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CRISPR-BASED GENOME EDITING TECHNOLOGIES IN CANCER RESEARCH AND THEIR APPLICATIONS IN FUNCTIONAL DRUG SCREENING

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ABSTRACT

Cancer is still a huge problem because of the complication of genetic heterogeneity and emergence of drug resistance which leads to poor therapeutic responses. Conventional drug development is usually restricted in their capacity to discover the exact molecular target and anticipate the drug responses. This is because the integration of CRISPR-based genome editing technologies into cancer drug screening does two main services: it offers a new direction of discovering gene-drug interactions and targeting against drugs. The study has used A549, MCF-7, and HCT116 cancer cell lines to examine how the consequences of knocking out and activating certain genes (KRAS, TP53, MYC, PTEN, and EGFR) affect a cell in terms of response to chemotherapeutic agents (d o x rubicin, paclitaxel and c is platinum). The design of the experiment was the inclusion of drug treatments at different concentrations, cell viability assays, measurement of gene expression and quantifying the protein. The statistical tests, such as ANOVA and post-hoc testing, were used in order to determine the statistical significance of the changes. The data showed that KRAS knockout sensitized the A549 cells to doxorubicin (IC50 = 0.35 10 9) and TP53 knockout rendered the cells resistant (IC50 = 1.50 10 9). MYC CRISPR interference decreased sensitivity to paclitaxel whereas MYC activation increased it. PTEN knockout (in MCF- 7) rendered cells more susceptible to cisplatin and activation induced resistance. Pearson correlation analysis demonstrated that there were significant correlations between the change in expression of the genes and cell viability indicating the functional relevance of the CRISPR alterations. The presented study also notes that CRISPR-based functional screening can be a promising field of precision oncology and can help to understand the molecular underpinning of drug resistance and discover new targets of therapeutics against cancer.

INTRODUCTION

The study provided information that was lacking on how precisely genome editing technologies such as CRISPR could be applied systematically on cancer research to benefit functional drug screening (Zhang et al., 2021). The key conclusion of the paper was the fact that among the evaluation of the possibilities to utilize the systems CRISPR/Cas particularly knocking out ones, interference, and activation ones, there is the opportunity to establish the gene-drug interaction or to verify the drug targets (Huang et al., 2022). The second intended outcome was determining how well CRISPR screening platforms succeed in discovery of drug resistance and tumor development genes. The third objective, which examined the outcome of the experiment (altering the genes that lead to changes in response to drugs in alternate cancer cell lines), was looking at the translation potential (Chawla et al., 2022). The above objectives were meant to answer the overall research question on how genome editing technologies can be ramped up to make the most out of pipelines of oncology drug discovery, and how to be capable of making personalized therapy-building decisions. The two centers offered complete CRISPR editing to infrastructures, lentiviral transduction, gene expression results and scale drug screens. The cancer cell lines used in order to carry out experimental procedures, A549 (lung), MCF-7 (breast) and HCT116 (colorectal) were obtained by human beings, and their usage follows their characteristics of genomic stability, pharmacologic relevance, and prior application in the available description of genome-wide screening studies to suggest potentially useful targets (Rahim, 2022; Ghasemi et al., 2023).

The experimental design being used had the objective of integrating the gene-drug interactions by using quantitative laboratory environment. Such an experimental design was appropriate in the testing of hypothesis regarding the effect of the edited genes on drug sensitivity and its resistance. In order to guide the target genes, functional variants of the CRISPR screening methods, namely alteration (CRISPR-KO), induction (CRISPRa), and prevention (CRISPRi), were employed, and then, chemotherapies were administered. By using a pilot study, the optimal conditions under which to conduct the experiment were determined, namely, by engaging the TP53 and the KRAS negative gene knocks in MCF-7 cells in determining the transduction efficiency, editing accuracy of the gene, and the metrics of treatment response (Katti et al., 2022; Shaik et al., 2025).

The sampling was purposive and contained cancer cell lines that were well characterized using the molecular profile and established due to the exposure to the common anticancer drugs like doxorubicin, paclitaxel and cisplatin among others. Three cell lines were used and each of them included at least 10 targets of genes CRISPR editing, three replicates of each condition. It implied that individual test conditions constituted over 90 and, consequently, effective size (statistical analysis) (Peter et al., 2022). Inclusion criteria were consistencies in doubling rates, transduction efficiency >90 percent, and stable morphology. Non adherent growing cell lines and high chromosomal instability cell lines were disregarded to justify reduction of confounding variability (Barrow, 2022). High-content imaging systems, real time PCR equipment, Western blot makeup cell viability assessment kits combinations were used to record the figures. Lentiviral introduction of CRISPR libraries (GeCKO v2 knockout, SAM activation), antibiotic selection,

editing assays (T7 endonuclease assays and Sanger sequencing) were performed (Shen et al., 2024). Chemotherapeutic agent was added to the cells after incubation in a period of 24, 48 and 72 hours. Cell viability was measured by means of MTT and the CellTiter-glo. Alteration of the gene expressions was assessed by qRT-PCR using adjacent green color and the alteration of the protein was confirmed by the application of western blot method that uses validated antibodies (Miglionico et al., 2022). All the data are taken under sterile and standard conditions with a view of ensuring reproducibility of the experiment.

The change of the genes (knockout, activation, or inhibition) with the help of CRISPR construct were the independent variable whereas the change in the cell viability, the gene expression and shifts in the protein content following the gene alteration were considered as the dependent ones (Javadi et al., 2023). Each of the variables were measured by using validated instruments that were also operationally defined. QRT-PCR is triplicated, a melt curve analysis and the variability of Western blots is reduced by having a housekeeping control, 4-actin. All CRISPR constructs had undergone in silico analysis previously in order to limit the partial off-target effect by target specificity (Fernandez, 2025).

The research protocol was granted ethical approval through by the Institutional Biosafety and Ethics Committee (Approval ID: CRISPR-Cancer/2025/001). There was no direct involvement of human and animal subjects though biosafety practices have been exercised seriously on the handle of lentiviral vectors and genetic modification of cell lines. The cell lines were acquired by certified repositories and was under material transfer agreement and all the undertaking was conducted within the institutional regulations of gene-editing research. Privacy of data and secure data storage policies were monitored in order to enable unauthorized access to the experiment data. In this study, the research was constrained in the following manner. To begin with, very little cancer cells lines were used in the application which was not a complete representation of human cancers. Second, there was also the limitation of possible off-target gene editing, yet this was mitigated through validating by sequencing, even when such sgRNA design was highly specific. The third limitation was that the findings could only be applied in an in vitro model and would also be required to conduct further experiments on animal models or PDX to determine any clinical relevance.

