CRISPR Genome-Wide Screening: Applications and Innovations

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ABSTRACT

Introduction: CRISPR genome-wide screening has revolutionized the landscape of biomedical research, providing an unprecedented ability to dissect gene functions and identify novel therapeutic targets. This technology is particularly relevant in cancer research, where the complex and adaptive nature of tumors often limits the effectiveness of traditional therapies. This study leverages the power of CRISPR-Cas9 genome-wide screening to investigate the potential of targeting redox homeostasis in lung cancer cells. Lung cancer remains a leading cause of cancer-related mortality globally, necessitating innovative therapeutic strategies to overcome drug resistance and improve patient outcomes. This research explores the vulnerability of lung cancer cells to oxidative stress as a means of identifying novel targets for pro-oxidant-based therapies.

The rationale behind this study is grounded in the observation that cancer cells exhibit altered redox balance compared to their normal counterparts, characterized by elevated levels of reactive oxygen species (ROS) and dysregulated antioxidant defenses. This inherent imbalance, often referred to as "redox stress," creates a vulnerability that can be exploited therapeutically. By systematically disrupting redox homeostasis in lung cancer cells using CRISPR-Cas9 genome-wide screening, this study aims to uncover new targets for selectively inducing oxidative stress and eliminating cancer cells. Reactive oxygen species (ROS)-based therapeutic strategies have shown promise, yet a significant challenge lies in identifying targetable weaknesses within individual cancer cells that can be effectively exploited. Genome-wide screening methods offer a powerful approach to identifying these novel targets and tailoring therapeutic interventions to the specific vulnerabilities of individual tumors.

Objectives: This study aims to advance the understanding of CRISPR technology and its applications in cancer research, emphasizing its potential to inform disease prevention strategies and improve animal health. Specifically, the study seeks to identify novel pro-oxidant-based therapies for the treatment of lung cancer by employing a CRISPR-Cas9 screening system in human lung cancer cell lines. This system is designed to work in combination with selective drugs targeting endogenous antioxidants, thereby disrupting redox homeostasis and selectively eliminating cancer cells. Our strategic goal is to find novel pro-oxidant-based therapies for the treatment of lung cancer by employing a CRISPR-Cas9 screening system in human lung cancer cell lines in combination with selective drugs targeting endogenous antioxidants, thus altering redox homeostasis. This comprehensive approach aims to unlock new avenues for targeted cancer therapy and improve patient outcomes.

Methodology: To achieve the study's objectives, a CRISPR-Cas9 screening system was implemented in the H460 human lung cancer cell line. These cells were engineered to stably express Cas9 nuclease, enabling targeted gene editing across the genome. Two sub-pools of CRISPR lentiviral gRNA libraries, targeting approximately 1000 cell cycle genes and 500 kinase genes, were utilized. These libraries were designed to introduce loss-of-function mutations in a comprehensive set of genes involved in cellular proliferation, cell cycle regulation, and signaling pathways.

The H460-Cas9 cells were infected with the gRNA libraries to generate a diverse population of cells, each harboring a unique gene knockout. Following infection, the cells were subjected to selection pressure using specific drugs known to modulate endogenous antioxidant pathways. This selection strategy aimed to enrich

for cells in which the knockout of certain genes conferred either enhanced sensitivity or resistance to the effects of redox-modulating drugs.

To identify the genes responsible for the observed changes in drug sensitivity, massive parallel sequencing of PCR-amplified lentiviral inserts was performed. This sequencing analysis enabled the determination of the gRNA repertoire and, consequently, the genes that were enriched or depleted in the selected cell populations. This information was then used to pinpoint candidate genes that play a critical role in modulating cellular redox balance and drug response. For validation, single genes of interest were knocked out using lentiviral Cas9-guide-RNA constructs to confirm their involvement in the observed phenotypes.

Key Findings: Initial screening efforts revealed several promising candidate genes that modulate the response of H460 cells to pro-oxidant stimuli. Further analysis focused on USP28, whose inactivation increased sensitivity to TH588, a drug that interferes with microtubule dynamics. Specifically, TH588 disrupts centrosome separation and spindle assembly, resulting in metaphase arrest and subsequent cell death.

Subsequent experiments, including colony forming assays and cell cycle analysis, were conducted to validate the effects of USP28 knockout on the drug response in H460 cells. These assays demonstrated that USP28 knockout significantly reduced the ability of H460 cells to proliferate under TH588-induced stress and promoted cell cycle arrest. Western blot analyses were used to determine whether USP28 knockout reduced cell proliferation through a TP53-dependent mechanism. These results provide clear evidence that USP28 plays a critical role in modulating the sensitivity of lung cancer cells to microtubule-disrupting agents and that its inactivation can enhance the efficacy of these drugs.

Conclusion: This study demonstrates the utility of a CRISPR-Cas9 screening system for identifying novel therapeutic targets in lung cancer. By targeting redox homeostasis and combining genome editing with selective drug pressure, the research has uncovered potential new strategies for exploiting cancer cell vulnerabilities. The identification of USP28 as a modulator of drug sensitivity highlights the potential for personalized therapies that target specific vulnerabilities of individual tumors. Future research should focus on further characterizing the role of USP28 in regulating microtubule dynamics and exploring the potential of USP28 inhibitors as a novel therapeutic strategy for lung cancer. The results of this study underscore the power of CRISPR genome-wide screening for accelerating the discovery of new cancer therapies.

Keywords: CRISPR-Cas9, Redox Homeostasis, Oxidative Stress, Reactive Oxygen Species (ROS).

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