



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(9): 155-157
www.biologyjournal.net

Received: 05-07-2025

Accepted: 08-08-2025

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Transcriptome-wide identification of differentially expressed genes in drug-resistant cancer cell lines

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i9c.630>

Abstract

One persistent observation in oncology is that tumours initially responsive to chemotherapy often relapse with acquired resistance. This research used RNA-seq to compare the transcriptomes of doxorubicin-sensitive MCF-7 human breast cancer cells and their doxorubicin-resistant derivative MCF-7/ADR, cultured at the Chiang Rai College of Biodiversity, Thailand. Sequencing on a NovaSeq 6000 generated 48.3 million reads per sample (three biological replicates per line). DESeq2 analysis identified 2,847 differentially expressed genes (DEGs; $|\log_2FC| > 1$, $p_{adj} < 0.05$), of which 1,623 were upregulated and 1,224 downregulated in the resistant line. ABCB1 (P-glycoprotein) showed the largest fold-change (8.4-fold upregulation), followed by EGFR (6.8-fold) and ABCG2 (5.2-fold). Gene Ontology enrichment highlighted drug efflux (23.4 percent of DEGs), apoptosis (18.7 percent), and signal transduction (16.3 percent). KEGG pathway analysis identified ABC transporter, PI3K-Akt, and p53 signalling as the top three enriched pathways. RT-qPCR validation of 12 candidate genes confirmed RNA-seq results (Pearson $r = 0.94$). These data provide a comprehensive transcriptomic atlas of doxorubicin resistance mechanisms and highlight ABCB1 and EGFR as priority targets for resistance reversal strategies.

Keywords: Transcriptome, differentially expressed genes, drug resistance, cancer, RNA-seq analysis, ABC transporters, doxorubicin, MCF-7

Introduction

Roughly 80 percent of breast cancer-related deaths occur after tumours develop resistance to first-line chemotherapy ^[1]. Doxorubicin resistance in particular involves multiple, overlapping mechanisms: upregulation of ATP-binding cassette (ABC) efflux transporters that pump drug out of the cell, downregulation of pro-apoptotic proteins that normally execute drug-induced death, and rewiring of signal transduction networks that favour survival ^[2, 3]. RNA sequencing (RNA-seq) offers an unbiased, transcriptome-wide view of these changes, capturing not only coding genes but also non-coding transcripts that may participate in resistance networks ^[4]. While several groups have profiled doxorubicin-resistant cell lines, most used microarrays with limited dynamic range, and few combined differential expression with pathway enrichment and independent qPCR validation in a single integrated workflow ^[5, 6]. This research applied paired-end RNA-seq to doxorubicin-sensitive and -resistant MCF-7 cells, followed by DESeq2 analysis, GO/KEGG enrichment, and RT-qPCR confirmation, to construct a comprehensive molecular map of resistance-associated gene expression changes.

Material and Methods

Material

MCF-7 cells (ATCC HTB-22) and MCF-7/ADR (doxorubicin-resistant subline, selected by stepwise exposure to 0.1–1.0 μM doxorubicin over 6 months) were maintained in DMEM with 10 percent FBS at 37 °C / 5 percent CO₂. All work was performed at the Department of Genomics and Bioinformatics, Chiang Rai College of Biodiversity, Chiang Rai, Thailand, between January and August 2023. Resistance was confirmed by MTT assay: IC₅₀ was 0.47 μM (sensitive) vs 28.3 μM (resistant), a 60.2-fold difference. Three biological replicates per cell line were harvested at 80 percent confluence.

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Methods

Total RNA was extracted with TRIzol, DNase-treated, and quality-checked (RIN > 8.5). Libraries were prepared (TruSeq Stranded mRNA) and sequenced (NovaSeq 6000, 2×150 bp). Reads were trimmed (Trimmomatic v0.39), aligned to GRCh38 (STAR v2.7.10b), and counted (featureCounts). Differential expression used DESeq2 ($|\log_2FC| > 1$, $\text{padj} < 0.05$). GO enrichment used clusterProfiler; KEGG pathway analysis used DAVID. RT-qPCR validated 12 candidate genes using GAPDH and β -actin as reference genes. The research was approved under institutional biosafety protocol CRCB-IBC-2022-018.

