



Investigating Signaling and Apoptotic Pathway Alterations in Oxaliplatin-Resistant Colorectal Cancer Cell Line

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INTRODUCTION

Globally, colorectal cancer (CRC) is the second most common cause of cancer-related mortality. Despite advanced clinical management, treatment resistance is observed, which leads to disease recurrence, adverse clinical outcomes, and poor overall survival of the patient.

The present study was undertaken to investigate the mechanisms of oxaliplatin resistance in CRC cell line and to identify novel molecular targets to overcome resistance.

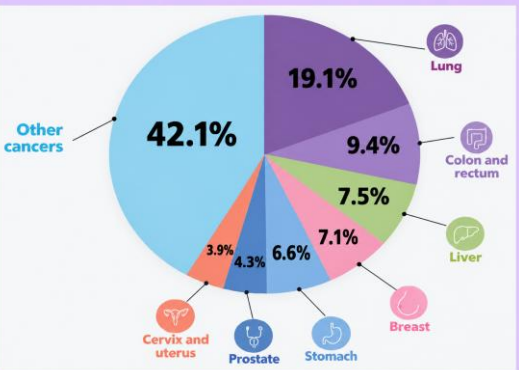


Figure 1: Global cancer-related mortality data 2026, showing CRC as the second most common cause of cancer-related death ^{1,2}

OBJECTIVES

- To establish stable oxaliplatin resistant clones from the CRC cell line (HCT-116).
- To compare cell cycle phases of resistant clones with parental (WT) controls.
- To compare the gene expression profile of resistant clones with WT controls to identify the potential survival pathways.
- To compare the protein expression profile of resistant clones with WT controls to validate the gene expression results at the protein level.