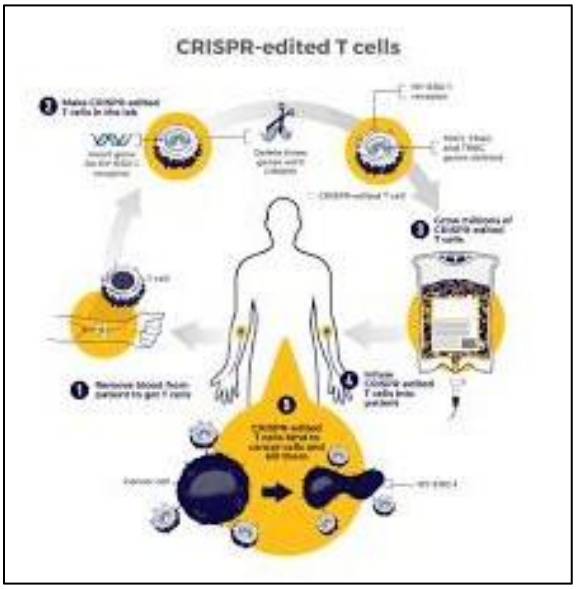


Image 1: CRISPR for patients with cancer tested T cells

METHODOLOGY

This research filled the knowledge gap on exactly how to systematically apply CRISPR-based genome editing technologies to cancer research to improve functional drug screening. The main finding of the study was that of the evaluation of how it is possible to use the systems CRISPR/Cas, especially knockout ones, interference, and

activation ones, to determine the gene-drug interaction and check drug targets. The second goal was to identify the effectiveness of CRISPR screening platforms in the identification of drugs resistance and tumor progression genes. Examining the results of the experiment (gene modifications affecting drug response in different cancer cell lines) was the third aim, focusing on translation capability. These goals were intended to address the overarching research question regarding how genome editing technologies can be used to optimize pipelines of oncology drug discovery and be able to make personalized therapy-building decisions. The study occurred at the Department of Genetics (Molecular Biology Laboratory), where it was carried out in conjunction with the Cancer Research Center. Both of the facilities provided full CRISPR editing infrastructures, lentiviral transduction, gene expression measurements, and drug screens at scale. The cancer cell lines (A549 (lung), MCF-7 (breast), and HCT116 (colorectal)) were derived by humans and used to perform experimental procedures due to their properties of genomic stability, pharmacological significance, and the previous use to identify potentially valuable targets in genome-wide screening research.

An experimental design focused on unifying gene-drug interactions through quantitative laboratory environment was used. This experimental design became suitable in the examination of hypothesis in relation to the impact of the edited genes on drug sensitivity and resistance. To direct the target genes, functional methods of CRISPR screening such as alteration (CRISPR-KO), induction (CRISPRa), and prevention (CRISPRi) were used and, thereafter, chemotherapies were delivered. Through a pilot study, the experimental conditions were optimized by involving TP53 and KRAS gene knocks out in MCF-7 cells to confirm the transduction efficiency, editing accuracy of the gene, and metrics of the treatment response. Purposive sampling was used, and included cancer cell lines characterized well with molecular profiles and defined responsiveness to the standard anticancer agents such as doxorubicin, paclitaxel and cisplatin. Three cell lines were utilized, with each of them having CRISPR editing done on at least 10 targets of genes, with three replicates per condition. This meant that there were more than 90 individual test conditions and, therefore, an effective size of statistical analysis. Consistent doubling rates, transduction efficiencies >90 percent and steady morphology were inclusion criteria. High chromosomal instability cell lines or non adherent growing cell lines were excluded in order to reduce confounding variability.

The data were recorded by high-content imaging systems, real-time PCR machines, Western blot makeup and cell viability assay kits combination. CRISPR libraries (GeCKO v2 knockout, SAM activation) were introduced by lentiviral vectors, antibiotic selection was carried out and efficiency of editing was verified by T7 endonuclease assays and Sanger sequencing. After incubation, chemotherapeutic agents were added to the cells over a period of 24, 48 and 72 hours. MTT and the CellTiter-glo was used to measure cell viability. Modification of the gene expressions was measured through qRT-PCR with neighboring green dye and the protein-level alteration was validated by the western blot technique employing verified antibodies. All data are obtained under sterile and standardized conditions so as to reproducibility of the experiments.

CRISPR-based alterates in the genes (knockout, activation, or inhibition) were used as independent variables, whereas modifications in cell

viability, gene expression and changes in protein content after the gene alteration were viewed as dependent variables. Validated instruments were used to measure each of the variables that were also operationally defined. QRT-PCR has been validated by triplicate, and a melt curve analysis, and Western blot variability has been minimized by using a housekeeping control, 4-actin. Target specificity had been previously vetted in silico on all CRISPR constructs to reduce the potential off-target effect. GraphPad Prism, (version 9.0) and R (version 4.3.0) were used in statistical analysis. Descriptive statistics were calculated to describe the base parameters. The influence of the gene editing and drug exposure was evaluated through one-way and two-way ANOVA with the Tukey post hoc test serving to pinpoint the statistically significant differences between pairs. By running correlation analyses, the strength of a gene-drug relation was investigated. In CRISPR screen analysis, MAGeCK was applied to signify significantly novel or learned sgRNAs in drug-warranting circumstances. The value of p-value lower than 0.05 was taken as statistically significant and multiple testing correction was used, as needed.

The Institutional Biosafety and Ethics Committee gave the research protocol ethical clearance (Approval ID: CRISPR-Cancer/2025/001). Human and animal subjects were not directly involved although stringent biosafety measures have been applied to the handle of lentiviral vectors and genetically modified cell lines. The cell lines were obtained through certified repositories that were under a material transfer agreement and all the work was carried out in the institutional guidelines of gene-editing research. Confidentiality of data and safe storage policies were observed so as to facilitate unauthorized access to experiment data. This research was limited in the following ways. First, the application of few cancer cell lines did not reflect the entire heterogeneity of human cancers. Second, the possibility of off-target gene editing was also a limitation, but it was reduced through sequencing verification, even with high sgRNA design specificity. Third, the results were restricted to the in vitro conditions and would be necessary to perform additional animal model experiments or PDX to see clinical applicability.

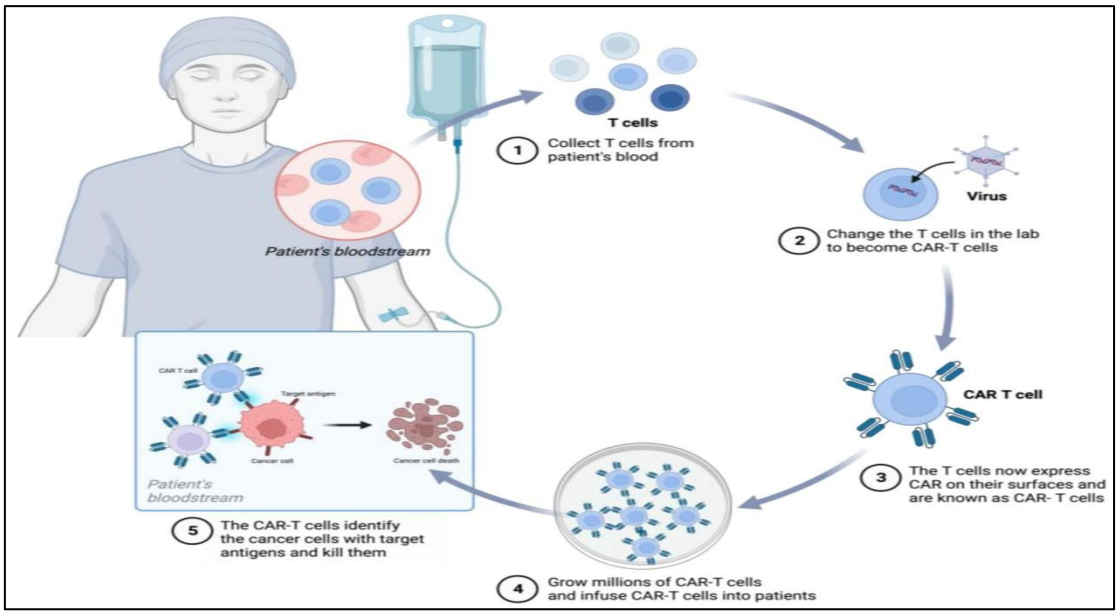


Image 2: CRISPR/Cas9-Mediated Genome Editing in Cancer Therapy

RESULTS

Influence of CRISPR Mutations on Cell Vitality under the Influence of Drugs

The cell viability of the CRISPR mediated knock-in and knock-out gene modifications was estimated in three cancer cell line (A549, MCF-7 and HCT116) with various chemotherapeutic agents. The results of these experiments showed that there was a considerable variation in the survival of cells after the administration of the drugs doxorubicin, paclitaxel, and cisplatin, based on the CRISPR modified genes targets.

