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The Effect of TP53 Mutations on Tumor Response to Standard Cancer Therapeutics

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Abstract

The human TP53 gene codes for a protein, p53, that regulates DNA repair and the cell cycle [9]. Therefore, TP53 plays an immensely important role in the proper growth and division of human cells. Mutations in TP53 are also associated with resistance to many standard cancer treatment drugs [6]. More than 50 percent of human cancers have mutations within TP53; it has become a major focus over the years in cancer research [10]. Organoids are *in vitro* cultures of primary cells established from human surgical samples [8]. They recapitulate genetics of human cancers better than do cell lines [14]. Some organoids in our lab were established from tumors that have a functional TP53 gene or are TP53 wild type (WT). Other organoids are from tumors that are TP53 mutant and have no functioning TP53 gene or p53 protein. The goal of this experiment was to figure out what chemotherapy drug sensitivity differences are caused by absence of functional TP53 gene, and therefore absence of p53 protein. To do this, our lab has used CRISPR/Cas9 gene editing technology to create TP53 knock out (KO) derivatives of TP53 WT organoids. This is called an isogenic pair of organoids, because they are identical to each other except for the TP53 KO that we engineer into the cells. Differences between the WT and KO cells' sensitivities to various cancer drugs can be solely attributed to the absence of functional TP53 gene, and not some other genetic difference between the cells. Overall, this experiment proved that colorectal cancer (CRC) organoids without a functional TP53 gene are more resistant to the cancer drugs Nutlin-3A, 5-Fluorouracil (5-FU), 6-Thioguanine, Irinotecan, Decitabine, and Thiotepa.

Introduction

TP53 and Its Role in Cancer Development

The human TP53 gene codes for a protein, p53, that regulates DNA repair and the cell cycle [10]. Therefore, TP53 plays an immensely important role in the proper growth and division of human cells and is one of the body's most important elements in anticancer defense. Normally, there are low levels of p53 protein in human body cells [11]. However, if an adverse event such as DNA damage occurs, the p53 protein can bring about cell cycle arrest, apoptosis, or senescence. TP53 is a tumor suppressor gene, so its role in regulating the cell cycle and causing its arrest during cellular stress helps to prevent tumor formation in the body [3]. Thus, mutations in the TP53 gene can lead to a decrease in the body's ability to suppress tumor formation. Mutations in TP53 are also associated with *de novo* resistance to many standard cancer treatment drugs including cisplatin, alkylating agents, antimetabolites, antiestrogens, anthracyclines, and EFGR-inhibitors [6]. More than 50 percent of human cancers have mutations within TP53; it has become a major focus over the years in cancer research [10]. More specifically, mutations within the TP53 gene are extremely common in CRC, and often contribute to the development of more aggressive and metastatic forms of CRC with poorer prognosis [11]. CRC is heavily researched because, worldwide, it is the third most common cancer among men, the second most common cancer among women, and the third leading cause of cancer deaths [16]. This study examines the effect of functional TP53 loss in a patient's CRC tumor cells on tumor response to eight standard cancer therapy drugs.

Organoid Culture

Researchers in the Cancer Genetics Lab (CGL) within the University of South Carolina College of Pharmacy grow colon and breast cancer organoids from cancer patients treated at local Prisma Health hospitals. Organoids are masses of primary cells grown three-dimensionally *in vitro* that resemble human organs and can be made using both normal and malignant tissue collected from patients [18]. They are miniature versions of *in vivo* tissue, and thus are extremely similar to and, in certain instances, histologically identical to, organs of the human body. Cancer cell lines have been growing artificially for years and years and have accumulated mutations that are unrelated to the person they came from [4]. Organoids, however, are taken from the human and grown in Matrigel right away, so they genetically match what was in the tumor of the human body. Because of this, organoids are much better models of human cancer compared to cell lines [8]. Organoids are ideal for modeling cancer progression and testing drug effectiveness in order to extrapolate data for the human body. Because organoids are such excellent models for human organs and tumors, they serve as a great link between research and clinical practice [18]. In this study, colon organoids collected from a patient with CRC were used to test drug effectiveness.

Gene Editing to Create an Isogenic Pair

In order to prove the consequences of any gene mutation, gene editing must be performed on cells and consequences measured. Genetic mutations occur naturally as humans or tumors evolve. Experimentally removing a gene, however, is different from studying a naturally occurring mutation because it is done in a lab and controlled by scientists. This is referred to as knocking a gene out and can be performed with

CRISPR/Cas9 gene editing technology. If gene editing was performed to mutate TP53 in cells to where the gene is no longer functional, these cells would be referred to as "TP53 KOs". Researchers in the CGL within the University of South Carolina College of Pharmacy have done this using CRISPR/Cas9 gene editing technology on a specific patient's CRC tumor sample collected from local Prisma Health hospitals. Although this is different than TP53 mutations that occur within cancer naturally, it is one of the best models to study the effect of TP53 mutations in cancer [16]. This is because, according to an analysis of 10, 225 patient samples from 32 cancers, 91% of cancers with a TP53 mutation contained a loss of function mutation in both alleles, and therefore a loss of p53 protein [3]. In the TP53 KOs, both alleles of the TP53 gene are removed; there is a loss of function in both alleles and therefore a loss of p53 protein. Some of the organoids are tumor cells that have a functional TP53 gene or are TP53 WT. This patient's tumor cells have been deidentified and coded as F130T. F130T refers to the tumor cells that have a functional TP53 gene (WT), while F130T-S6 refers to the tumor cells engineered by the CGL that do not have a functional TP53 gene (KO). This is called an isogenic pair of organoids, because they are identical to each other except for the TP53 KO that has been engineered into the cells. This is the isogenic pair used in this study. When comparing tumor cells to normal cells, there are an immense number of genetic differences between the two; it would not be possible to attribute the cause of change in tumor response to cancer drugs to one gene [13]. With an isogenic pair, however, differences between the cells' sensitivities to various cancer drugs can be solely attributed to the absence of functional TP53 gene, and not some other genetic difference between the cells. Because the presence or absence of the TP53 gene is the only difference between these organoids,

this allows a cause-and-effect relationship to be established between TP53 loss and drug sensitivity rather than just a correlation. Our lab has sequenced the F130T organoid; refer to Table 1 for a list of some of the cancer-causing somatic mutations in the F130T organoid. Additionally, refer to Figure 1 to see the frequency of these mutations across 32 different types of cancer. Notice that the cancer type with the highest frequency of these mutations is CRC cancer cell lines, at just below 80 percent.