METHODS

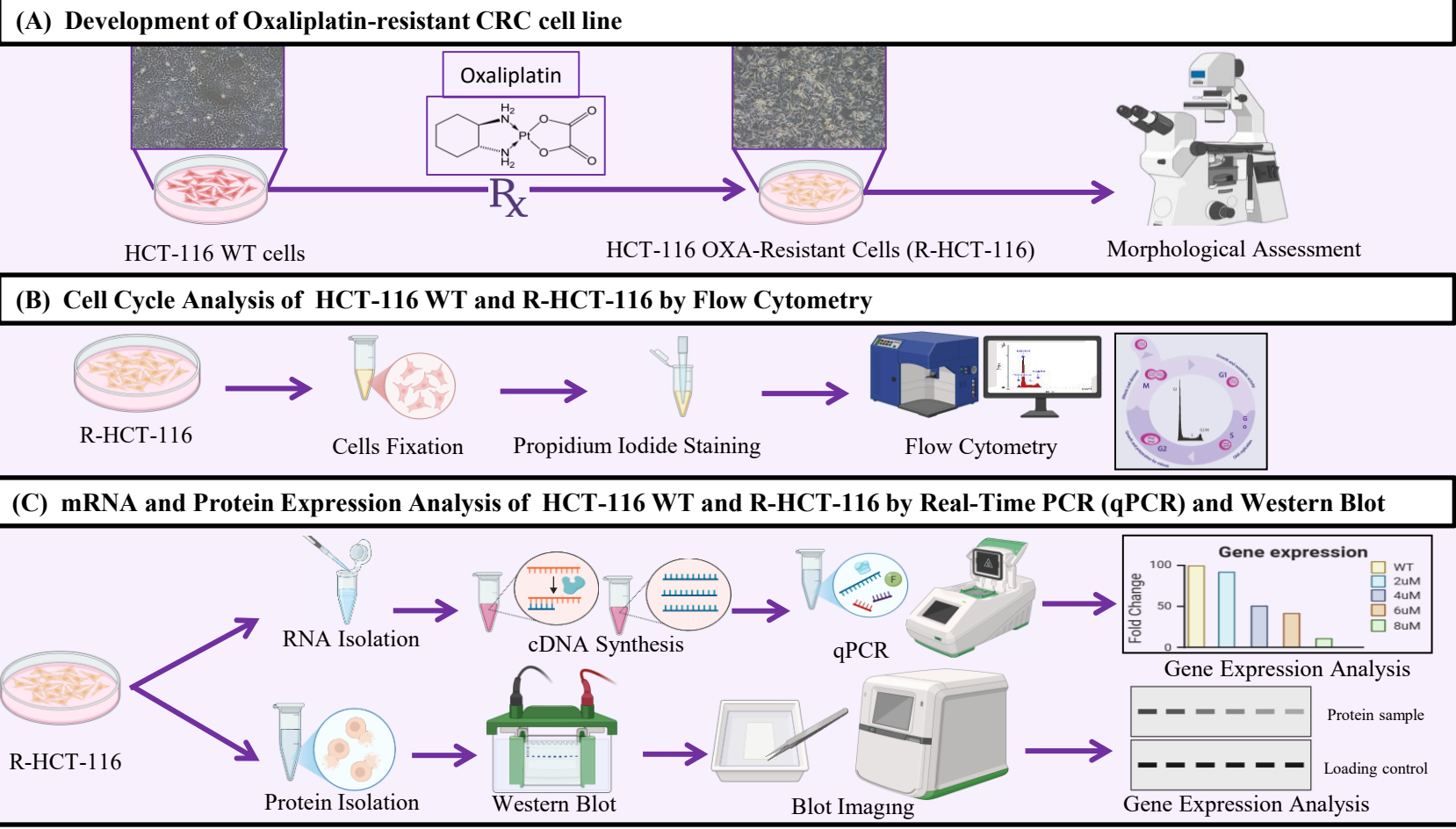


Figure 2: (A) HCT-116 cells were sequentially exposed to increasing doses of oxaliplatin (OXA; 2 µM to 8 µM) to generate resistant clones (R-HCT-116). Cells were treated for 72 hours, followed by a 48-hour recovery period prior to the subsequent higher dose. The treatment cycle continued until post-treatment cell death was significantly reduced and the cells regained their healthy morphology. The R-HCT-116 were also cryopreserved. (B) Cell cycle analysis was performed using flow cytometry (FACS Aria II), in which fixed cells were washed, treated with RNase A, and stained with propidium iodide (PI) before analysis of DNA content. (C) Total mRNA was isolated to assess differential expression of genes involved in the survival of the OXA-resistant clones. Statistical analysis was done using one-way ANOVA with Tukey post-hoc test, and all gene expression data were compared to WT cells. For protein expression analysis, the total protein was isolated using RIPA lysis buffer, quantified using the Bradford assay, separated by SDS-PAGE, followed by Western Blotting. After probing the blot with the primary antibody, the blot was incubated with an HRP-conjugated secondary antibody. The protein bands were visualized using a chemiluminescence assay, and the protein samples were normalized to β-actin.

