DDR Cell Panel For Novel Drug Discovery

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Introduction

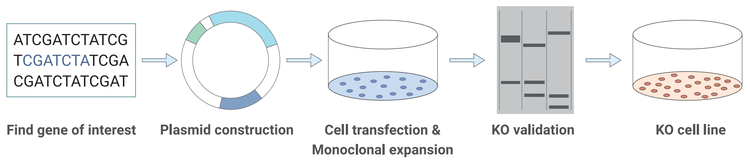
DNA damage represents a critical threat to cellular viability, as improperly repaired DNA damage can lead to cellular senescence, apoptosis, or tumorigenesis. The DNA damage response (DDR) encompasses a series of cellular processes that detect and repair genomic lesions. Targeting DDR pathways and inhibiting DNA repair mechanisms have emerged as promising strategies in cancer therapy, and significant progress has been made in the discovery of DDR-related inhibitors. However, drug resistance has become an increasingly prevalent challenge in this field. To better understand compound potency across various cancer cell lines, we generated drug-sensitive and drug-resistant cell lines by knocking out key DDR genes (e.g., BRCA1/2, XRCC1) and culturing cells under selective pressure from different DDR-targeting compounds. Additionally, together with wild-type (WT) cells commonly used in DDR-related drug discovery, we established a DDR cell panel encompassing 14 distinct cancer types. This panel has been rigorously validated through in vitro proliferation assays and in vivo efficacy studies. Furthermore, sequencing and bioinformatic analyses have been employed to elucidate the mechanisms underlying drug sensitivity and resistance. Our findings demonstrate that this DDR cell panel offers a rapid and comprehensive platform for evaluating DDR inhibitors, thereby facilitating the efficient discovery of novel therapeutics in cancer treatment.

DDR Targets

DDR Pathways	Targets
HR	ATM, ATR, BLM, RECQ1, RECQ4, RECQ5,HELQ
NHEJ and TMEJ	DNA-PK, WRN, POLQ
SSB and BER	PARP1/2/3/5A/5B/6/7/10/11/12/14/15,PARG, Topo I, APE1,XRCC1
Cell Cycle	WEE1/2, MYT1, p53 Y220C, CHK1/2, CDK family
Others	POLA, POLG, POLH, POLN, MAT2A, PRMT5,TREX1/2, DHX9

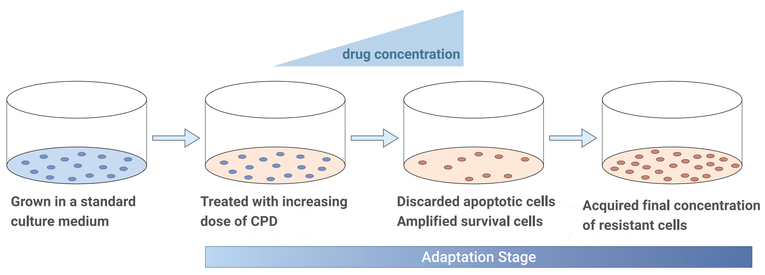
ICE bioscience has put great effort to the DDR field. We have developed and validated the enzymatic assays which cover the DDR targets listed above. Meanwhile, as cell-based assays being the natural down-stream study, we have constructed and generated various drug-sensitive and –resistant cell lines to facilitate the drug screening process.

Gene Knock-Out by CRISPR/Cas9 System and Resistant Cell Line Generation



6-8 weeks

The workflow of KO cell line generation.