Chromosome	GrCH38 Position	Cosmic Database ID	Reference Allele	Mutant Allele	F130-Normal Genotype	F130-Tumor Genotype	Gene name	Gene section	Nt change	AA change
1	9717612	COSV100740205	G	A	GG	GA	PIK3CD	Exon 8	c.1006G>A	D336N
1	22784695	COSV101006788	G	A	GG	GA	EPHB2	Exon 3	c.430G>A	D144N
2	25282524	COSV53085629	G	A	GG	GA	DNMT3A	Exon 4	c.365C>T	A122V
2	239081104	COSV61859359	G	A	GG	GA	HDAC4	Exon 22	c.2726C>T	A909V
3	184577730	COSV57808583	C	A	CC	CA	EPHB3	Exon 7	c.1552C>A	R518S
5	1293360	COSV57248775	G	A	GG	GA	TERT	Exon 2	c.1526C>T	T509M
5	1294162	COSV57211038	C	T	CC	CT	TERT	Exon 2	c.724G>A	A242T
5	112838399	COSV57328770	C	A	CC	CA	APC	Exon 16	c.2805C>A	Y935*
5	112839942	COSV57321313	C	T	CC	CT	APC	Exon 16	c.4348C>T	R1450*
6	41061597	COSV55143998	G	A	GG	GA	APOBEC2	Exon 2	c.401G>A	R134H
9	136496796	COSV53079510	G	A	GG	GA	NOTCH1	Exon 34	c.6943C>T	Q2315*
12	11884558	COSV56766903	G	A	GG	GA	ETV6	Exon 6	c.1123G>A	G375R
12	25245350	COSV55497419	C	A	CC	CA	KRAS	Exon 2	c.35G>T	G12V
21	44258593	COSV54264177	G	A	GG	GA	DNMT3L	Exon 6	c.446C>T	S149F
22	41178811	COSV99606981	C	A	CC	CA	EP300	Exon 31	c.7100C>A	P2367Q

Table 1: List of mutations in the F130T CRC organoid.

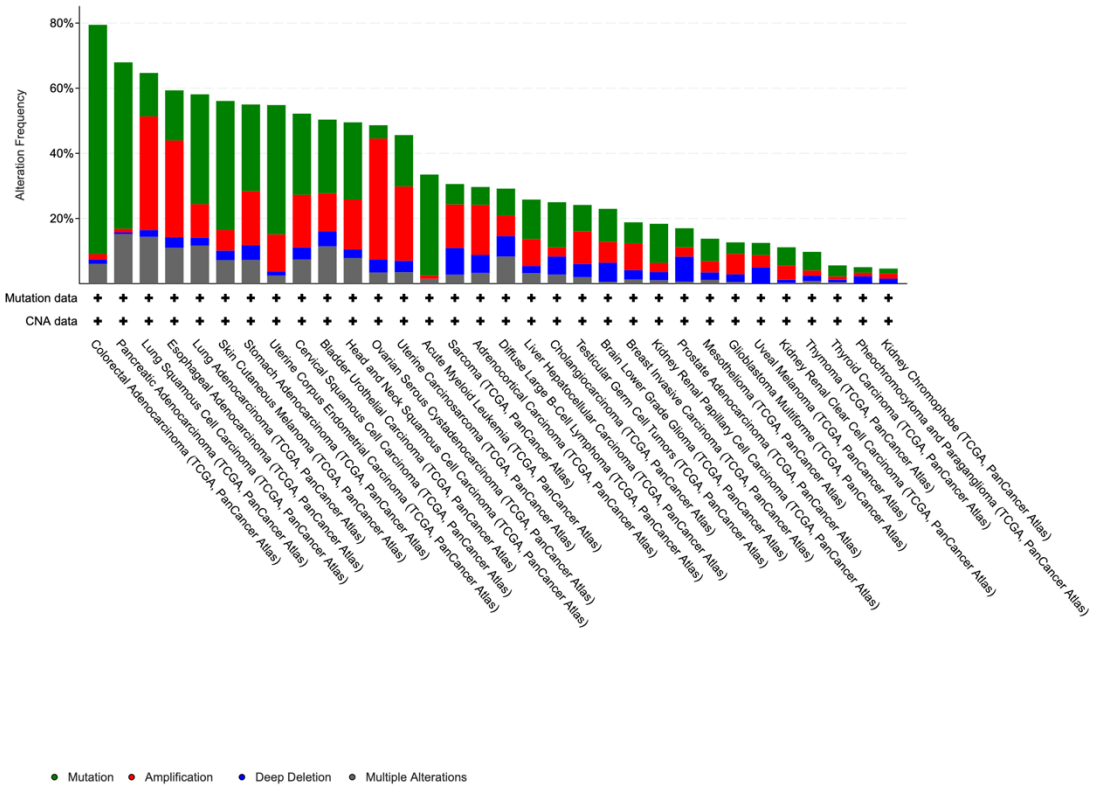


Figure 1: Frequency of mutations in the genes from Table 1 across 32 types of cancer. Data from a combined study of 48,081 samples from cBioPortal.org

