Screening Therapeutics for TP53 Synthetic Lethality

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The tumor suppressor gene TP53 is inactivated by somatic mutation in over 50% of human cancers. A common G/C germline polymorphism encodes either Arg or Pro at codon 72, and may affect resistance to certain first-line chemotherapies. Our aim was to utilize isogenic derivatives of RKO colon cancer cells, with defined TP53 genotypes, to screen FDA-approved compounds for those that show TP53-dependent differential toxicity.

RKO Colon Cancer Cells with engineered derivatives of the TP53 gene were used for cell screening.

RKO Colon Cancer Cells	
RKO Cell Lines	TP53 Codon 72 Polymorphism
Parental RKO, Wild Type (WT)	Heterozygous Arg-Pro
RKO Knockout (KO)	Homozygous Null
RKO Isogenic Derivative, Arg (R)	Hemizygous Arg
RKO Isogenic Derivative, Pro (P)	Hemizygous Pro

Using the Approved Oncology Drug Set from the NCI, a 119 compound set of the most current FDA-approved chemotherapeutics, we screened Parental RKO colon cancer cells, and engineered isogenic derivatives with alterations to the TP53 gene. Cells were plated in 96 well plates, compound was added at a final concentration of 10uM in 0.1% DMSO and incubated for 72 hours. The cell permeable redox indicator resazurin was used to monitor cell viability. Compounds of interest were later screened in patient derived organoid cells (tumor and normal paired sets) as noted above.

EXPERIMENTAL DATA

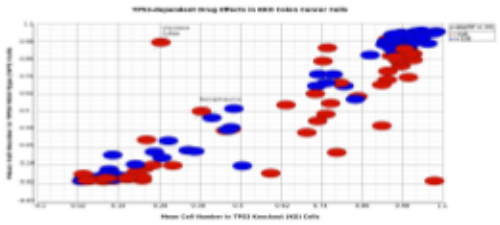


Figure 1. Cell viability values as a function of TP53 status (Knockout KO; x-axis, Wild Type WT; y-axis). The red points are drugs with significantly differential effects on cell viability (p-value < 0.05).

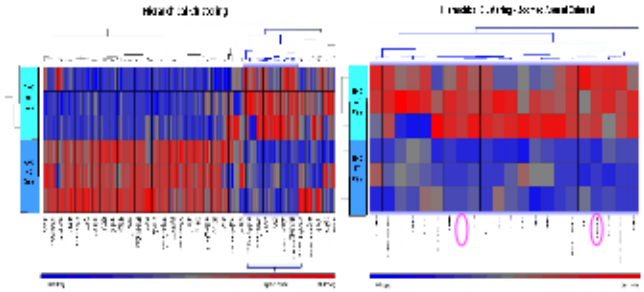
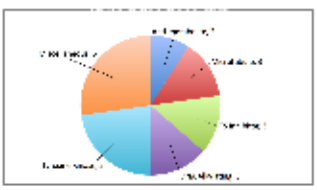


Figure 2. Unsupervised hierarchical clustering of cells and drugs according to viability after treatment. The left panel shows all 119 NCI compounds, and the right panel shows those compounds that were more detrimental to the TP53 null cells than to TP53 WT cells. Mercaptopurine and vincristine were chosen for further analysis.

The 22 compounds isolated by hierarchical clustering as harmful to the RKO KO cells (TP53 homozygous null) were analyzed for common mechanisms of action.

Figure 3. Classification of compounds for validation. The drugs vincristine sulfate (inhibitor of microtubule polymerization) and mercaptopurine (an antimetabolite purine antagonist) showed the some of the greatest differences in cell viability between the parental WT and the KO cells.



In addition to discovering compounds showing selectivity toward TP53-null cells, we noted several compounds with wild type, Arg72 vs. Pro72 dependent viability differences. Such compounds may show differential toxic profiles depending on TP53 germline genotype. Because this allele frequency varies by ethnic ancestry, these differences may be important toward addressing racial disparity in cancer treatment outcomes.

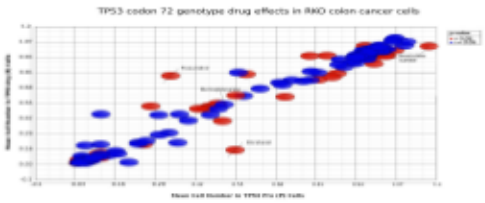


Figure 4. Cell viability as a function of codon 72 genotype (Pro72; x-axis, Arg72; y-axis). The red points are significantly differentially toxic (p-value < 0.05).

The compounds that were most sensitive in WT vs. KO screening also showed significant differences in toxicity toward Arg72 vs. Pro72 cells.

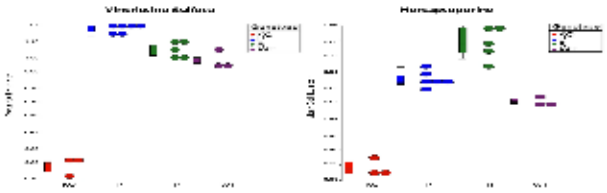


Figure 5. Viability cell triplicates of compounds on each cell line. Vincristine sulfate (left panel) shows a detrimental effect to KO vs. WT cells but also shows that the Pro cell line has less toxicity than the Pro72 or WT cells. Mercaptopurine (right panel) shows similar WT vs. KO viability result but the Arg cells are less affected than either the Pro72 cells or WT.

The lead compound of mercaptopurine was validated in patient organoids.

Patient organoids (3-dimension cell clusters which recapitulate the architecture of the parental tissue) were created from normal and tumor colon cancer tissue obtained through collaboration with the Department of Surgery at the University of Alabama at Birmingham. The patient cell lines were sequenced and analyzed for somatic mutations at TP53. Patient organoid P11042015 was found to carry an Arg213>stop mutation as well as a loss of heterozygosity.

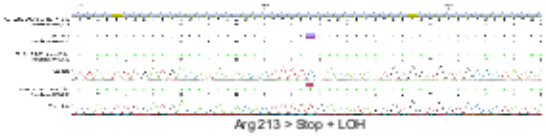


Figure 6. Identification of a TP53 somatic mutation in tumor organoid from patient P11042015.

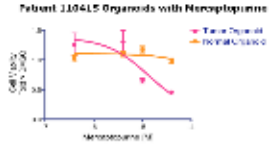


Figure 7. Patient P11042015 tumor and normal derived organoids were treated with mercaptopurine in 0.5% DMSO for 72 hours and cell viability tested by resazurin. This patient has a known TP53 mutation at codon 72. The synthetic lethality shown in the RKO cells repeated in the patient derived lines.

Compound screening in isogenic derivatives of RKO cells has confirmed the classification of several drugs known for their TP53 null chemoresistance in colon cancer, and discovered novel therapeutic approaches for TP53 mutant tumors. Also noteworthy is the discovery of classes of chemotherapeutics that could be selectively beneficial to specific subsets of colon cancer patients in the clinic, depending on TP53 germline sequence.