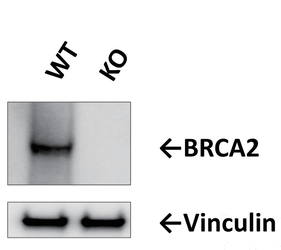


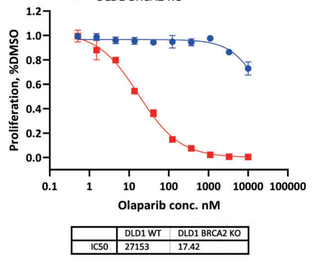
8-12 weeks

The workflow of drug resistant cell line generation

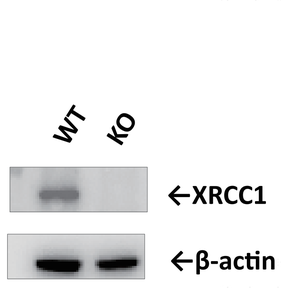
Results

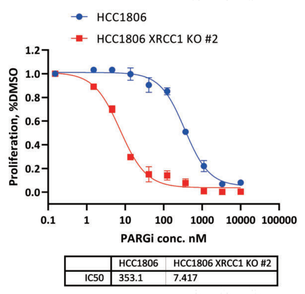
1.KO Cell Line Generation





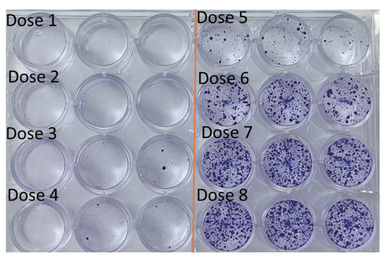
	DLD1 WT	DLD1 BRCA2 KO
IC50	27153	17.42



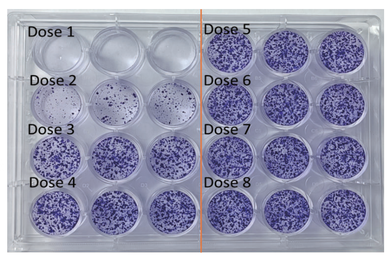


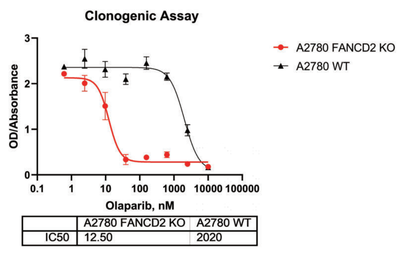
	HCC1806	HCC1806 XRCC1 KO #2
IC50	353.1	7.417

A2780 FANCD2 KO



A2780 FANCD2 KO

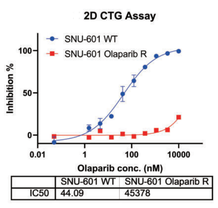




	A2780 FANCD2 KO	A2780 WT
IC50	12.50	2020

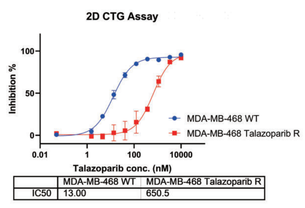
We have successfully knocked out DDR related genes in various parental cancer cell lines. These KO cell lines have been validated using both Western Blot and sequencing. In addition, we have also performed cell proliferation assay to validate the functional significance of the knock-out genes.

2.Resistant Cell Line Generation



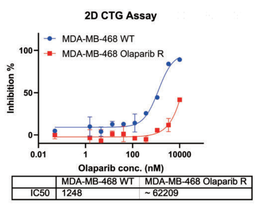
	SNU-601 WT	SNU-601 Olaparib R
IC50	44.09	45378

SNU-601 Olaparib R



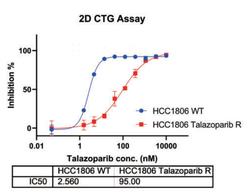
	MDA-MB-468 WT	MDA-MB-468 Talazoparib R
IC50	13.00	650.5

MDA-MB-468 Talazoparib R



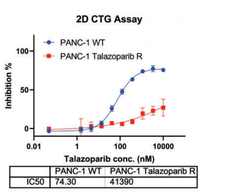
	MDA-MB-468 WT	MDA-MB-468 Olaparib R
IC50	1248	~ 62209

MDA-MB-468 Olaparib R



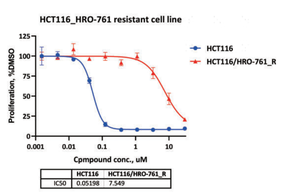
	HCC1806 WT	HCC1806 Talazoparib R
IC50	2.560	95.00

HCC1806 Talazoparib R



	PANC-1 WT	PANC-1 Talazoparib R
IC50	74.30	41390

PANC-1 Talazoparib R



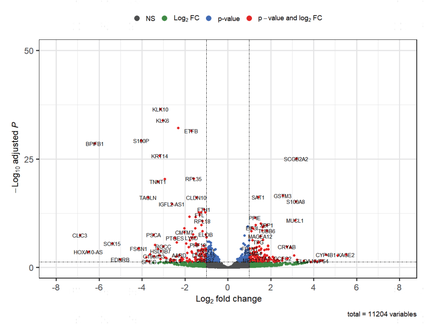
	HCT116	HCT116/HRO-761_R
IC50	0.05198	7.549

HCT116 HRO-761 R

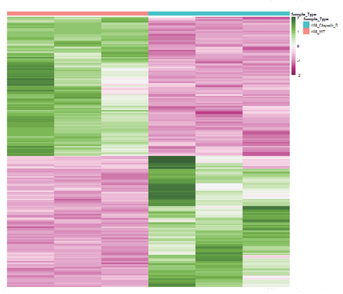
Besides generating KO cell lines which sensitizes to certain DDR inhibitors, we have also constructed cell lines which are resistant to anti-cancer drugs. The validation data of 4 cell lines have been listed above.

