Developing novel resistant models to diverse ADC drugs to accelerate new generation drug discovery

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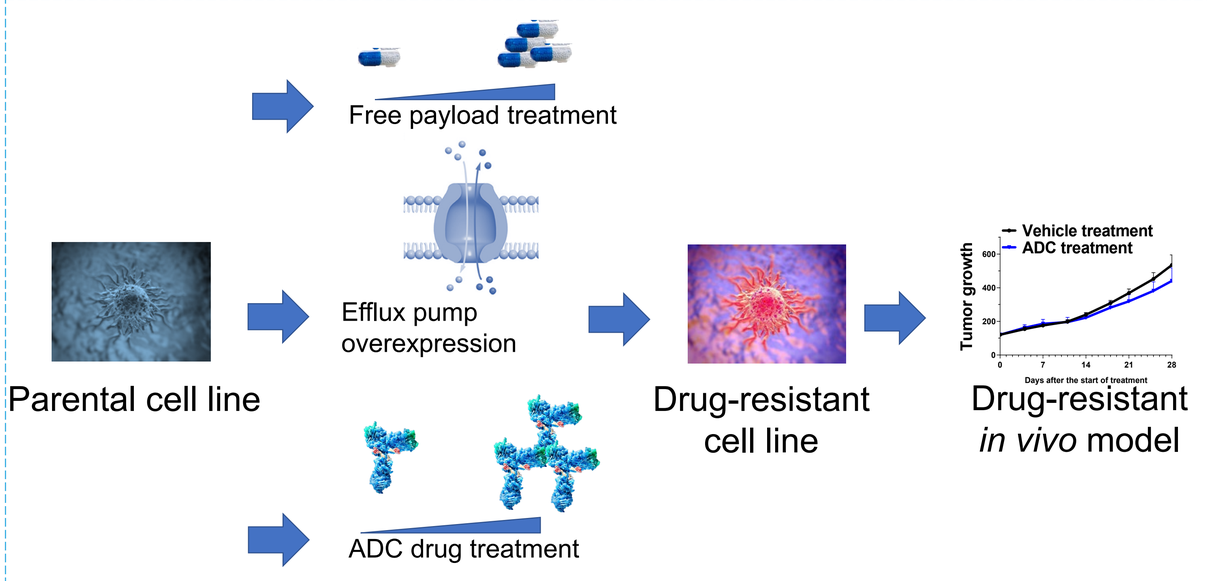
Introduction

In recent years, ADC drugs have demonstrated impressive therapeutic effects in cancer treatment, particularly for solid tumors. Among these, DS-8201, an ADC drug targeting HER2, has been notably approved for multiple cancer treatments, showcasing its remarkable efficacy. However, resistance to ADC therapy remains a significant challenge that limits its long-term effectiveness. Consequently, research on ADC resistance mechanism has been increasing. To address this, we have developed various ADC-resistant cell lines successfully through processes such as drug induced (ADC or free payload) or efflux pump overexpression. Resistant properties of these models have been validated both *in vitro* and *in vivo.* For some of the drug induced resistant models, we also disclosed their potential resistance mechanism. One interesting finding in an ADC-induced model is the surface marker of target antigen dose not change while the metabolic status of payload is changed. These drug resistant models and comprehensive findings provide crucial tools that can assist development and optimization of next-generation ADC drugs in the future significantly.

Construction strategy



Scheme of ADC drug resistant model construction.