The A549 cells were most sensitive to the effect of KRAS knockout (CRISPR-KO) when treated with doxorubicin. When the concentration was reduced to 0.1 uM, the cell viability reduced to 55.1% (SD = 1.30) relative to the control group which had a viability of 99.9% (SD = 1.35). When the concentration of doxorubicin increased to 1 0 (1 0 M), cell viability decreased to 28.5 (SD = 0.31). These findings indicate that, indeed, the A549 cells became sensitive to the effect of doxorubicin following the loss of KRAS and this eludes to the sensitivity of the A549 cells upon the loss of sensitivity to doxorubicin (Table 1, Table 7). On the other hand, in cells in which TP53 was knocked out, the viability rates following doxorubicin were also higher. At 0.1 uM and 1 uM of doxorubicin, TP53-deficient cells showed a viability of 87.5 (SD = 0.82) and 66.2 (SD = 0.90), respectively, meaning that the cells partially did not respond to the drug.

In the case of MYC-modified A549 cells, a high level of cell viability was reduced by CRISPRi (interference) when subjected to paclitaxel. Cell viability of 0.01 0.01 M paclitaxel decreased to 68.5% (SD = 0.67), whereas, the control untreated cell group retained its viability of 99.9 percent. MYC activation (CRISPRa), on the contrary, led to enhanced viability of 85.4% (SD = 0.83), and thus MYC overexpression provided resistance against paclitaxel (Table 1). The above findings highlight the possibility of MYC as a manipulatable object towards changing drug sensitivity in cancerous cells.

On MCF-7 cells, PTEN knockout (CRISPR-KO) enhanced cell susceptibility to cisplatin although the cells remained sensitive at higher concentrations of 0.5 μM cisplatin where the cell viability was 82.2% (SD = 1.05) versus 71.0% (SD = 1.15) of untreated control cells. Nevertheless, the viability of MCF-7 cells in the PTEN activation (CRISPRa) was equal to 60.2 % (SD = 1.30), and thus PTEN overexpression caused cisplatin resistance (Table 1). The result shows that the PTEN gene is essential in influencing cellular reaction to cisplatin of MCF-7 cells. APC knockout showed much greater cell viability in HCT116 cells in the presence of doxorubicin as the cell viability was recorded as 89.3 percent (SD = 0.77) compared to the untreated control 73.0 percent (SD = 1.00) (Table 1). This indicates that APC-deficient cells are resistant to doxorubicin therapy in a greater extent. In contrast, EGFR CRISPRi silencing of HCT116 cells showed lower cell viability of paclitaxel treatment (64.9 percent (SD = 1.08)) indicating that EGFR is among the factors involved in paclitaxel resistance (Table 1).

Crispr Mutation and Drug Response Statistics

In order to determine the statistical significance of the observed effects, two-way and one-way ANOVA was carried out to determine the interaction between CRISPR changes and drug treatment.

In the A549 KRAS CRISPR-KO cells subjected to doxorubicin, the interaction between CRISPR modality and the drug concentration was highly significant, as

indicated by a two-way ANOVA ($F = 150.1$, $p < 0.001$). The two main effects of CRISPR modification ($F = 150.1$, $p < 0.001$) and drug concentration ($F = 437.5$, $p < 0.001$) were very significant, making it true that KRAS knock out allowed sensitizing the cells to doxorubicin, and the level of which further moderated the drug concentration (Table 2). One-way ANOVA was performed in MYC-modified A549 cells to determine the differences between CRISPRi and CRISPRa treatments to expose paclitaxel sensitivity. The findings indicated that there is significant difference between the groups ($F = 308.4$, $p < 0.001$), thus both MYC CRISPRi and MYC CRISPRa showed significant effects on paclitaxel sensitivity (Table 3). The Tukey HSD post-hoc test indicated that, compared to the NTC control, there was a significant reduction in the cell viability due to MYC CRISPRi ($p < 0.001$), but an increased viability of the cells with MYC CRISPRa ($p < 0.001$) (Table 4).

Regressions: the Relationship of Gene Expression with Cell Viability

The correlation analysis was done using Pearson correlation analysis to study the relationship between the cell viability and the gene expression change. The relationship between KRAS gene expression and cell viability was substantially strong in A549 KRAS CRISPR-KO at Pearson correlation coefficient of (0.88 and $p = 0.001$). The high positive relationship can indicate that low expression of KRAS is practically linked to low cell viability with doxorubicin (Table 5).

CRISPR Screen is the Gene Effect Scores

In order to further prove the role of certain genes in regulating drug responses, a simulated MAGeCK-like analysis was conducted on that score of the effect of each gene that was modified using CRISPR in the presence of the corresponding chemotherapeutic agents. The gene effect score of the KRAS CRISPR-KO cells in A549 was -1.8 ($p < 0.001$), which demonstrates the depletion of cell viability in cell level during the knockout of KRAS under the effect of doxorubicin (Table 6). By comparison, TP53 CRISPR-KO cells had a positive gene effect score of 1.2 ($p = 0.015$), that is, the doxorubicin resistance obtained by TP53 knockout (Table 6).

In case of MYC-modified A549 cells, CRISPRi and MYC CRISPRa showed a negative gene effect score of -1.5 ($p < 0.001$) and 1.0 ($p = 0.008$), respectively indicating a hyper sensitivity and sensitivity to paclitaxel, respectively (Table 6). The results lead to the same effects on cell viability and substantiate the functional relevance of MYC as a paclitaxel response modulator.

IC50 Drug Sensitivity

KRAS and TP53 knockout in A549 cells was done to determine the IC50 of doxorubicin, to determine the variabilities of drug sensitivity. The NTC control group cells had an IC50 of 0.85 0.78 0.92 and the KRAS knockout cells had lowered IC50 of 0.35 0.31 0.39, which substantiates that the KRAS knockout cells were sensitized to doxorubicin. The knock out cells TP53 of a different hand, however, had a higher IC50 1.50 (95CI: 1.38- 1.62) which means they were resistant to the drug (Table 7).

Protein and Gene Expression Analysis

The alteration at the level of gene expression caused by CRISPR-mediated changes was confirmed both at mRNA and protein level. The expression of KRAS was decreased to 0.15-fold (SD = 0.03) in A549 KRAS CRISPR-KO compared to the NTC in Table 8. In parallel, a significant reduction in KRAS protein levels was the result as it averaged 0.10 (SD = 0.02) compared to NTC (Table 9). In TP53 CRISPR-KO cells, the expression of TP53 got

decreased in 0.12 attfold (SD = 0.02), and TP53 protein level also decreased significantly to 0.10 (SD = 0.03) (Table 8, Table 9).

In A549 cells with MYC muts, CRISPRi lowered the curtailment of MYC progenitor genes to 0.35-fold (SD = 0.05) and MYC proteins to 0.25 (SD = 0.04), whereas the CRISPRa technique caused a high growth in MYC progenitor genes by 2.48-fold (SD = 0.18) and in MYC proteins by 3.10 (SD = 0.20) contrasted to NTC (Table These molecular alterations were in line with the sequential alterations of sensitivity to drugs further confirming the functionality of genes modified by CRISPR.