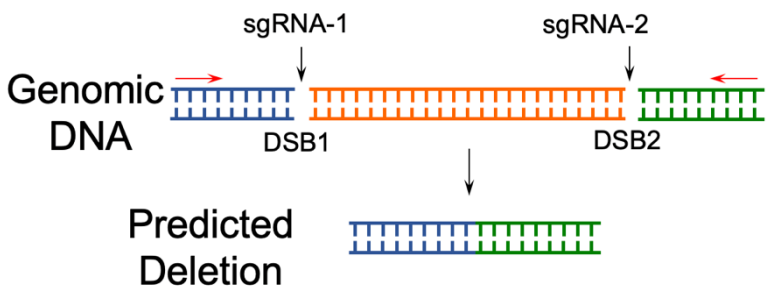


Figure 2: Depiction of how TP53 KO (F130T-S6) CRC organoids were engineered using CRISPR/Cas9 gene editing technology.

Cancer Drug Stock and Past Studies

The CGL has a stock of 127 FDA approved chemotherapy drugs obtained from the National Cancer Institute (NCI). Specifically, this set of drugs is referred to as NCI Approved Oncology Drug Set IV. In a previous experiment, researchers in the CGL performed drug assays on human embryonic stem cells (hESCs) that were either TP53 WT or TP53 KO with this drug set [12]. Liu, et al. (2019) found various drugs that killed TP53 WT cells but did not harm TP53 KO cells. Liu, et al. (2019) then discovered that inhibition of genes associated with spindle assembly checkpoints resensitized these TP53 KO hESCs to many of the chemotherapy drugs that they were previously resistant to. Our project expands on Liu, et al.'s (2019) experiment by performing drug assays on human tumor cells in the form of organoids rather than hESCs. In order to determine drugs to include in the drug assay for our experiment, we analyzed the results of Liu, et al.'s (2019) experiment and determined which drugs showed the most significant difference in sensitivity between the TP53 WT hESCs and TP53 KO hESCs. Eight drugs were chosen to be tested in this study and they were: Nutlin, Carboplatin, Decitabine, Fluorouracil (5-FU), Irinotecan hydrochloride (Irinotecan), Olaparib, Thioguanine, and Thiotepa. Nutlin

served as the positive control because it is known to harm TP53 WT cancer cells but not TP53 KO cancer cells. The CGL eventually wants to model Liu, et al.'s (2019) experiment but perform it with human organoids rather than hESCs. This project begins that process by first trying to find which drugs and drug concentrations TP53 KO cancer cells are resistant to.

The PRISM Lab and DepMap

Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) is a technology that was developed by the Broad Institute of MIT and Harvard [2]. PRISM allows for quick screening of more than 750 human cancer cell line models. Specifically, it is used to screen cell viability of these cancer cell line models against cancer drugs. This is done by barcoding each cell line and pooling the cell lines together. The cell lines are then treated with cancer drugs for around five days, lysed, and their mRNA is isolated and amplified. Cell line sensitivities can then be produced in treatment versus control conditions. These cell lines are also genomically characterized, so different genetic mutations can be observed as well. Genomic information used with PRISM sensitivity data is from the Broad Institute's Cancer Dependency Map (DepMap). The generation of this PRISM cell line sensitivity data against genomic data is a correlation, rather than a cause-and-effect relationship, as the scientists did not engineer the genomic mutations into the cell lines themselves. The goal of PRISM and DepMap is to provide the scientific research community access to cancer dependency and drug sensitivity data. For this experiment, we analyzed the PRISM and DepMap data for the eight drugs used (if the PRISM drug sensitivity data was available) for CRC cell lines with and without TP53 mutations.

Question, Objective, and Significance of Study

The main question that this project is trying to answer is does loss of functional TP53 gene in CRC organoids cause a change in tumor response to standard cancer treatment drugs compared to TP53 WT CRC organoids? Relating to this, the objective and goal of the study is to find drugs and drug concentrations that TP53 KO CRC organoids (F130T-S6) are more resistant or more sensitive to than TP53 WT CRC organoids (F130T). We hope to find what drug sensitivity differences are caused by TP53 loss specifically in CRC tumors, and characterize the p53-dependency of these drugs.

This study has an immense amount of clinical significance because of the use of human organoids. Patients with or without TP53 mutations in their tumors could benefit from the data of this experiment to know which drugs may be more effective or less effective in treating their cancer. Additionally, a drug assay in an isogenic pair of CRC TP53 WT versus KO organoids is a novel experiment. A thorough literature review shows no other studies in which a pair of TP53 WT versus KO isogenic CRC organoids has been engineered in the lab and tested with various chemotherapy drugs. Many studies have conducted drug assays on other CRC cancer cell lines [18]. We hope to be the first to *prove* the drugs' p53 dependencies and establish a cause-and-effect relationship between the absence of functional TP53 gene and resistance to certain drugs.

