All-in-one CRISPR-knockout screening for identifying drug-sensitive genes linked to apoptosis and vascular mimicry in colorectal cancer cells.

Over the past decade, CRISPR (clustered regularly interspaced short palindromic repeats) has revolutionized the biomedical field by enabling more precise DNA editing than before. This breakthrough technology allows researchers to investigate complex genetic interactions, especially in diseases like cancer. By editing specific genes, scientists gain unprecedented insights into the molecular basis of malignancies. Moreover, CRISPR helps to accelerate the identification of novel drug targets, characterize new compounds, and pinpoint patients who would benefit most from treatment, ultimately reducing the failure rates in the drug discovery pipeline[1].

One of the most intensively researched areas for drug discovery is the development of therapeutics that can weaken tumor survival, promote cytotoxicity, and impair the angiogenic processes in tumor cell survival and metastasis.

A team of scientists from TaiRx, Inc. has been developing a novel anti-cancer drug, CVM-1118 (foslinanib)[2], for neuroendocrine and hepatocellular cancers which is currently in Phase 2 clinical trials. This drug quickly metabolized in the body to CVM-1125 and induces apoptosis in cancer cells. It also inhibits vascular mimicry, a characteristic feature of aggressive cancer cells that is not currently druggable.

Methods

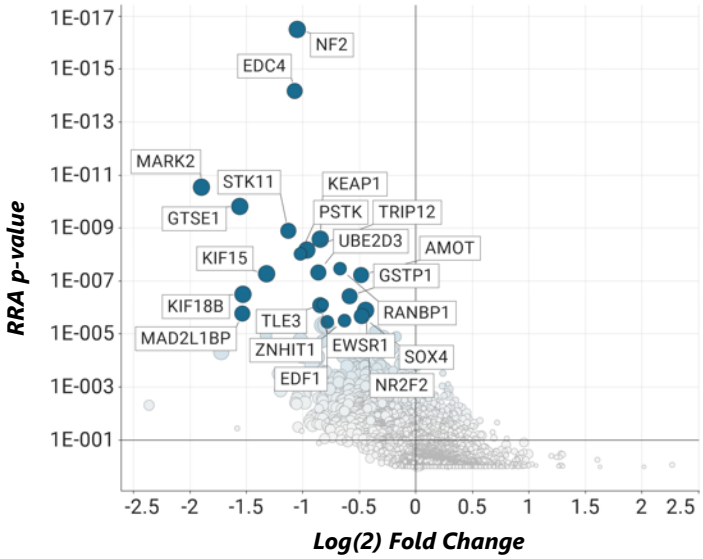
To investigate potential efficacy enhancements of CVM-1125, a whole-genome CRISPR-knockout (CRISPRko) screen was performed using the colorectal cell line COLO205 (ATCC), known for its responsiveness to the drug. The screen aimed to identify genes that increase sensitivity to CVM-1125.

Cells were transduced with Revvity's high-complexity All-in-one CRISPRko library, with eight guides per gene designed using the Edit-R™ algorithm to maximize the screen power and improve the chances of identifying all relevant mechanisms. Populations of cells at 300X coverage were maintained in duplicate in the presence or absence of low dose CVM-1125 (giving 10-20% growth inhibition compared to control-treated cells) for approximately 12 population doublings of the control population.

Results

The screen identified genes that, when perturbed, induced sensitivity to the compound, resulting in a drop out of these cells from the end-point (Figure 1). The high-quality data showed great overlap between two benchmark algorithms, DrugZ[1] and MAGeCK RRA[3] (overlapping hits shown in orange text in the table). Highly significant P-values and false discovery rates for these putative hits bolster confidence in these genes being of consequence.

Top sensitivity hits in CVM-1125 treated COLO 205 cells using RRA



GENE	αRRA	P-Value	FDR	LFC
NF2	3.12E-17	2.67E-07	0.000413	-1.0519
EDC4	6.57E-15	2.67E-07	0.000413	-1.0761
MARK2	2.75E-11	2.67E-07	0.000413	-1.8999
GTSE1	1.51E-10	2.67E-07	0.000413	-1.5603
STK11	1.23E-09	2.67E-07	0.000413	-1.1317
KEAP1	2.63E-09	2.67E-07	0.000413	-0.85234
TRIP12	6.45E-09	2.67E-07	0.000413	-0.97084
PSTK	9.47E-09	2.67E-07	0.000413	-1.025
RANBP1	3.29E-08	2.67E-07	0.000413	-0.67668
UBE2D3	4.74E-08	2.67E-07	0.000413	-0.86499
KIF15	5.23E-08	2.67E-07	0.000413	-1.3265
AMOT	5.93E-08	2.67E-07	0.000413	-0.49001
KIF18B	3.07E-07	2.40E-06	0.003182	-1.5332
GSTP1	3.68E-07	2.40E-06	0.003182	-0.59108
TLE3	7.51E-07	6.68E-06	0.008251	-0.82525
EWSR1	8.07E-07	8.28E-06	0.009592	-0.85126
NR2F2	1.26E-06	1.10E-05	0.011939	-0.44947
MAD2L1BP	1.64E-06	1.36E-05	0.014026	-1.541
SOX4	2.00E-06	1.63E-05	0.015894	-0.48306
ZNHIT1	3.02E-06	2.48E-05	0.02302	-0.6348

Figure 1: Top Sensitivity hits in CVM-1125 treated COLO205 cells using MAGeCK RRA. The graph shows log-fold change plotted against the RRA-adjusted p-value. Top 20 genes are highlighted in blue. Top genes are also listed in the table to the right, with RRA-adjusted p-value, p-value, false discovery rate (FDR), and log-fold change (LFC). Genes that also appeared in the top 20 by DrugZ are highlighted in orange.

A deeper dive into pathway analysis revealed that many genes causing drug sensitivity are linked to the mTOR pathway. Scientists at TaiRx, Inc. validated the significance of two mTOR top hits, STK11 and NF2, using an orthogonal technology, shRNA knockdown, in both COLO205 and HCT-116 cells[2]. This confirmed that knockdown of either gene significantly reduced cell viability in response to CVM-1125. Significantly, STK11 is often mutated in NSCLC, and two NSCLC cancer lines carrying such mutations were highly sensitive to CVM-1125.

Conclusion

This study reveals the power of CRISPR screening for identifying key genes and entire pathways of importance and providing a comprehensive understanding of biological interactions. This approach identifies robust, actionable hits that can be directly progressed into validation studies, and provides the results that are statistically significant and biologically relevant. Moreover, this technology aids pharmaceutical companies in targeting new therapeutics tailored to the unique genetic makeup of individual tumors. This patient-specific strategy enhances the effectiveness of drugs and boosts the overall success rate of drug development, marking a significant stride in personalized medicine.

Role of revvity preclinical services

TaiRx, Inc. partnered with Revvity Preclinical Services team (formerly Horizon Discovery) to conduct a comprehensive whole-genome CRISPRko screen. The team of Lifen Shen and colleagues has now successfully published the data[2].

References

1. Colic, M. et al., Identifying chemogenetic interactions from CRISPR screens with drugZ. Genome Medicine 11, 52 (2019).
2. Shen, L. et al., CVM-1118 (foslinanib), a 2-pheyl-4-quinolone derivative, promotes apoptosis and inhibits vasculogenic mimicry via targeting TRAP1. Pathology & Oncology Research 29:1611038 (2023).
3. Li, W. et al., MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. Genome Biology 15, 554 (2014).

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