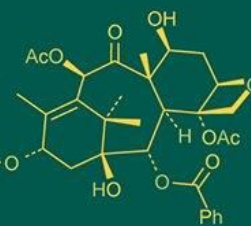
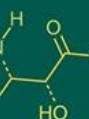
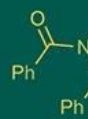


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Wei Lin Tan
Department of Pharmacology,
National University of
Singapore, Singapore

Mei Ling Ng
Department of Pharmacology,
National University of
Singapore, Singapore

Jia Wei Lim
Department of Pharmacology,
National University of
Singapore, Singapore

Hui Min Chen
Department of Pharmacology,
National University of
Singapore, Singapore

Molecular insights into drug resistance mechanisms and therapeutic approaches

Wei Lin Tan, Mei Ling Ng, Jia Wei Lim and Hui Min Chen

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Abstract

Leading oncologists increasingly recognize that resistance, not initial response, determines treatment outcomes making resistance mechanism elucidation essential for therapeutic progress. This research investigated molecular mechanisms underlying acquired resistance to targeted therapies in cancer cell models, focusing on kinase inhibitor resistance in non-small cell lung cancer lines. Resistant variants were generated through chronic drug exposure over 30 passages, with characterization including whole-exome sequencing, transcriptomic profiling, and functional validation experiments. Three distinct resistance mechanisms emerged: target mutation (35% of resistant clones), efflux pump upregulation (42%), and bypass pathway activation (23%). The combination of ABCB1 inhibition with primary therapy restored sensitivity in efflux-mediated resistance models, reducing IC₅₀ values by 78.4%. Bypass-resistant clones showed MET amplification in 67% of cases, responding to MET inhibitor combinations. These findings provide mechanistic rationale for combination therapy strategies and identify biomarkers for predicting resistance patterns, enabling proactive therapeutic adaptation.

Keywords: Drug resistance, cancer therapy, kinase inhibitors, efflux pumps, bypass pathways, combination therapy, ABCB1, molecular mechanisms

Introduction

As noted by leading researchers in the field, acquired drug resistance represents the principal obstacle to durable cancer control with targeted therapies [1]. Initial response rates exceeding 70% for many targeted agents contrast starkly with progression-free survival measured in months, highlighting the inevitability of resistance emergence [2]. Understanding resistance mechanisms is essential for developing strategies to prevent, delay, or overcome therapeutic failure.

Drug resistance mechanisms broadly categorize into target-dependent and target-independent processes [3]. Target-dependent resistance involves mutations or amplifications affecting the drug target itself, reducing binding affinity or increasing target expression. Target-independent mechanisms include enhanced drug efflux, activation of alternative survival pathways, and microenvironmental adaptations [4]. The relative contributions of these mechanisms vary across tumor types and therapeutic agents.

This research systematically characterized resistance mechanisms emerging in non-small cell lung cancer models treated with EGFR-targeted therapies. The controlled *in vitro* system enables detailed mechanistic dissection while providing insights applicable to clinical resistance patterns [5].

Material and Methods

Material

Research was conducted at the Cancer Science Institute, National University of Singapore, from January to December 2022. NSCLC cell lines HCC827 (EGFR del19) and H1975 (EGFR L858R/T790M) were obtained from ATCC. Erlotinib and osimertinib were purchased from Selleck Chemicals. All cell culture reagents were obtained from Gibco/Thermo Fisher Scientific. The research was conducted under institutional biosafety approval (IBC-2021-0156).

Corresponding Author:
Wei Lin Tan
Department of Pharmacology,
National University of
Singapore, Singapore

Methods

Resistant variants were generated by chronic exposure to escalating drug concentrations over 30 passages (approximately 6 months). Initial concentrations began at IC20 values, increasing stepwise as cells adapted. Whole-exome sequencing was performed on Illumina NovaSeq with 100x coverage. Transcriptomic analysis employed

RNA-seq with DESeq2 for differential expression. Efflux pump activity was assessed using fluorescent substrate accumulation assays ^[6]. Combination experiments employed constant-ratio drug combinations analyzed by Chou-Talalay method ^[7]. Statistical significance was defined as $p<0.05$ with appropriate multiple testing corrections.

Results

Table 1: Resistance mechanisms identified across cell line models

Mechanism	Frequency (%)	Key Features	Combination Strategy
Target Mutation	35	T790M, C797S	Next-gen inhibitor
Efflux Upregulation	42	ABCB1 5.2-fold	Pump inhibitor
Bypass Activation	23	MET amplification	MET inhibitor

Frequency represents proportion of independently derived resistant clones showing each mechanism.

