Exploration of gene expression changes in cisplatin-resistant high grade serous ovarian cancer

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Background

Ovarian cancer (OC) has a low survival rate, especially in late-stage cases where it spreads to nearby tissues. Treatment usually involves surgery to remove cancerous growths, followed by drug therapy like cisplatin. However, drug resistance is a major issue, limiting the effectiveness of chemotherapy. In this study, we analyzed gene expression patterns in drug-naïve (A2780) and cisplatin-resistant (A2780CisR) ovarian cancer cell lines, identifying significant changes in the smooth muscle actin gene ACTA2 and other proteins involved in actin network arrangement. This discovery could lead to potential therapeutic targets for further investigation and validation in overcoming cisplatin resistance in ovarian cancer.

Methods and Results

In this study, we employed normalized count data. Subsequently, we conducted differential gene expression analysis utilizing the DESeq2 package, a robust statistical tool for detecting genes that exhibit significant differential expression. As a result, we observed that genes displaying high log2 fold change (log2fc) and low log2 fold change (log2fc) were depicted in **Figure 1A**. Upon closer examination of the biological characteristics of these identified genes, particular attention was directed towards ACTA2, a gene of interest known to play a crucial role in cytoskeletal regulation. To further validate our findings, we conducted gene set enrichment analysis (GSEA). **(Figure 1B)**

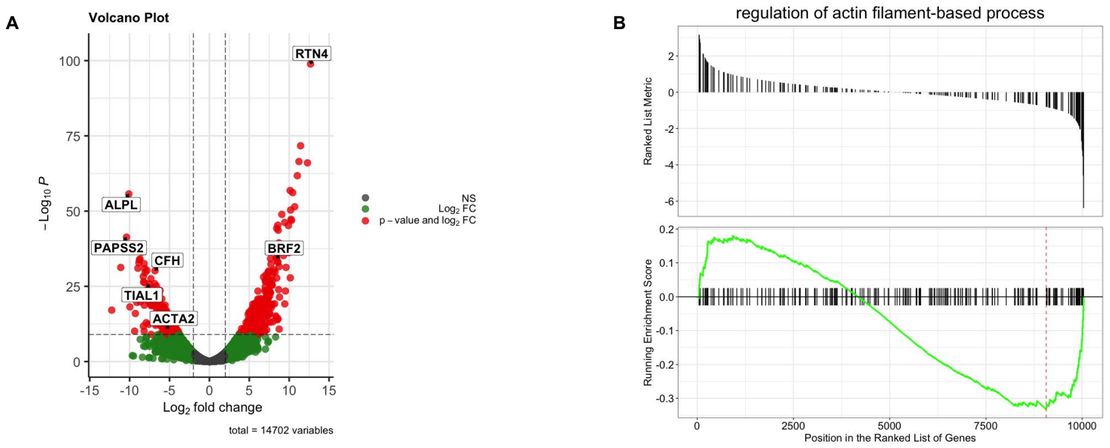

Figure 1. (A) Volcano Plot of Differentially Expressed Genes with Log2 Fold Change Values Identified by DESeq2. **(B)** Percentage of reads passed all filters.

Subsequently, we performed Gene Ontology (GO) enrichment analysis to investigate the distribution of genes within specific pathways and identify genes associated with cytoskeleton regulation (**Figure 2A**). **Figure 3A** demonstrates a significant downregulation of ACTA2 log2 fold change (log2fc), particularly in the context of actin filament-based processes.

