PARPi Resistant Cell Line Generation and DDR Cell Panel Development

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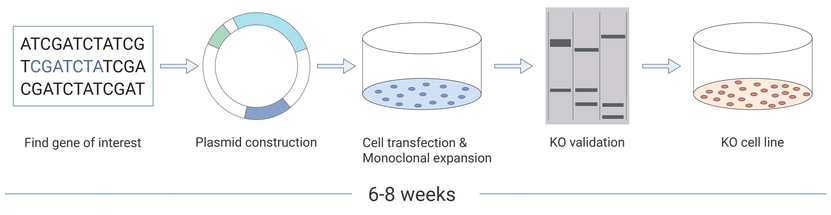
Introduction

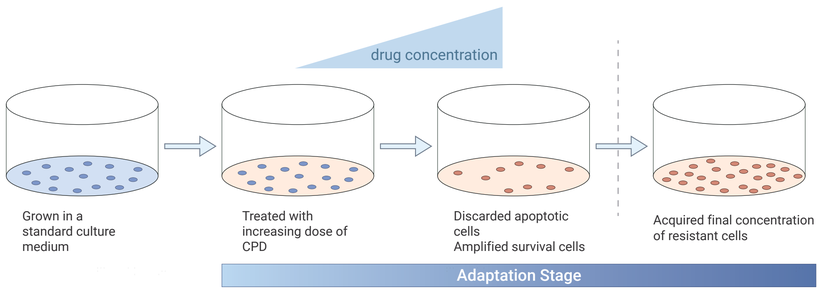
The DNA damage response (DDR) encompasses a series of cellular processes that detect and repair genomic lesions. Targeting DDR pathways and inhibiting DNA repair mechanisms have emerged as promising strategies in cancer therapy, and significant progress has been made in the discovery of DDR-related inhibitors. 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 PARP inhibitors. 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.

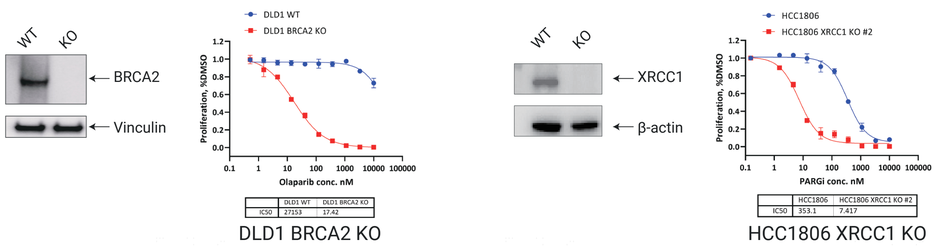
DDR Related Specific Cell Line Generation

The workflow of KO cell line generation using Gene knock-out by CRISPR/Cas9 system.

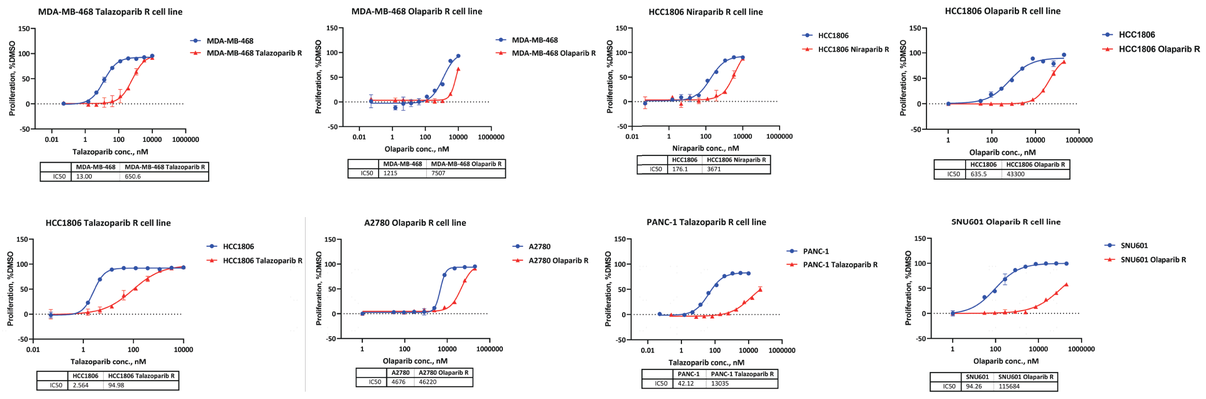


The workflow of drug resistant cell line generation.





