Novel drug resistant CDX models for anti-cancer drugs discovery

Hai-Ting Dai, Tie-Jun Bing, Zhi-Da An, Jie Yang, Tian-Yu Pang, Jia-Wei Gao, Yun-Yi Bo, Wen-Jen Yu

Innovative CRO+Explorer
ICE Bioscience

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Abstract

Oncogene BRAF is located on chromosome 7q34, consisting of 766 amino acids and 22 exons, encoding a serine/threonine protein kinase belonging to the RAF family and plays a critical role in the MAPK signaling pathway. The mutation of BRAF was documented in nearly 8% of all human cancers including melanoma (60%), thyroid (60%), and lung adenocarcinoma (10%). The most common mutation of BRAF is V600E (Class I), which was found in more than 70% in these cancers. Despite the clinical success of approved small molecule inhibitors of BRAF V600E (vemurafenib, dabrafenib and encorafenib), this remains an area of unmet medical need because of primary or acquired drug resistance. The construction of BRAF drug-resistant cell lines plays a pivotal role in cancer research. It serves as a crucial tool for discovery of the mechanisms of drug resistance. In this study, we have developed a systematic approach for constructing drug-resistant cell lines, inducing resistance through the gradual escalation of drug concentrations. The validation of drug resistance involves morphological recordings of parent and drug-resistant cell lines, drug resistance index in vitro through IC50 measurements and in vivo through the inhibition of tumor growth (TGI) which compare to those in the parental cell line. We successfully established Vemurafenib and Dabrafenib resistant A375 cell lines (A375 Vemurafenib R, A375 Dabrafenib R) with 21 and 31.5 folds of resistance index, respectively. The in vivo drug resistance efficacy was also tested by implanting Vemurafenib resistant cells subcutaneously into NOD-SCID mice and showed that A375 Vemurafenib R exhibited robust tolerance to 50 mg/kg Vemurafenib treatment (P.O.21 days, 50mpk, TGI=17.4%), whereas the parental cells were nearly completely inhibited (P.O.21 days, 50mpk, TGI=92.3%). In addition, single-cell sequencing of A375 Vemurafenib R revealed significant gene expression differences in the MAPK and EGFR signaling pathways, aiding in the identification of new targets, and signaling pathways associated with drug resistance, thereby providing potential targets for new drug development.

Results

• Establishment of drug-resistant cell lines

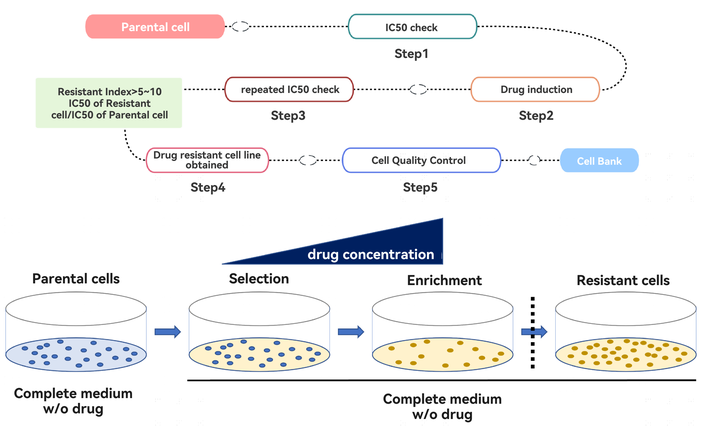


Figure 1: Schematic diagram of establishment of drug-resistant cell line
A375 drug resistant cell lines (A375 R) were generated by incubation with conditioned medium contained escalated drugs concentration for 4-6 months which mimic the process of drugs resistance tumors during the clinical chemotherapy.

• Properties of cell proliferation in parental and drug-resistant A375 cell lines

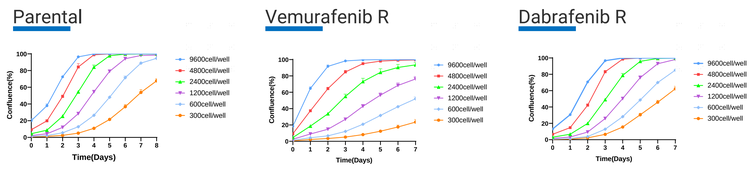
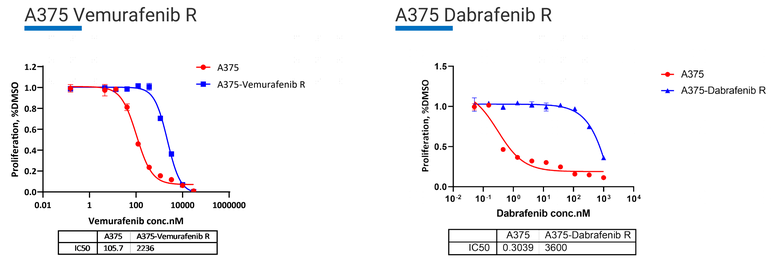


Figure 2: Proliferation of parental and drug-resistant A375 cell lines
Proliferation of parental and drug-resistant A375 cell lines were checked in different cell densities and indicated that longer doubling time was found in drugs resistant cells compared to that of parental cells.

• Drug resistant index verification



	A375	A375-Vemurafenib R
IC50	105.7	2236

	A375	A375-Dabrafenib R
IC50	0.3039	3600

Figure 3: Drug resistance index was determined using IC50
Drug resistance index was determined by comparison the IC50 between parental and resistant cell lines, the drug resistance index of A375 Vemurafenib R and A375 Dabrafenib R is 21.15 and 11846, respectively.