Methodology

Organoid Cell Culture

To prepare for drug assay performance, the first step in this experiment was to practice growing the organoids. Organoids are very delicate, sensitive cells, and successfully growing and passaging them requires practice. This was done for around two

and a half months, and the organoids were split 1:4 every one and a half to two weeks. Organoids were grown in a 12-well plate with two mL of media in each well. Tumor organoids grow in ENRA media, which is made in a 500 mL model of Advanced Dulbecco's Modified Eagle Medium (DMEM)M/F12. 132.5 mLs of media are removed in order to make room for the additives. 40 mL of R-spondin conditioned media and 40 mL of Noggin conditioned media are added. 10 mL of B27, 5 mL of N2, 5 mL of Nicotinamide, 5 mL of PenStrep, 5 mL of Hepes, 5 mL of GlutaMax, 1 mL of Primocin, 500 uL of Fungizone, 650 uL of N-acetylcysteine, 50 uL of [leu-15]-Gastrin I, 50 uL of EGF, and 50 uL of A83-01 are all added as well. Once plated, the organoids were maintained at 37 ℃ and 5% CO_2 in an incubator. Media was changed every other day. Approximately two days before passaging or freezing and two days after passaging or thawing, Rho-associated protein kinase (ROCK) inhibitor was added to the ENRA media (1 uL of ROCK inhibitor per 1 mL of ENRA media).

Donut Organoid Plating

Once organoid cell culture was sufficient, F130T and F130T-S6 organoids were plated in a donut style in 96-well clear-bottom plates. There were four plates total with four drugs tested in triplicate on each plate. Two plates were F130T organoids (drugs 1-4 on one plate and 5-8 on another) while the other two plates were F130T-S6 organoids (drugs 1-4 on one plate and 5-8 on another) (reference Figure 2 for plate set up). In order to plate a full 96-well plate of donut organoids, one confluent well from a 12-well plate is needed. Thus, two confluent wells of F130T organoid cells from a 12-well plate and two confluent wells of F130T-S6 organoid cells from a 12-well plate were needed for this experiment. Once organoids are detached from the 12-well plate using TrypleE and

ROCK inhibitor, a “slurry” of ENRA media:matrigel is made in a ratio of 3:4 and resuspended with the cell pellet. The slurry is kept on ice and a multichannel pipette is used to put 20 uL of slurry into each well. While dispensing the slurry into wells, the hand is moved in a circular motion around the wall of the well to create a mini ring. Once all of the wells are plated, a p20 pipette tip is used to ensure the slurry is around the entire rim of the well and the mini ring is complete. The plates are then incubated at 37℃ for 15 minutes to solidify. Before adding any drug, ENRA media with ROCK inhibitor is added to the wells for the first two media changes to let the organoids recover. Media is changed every other day and cells are maintained at 37 ℃ and 5% CO_2 in an incubator. Because the organoids are plated in a donut shape, a benchtop pipettor can be used to aspirate media off and add fresh media on. In this experiment, a Sorenson Bioscience® BenchTop Pipettor was used and 200 uL of media with ROCK inhibitor was added to each well (1 uL of ROCK inhibitor per 1 mL of ENRA media).

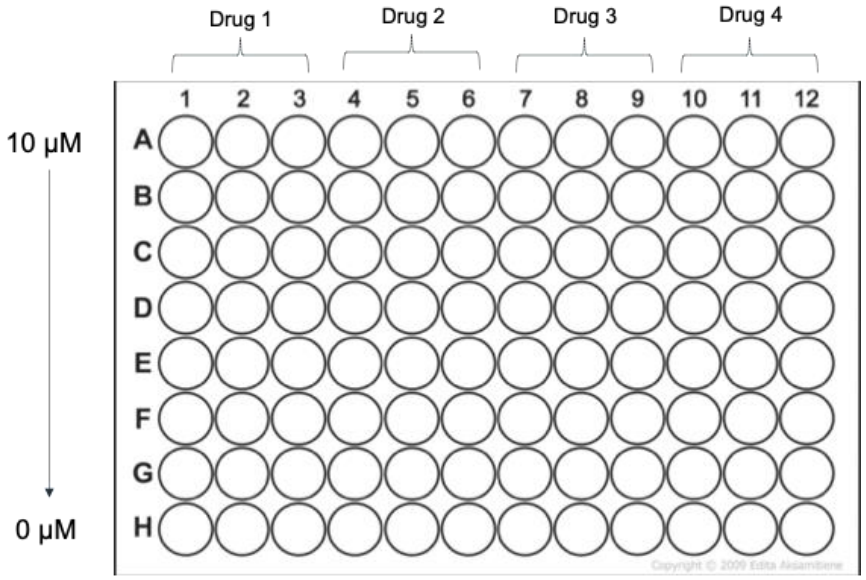


Figure 3: Plate setup for the drug assays. There were four plates total: two for drugs 1-4 and 5-8 for F130T and two for drugs 1-4 and 5-8 for F130T-S6.

Serial Dilution for Drug Concentration

For the eight drugs tested in this experiment, each drug was tested at eight concentrations which were 10 uM, 5 uM, 2.5 uM, 1.25 uM, 0.625 uM, 0.3125 uM, 0.15625 uM, and 0 uM. In order to make the correct concentrations for each drug, a serial dilution for each drug was performed. Drug dilutions were made in ENRA media in 50 mL conical tubes and then transferred to a 96-well deep well plate. Each drug stock is stored at -20℃ in DMSO at a concentration of 10 mM. For each drug, 20 mL of ENRA media were added to the first 50 mL conical and 10 mL of ENRA media to seven other 50 mL conical tubes. 20 uL of drug from the stock solution were added into the first conical tube, then the tube capped and inverted to thoroughly mix the solution. 10 mL from the first conical were then added to the second conical tube and mixed again. These steps were repeated for tubes 1-7. In the eighth tube, 10 uL of DMSO were added to the 10 mL of media to have a final drug concentration of 0 uM. 1 mL of each drug concentration was transferred to the deep well plate according to the plate setup in Figure 2. For the third media change with the donut organoids, the Sorenson Bioscience® BenchTop Pipettor was used to aspirate off old media and add on fresh media + drug from the deep well plate. 200 uL of media + drug were added to each well according to the plate map in Figure 2. Media + drug was changed every other day until the drugs had been on the organoids for 10 days.