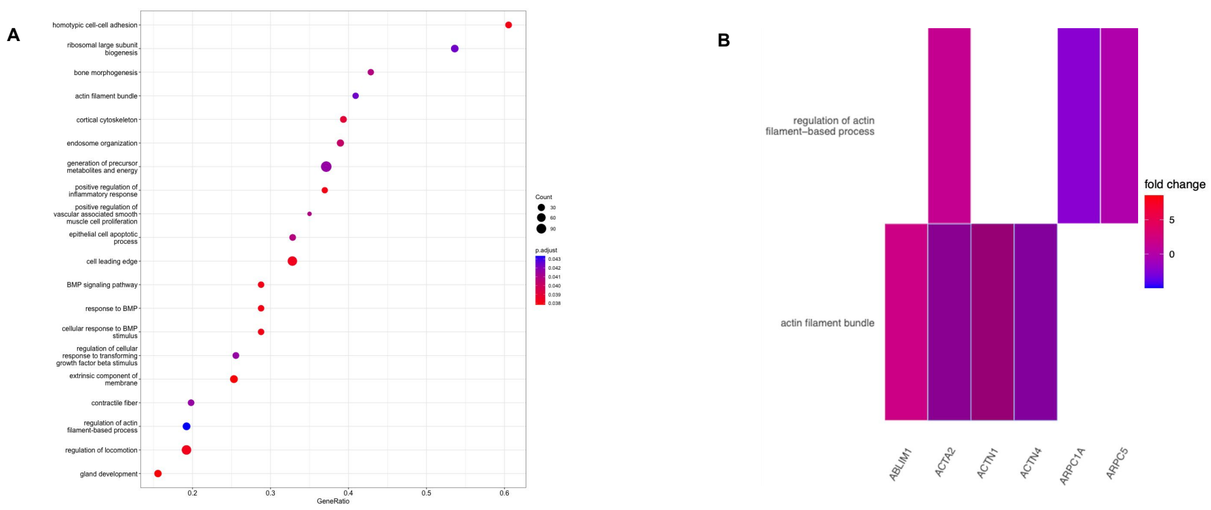

Figure 2. (A) Bar plot illustrating genes associated with various enriched terms. **(B)** Heatmap representing the downregulation of ACTA2 in relation to actin filament processes.

Next, Western blotting and immunofluorescent imaging validated the increased expression of ACTA2 and proteins associated with actin cytoskeleton regulation like ezrin, coffilin2 and myosin light-chain (MLC) in cisplatin-resistant cells (A2780CisR). **(Figure 3 and Figure 4)**