3. Bioinformatic Analysis

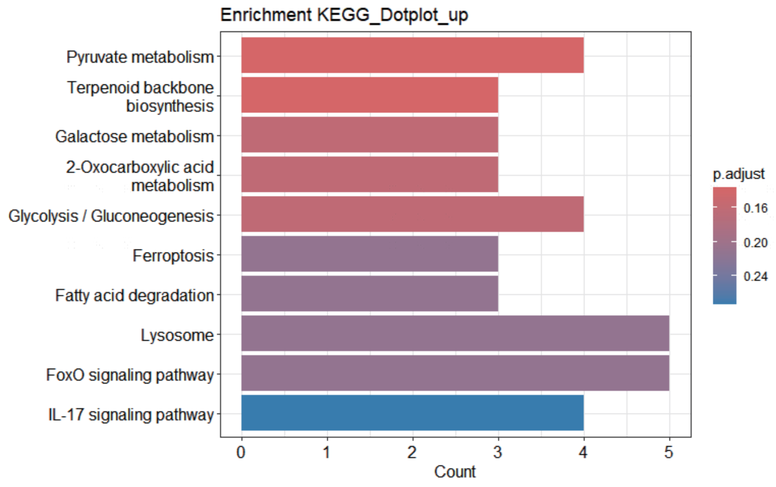
For generated drug resistant cell lines, we perform RNAseq-based bioinformatic analysis to investigate the mechanism. Utilizing our in-house algorithm, we can provide detailed information about differential gene expression, enriched pathway, and featured gene profiling. Representative data for MDA-MB-468 olaparib resistant cell line is shown below.



