



Investigating survival pathway alterations in oxaliplatin-resistant triple-negative breast carcinoma cell line

Zainab Saleem^{*1}, Noor Fatima¹, Azhar Hussain², Kulsoom Ghias^{2,3}, Sheerien Rajput^{1,2}

¹Centre for Regenerative Medicine and Stem Cell Research, ²Department of Biological and Biomedical Sciences, ³Faculty of Arts and Sciences, Aga Khan University (AKU), Pakistan

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Introduction

- Breast cancer (BCa) is the most commonly diagnosed cancer in women.
- Triple-Negative Breast Cancer (TNBCs), represents 10–20% of all breast cancers. They lack ER, PR, and HER2 expression
- TNBCs show:

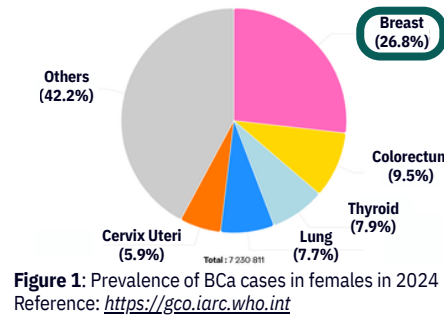


Figure 1: Prevalence of BCa cases in females in 2024
Reference: <https://gco.iarc.who.int>

- high recurrence
 - poor survival
 - metastasis
 - variable treatment responses
- While several therapeutic agents have been investigated to improve TNBC patient survival, drug resistance remains common, arising from complex disruptions in cell signalling and apoptotic pathways.

Objectives

- To establish stable oxaliplatin resistant clones from TNBC cell line (MDA-MB-231).
- To compare the gene expression profile of resistant clones with parental controls (wild-type) to identify the potential survival pathways.
- To compare cell cycle phases of resistant clones with parental clones.
- To compare the protein expression profile of resistant clones with parental clones

Methods

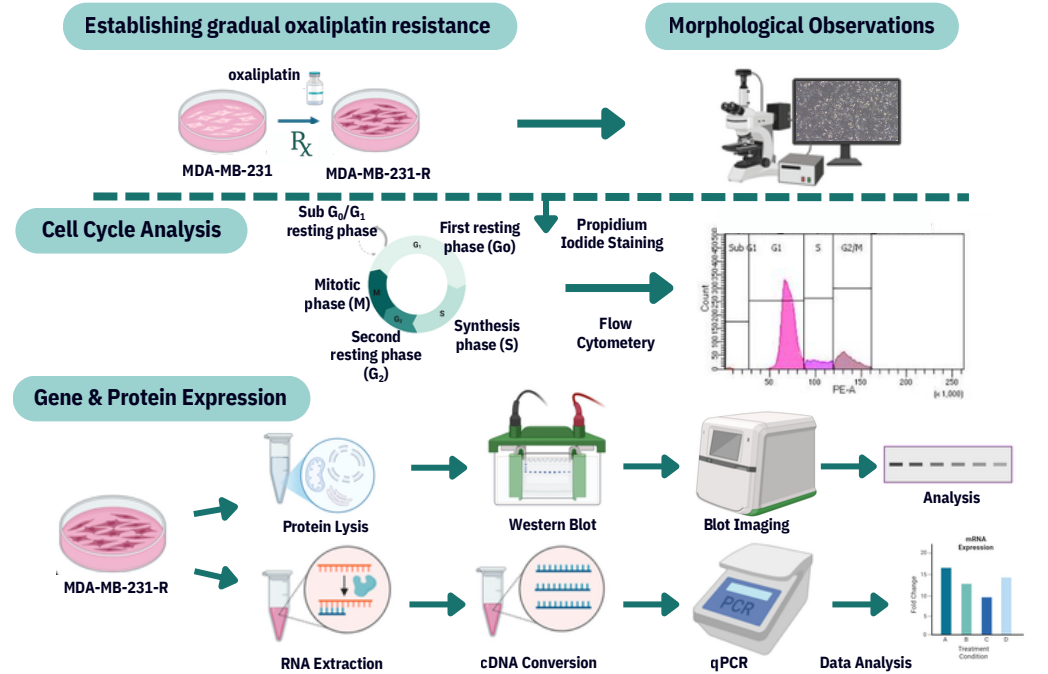


Figure 2: Oxaliplatin resistance was developed in MDA-MB-231 cells by gradual dose escalation (2–8 μ M). Cells were treated for 72 hours following a 48-hour recovery phase. Morphological changes and cell viability were examined using phase contrast microscopy. Flow cytometry was performed on FACS Aria II to analyze cell cycle distribution and apoptotic cell populations after cells were stained with Propidium Iodide. For protein analysis, total protein was extracted from parental and resistant cells (MDA-MB-231-R) using RIPA lysis buffer, quantified through Bradford assay, separated through SDS-PAGE, and transferred onto PVDF membranes. Membranes were probed with primary antibodies against target proteins, followed by HRP-conjugated secondary antibodies, and bands were detected by chemiluminescence, normalized to β -actin. RNA from MDA-MB-231-R was extracted and reverse transcribed into cDNA, followed by analysis of gene expression differences. The bars in the data analysis graph represent mean fold change. Statistical significance relative to WT was assessed using ANOVA with Tukey's post-hoc test, with p-values reflecting differences in normalized relative mRNA expression.