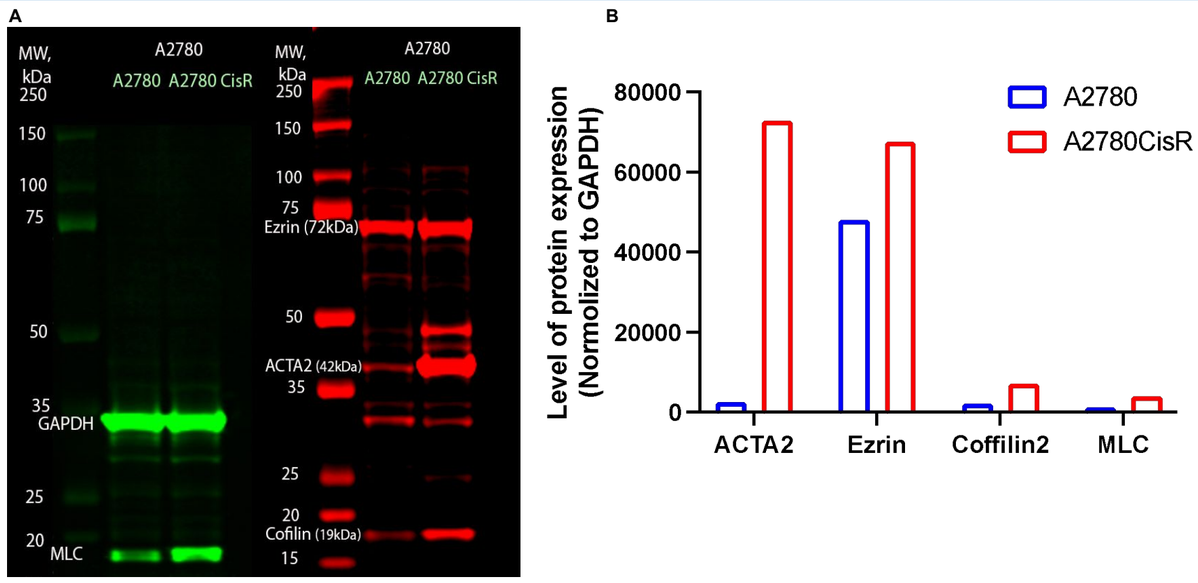

Figure 3. (A) Samples of parental (A2780) and chemo-resistant (A2780CisR) cells were subjected to Western Blot. The membrane stained with antibodies against Ezrin, MLCACTA2, coffilin2, was imaged using Odyssey CLx LI- COR Imaging system **(B)** densitometric analysis of immunoblot images was performed using LI-COR Image STUDIO, normalized to GAPDH and visualized as a bar graph.

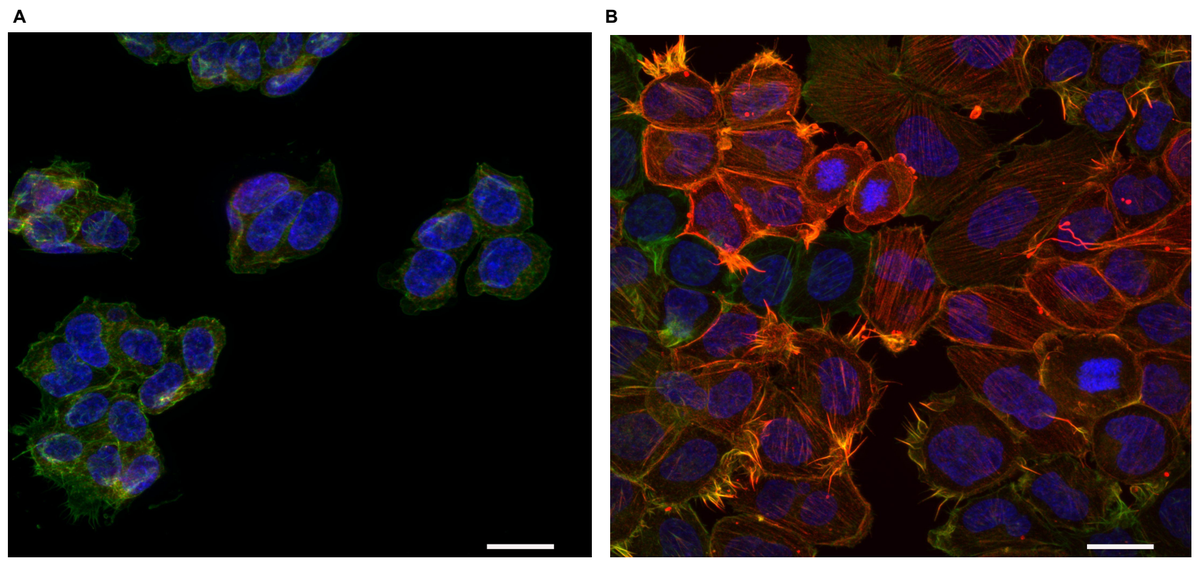

Figure 4. (A) Immunofluorescent images of OC cells A2780 and **(B)** cisplatin-resistant OC cells, A2780CisR acquired using the Opera Phenix™ High Content Screening System (63x magnification) reveal differences in actin network arrangement, cellular shape and intensity of fluorescent ACTA2 staining. Actin filaments are labeled green, the nucleus - blue, and ACTA2 – red. Scale bar = 20 μm.

Our initial results highlight the potential of using cell mechanical features to assess ovarian cancer cell sensitivity, suggesting valuable implications for diagnosis. To confirm our findings, we plan to conduct RNA-Seq experiments, to compare the gene expression profiles of treated and untreated ovarian cancer cells. This approach will provide a detailed molecular understanding, contributing to the validation of our hypotheses and informing potential therapeutic strategies for ovarian cancer.

Conclusions

In conclusion, this study highlighted the challenges of late-stage ovarian cancer and identified ACTA2 as a potential player in cisplatin resistance. The use of deep learning algorithms shows promise in assessing drug effectiveness and advancing personalized medicine for ovarian cancer. These findings have significant implications for improving diagnostics and treatment strategies.

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