Accelerating DDR related drug discovery through a customized cell panel

Cong Huang, Xiaoqing Li, Zhengtai Li, Yan Tan, Huawei Pan, Lizhen Zhou, Kejun Mao, Yanyan Qin, Zhida An, Wanxiao Song, Yifan Li, Shuyu Jin, and Tiejun Bing

ICE Bioscience, INC. Building 14, Yard 18, Kechuang 13th Street, Beijing, China 100176 Email: bingtj@ice-biosci.com

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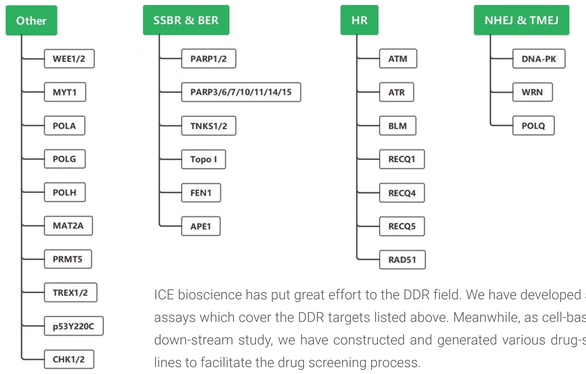
Abstract #414

Introduction

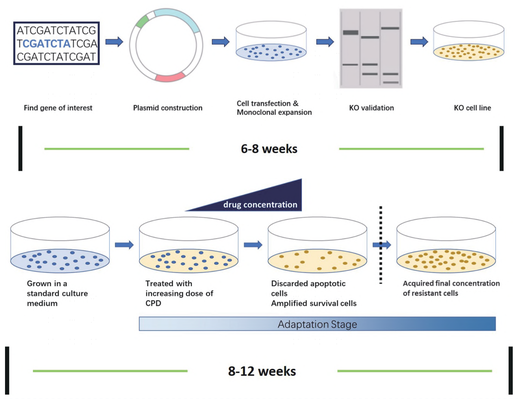
DNA damage is a major threat to cell survival. Not properly repaired DNA damage can lead to cell senescence, apoptosis or tumor development. DDR (DNA Damage Repair) is a collection of bioprocesses which cells utilize in order to identify and correct the DNA damage of the genome. The usage of various compounds for targeting DNA damage responses as well as attenuating DNA repair is also an approach for cancer therapy that has been widely studied in recent years. Despite the success of drug discovery of DDR related inhibitor, drug resistance has become an emerging issue of the field. Here we have constructed multiple cancer cell lines which are either DDR gene, such as XRCC1 and FANCD2, knock-out or resistant to certain DDR inhibitors. Together with the corresponding parental cell lines, we have generated a DDR cell panel which covers 12 different cancer types. We have first validated those DDR inhibitor sensitive or resistant cell lines with functional cell proliferation assays. Additionally, RNAseq has been performed for all constructed resistant cell lines. In combination with a thorough bioinformatic analysis using our in-house generated algorithms, we can provide the genetic background information of the resistant cell lines and top features that potentially contribute to the resistance mechanism. The representative data in this study has shown that LINC02709, BDKRB1, and PRKCQ are the top featured genes in A375 Vemurafenib resistant cell. Meanwhile, cell cycle, ECM-receptor interaction, and DNA replication are the most enriched KEGG terms. Lastly, we have validated this cell panel against multiple inhibitors targeting different DDR vital proteins such as ATM, PARP, POLQ etc. The result has shown that our unique DDR cell panel is capable of providing fast and comprehensive evaluation of DDR related inhibitors, thus facilitate faster and more efficient discovery of DDR inhibitors in the cancer therapy field.

DDR targets



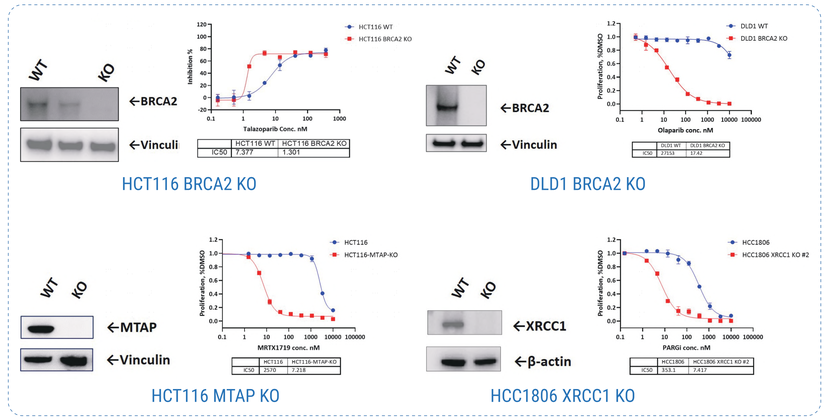
ICE bioscience has put great effort to the DDR field. We have developed and validated the enzymatic assays which cover the DDR targets listed above. Meanwhile, as cell-based assays being the natural down-stream study, we have constructed and generated various drug-sensitive and –resistant cell lines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