CellTiter-Glo® Luminescent Cell Viability Assay

After organoids were treated with drugs for 10 days, a cell viability assay was performed. The specific assay performed in this experiment was the CellTiter-Glo® Luminescent Viability Assay. CellTiter-Glo® mixture and DMEM media (with no

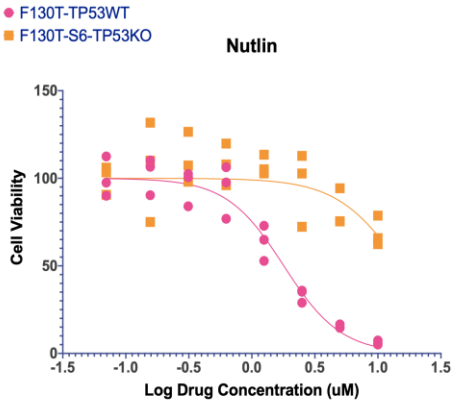
additives) were mixed together in a ratio of 1:1. 200 uL of this CellTiter-Glo® and DMEM mixture were added to each well after the old media + drug had been aspirated. The mixture was also added into one row of a blank 96-well clear-bottom plate to account for background luminescence. The plates were then read in a machine with the ability to read multiwell plates.

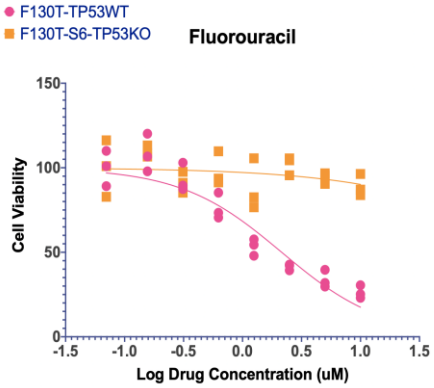
Data Analysis

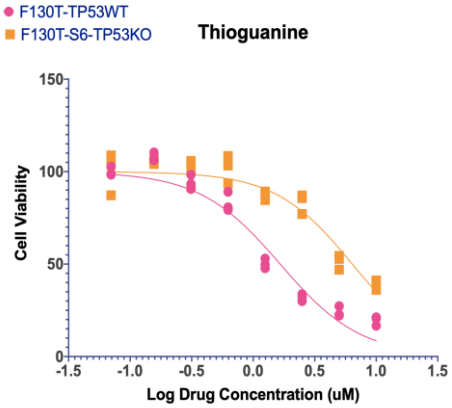
For data analysis in this study, Microsoft Excel and GraphPad Prism 9.0.0 were used. Graphs were generated with GraphPad Prism 9.0.0.

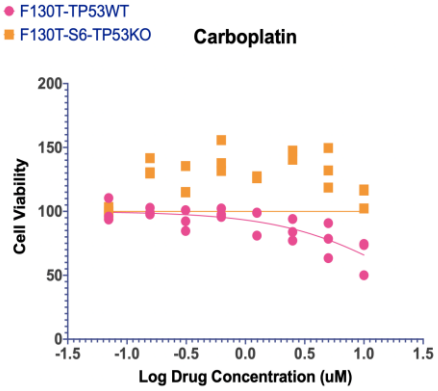
Results

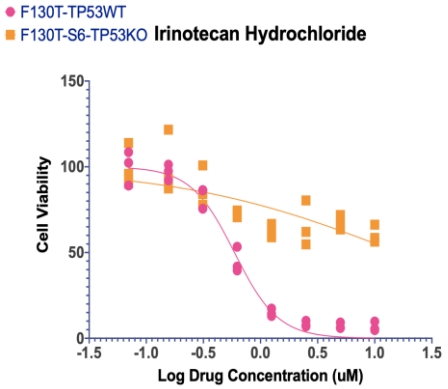
Nutlin was used as a positive control in this experiment. F130T-S6 (TP53 KO) tumor organoid cells were more resistant to Nutlin, 5-FU, Irinotecan, Thiotepa, Thioguanine, and Decitabine. Carboplatin began to show some effectiveness against F130T (TP53 WT) tumor organoid cells, but overall, there was not a significant difference in drug sensitivity between F130T and F130T-S6. Additionally, there was not a significant difference in drug sensitivity between F130T and F130T-S6 for Olaparib. Reference Figure 4. The half maximal inhibitory concentration (IC_{50}) for each drug was also calculated. In this experiment, the IC_{50} of these drugs is the drug concentration that was needed to inhibit (kill) half of the cells present [1]. IC_{50} is widely used in research as a measure of drug sensitivity or drug potency. IC_{50} for the drugs tested in this study increased for F130T-S6 cells compared to F130T cells. IC_{50}s for Carboplatin F130T-S6, Olaparib F130T-S6, and Thiotepa F130T-S6 were unstable and unable to be calculated. Reference Table 2.

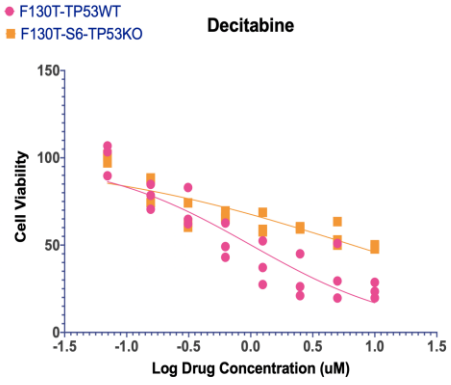

F130T-TP53WT
F130T-S6-TP53KO
Nutlin
Cell Viability
Log Drug Concentration (uM)


F130T-TP53WT
F130T-S6-TP53KO
Fluorouracil
Cell Viability
Log Drug Concentration (uM)