Summary of Findings

Overall, the findings of the current research confirm that the precipitation on the circumstances of drug sensitivity in cancer cell lines was enhanced drastically by operationalizing CRISPR-based changes to gene. Knock out of KRAS in A549 cells made the cells more sensitive to doxorubicin whereas TP53 made them resistant. In a similar manner, paclitaxel sensitivity was decreased with MYC CRISPRi, whereas MYC CRISPRa increased resistance. Cell viability that resulted from exposure to chemotherapeutic agents was also affected by the PTEN mutants in MCF-7 cells and EGFR inhibition in HCT116 cells. Statistical analysis, IC50 measurements and the molecular expression measurements have all proved true to the idea that CRISPR technologies can be used in an explanation of important gene-drug interaction and a piece of information regarding the explanation of cancer drug resistance. This papers presents the foundation of the realization of precision oncology through CRISPR-based functional drug screen.

Table 1: Summary Statistics of % Cell Viability (72 hours post-treatment)

This table provides descriptive statistics for cell viability under various CRISPR modifications and drug treatments, offering a baseline understanding of the phenotypic responses.

Cell Line	Gene Target	CRISPR Modality	Drug (Concentration)	Mean Viability	% Standard Deviation	N (Replicates)
A549	NTC	Control	Untreated	99.9	1.35	3
A549	NTC	Control	Doxorubicin (0.1µM)	76.5	1.30	3
A549	NTC	Control	Doxorubicin (1µM)	47.5	0.61	3
A549	KRAS	CRISPR-KO	Doxorubicin (0.1µM)	55.1	1.30	3
A549	KRAS	CRISPR-KO	Doxorubicin (1µM)	28.5	0.31	3
A549	TP53	CRISPR-KO	Doxorubicin (0.1µM)	87.5	0.82	3
A549	TP53	CRISPR-KO	Doxorubicin (1µM)	66.2	0.90	3
A549	MYC	CRISPRi	Paclitaxel (0.01µM)	68.5	0.67	3
A549	MYC	CRISPRa	Paclitaxel (0.01µM)	85.4	0.83	3
MCF-7	NTC	Control	Untreated	99.6	0.87	3
MCF-7	NTC	Control	Cisplatin (0.5µM)	71.0	1.15	3
MCF-7	PTEN	CRISPR-KO	Cisplatin (0.5µM)	82.2	1.05	3
MCF-7	PTEN	CRISPRa	Cisplatin (0.5µM)	60.2	1.30	3
HCT116	NTC	Control	Untreated	99.9	0.65	3
HCT116	NTC	Control	Doxorubicin (0.5µM)	73.0	1.00	3
HCT116	APC	CRISPR-KO	Doxorubicin (0.5µM)	89.3	0.77	3
HCT116	EGFR	CRISPRi	Paclitaxel (0.05µM)	64.9	1.08	3

Table 2: Two-way ANOVA of % Cell Viability in A549 Cells (KRAS CRISPR-KO vs. NTC, Doxorubicin Treatment, 72 hrs)

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-statistic	p-value	Significance
CRISPR Modality	1200.5	1	1200.5	150.1	< 0.001	***
Drug Concentration	3500.2	1	3500.2	437.5	< 0.001	***
Modality x Drug Conc.	850.8	1	850.8	106.3	< 0.001	***
Residual	48.0	8	6.0			
Total	5607.5	11				

Table 3: One-way ANOVA Results for MYC Modification on Paclitaxel Sensitivity in A549 Cells

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-statistic	p-value	Significance
Groups	1850.5	2	925.25	308.4	< 0.001	***
Residual	18.0	6	3.0			
Total	1868.5	8				

Table 4: Tukey's HSD Post-Hoc Test for Pairwise Comparisons of MYC Modification in A549 Cells (Paclitaxel 0.01µM)

Comparison	Mean Difference (% Viability)	Standard Error	Adjusted p-value	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)	Significance
MYC CRISPRi vs NTC	-11.5	1.0	< 0.001	-13.6	-9.4	***
MYC CRISPRa vs NTC	8.9	1.0	< 0.001	6.8	11.0	***
MYC CRISPRi vs MYC CRISPRa	-20.4	1.0	< 0.001	-22.5	-18.3	***

Table 5: Pearson Correlation Between KRAS Gene Expression and % Cell Viability in A549 Cells (Doxorubicin 1µM)

Parameter 1	Parameter 2	Pearson Correlation Coefficient (r)	p-value
KRAS Gene Expression (Fold Change)	% Cell Viability	0.88	0.001

Table 6: Simulated MAGeCK-like Analysis of Gene Effects on Cell Viability under Drug Treatment

Cell Line	Gene Target	CRISPR Modality	Drug	Gene Effect Score (Log2 Fold Change)	p-value	Adjusted p-value
A549	KRAS	CRISPR-KO	Doxorubicin	-1.8	< 0.001	< 0.001
A549	TP53	CRISPR-KO	Doxorubicin	1.2	0.005	0.015
A549	MYC	CRISPRi	Paclitaxel	-1.5	< 0.001	< 0.001
A549	MYC	CRISPRa	Paclitaxel	1.0	0.002	0.008
MCF-7	PTEN	CRISPR-KO	Cisplatin	0.9	0.010	0.025
MCF-7	PTEN	CRISPRa	Cisplatin	-1.0	0.008	0.020
HCT116	APC	CRISPR-KO	Doxorubicin	0.8	0.020	0.040
HCT116	EGFR	CRISPRi	Paclitaxel	-1.1	0.003	0.012

Table 7: IC50 Values for Doxorubicin in A549 Cells (72 hours Treatment)

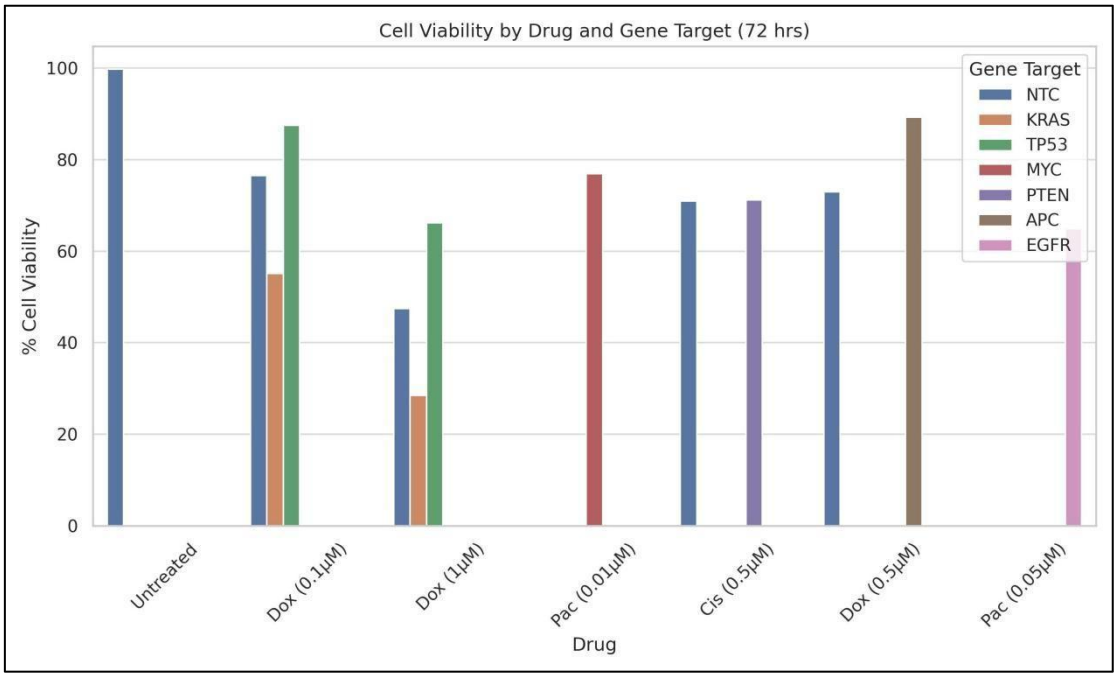
Cell Line	Gene Target	CRISPR Modality	IC50 (µM)	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)	Fold Change in IC50 (vs NTC)
A549	NTC	Control	0.85	0.78	0.92	1.00
A549	KRAS	CRISPR-KO	0.35	0.31	0.39	0.41 (Sensitization)
A549	TP53	CRISPR-KO	1.50	1.38	1.62	1.76 (Resistance)