KO cell line generated in ICE



PARPi resistant cell line generated in ICE

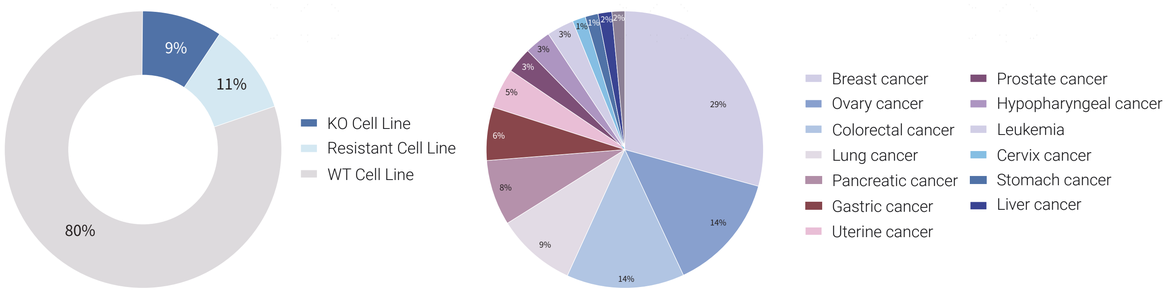
DDR Cell Panel

DDR targets

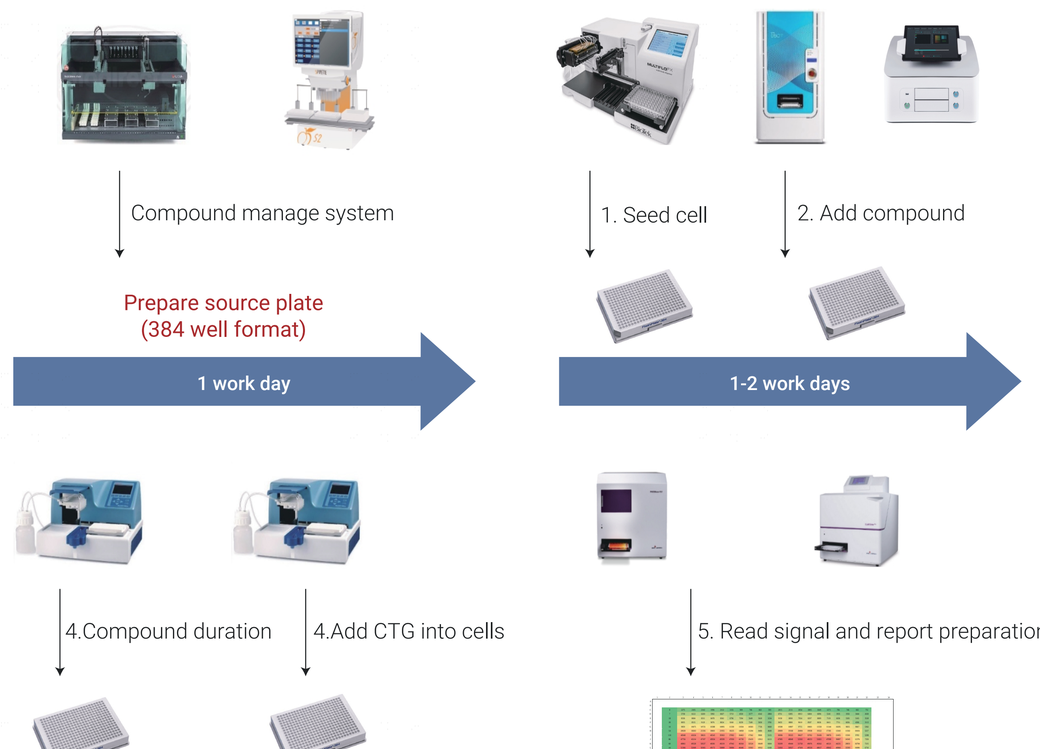
DDR Pathways	Targets
HR	ATM, ATR, BLM, RECQ1, RECQ4, RECQ5, HELQ
NHEJ and TMEJ	DNA-PK, WRN, POLQ
SSB and BER	PARP1/2/3/5A/5B/6/7/10/11/12/14/15, PARG, Topo I, APE1, XRCC1
Cell Cycle	WEE1/2, MYT1, p53 Y220C, CHK1/2, CDK family
Others	POLA, POLG, POLH, POLN, MAT2A, PRMT5, TREX1/2, DHX9

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.