F130T-TP53WT
F130T-S6-TP53KO
Thioguanine
Cell Viability
Log Drug Concentration (uM)


F130T-TP53WT
F130T-S6-TP53KO
Carboplatin
Cell Viability
Log Drug Concentration (uM)


F130T-TP53WT
F130T-S6-TP53KO
Irinotecan Hydrochloride
Cell Viability
Log Drug Concentration (uM)


F130T-TP53WT
F130T-S6-TP53KO
Decitabine
Cell Viability
Log Drug Concentration (uM)

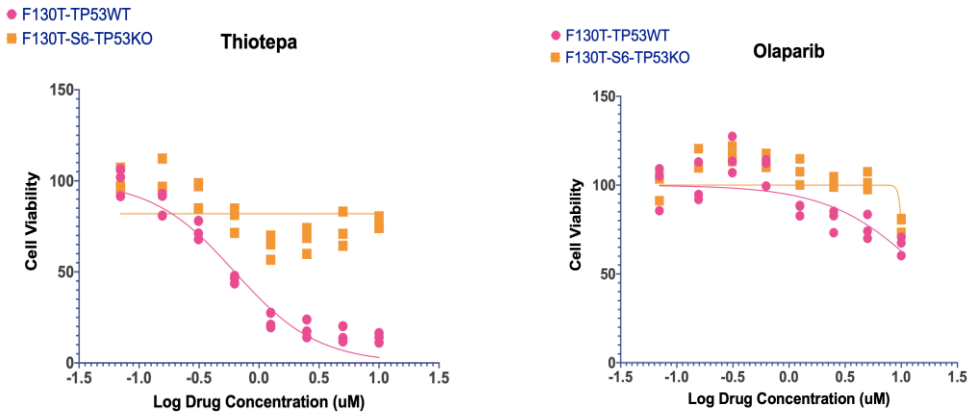


Figure 4: Viability of TP53 WT and TP53 KO CRC organoids after being treated with various drugs.

	IC50s (uM)							
	Nutlin	Fluorouracil	Thioguanine	Carboplatin	Irinotecan Hydrochloride	Decitabine	Olaparib	Thiotepa
F130T	1.794	2.148	1.657	21.49	0.5917	1.006	16.76	0.6265
F130T-S6	15.18	462.8	6.394	*	16.05	6.627	*	*

* = unstable/could not calculate

Table 2: IC_{50}s for TP53-WT and TP53-KO CRC organoids for the eight drugs tested in this experiment.

DepMap and PRISM data was analyzed for CRC cell line sensitivities with and without TP53 mutations to Decitabine, Irinotecan, and Thioguanine, and Nutlin (reference Figure 5, A-D). Data points to the left of the y-axis were more sensitive to the drug while data points to the right of the y-axis were more resistant to the drug. According to this data, there were not significant differences in drug sensitivity between TP53 mutated and TP53 WT CRC cell lines. Most of the CRC cell lines, with and without TP53 mutations, seemed to be relatively sensitive to Decitabine, Irinotecan, and Thioguanine. With the DepMap data for Nutlin, however, there is a stronger correlation and a relatively significant difference in response to drug between TP53 mutated CRC cell lines and TP53 non-mutated CRC cell lines.