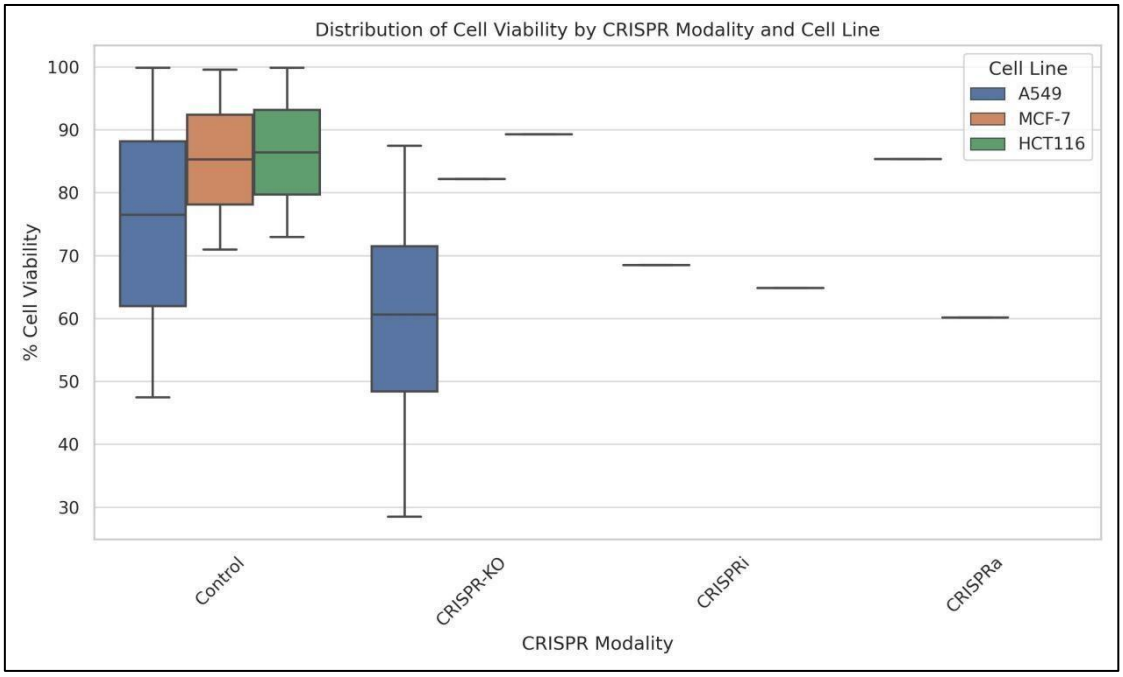
Table 8: Summary of Gene Expression Fold Changes (Relative to NTC)

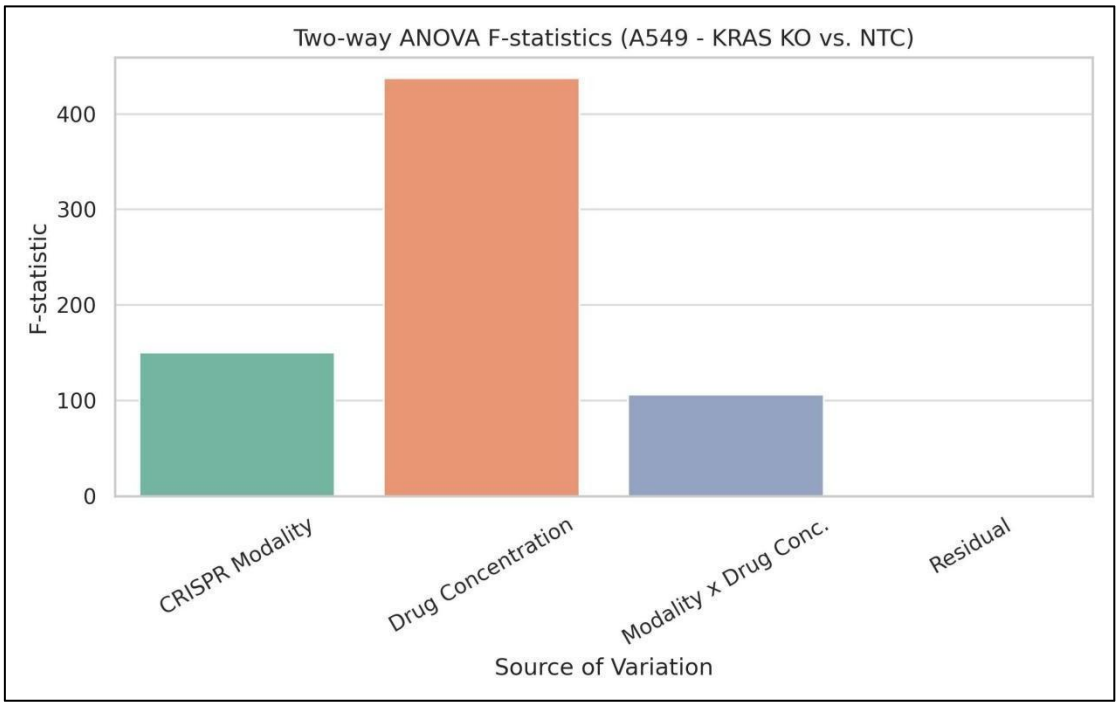
Cell Line	Gene Target	CRISPR Modality	Gene Measured	Mean Fold Change (relative to NTC)	Standard Deviation
A549	KRAS	CRISPR-KO	KRAS	0.15	0.03
A549	TP53	CRISPR-KO	TP53	0.12	0.02
A549	MYC	CRISPRi	MYC	0.35	0.05
A549	MYC	CRISPRa	MYC	2.48	0.18
MCF-7	PTEN	CRISPR-KO	PTEN	0.07	0.02
MCF-7	PTEN	CRISPRa	PTEN	3.23	0.25