• Drug resistant CDX models

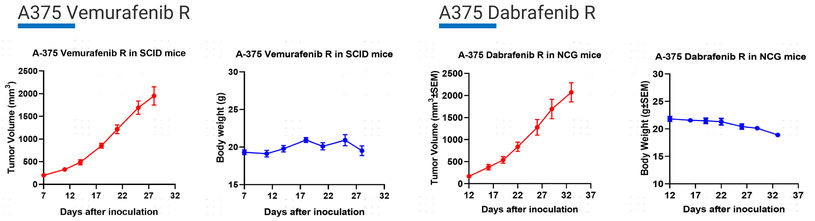


Figure 4: CDX modes of A375 Vemurafenib R and A375 Dabrafenib R cell lines
The tumor growth and bodyweight curves of A375 Vemurafenib R and Dabrafenib R cell lines are shown as a good model for new drug development on BRAF mutation.

• Efficacy evaluation of A375 drug resistance CDX models

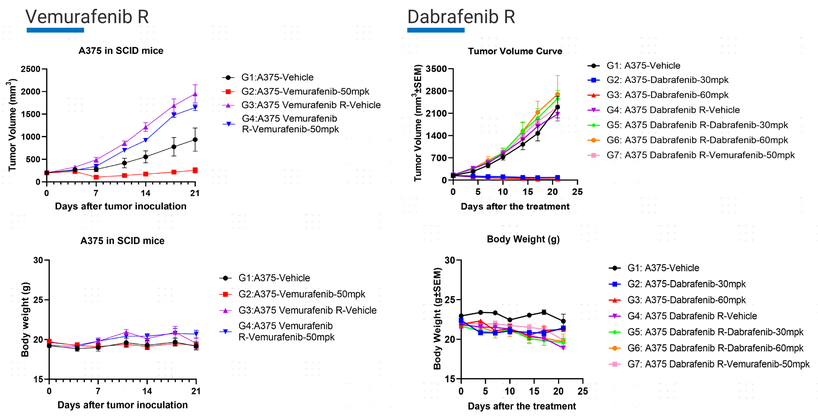
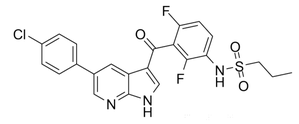


Figure 5: Efficacy of Vemurafenib and Dabrafenib on specific drug resistant CDX models
The drug resistance of A375 Vemurafenib and Dabrafenib R CDX models were shown and indicated that each cell lines performed significant drug resistance to Vemurafenib and/or Dabrafenib compared to that in parental cell line.

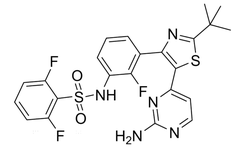
• Transcriptome sequencing analysis of drug-resistant strains

Vemurafenib R

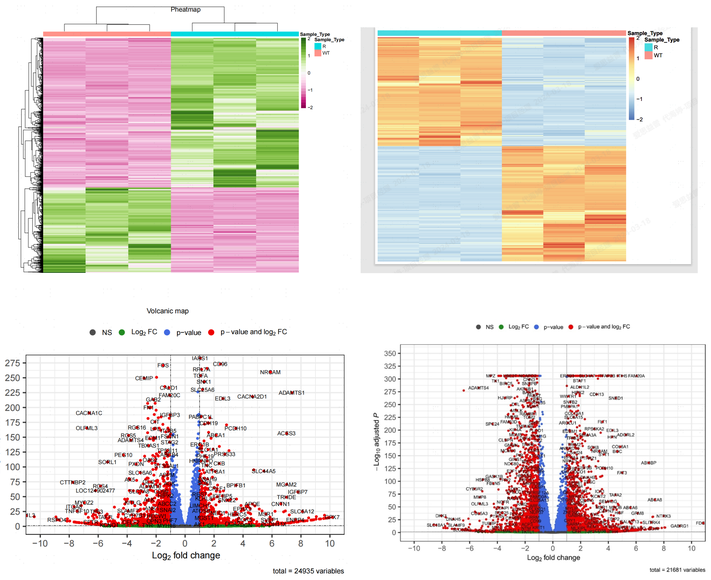


Vemurafenib Chemical Structure

Dabrafenib R



Dabrafenib Chemical Structure



Number of differential genes: 2402
Number of upregulated genes: 792
Number of downregulated genes: 1610

Number of differential genes: 4838
Number of upregulated genes: 2493
Number of downregulated genes: 2345

Figure 6: Transcriptome analysis of drug resistant cell lines with parental cell line.
Transcriptome analysis of drug-resistant cells indicates thousands of genes were influenced under drug resistant challenge. The differential genes expression profile may light a new hope for the anti-drug resistant cancer therapies.

Summary

Drug resistant cell lines and CDX models are getting more important for anticancer therapy. In this study, A375 Vemurafenib and A375 Dabrafenib resistant cell lines were established by escalation the drug concentrations in the culture medium to obtain the drug resistance. Drug resistant assays were performed not only in vitro cell proliferation but also in vivo CDX models treated with indicated drugs. Transcriptome analysis was also shown the differential gene expression levels between parental and resistant cell lines and suitable for the future development of anticancer drug in BRAF mutations.

References

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