RESULTS

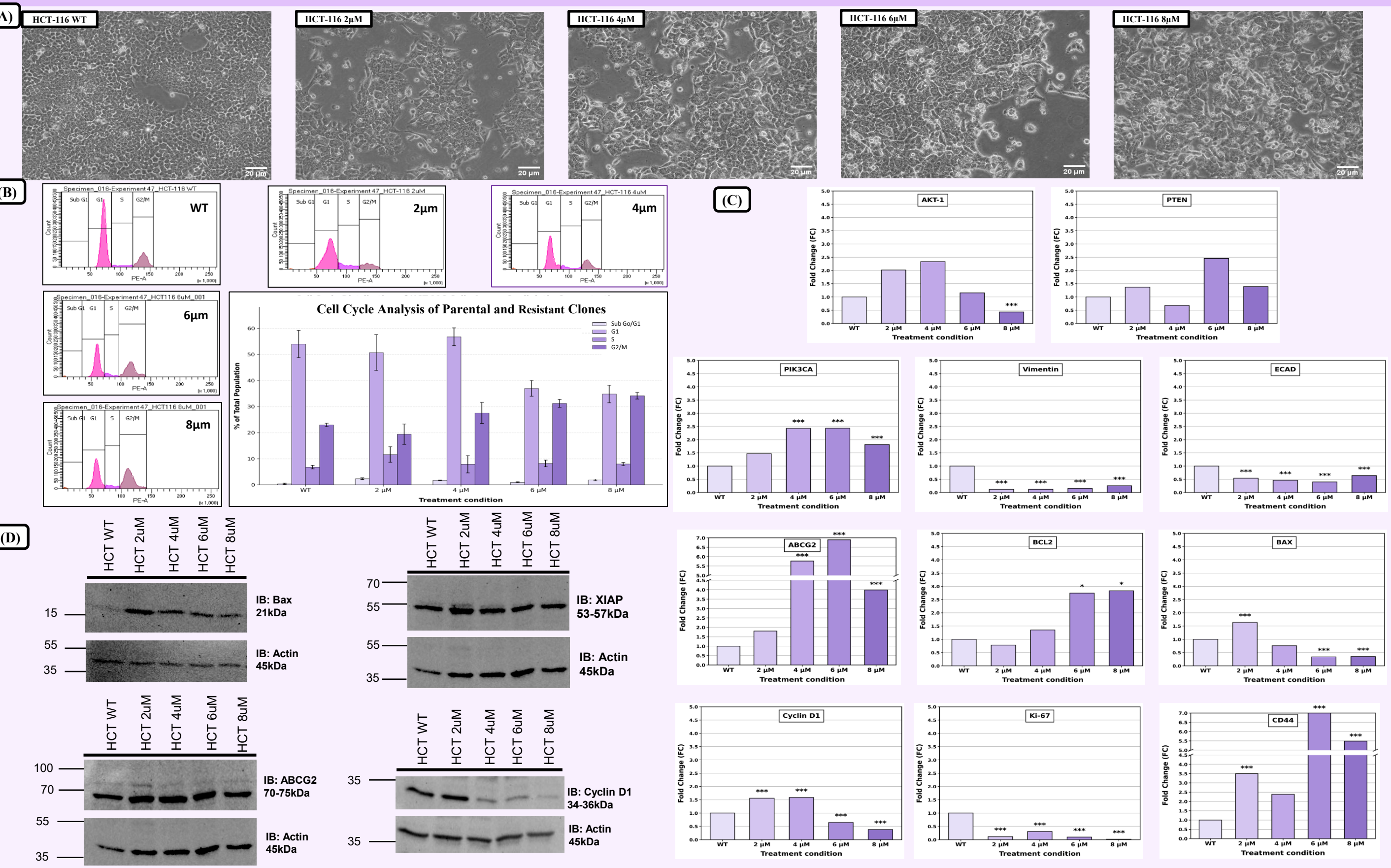


Figure 3: (A) R-HCT-116 showed varied epithelial morphology compared to the WT cells. Image magnification: 20X. (B) Cell cycle analysis revealed significant G2/M phase accumulation compared to the WT control cells, as shown in the histograms. The cell cycle data was analyzed and shown in bar graphs to compare the different treatment conditions to WT cells. (C) The gene expression analysis revealed significant downregulation of both Ki67 and cyclin D1 ($p < 0.001$). Within the PI3K/AKT axis, PTEN was non-significantly upregulated, whereas AKT expression was non-significantly downregulated. Furthermore, BCL2 expression was significantly upregulated ($p < 0.05$), while BAX was significantly downregulated ($p < 0.001$). Expression of both E-cadherin ($p = 0.001$) and vimentin was significantly decreased ($p < 0.001$), and ABCG2 was significantly upregulated ($p < 0.001$). CD44 is the stem cell marker of HCT116 cell line, and it was significantly upregulated ($p < 0.001$). All gene expression data were compared to the WT cells. These data are a representation of one set of experiments. (D) Western blot analysis showed increased ABCG2 and Bax expression, along with sustained XIAP levels, in R-HCT-116 clones (2–8 µM) compared to WT cells. Cyclin D1 expression progressively decreased with increasing oxaliplatin concentration, consistent with reduced proliferative capacity. Actin was used as the loading control.

CONCLUSION

These findings indicate that proliferative quiescence was induced in R-HCT-116 cells, as supported by G2/M arrest and reduced expression of Ki67 and cyclin D1. Simultaneous downregulation of E-cadherin and vimentin showed that resistance has developed independently of classical EMT, with stem cell phenotype as shown by increased CD44 expression. Cell survival is sustained *via* active ABCG2-mediated drug efflux as observed from the mRNA and protein expression data, with a robust, transcriptional-level AKT-independent BCL2/BAX apoptotic barrier.

REFERENCES

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