Volcano plot

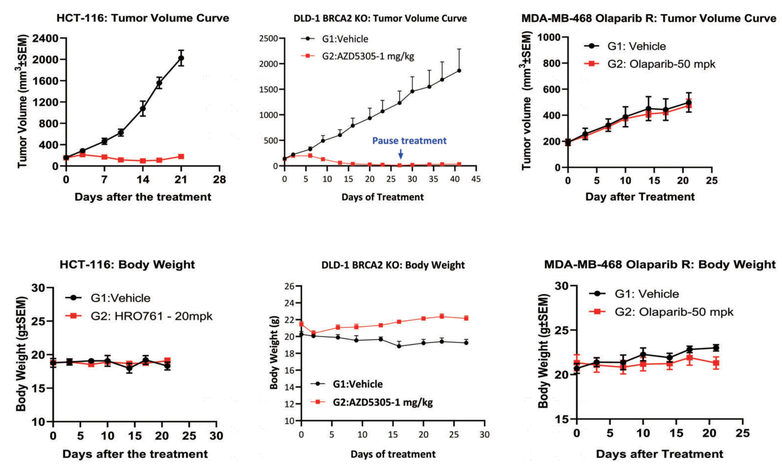


Heatmap analysis



Barplot of enriched KEGG terms

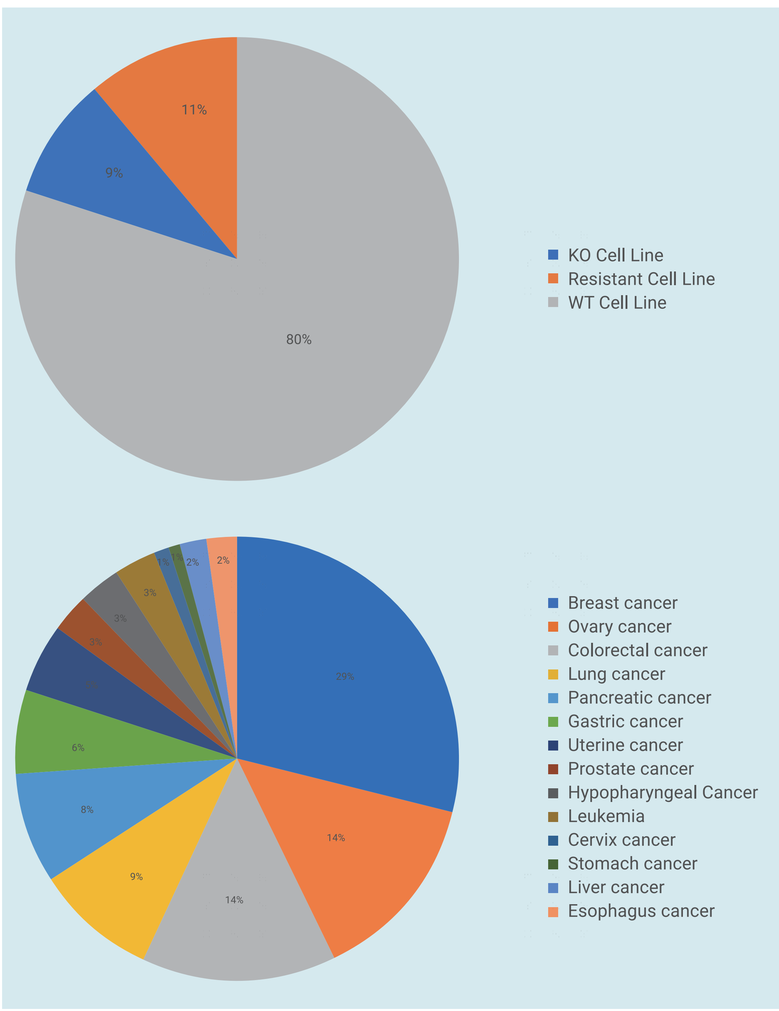
4. In Vivo Modeling



We have established and validated WT, drug-sensitive, and drug-resistant cell lines in the in vivo models.

5. DDR Cell Panel

Breast Cancer	HCC1937	**Cervix Cancer**	LOVO	SNU-601 Olaparib R	NCI-H358	OVCAR-8	**Prostate Cancer**
BT-20	Hs 578T	C-33A	RKO	SNU-638	NCI-H460	SK-OV-3	22Rv1
BT-549	MCF7	**Colorectal Cancer**	**Esophagus Cancer**	**Leukemia**	NCI-H460 Paclitaxel R	UWB1.289	DU145
CAL51	MDA-MB-231	DLD-1	TE-1	HL-60	NCI-H1703	UWB1.289+BRCA1	**Stomach Cancer**
HCC1428	MDA-MB-436	DLD-1 BRCA2 KO	**Hypopharyngeal Cancer**	K-562	**Ovarian Cancer**	**Pancreatic Cancer**	MKN1
HCC1806	MDA-MB-453	DLD-1 WRN KO	FaDu	**Liver Cancer**	A2780	AsPC-1	**Uterine Cancer**
HCC1569	MDA-MB-468	HCT-15	FaDu ATM KO	Hep G2	A2780 Cisplatin R	BxPC-3	MFE-296
HCC1806 Niraparib R	MDA-MB-468 Olaparib R	HCT-116	**Gastric Cancer**	**Lung Cancer**	A2780 FANCD2 KO	Capan-1	HEC-1-A
HCC1806 Talazoparib R	MDA-MB-468 Talazoparib R	HCT-116 BRCA1 KO	SNU-1	H1299	COV362	PANC-1	HEC-59
HCC1806 XRCC1 KO	T47D	HT29	SNU-601	NCI-H23	OVCAR-3	PANC-1 Talazoparib R	



Our DDR cell panel includes WT, drug-sensitive, and drug-resistant cells across 14 cancer types. These cell lines can provide comprehensive evaluation of the compound efficacy.

Summary

DDR is an essential cellular process that supports cell growth and proliferation, offering critical therapeutic targets. Inhibition of DDR pathways can effectively lead to the elimination of tumor cells, making DDR inhibitors a focal point of interest for both the academic community and the pharmaceutical industry. Cell panel screening is a robust tool for assessing the effects of compounds. In this study, we present a specialized DDR cell panel, comprising various engineered and drug-resistant cell lines. The use of this cell panel screening approach offers several key insights: 1. It enables the assessment of compound potency and efficacy across multiple tumor types, significantly aiding in indication evaluation; 2. It provides insights into the mechanisms by which compounds counteract tumor resistance; 3. It offers crucial information on the selectivity of compounds against different DDR pathways.