Results

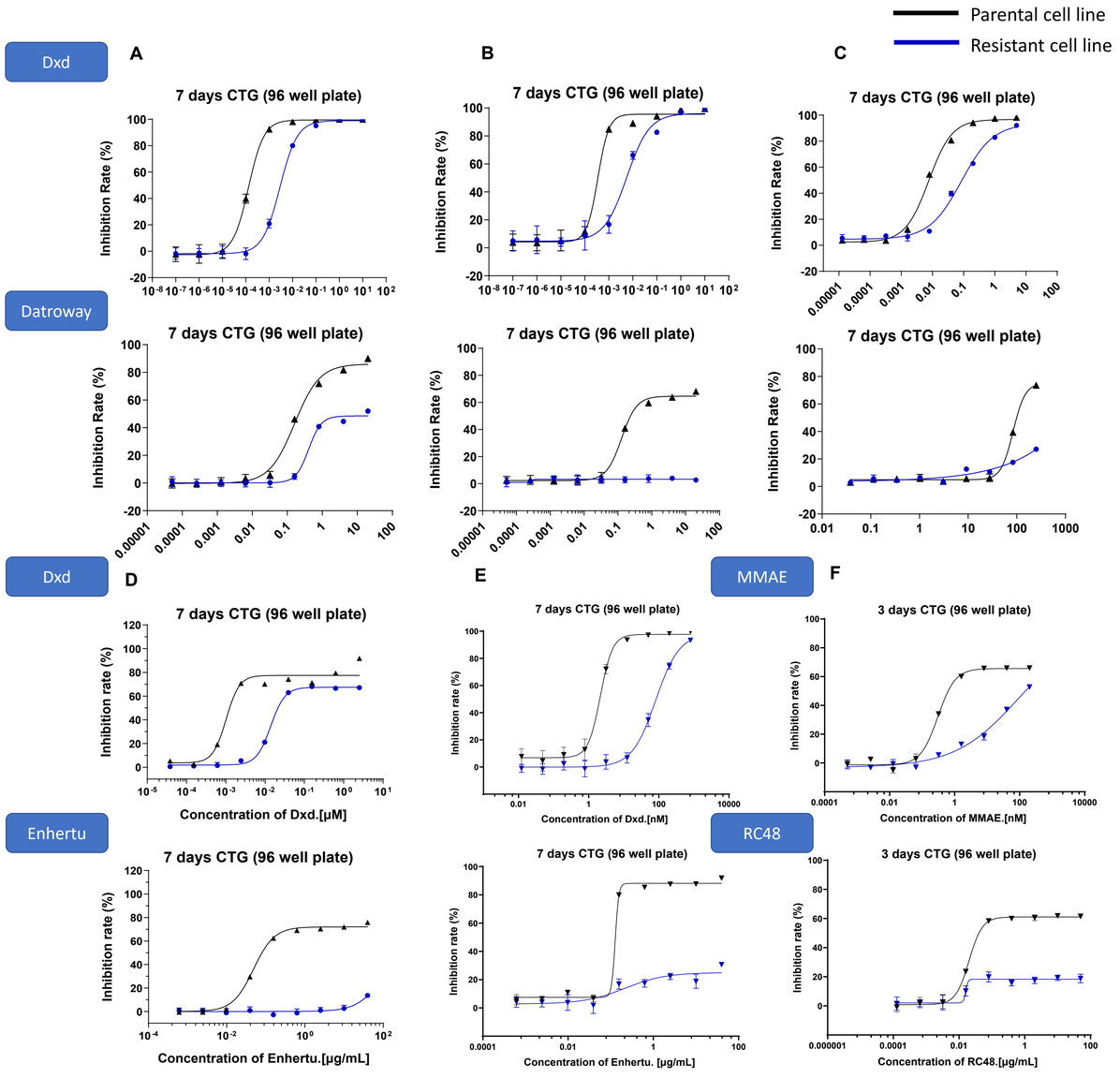


Figure 1. Evaluation of payload-resistant tumor cells. CTG analysis of Dxd-induced resistant HCC1806 cells (A), Dxd-induced resistant HCC827 cells (B), Dxd-induced resistant HCC4006 cells (C). CTG analysis of ABCG2-overexpressed HCC1954 cells (D), ABCG2-overexpressed N87 cells (E), ABCB1-overexpressed N87 cells (F).

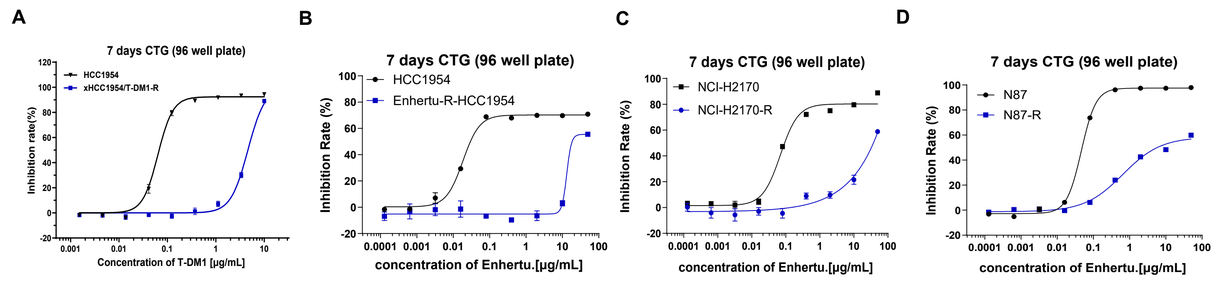


Figure 2. Evaluation of ADC-induced resistant tumor cells. CTG analysis of T-DM1-induced resistant HCC1954 cells (A), Enhertu-induced resistant HCC1954 cells (B), H2170 cells (C) and N87 cells (D).

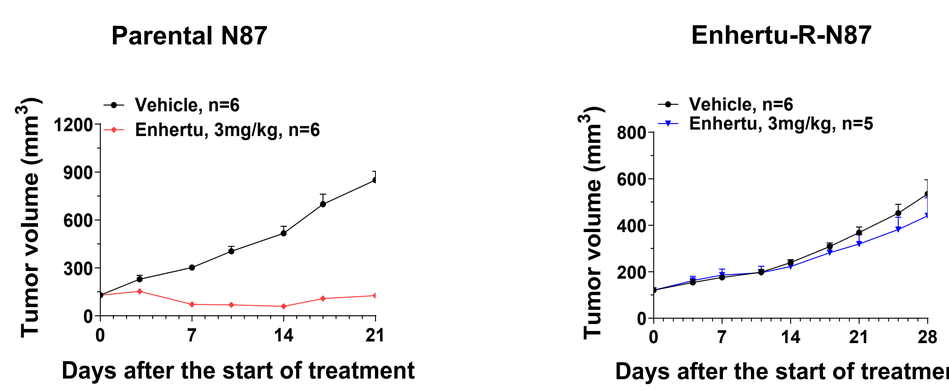


Figure 3. *In vivo* evaluation of Enhertu-resistant N87 model.