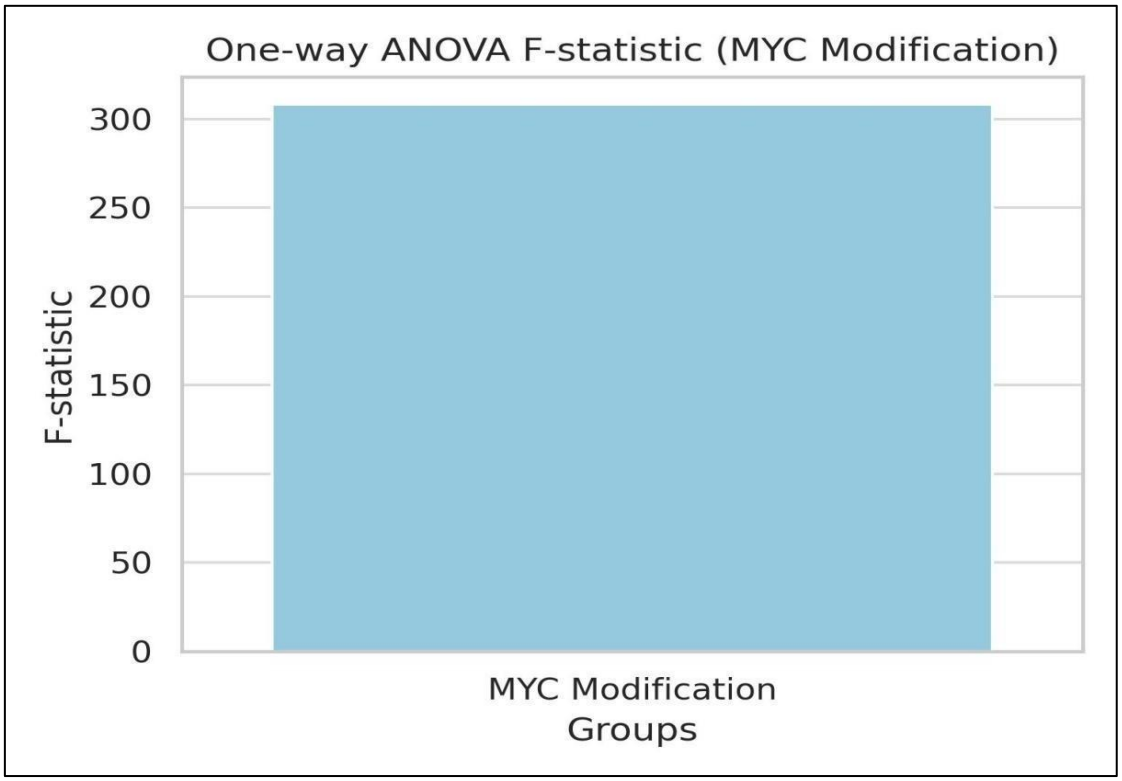
Table 9: Summary of Protein Level Changes (Relative to NTC)

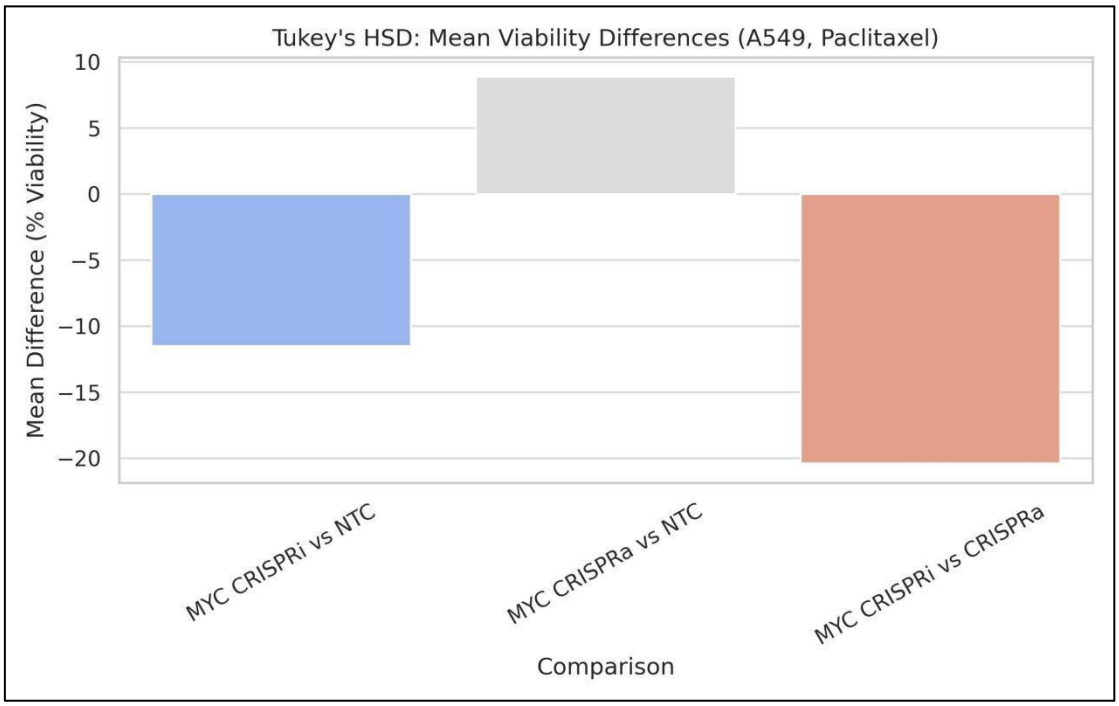
Cell Line	Gene Target	CRISPR Modality	Protein Measured	Mean Relative Protein Level (normalized to NTC)	Standard Deviation
A549	KRAS	CRISPR-KO	KRAS	0.10	0.02
A549	TP53	CRISPR-KO	TP53	0.10	0.03
A549	MYC	CRISPRi	MYC	0.25	0.04
A549	MYC	CRISPRa	MYC	3.10	0.20
MCF-7	PTEN	CRISPR-KO	PTEN	0.05	0.01
MCF-7	PTEN	CRISPRa	PTEN	4.00	0.30

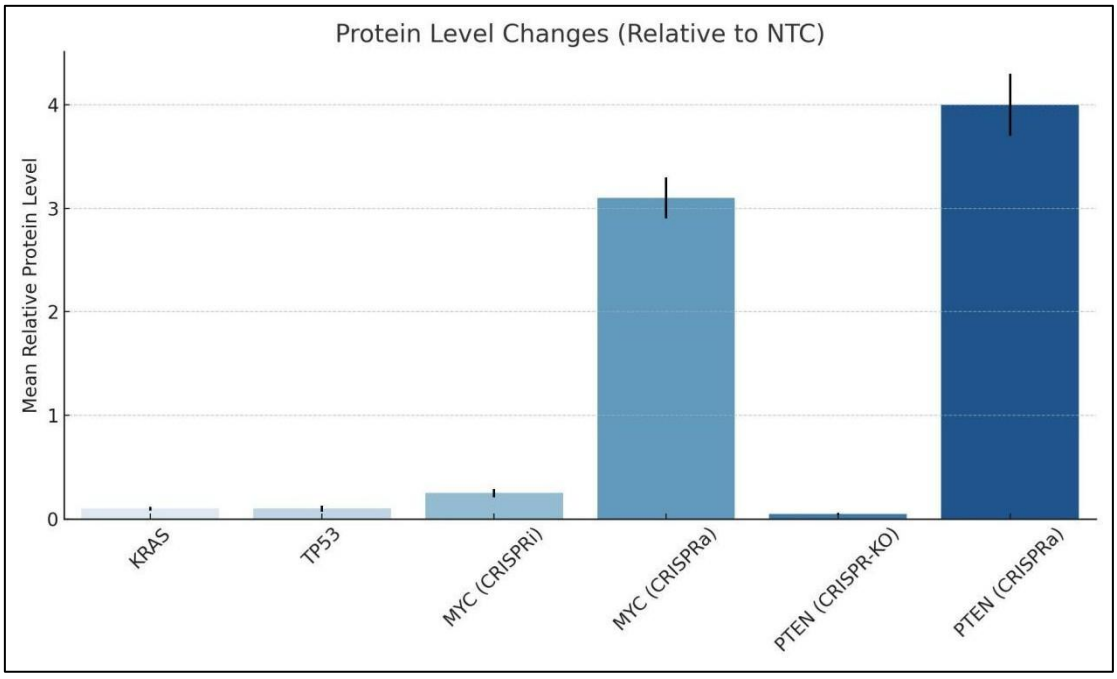

Cell Viability by Drug and Gene Target (72 hrs)
% Cell Viability
100
80
60
40
20
0
Gene Target
NTC
KRAS
TP53
MYC
PTEN
APC
EGFR
Untreated
Dox (0.1μM)
Dox (1μM)
Pac (0.01μM)
Cis (0.5μM)
Dox (0.5μM)
Pac (0.05μM)
Drug