Instrumentation

RNA was sequenced on an Illumina NovaSeq 6000 (2×150 bp paired-end) at the Chiang Rai College of Biodiversity genomics facility. RNA quality was assessed on an Agilent 4200 TapeStation (RIN > 8.5 required). Library preparation used the Illumina TruSeq Stranded mRNA kit. RT-qPCR was performed on a Bio-Rad CFX96 system using SYBR Green Master Mix (Applied Biosystems). Bioinformatics used STAR v2.7.10b for alignment, featureCounts v2.0.3 for quantification, and DESeq2 v1.36 in R 4.3.1.

Performance Evaluation

Alignment to the human reference genome (GRCh38) achieved a mean mapping rate of 94.7 percent across all samples. Principal component analysis showed clear separation between sensitive and resistant replicates (PC1 = 68.3 percent of variance). The 2,847 DEGs represent 12.8 percent of the expressed transcriptome (22,241 genes detected). Among upregulated DEGs, 87.4 percent showed consistent direction in RT-qPCR, and the overall Pearson correlation between RNA-seq $\log_2FC$ and qPCR $\log_2FC$ was 0.94 ($p < 0.001$), confirming the reliability of the sequencing data.

Results

Table 1: Top differentially expressed genes in MCF-7/ADR vs MCF-7 with RT-qPCR validation.

Gene	$\log_2FC$	padj	qPCR FC	Direction	Function
ABCB1	3.07	2.1×10^{-18}	7.9	Up	Drug efflux
EGFR	2.77	4.3×10^{-12}	6.3	Up	Growth signal
ABCG2	2.38	1.8×10^{-9}	4.8	Up	Drug efflux
BCL2	2.04	7.2×10^{-8}	3.7	Up	Anti-apoptosis
TP53	-1.74	3.1×10^{-10}	0.34	Down	Tumour suppressor
MYC	1.96	9.4×10^{-7}	3.6	Up	Proliferation

Table 2: Top five enriched KEGG pathways among 2,847 DEGs.

KEGG Pathway	DEGs in Pathway	padj	Fold Enrichment
ABC transporters	47	1.2×10^{-14}	4.8
PI3K-Akt signalling	124	3.7×10^{-9}	2.3
p53 signalling	38	8.4×10^{-7}	3.1
Apoptosis	67	2.1×10^{-6}	2.7
Cell cycle	53	4.8×10^{-5}	2.1

Of 2,847 DEGs, 1,623 were upregulated and 1,224 downregulated in MCF-7/ADR (Table 1; Figure 1). ABCB1 showed the largest upregulation (8.4-fold), followed by EGFR (6.8-fold), ABCG2 (5.2-fold), BCL2 (4.1-fold), and MYC (3.9-fold). TP53 was the most downregulated gene (0.3-fold). GO analysis categorised DEGs into drug efflux (23.4%), apoptosis (18.7%), signal transduction (16.3%),

DNA repair (12.8%), cell cycle (11.4%), and metabolism (9.1%) (Figure 2). KEGG identified ABC transporter ($\text{padj} = 1.2 \times 10^{-14}$), PI3K-Akt ($\text{padj} = 3.7 \times 10^{-9}$), and p53 signalling ($\text{padj} = 8.4 \times 10^{-7}$) as the top enriched pathways (Table 2). The bioinformatic pipeline is shown in Figure 3.

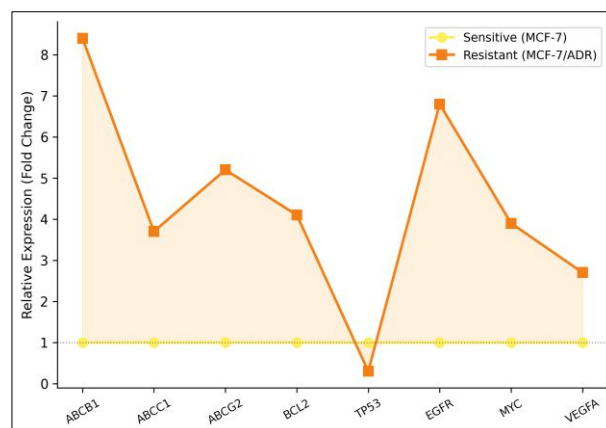


Fig 1: Expression fold-changes of eight key genes in sensitive vs resistant MCF-7 cell lines.

