#2955 Systematic mechanistic profiling of ADC-resistant cancer cell lines

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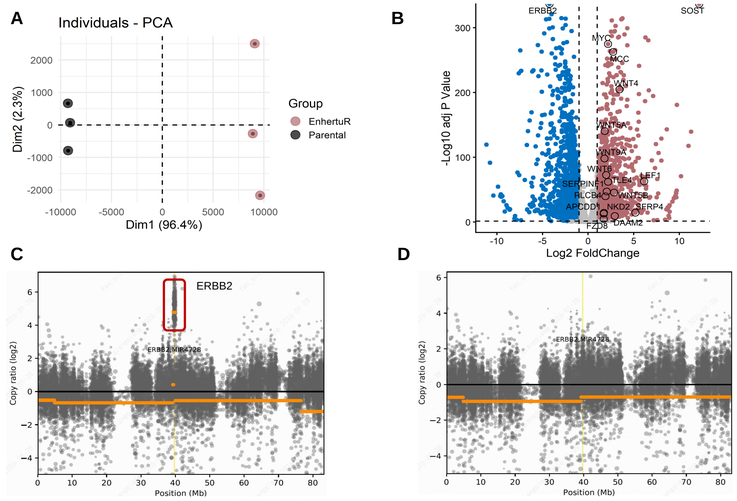
Summary

ADC has emerged as a hotspot for targeted therapy development across various cancers. However, resistance severely limits its clinical efficacy, making resistant cell lines crucial models for dissecting resistance mechanisms. WuXi Biology has established several ADC-resistant cell lines through chronic, escalating ADC exposure. Among these, most models exhibited over 10-fold increases in IC50 compared to their parental cells. To further investigate underlying mechanisms, we applied RNA-Seq and WES profiling on several resistant models paired with their parental controls, including HCC1954 and H2170 Enhertu-resistant cells.

RNA-Seq analysis revealed that in HCC1954 Enhertu-resistant cells ERBB2 expression was dramatically (~30-fold) reduced at mRNA level, with WES data also indicating a significant loss of ERBB2 copy number in resistant group. Additionally, overexpression of genes related to the WNT signaling pathway was observed in HCC1954 Enhertu-resistant models. This suggests that the downregulation of ERBB2, together with the upregulation of WNT signaling, may contribute to Enhertu resistance in this model.

Interestingly, only 4-fold decrease of ERBB2 expression was found in H2170 Enhertu-resistant cells, and there were no significant changes in WNT signaling related genes. Instead, we observed an abnormal upregulation of protease inhibitor family members, such as SERPINB3, in H2170 Enhertu-resistant cells. This indicates that proteolysis dysregulation in this resistant model may impair linker cleavage and ADC payload release, leading to resistance. Overall, our research sheds light on diverse ADC resistance mechanisms across different cancer cell lines. Moving forward, we will continue to conduct systematic mechanistic profiling and validation on more resistant models. We hope our research will contribute to the discovery of new routes overcoming ADC drug resistance in cancer therapy.

Results



Results

Fig.1 Genomic profiling of HCC1954 Ehhertu-resistant model by RNA-Seq and WES

A. PCA results of 6 samples indicate high reproducibility within groups

B. Volcano plot shows a significant downregulation of ERBB2 expression (log2 FC ≈ -5), while WNT signaling pathway associated genes are significantly overexpressed in the resistance group

C & D. Copy number variation analysis shows that the loss of ERBB2 copy number occurred in the resistance group (D) compared to the parental group (C)