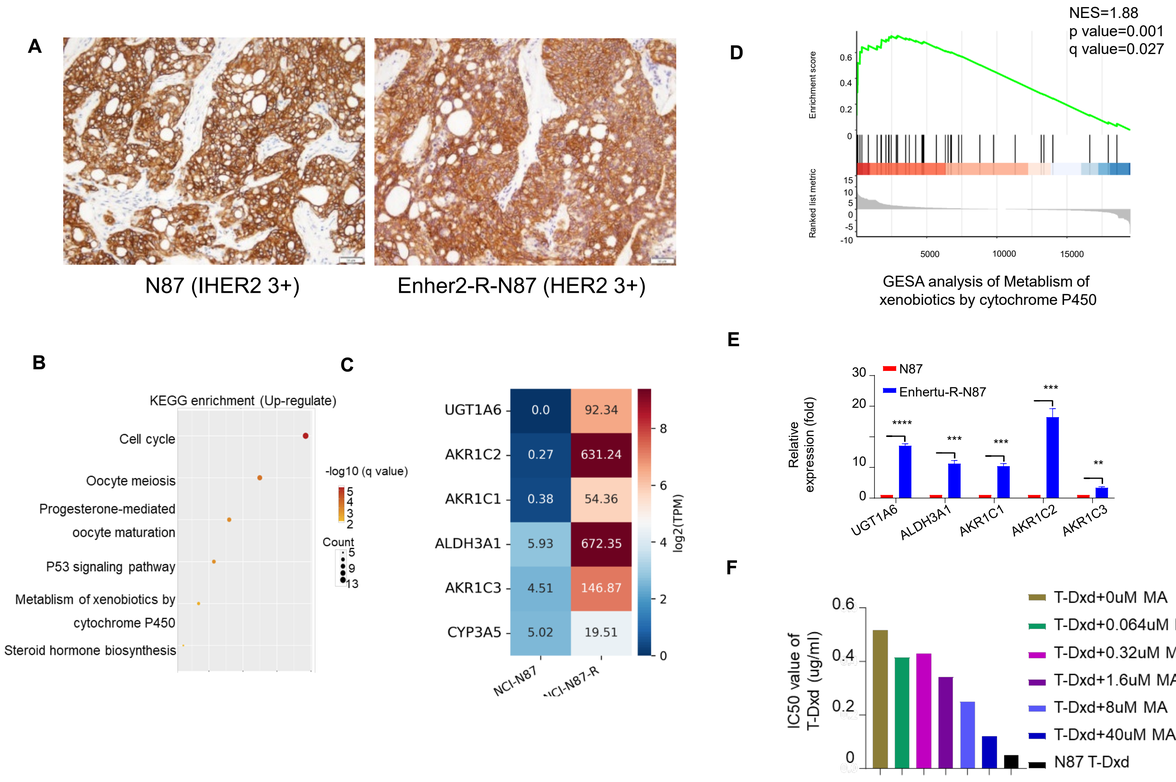


Figure 4. Mechanism exploration of Enhertu-resistant N87 model.
(A) IHC analysis of HER2 expression in tumors. (B) KEGG enrichment analysis. (C) Genes with differential levels of metabolism of xenobiotics by cytochrome P450 signal. (D) GSEA analysis of metabolism of xenobiotics by cytochrome P450 signal. (E) qPCR validation of changed genes. (F) CTG analysis of combination MA (AKR1C inhibitor, Mefenamic acid) and Enhertu.

Summary table of developed resistant models

Model Name	Cancer Type	Target	Validated Resistance	Status
Enhertu-R-N87	Gastric	HER2	Enhertu, Dxd	Fully validated
Enhertu-R-HCC1954	Breast	HER2	Enhertu, T-DM1, RC48	Fully validated
Enhertu-R-H2170	Lung	HER2	Enhertu, RC48	Fully validated
T-DM1-R-HCC1954	Breast	HER2	T-DM1	Fully validated
RC48-R-HCC1954	Breast	HER2	Enhertu, T-DM1, RC48	*In vitro* only
Dxd-R-HCC1806	Breast	TROP2	Dxd, Datroway	Fully validated
Dxd-R-HCC827	Lung	TROP2	Dxd, Datroway	Fully validated
Dxd-R-HCC4006	Breast	HER3	Dxd, HER3-Dxd	Fully validated
HCC1954-hABCG2	Breast	HER2	Enhertu, Dxd	Fully validated
N87-hABCG2	Gastric	HER2	Enhertu, Dxd	Fully validated
N87-hABCB1	Gastric	HER2	MMAE, RC48	Fully validated

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