Gene knock-out by CRISPR/Cas9 system and Resistant cell line generation



Results

1.KO cell line generation

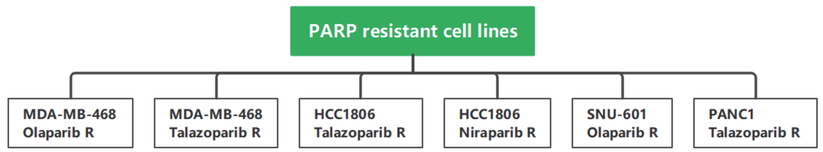


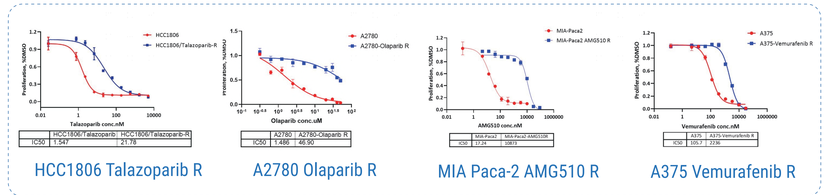
HCT116 BRCA2 KO DLD1 BRCA2 KO

HCT116 MTAP KO HCC1806 XRCC1 KO

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



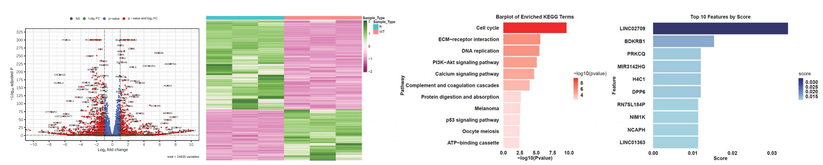


HCC1806 Talazoparib R A2780 Olaparib R MIA Paca-2 AMG510 R A375 Vemurafenib R

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

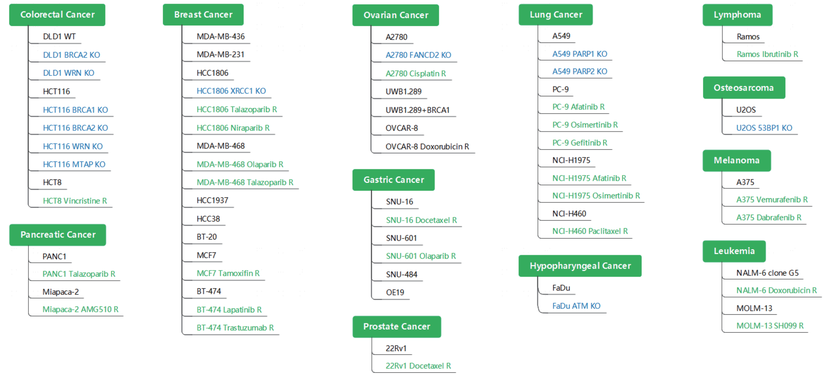
For generated drug resistant cell lines, we perform RNAseq-based bioinformatic analysis to investigate the mechanism. Utilizing our in-house algorithm, we can provide detailed information about differential gene expression, enriched pathway, and featured gene profiling. Representative data for A375 Vemurafenib resistant cell line is shown below.

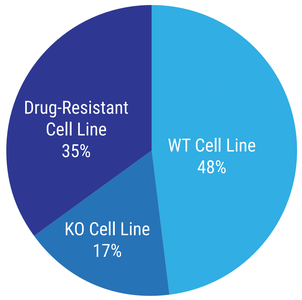


Volcano plot Heatmap analysis Barplot of enriched KEGG terms Top 10 features by score

4. DDR cell panel test

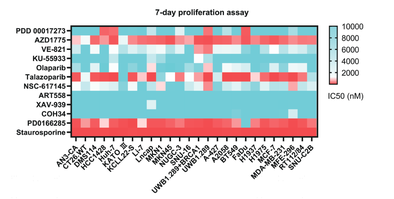
We established a DDR cell panel which consists resistant and engineered cell lines across different tumor types. We then performed a panel test for compounds targeting key components of DDR pathways as shown below.

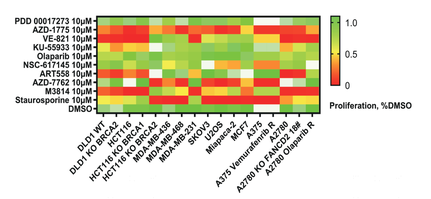




Compound Name	Target
PDD 00017273	PARG
AZD-1775	Wee1
Staurosporine	Pan Kinase
VE-821	ATR
KU-55933	ATM
Olaparib	PARP1/2
NCS-617145	WRN
ART558	POLQ
AZD-7762	Chk1/2
M3814	DNA-PK

Based on the result, our DDR cell panel can deliver strong target/pathway indication for different DDR inhibitors. We have shown partial results below for single dose and cell proliferation test heatmap.





Summary

DDR is a vital process which supports cell growth and proliferation. It provides valuable potential targets which, upon inhibition, can lead to tumor cell elimination. Therefore, DDRi development is of great interest of academic society and pharmaceutical companies. Cell panel screen is a powerful tool for evaluating compounds` effect. Here we have presented a unique specialized DDR cell panel, which includes various engineered and drug resistant cell lines. The utilization of such cell panel screen will provide the following information:

1. It provides both evaluation of compounds` potency and efficacy across different tumor types, which will greatly help the indication evaluation;

2. It can provide information for how compounds act against tumor resistance;

3. It can provide the selectivity information of the compounds against different DDR pathways.