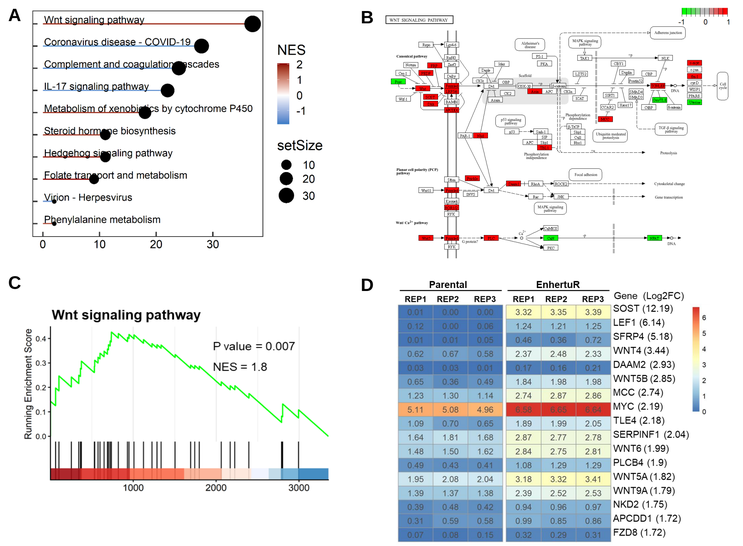


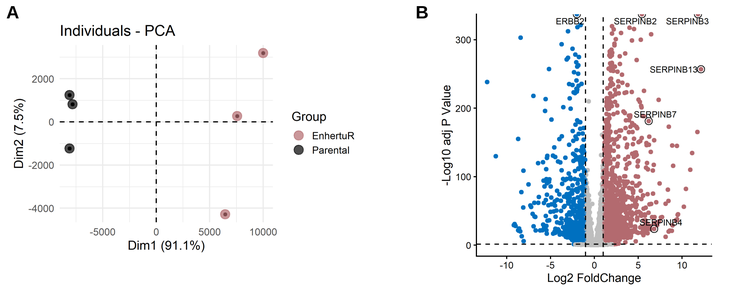
Fig.2 Overexpressed genes are highly enriched in WNT signaling pathway in HCC1954 Enhertu-resistant (EnhertuR) cells

A. Top 10 enrichment results; WNT signaling pathway is significantly enriched in the resistance group

B. WNT signaling pathway with corresponding genes' expression

C. Gene set enrichment analysis of WNT signaling pathway

D. Heatmap plot of gene expression from WNT signaling pathway in samples



Results

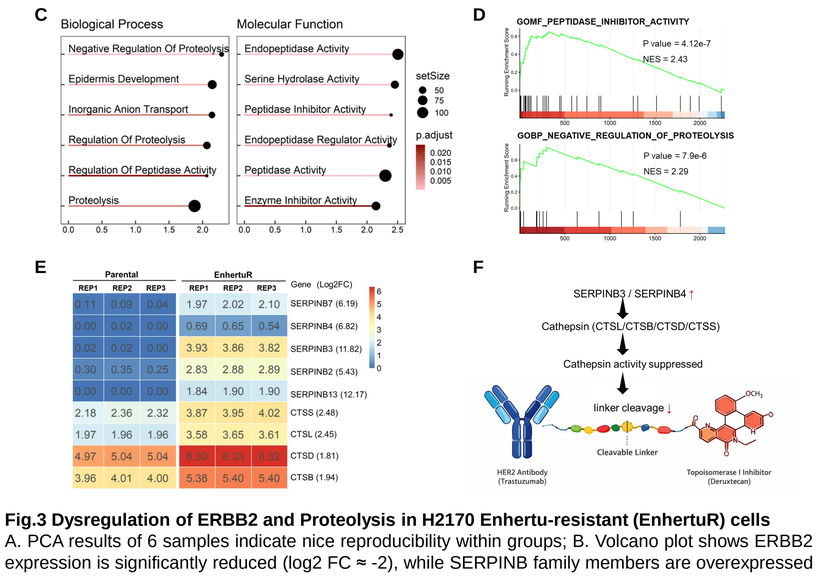


Fig.3 Dysregulation of ERBB2 and Proteolysis in H2170 Enhertu-resistant (EnhertuR) cells

A. PCA results of 6 samples indicate nice reproducibility within groups; B. Volcano plot shows ERBB2 expression is significantly reduced (log2 FC ≈ -2), while SERPINB family members are overexpressed in the resistance group; C. Enrichment analysis reveals that overexpressed genes in the resistance group are significantly enriched for the regulation of proteolysis and peptidase activity; D. Gene set enrichment analysis of peptidase inhibitor activity (Top) and negative regulation of proteolysis (Bottom); E. Heatmap plot of gene expression associated with the regulation of proteolysis; F. Hypothesis of SERPINBs and CTSs in EnhertuR resistance.

Discussion

For ERBB2-targeting ADCs such as Enhertu, plenty of preclinical and clinical studies have identified multiple mechanisms, including antigen loss or mutation, impaired ADC internalization or trafficking, altered payload metabolism, DNA repair upregulation, and tumor microenvironment factors. In our mechanistic profiling research, besides the reduced antigen expression, WNT pathway alterations were noted in HCC1954 Enhertu-resistant model, which aligns with other reports showing activation of WNT/β-catenin correlates with reduced ERBB2 expression and alters endosomal trafficking and lysosomal acidification, reducing payload release and cytotoxicity, suggesting WNT signaling might promote resistance in this HCC1954 model.

While in H2170 Enhertu-resistant cells, SERPINB3, a serine/cysteine protease inhibitor, is significantly overexpressed in resistance group. SERPINB3 (also called SCCA1, squamous cell carcinoma antigen 1) is a member of the clade B serine protease inhibitor (SERPIN) family. It is a non-secreted (intracellular) serpin with several biochemical and cellular activities that contribute to tumor progression and therapy resistance. By interacting with lysosomal proteases such as cathepsins, SERPINB3 can affect lysosomal integrity and reduce payload liberation, lowering effective intracellular drug concentration. Following validation work need to be conducted to further explore SERPINB3's role as a candidate contributor in this H2170 Enhertu-resistant model.

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