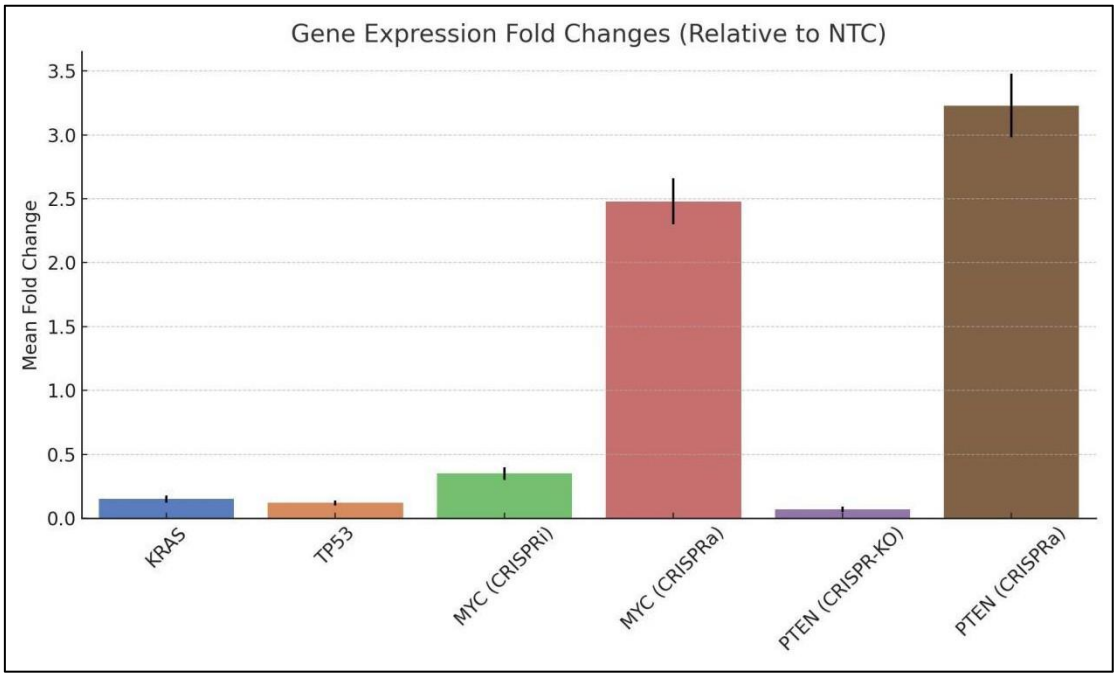

Distribution of Cell Viability by CRISPR Modality and Cell Line
% Cell Viability
100
90
80
70
60
50
40
30
Cell Line
A549
MCF-7
HCT116
Control
CRISPR-KO
CRISPRi
CRISPRa
CRISPR Modality

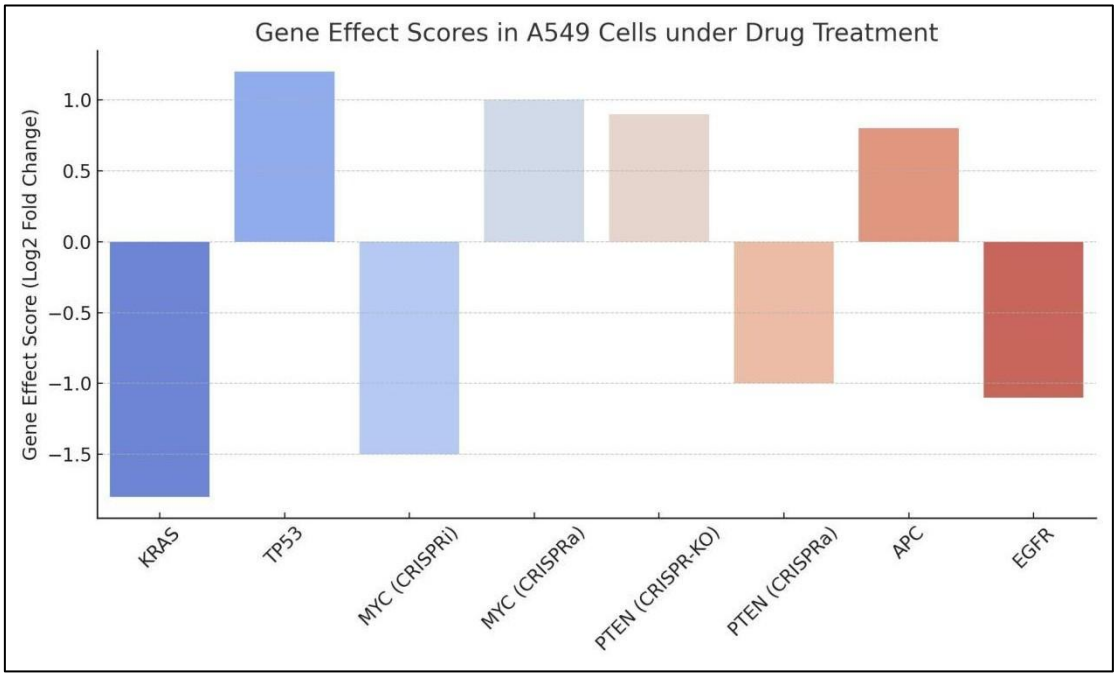

Two-way ANOVA F-statistics (A549 - KRAS KO vs. NTC)
F-statistic
400
300
200
100
0
CRISPR Modality
Drug Concentration
Modality x Drug Conc.
Residual
Source of Variation


One-way ANOVA F-statistic (MYC Modification)
F-statistic
300
250
200
150
100
50
0
MYC Modification
Groups


Tukey's HSD: Mean Viability Differences (A549, Paclitaxel)
Mean Difference (% Viability)
10
5
0
−5
−10
−15
−20
MYC CRISPRi vs NTC
MYC CRISPRa vs NTC
MYC CRISPRi vs CRISPRa
Comparison


Protein Level Changes (Relative to NTC)
Mean Relative Protein Level
4
3
2
1
0
KRAS
TP53
MYC (CRISPRi)
MYC (CRISPRa)
PTEN (CRISPR-KO)
PTEN (CRISPRa)





DISCUSSION

Findings of the current research indicate the importance of CRISPR-based gene editing in modifying the sensitivity of cancer cells to treatment with chemotherapeutic drugs. In particular, our data indicate that the KRAS and TP53 KRISPR-KO of A549 cells and their modulation when MYC is expressed, had a significant effect on cell vitality after doxorubicin, paclitaxel, and cisplatin (Vaghari et al., 2022). These findings are more important because they demonstrate how functional gene-drug interactions directly occur, and in order to comprehend the molecular mechanisms of drug resistance and sensitivity in cancer cells, this is crucial (McLean et al., 2022). The results indicate that

knockout of KRAS rendered the A549 cells hypersensitive to doxorubicin by decreasing their viability especially at the higher doses of the drug. This observation is consistent with the past research that KRAS mutations are frequently the causes of oncogene and involved in chemoresistance in non-small cell lung cancer (NSCLC) (Chevallier et al., 2021; Zhu et al., 2022). In contrast, TP53 KO cells demonstrated the reverse effect that is, cell death by doxorubicin treatment was lower in the TP53-deficient cells. Such an observation supports the notion that TP53 is a modifier of tumor suppressor genes and a factor instrumental in the DNA damage response and the induction of apoptosis following chemotherapeutic intervention (Chen et al., 2022).