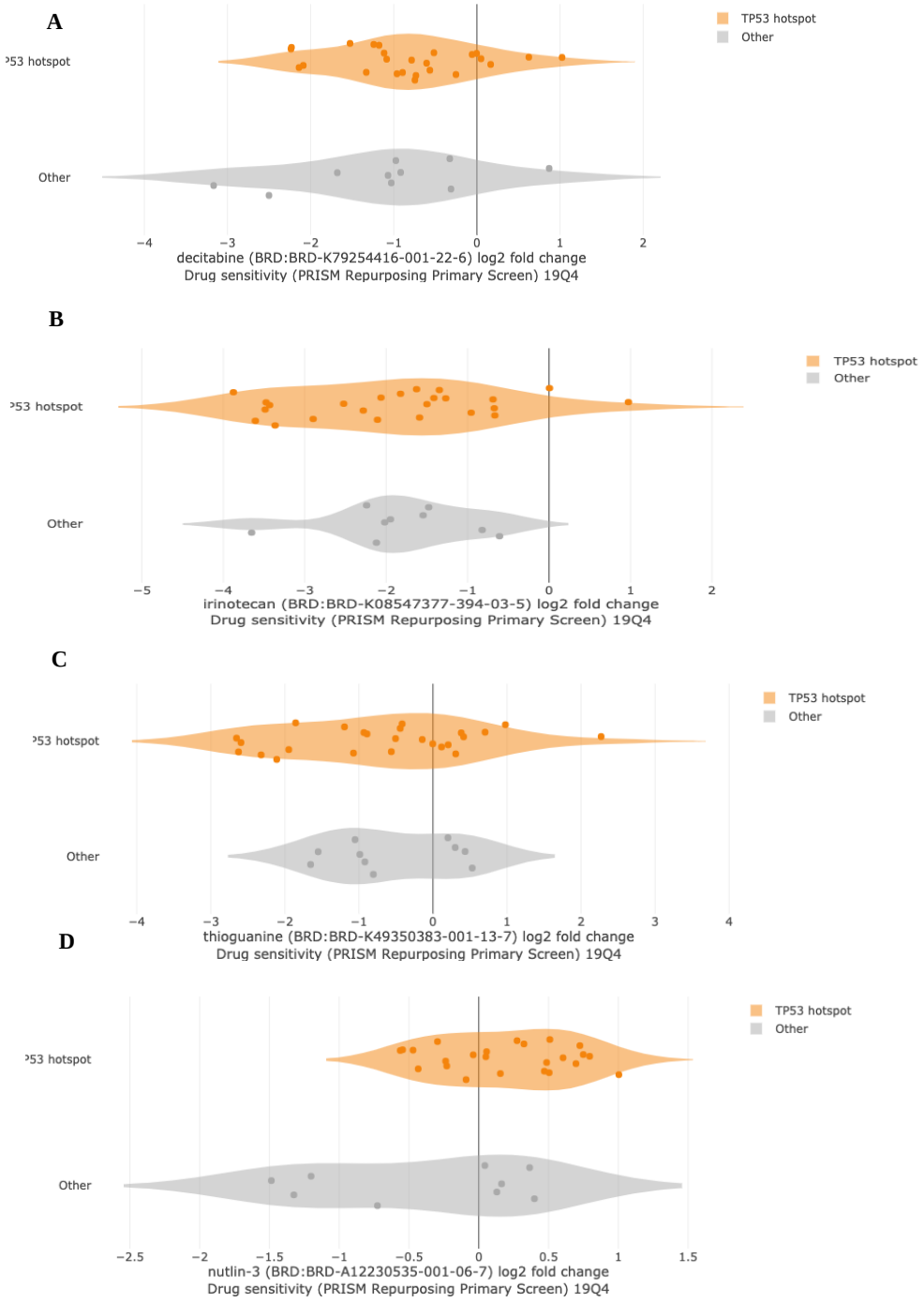


Figure 5, A-D: PRISM and DepMap drug sensitivity data for CRC cell lines with and without TP53 mutations.

Discussion and Conclusion

The results of this experiment show that CRC organoids without functional TP53 gene are more resistant to the cancer drugs Nutlin, 5-FU, Thioguanine, Irinotecan, Decitabine, and Thiotepa. This can be observed on the graphs displaying drug concentration versus cell viability as well as with the IC_{50}s. For the cancer drugs Carboplatin and Olaparib, there was not a significant difference in sensitivity detected between the TP53 WT tumor organoid cells and the TP53 KO tumor organoid cells. In the future, higher concentrations of these drugs should be tested on the same isogenic pair of organoids to determine if there is a difference in tumor response to drug. While past studies have found similar patterns in sensitivity to drug in other cell lines, this is the first study to prove this difference in response within an isogenic pair of CRC organoids. This is extremely significant because, as previously described, organoids are excellent models for human organs and tumors [8]. Thus, the findings from conducting research with organoids can be more easily applied to clinical practice. In the case of this study, this would involve the treatment of patients' tumors. For people without functional TP53 gene in their tumor, Nutlin, 5-FU, Thioguanine, Irinotecan, Decitabine, and Thiotepa would not be effective in treating their tumor. People who do not have TP53 mutations in their tumor, however, would benefit from being treated with these drugs. Further experiments should be conducted with other cancer drugs to see if comparable results are obtained with drugs that work similarly to the ones tested in this experiment.

Data from the PRISM and DepMap analysis also demonstrate the significance of the results of this study. According to the correlative data from PRISM and DepMap, no significant differences in drug sensitivity between TP53 mutated and TP53WT CRC cell

lines were seen. This data is, however, correlative data and suggests a correlation of drug sensitivities versus genetic mutations. In our experiment, we proved that TP53 loss causes resistance to these drugs by engineering the mutation ourselves. The data for Decitabine, Irinotecan, and Thioguanine demonstrate how correlative experiments are not always ideal models. The Nutlin PRISM and DepMap data, however, shows a correlation similar to the relationship we found; CRC cell lines with TP53 mutations seem to be more resistant to Nutlin then CRC cell lines without TP53 mutations. This data demonstrates that correlative experiments can occasionally be good models. Because our experiment shows a cause-and-effect relationship, it is a better, more consistent model. This is very significant and this PRISM and DepMap data should be analyzed in conjunction with further drug assays.

Additionally, our TP53 KO isogenic pair of organoids is a good model for testing the effect of TP53 mutations on drug response because of the amount of naturally occurring TP53 mutations that are inactivating, or loss of function, mutations. Based on a PanCancer Atlas study containing 594 samples of CRC tumors analyzed on cBioPortal.org, 58.8 percent of the samples had a mutation in TP53. 75 of these mutations were truncating, 171 missense shallow deletions, and 3 inframe shallow deletions. This means that 249 out of the 315 mutations in TP53 were inactivating mutations, just like our TP53 KO organoid that we engineered. Refer to Table 3.

	TP53 Mutations in CRC Cell Lines
Somatic Mutation Frequency	58.80%
Total Mutations	315
Truncating	75
Total Missense	235
Missense (ShallowDel)	171
Total Inframe	5
Inframe (ShallowDel)	3
Inactivating Mutations	249

Table 3: TP53 mutation data from PanCancer Atlas study of CRC

The eight drugs chosen to be tested in this experiment were based on the experiment conducted by Liu, et al. (2019). In Liu, et al.'s (2019) study, sensitivity of TP53 KO hESCs to all 127 drugs from the NCI Approved Oncology Drug Set IV were investigated [12]. Of these 127 drugs, Liu, et al. (2019) identified 27 drugs that the TP53 KO hESCs were resistant to. When analyzing these 27 drugs, the drugs that seemed to produce some of the most significant differences in sensitivity between TP53 KO hESCs and TP53 WT hESCs were Nutlin, 5-FU, Thioguanine, Irinotecan, Decitabine, Thiotepa, Carboplatin, and Olaparib. The results of our experiment demonstrate very similar trends to the results of Liu, et al.'s (2019) experiment as seen in Figure 6, A-E.