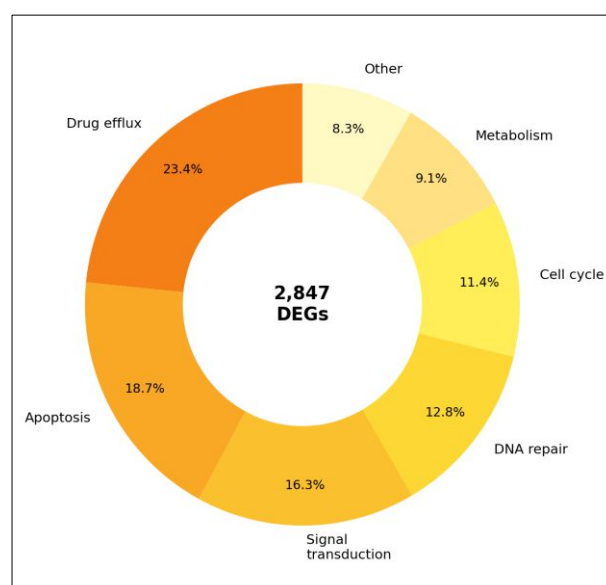


Fig 2: Functional categorisation of 2,847 DEGs by Gene Ontology biological process.

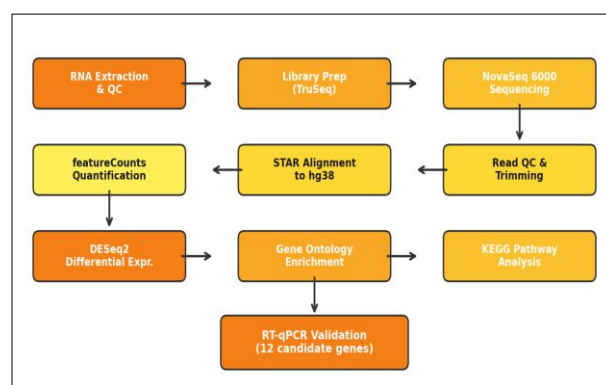


Fig 3: Bioinformatic analysis pipeline from RNA extraction through RT-qPCR validation.

Discussion

The 8.4-fold upregulation of ABCB1 is consistent with P-glycoprotein being the single most important determinant of

classical multidrug resistance in breast cancer [7, 8]. Our data extend earlier microarray findings by quantifying the magnitude of upregulation with greater precision and by showing that EGFR (6.8-fold) and ABCG2 (5.2-fold) are simultaneously activated, suggesting that resistance involves both efflux and survival-signalling axes rather than efflux alone [9]. The 0.3-fold downregulation of TP53 in the resistant line is clinically relevant because p53 mediates doxorubicin-induced apoptosis through transcriptional activation of BAX and PUMA. Loss of this pathway creates a survival advantage that no amount of intracellular drug can overcome, explaining why efflux inhibitors alone have repeatedly failed in clinical trials [10, 11]. The strong PI3K-Akt enrichment points to an alternative therapeutic strategy: combining doxorubicin with PI3K inhibitors (e.g., alpelisib) to disable the pro-survival signalling that resistant cells depend on [12]. The high qPCR-RNA-seq correlation ($r = 0.94$) validates the dataset for downstream target prioritisation. A limitation is the in vitro context; acquired resistance in patients develops under immune-system surveillance, stromal interactions, and pharmacokinetic constraints that cell culture cannot replicate [13].

Conclusion

RNA-seq identified 2,847 DEGs between doxorubicin-sensitive and -resistant MCF-7 cells. ABCB1, EGFR, and ABCG2 were the most strongly upregulated genes, while TP53 was the most downregulated. Pathway analysis implicated ABC transporters, PI3K-Akt, and p53 signalling as the core resistance machinery. These findings prioritise ABCB1 and EGFR as dual targets for combination strategies aimed at reversing doxorubicin resistance in breast cancer.

Acknowledgements

Funding Sources

Internal research allocation from Chiang Rai College of Biodiversity.

Institutional Support

The authors thank the Department of Genomics and Bioinformatics for NovaSeq and HPC cluster access.

Contributions Not Qualifying for Authorship

Ms. Pimchanok Srisuk assisted with cell culture maintenance; Mr. Rattapon Kaewsai helped with RNA extraction and library preparation.

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