resistance evolution while identifying druggable vulnerability nodes. Such functional genomics revelations empower rational design of mechanism-driven interventions to either neutralize resistance orchestrators or exploit synthetic lethal dependencies – effectively redefining therapeutic paradigms for treatment-refractory malignancies through precision oncology frameworks that maximize therapeutic indices and durability of clinical benefit. Looking ahead, the development of this technology will focus on enhancing editing efficiency and safety. Through optimizing tool design and screening methods, it will enable more precise and efficient identification of drug resistance genes, while combining with multi-omics analysis to advance the development of personalized resistance intervention strategies, to lay a more solid theoretical and clinical foundation for ultimately overcoming tumor drug resistance.

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