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	

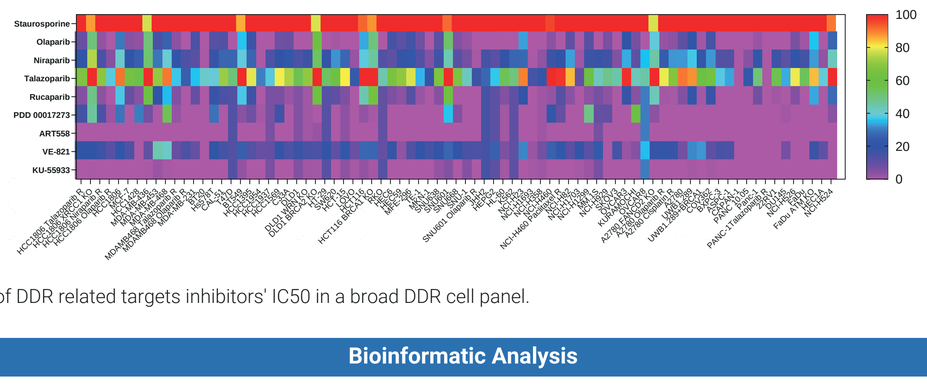


Our DDR cell panel includes WT, drug-sensitive, and drug-resistant cells across 14 cancer types. These cell lines can provide comprehensive evaluation of the compound efficacy.



High throughput work flow of DDR cell panel platform.

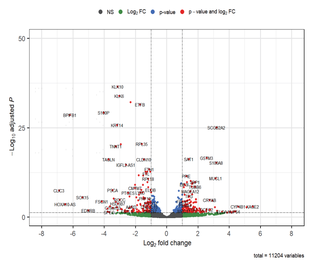
Drug Name	Target	Highest Phase
Olaparib	PARP1/2	Launched
Niraparib	PARP1/2	Launched
Talazoparib	PARP1/2	Launched
Rucaparib	PARP1/2/3	Launched
PDD 00017273	PARG	Preclinical
ART558	POLQ	Terminated
VE-821	ATR	Preclinical
KU-55933	ATM	Preclinical



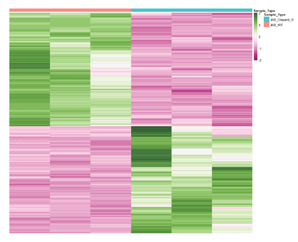
The evaluation of DDR related targets inhibitors' IC50 in a broad DDR cell panel.

Bioinformatic Analysis

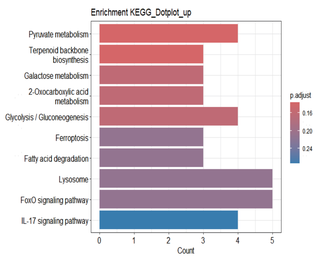
Volcano plot



Heatmap analysis

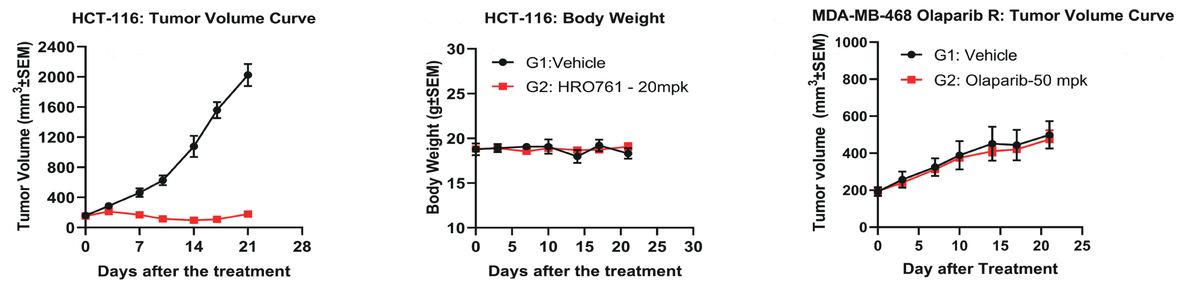


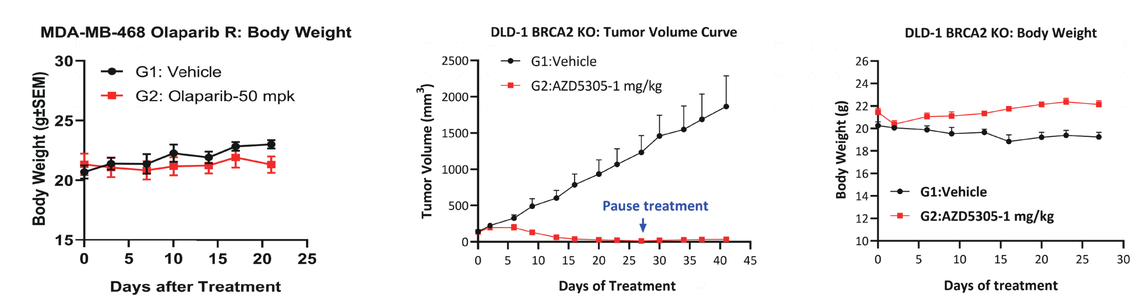
KEGG



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.

In Vivo Modeling





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

Conclusions

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.

Acknowledgements

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