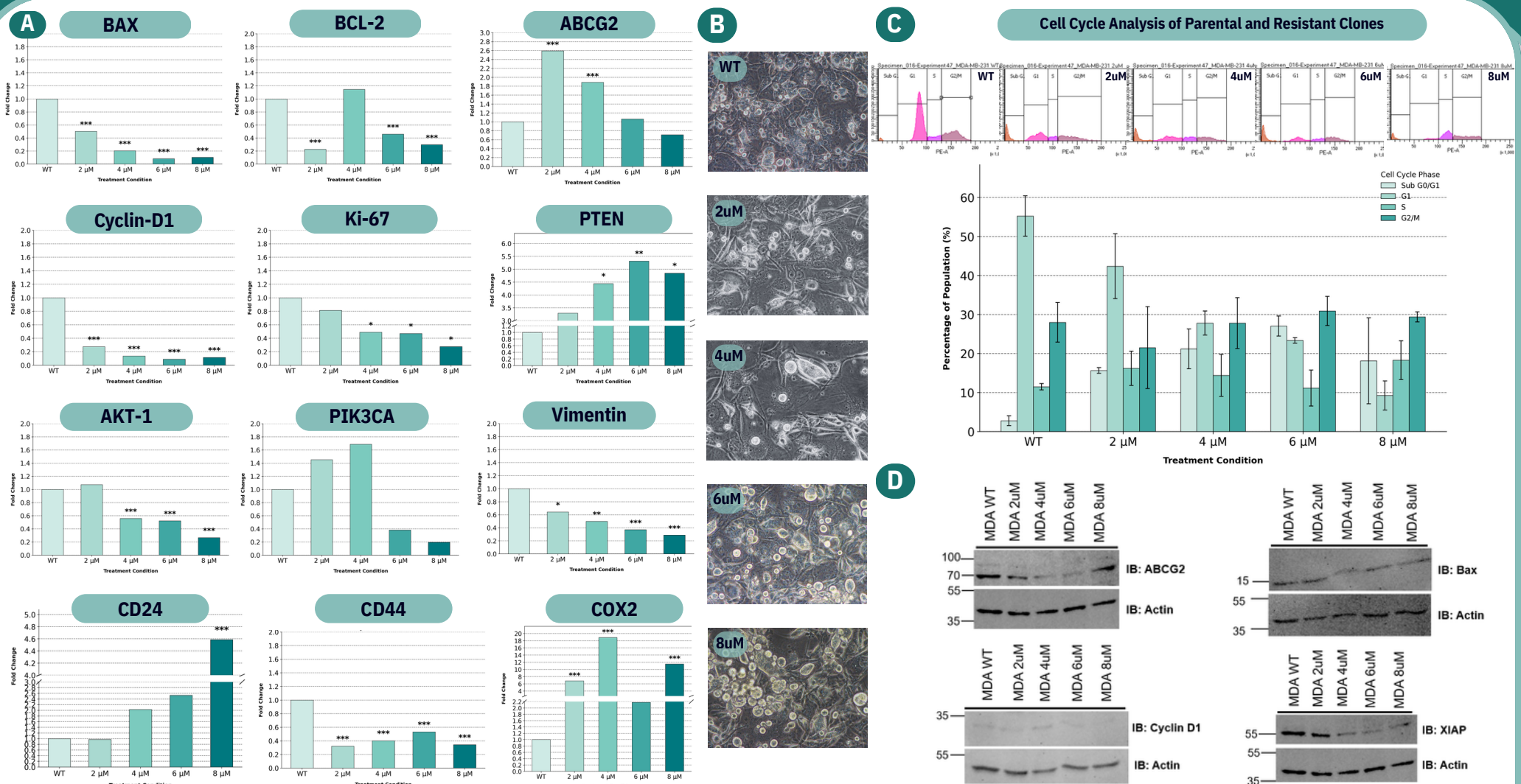


Figure 3: (A) mRNA expression analysis to identify differential gene expression of selected genes. Genes involved in apoptosis (BAX, BCL2). The gene involved in drug efflux/resistance (ABCG2) was upregulated at first before going back to normal. Genes associated with cell cycle progression/proliferation (Cyclin D1, Ki-67) were downregulated. Genes associated with PI3K/AKT signaling (AKT1, PIK3CA) were downregulated alongside PTEN upregulation. The gene associated with the epithelial-mesenchymal transition (Vimentin) was downregulated. The cancer stem cell marker, CD44 was downregulated while CD24 was upregulated. Inflammatory signalling marker, COX2 was significantly upregulated, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The data shown is a representation of one set of experiments. (B) Phase contrast microscope images showing morphology of wild-type (WT) and treated cells after a selected number of hits of oxaliplatin. Images were taken at 40X magnification. (C) Cell cycle analysis revealed MDA-MB-231-R cells in G2/M cell cycle arrest, suggesting cell cycle dysregulation compared to parental cells. (D) Target Protein Expression following drug treatment in MDA-MB-231 cells showed decreased ABCG2, XIAP and Cyclin D1 expression, consistent with the gene expression results. BAX expression was seen to be consistent across conditions. B-Actin was used as an internal loading control. Molecular weight markers (kDa) are indicated on the left.

Conclusions

- MDA-MB-231-R cells established using 8 μ M oxaliplatin adapt a slow-cycling, epithelialoid morphology independent of PI3K/AKT signaling and EMT.
- Cell cycle analysis showed G2/M cell cycle arrest in MDA-MB-231-R cells, supported by downregulation of Ki-67 and Cyclin, indicating cell cycle dysregulation and the emergence of a slow-cycling, drug-tolerant persister population compared to parental cells.
- CD44 downregulation with CD24 upregulation, indicate loss of the classical CD44⁺/CD24⁻ stem-like phenotype, while ABCG2 downregulation suggests an adaptation of the resistant clone (MDA-MB-231-R) to the concentration of oxaliplatin used.
- COX2 upregulation with dysregulated BCL2/BAX confirmed that inflammatory signaling and disrupted apoptosis contribute to resistance, making COX2 a potential therapeutic target for resistant TNBC with a potential for a future clinical trial.