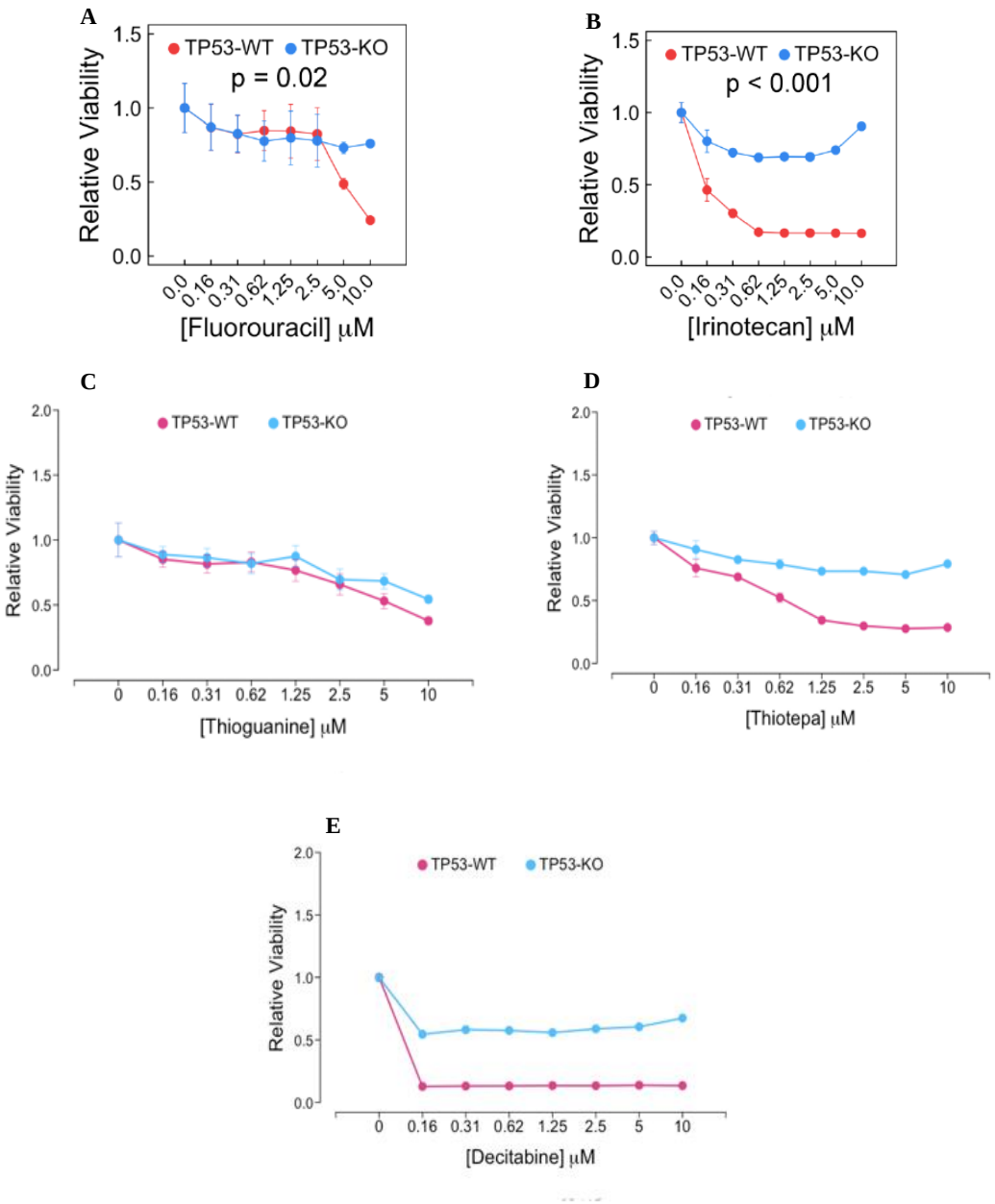


Figure 6, A-E: Viability of TP53 WT and TP53 KO hESCs after being treated with various drugs. Obtained from Liu, et al. (2019).

As stated previously, Liu, et al.'s (2019) experiment works with hESCs, while our study works with CRC organoids, which are more clinically applicable. This experiment is a beginning step towards what Liu, et al. (2019) did with resenstizing TP53 KO hESCs to standard cancer drugs. In the future, these same CRC organoids should be treated with the rest of the 119 drugs in the NCI Approved Oncology Drug Set IV to determine all of the drugs that the TP53 KO organoids are resistant to. Then, spindle assembly checkpoint inhibition, as in Liu, et al.'s (2019) study, should be performed on the TP53 KO organoids, and they should again be treated with the drugs they were resistant to in order to determine if spindle assembly checkpoint inhibition resensitizes the TP53 KO organoids to these cancer drugs.

Possible limitations of this experiment include human error when making drugs dilutions and when plating organoids. Additionally, only a small number of drugs were tested, and more drugs need to be tested in the future. This is mainly due to error in analysis of the first attempt at the drug assay. Mistakes were made in the incubation time when using the resazurin assay kit, so the drug assay had to be repeated and CellTiter-Glo® Luminescent Viability Assay was used to analyze cell viability the second time. Because of this, there was not sufficient time to test more than the eight drugs that were chosen.

Overall, this experiment was successful in answering the main question posed and achieving the main objectives. We proved that loss of functional TP53 gene in CRC organoids does cause a change in tumor response to standard cancer treatment drugs compared to TP53 WT CRC organoids. Additionally, we found the drugs and drug concentrations that TP53 KO CRC organoids (F130T-S6) are more resistant to than TP53

WT CRC organoids (F130T). We found what drug sensitivity differences are caused by TP53 loss specifically in CRC tumors, and characterized these drugs as p53-dependent; in order to function and kill CRC tumor cells, the drugs Nutlin, 5-FU, Thioguanine, Irinotecan, Decitabine, and Thiotepa require the presence of the p53 protein.

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