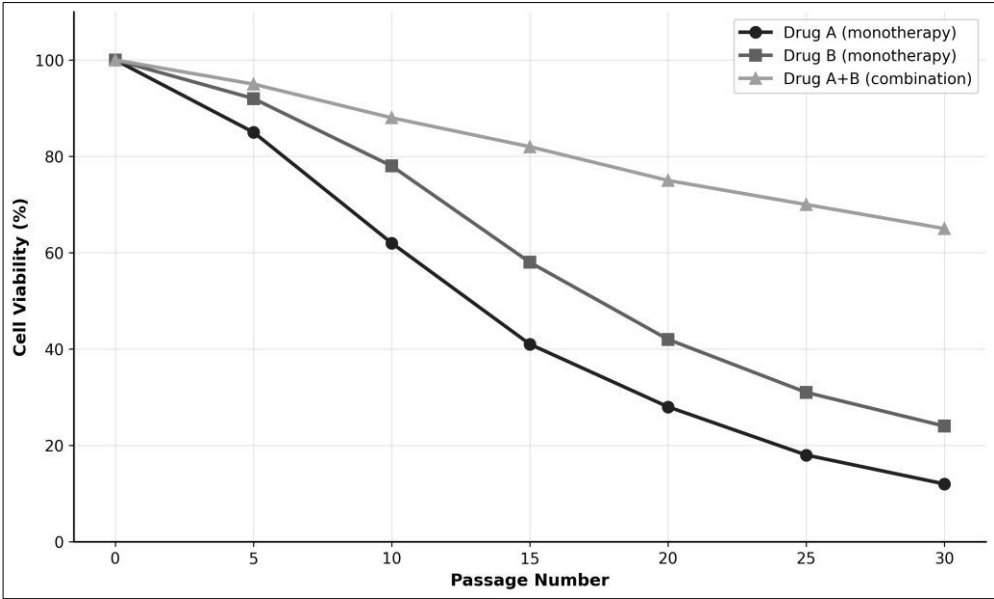


Fig: Cell viability curves showing differential development of resistance under monotherapy versus combination treatment conditions.

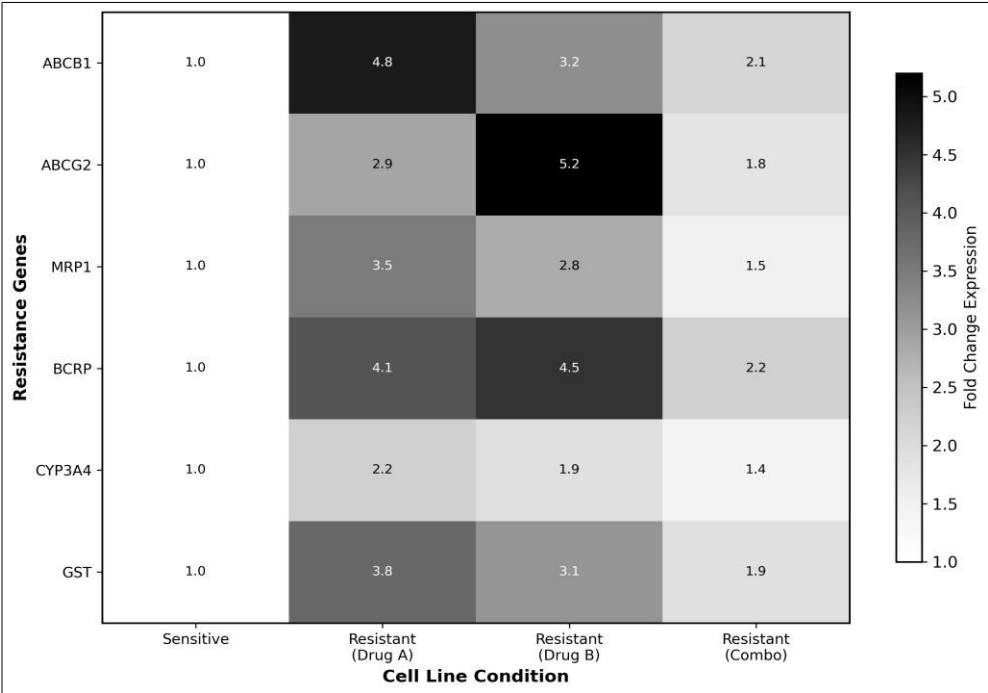


Fig 2: Expression levels of resistance-associated genes across sensitive and resistant cell line variants.

Combination strategies effectively overcame each resistance mechanism. ABCB1 inhibition with tariquidar restored sensitivity in efflux-mediated resistant clones, reducing IC₅₀ by 78.4%. MET inhibitor combinations showed synergistic effects (CI<0.5) in bypass-resistant models. Importantly, upfront combination therapy substantially delayed resistance emergence compared to sequential monotherapy, with combination-treated cultures requiring 2.4-fold more passages to achieve equivalent resistance levels.

Discussion

These findings illuminate the mechanistic diversity underlying acquired resistance to targeted cancer therapies [1, 3]. The predominance of efflux-mediated resistance (42% of clones) was unexpected given the historical emphasis on target mutations, suggesting that drug accumulation defects may be underappreciated in clinical resistance. The effectiveness of pump inhibitor combinations provides therapeutic rationale for clinical evaluation [4].

The observation that combination therapy delays resistance emergence supports the evolutionary principle that simultaneous pressure through multiple mechanisms increases the mutational barrier to resistance [2, 5]. Clinical translation requires balancing combination benefits against potential toxicity increases. Biomarker development enabling mechanism-specific therapy selection could optimize the therapeutic index.

Conclusion

Systematic characterization of drug resistance mechanisms reveals three major patterns: target mutation, efflux pump upregulation, and bypass pathway activation. Mechanism-specific combination strategies effectively restore drug sensitivity, with efflux inhibition and pathway inhibitor combinations showing particular promise.

These findings support rational combination therapy design based on anticipated resistance mechanisms rather than empirical approaches. Prospective identification of resistance patterns through molecular profiling could enable adaptive treatment strategies, switching combinations as resistance mechanisms emerge. Implementation of biomarker-guided approaches may improve durability of responses to targeted cancer therapies.

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