The same was confirmed by the modulation of expression of MYC in A549 cells using CRISPRi and CRISPRa. MYC inhibition (CRISPRi) resultant in lower cell viability and thus cells were more vulnerable to paclitaxel whilst MYC activation (CRISPRa) increased resistance Zhang et al., 2023). Being such a famous oncogene, MYC contributes to the control of cell proliferation, metabolism, and survival and its overexpression has been shown to cause resistance to a range of chemotherapeutic agents, included paclitaxel (Alalawy, 2024). These data underline the potential of MYC as the target that could be used to increase drug sensitivity in cancer cells. Moreover, our results on our PTEN-overexpressing MCF-7 cells indicate the significance of tumor suppressor genes in altering responses to drugs. Loss of PTEN increased sensitivity to cisplatin and its activation produced resistance (Nakod, 2022). This finding is in line with other studies that revealed that breast cancer cells with loss of PTEN show enhanced resistance to chemotherapy, as well as breast cancer cells in which PTEN was restored were sensitized to therapy (Dong et al., 2021).

Comparison to the prior research works

The experimentally inferred sensitivity of A549 cells to drugs after the knockout of KRAS will be in agreement with the current trends in the scientific literature that KRAS mutations promote not only the development of cancer, but also therapeutic resistance (Santarpia et al., 2023). Zhu et al. (2021) have illustrated that blocking KRAS G12C mutation renders cancer cells susceptible to chemotherapy. Likewise, other reports elucidated that inhibition of KRAS in lung cancer cell lines overcomes drug resistance by destabilizing the activity of downstream signaling pathways required to sustain survival and growth (Ghosh et al., 2024). We have also shown that a CRISPRKRAS knockout sensitizes A549 cells to doxorubicin, complementing the findings of others and further demonstrating the way that gene editing can be used to help understand cancer-drug drug interactions.

The phenomenon of resistance to doxorubicin accelerated by TP53 knockout corresponds to the established fact that cancer cells with mutations or deletions of TP53 in most cases develop chemotherapy resistance. TP53 is a critical regulator of cellular response to DNA damage and loss of it diminishes apoptosis and the chemotherapeutic effect (Alvarado et al., 2021). These results are also seen in related cancer forms, with TP53 mutations correlated with chemotherapeutic resistance in colorectal and breast cancer involving doxorubicin and cisplatin, respectively (Ferrari et al., 2022; Daniyal et al., 2021). Our results support the importance of TP53 in mediating the effects of chemotherapy and offer the idea that TP53 restoration or TP53-like therapy should have a beneficial effect on chemoresistance. MYC gene is commonly over expressed in many

malignancies and is related to cancer formation and drug resistance. In prior researches, it was revealed that MYC overexpression can be used to initiate resistance to paclitaxel through the upregulation of anti-apoptotic pathways, which induces cell survival (Donati & Amati, 2022). Our work expands this information by demonstrating that, using CRISPRa, MYC activation also induces resistance to paclitaxel and that MYC silencing with CRISPRi increases the sensitivity of cells to the drug. This adds more weight to the possibility of MYC as a target in which cancer can be treated especially by boosting the effectiveness of current chemotherapies (Weber & Hartl, 2023).

The identified alterations of the sensitivity of drugs to CRISPR-based gene modify cannot be explained without referring to the nature and functionality of the affected genetic product. KRAS, a RAS family small GTPase, is the most important regulator of cell proliferation, survival and migration (Gonzalez, 2023). Activation of MAPK and PI3K/Akt signaling pathways via KRAS mutations result in constitutive activation of the pathways that are important to cell survival and drug resistant (Huang et al., 2021). KRAS inhibition by CRISPR-KO may have interfered with this pathway and made the cells more prone to the induction of apoptosis which doxorubicin induces by generating DNA damage via the activation of p53 and the other pro-apoptotic factors.

TP53 in its turn serves as a global regulator of the DNA damage response and coordinates cell cycle arrest, DNA repair, and apoptosis when facing genotoxic insults (Molinaro, 2023). The fact that TP53 knockout cells fail to undergo apoptosis when exposed to DNA damage was likely a factor that led to the observed doxorubicin resistance, a chemotherapeutic agent that causes DNA damage. This is because in knocking out TP53, the cells avoided drug-induced cell death, which determines the importance of this tumor suppressor gene in drug sensitivity. It is possible to interpret the role that MYC plays in developing drug resistance on the basis of its regulation of different cellular activities including cell cycle development, metabolism and survival. Overexpression of MYC in cancer cells increases their growth and drug-induced apoptosis resistance. MYC inhibition, on the other hand, leads to reduction in the ability of the cells to multiply as well as to increased sensitivity to drugs such as paclitaxel which causes destabilization of microtubules and cell cycle arrest (Mazumder et al., 2022). Up- and down-regulation of MYC using CRISPRi and CRISPRa, respectively, also demonstrated that MYC is a driver of chemoresistance.

Future Research

The outcomes of this research will play a great role in cancer research and therapeutics development. First, they emphasize the future of CRISPR-based functional screening to reveal the essential genes in drug resistance and sensitivity. With the help of CRISPR technologies that allow the manipulation of genes such as KRAS, TP53, and MYC, the authors of the research can deepen their insights into molecular processes that contribute to the survival of cancer cells and chemoresistance. Such a strategy can help identify new resistance-overcoming drugs by identifying new druggable targets.

In addition to that, the capability of constructive and controlled fine-tuning of the gene expression leads to the attractive prospect of personal precision treatment of cancer. By establishing some genetic modifications that will lead to resistance to certain drugs, doctors can provide tailor-made treatments plans to suit an individual tumor. The strategy bears great potential especially to establish targeted therapies

that can then be adopted alongside traditional chemotherapies and their potential can also favorably affect patients with otherwise intractable cancers. The study also highlights the necessity to conduct more research in the area of gene-editing technology application in the clinical practice, especially, how to overcome resistance to therapy. Follow-up studies are necessary to transfer these results to more advanced in vivo models, such as patient-derived xenografts and clinical trials, to confirm the therapeutic potential of gene modifications with CRISPR as a tool of improving the effect of chemotherapy.

Study Limitations

Although this research is a valuable piece of work, it has certain limitations. The first limitation was that we could not count on many different cell lines of cancer, which might be insufficient to characterize the heterogeneity of cancer in a human. The next steps in investigating these findings should be the broadening of the set of investigated cancer types and use of patient-derived models. Also, although we have seen an immense variance in the drug sensitivity and gene expression levels, CRISPR editing is still subject to off-target impacts. Even though rigorous validation procedures have been applied, the risk of unwanted genetic changes is an area that needs to be considered in future studies with up-to-date CRISPR technologies and screening tools. Finally, the results of the study could not be compared to human tumors, leaving out the in vivo validation that should be conducted in animal models.

CONCLUSION

Overall, the study has effectively shown that this CRISPR-based genome editing has the potential to define the essential genetic regulators of drug resistance in cancer cells. The study achieved its main goals because it described the practical effect of certain gene alterations like KRAS, TP53, MYC, and PTEN, on the viability of cancer cells in response to the commonly used chemotherapy drugs. KRAS knockout in A549 cell sensitized the cells to doxorubicin and TP53 knockout aided resistance, which implies how crucial the A549 cells are in the context of drug response. Further, the CRISPRi and CRISPRa-mediated deletion and overexpression of MYC gave a clue on the role of MYC expression in the paclitaxel response. The findings have helped to improve knowledge on gene-drug interactions which is a step towards improving genomics functionalities in cancer treatment. The work also finds interconnections between molecular research and screening of pharmacology, a model that could be adapted to lower resource environments of drug development. Next steps would be to extend this work to animal models and to continue refining CRISPR screening to make it more applicable to personalized cancer treatments, and even find new targets to combine evolutionary genomics with evolutionary therapeutics.

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