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ORIGINAL RESEARCH

Targeting ANXA4 to Overcome Cisplatin Resistance in Ovarian Cancer: A Bioinformatics and in Vitro Study

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Background: Ovarian cancer is a leading cause of gynecologic cancer-related deaths, with cisplatin (DDP) resistance posing a significant challenge to effective treatment. Understanding the molecular mechanisms underlying DDP resistance is crucial for developing new therapeutic strategies.This study aimed to explore the molecular mechanisms of DDP resistance in ovarian cancer, focusing on identifying key genes involved in this process.

Methods: Differential gene expression analysis was conducted using three GEO datasets to identify genes associated with DDP resistance in ovarian cancer cells. Functional enrichment analysis was performed to elucidate the biological pathways involved. ANXA4 was identified as a key gene, and its role was further investigated through in vitro experiments, including gene silencing and overexpression assays, to assess its impact on cell viability, apoptosis, and epithelial-mesenchymal transition (EMT) markers.

Results: A total of 33 common differentially expressed genes (DEGs) were identified, with ANXA4 significantly upregulated in DDP-resistant ovarian cancer cells. Functional analysis revealed that these DEGs, including ANXA4, were involved in pathways related to cell survival, proliferation, and apoptosis. In vitro experiments showed that silencing ANXA4 decreased cell viability, increased apoptosis, and reversed EMT markers in DDP-resistant cells. Conversely, ANXA4 overexpression enhanced resistance to DDP, as evidenced by increased cell viability, reduced apoptosis, and upregulation of EMT markers.

Conclusion: ANXA4 plays a critical role in promoting DDP resistance in ovarian cancer by enhancing cell survival, inhibiting apoptosis, and maintaining EMT characteristics. Targeting ANXA4 may offer a novel therapeutic strategy to overcome chemoresistance and improve treatment outcomes in patients with ovarian cancer. Future studies should validate these findings in vivo and explore the precise molecular mechanisms by which ANXA4 modulates DDP resistance.

Keywords: ovarian cancer, cisplatin resistance, ANXA4, cell viability, apoptosis, epithelial-mesenchymal transition

Introduction

Ovarian cancer is the sixth most common cancer in women worldwide, and the fifth leading cause of cancer death among females.[1,2] Because there is no specific and effective early screening method for ovarian cancer, most patients are diagnosed at an advanced or end stage. Chemotherapy is the main treatment for advanced ovarian cancer patients, but effective chemotherapy drugs are relatively few. According to the NCCN guidelines, the standard chemotherapy regimen for ovarian cancer is basically based on platinum and paclitaxel, with platinum drugs, especially carboplatin, playing a greater role.[3,4] In clinical patients, most patients' conditions can be relieved by platinum drug chemotherapy, but about 30% of patients have no response to the basic platinum drugs.[5] The generation of platinum drug resistance leads to the reduction of chemotherapy effect of patients, which becomes a major obstacle in the treatment of ovarian cancer.[6]

Cisplatin (DDP) is a commonly used platinum drug at present. Its mechanism of action is that when it enters tumor cells, it can interact with special sites of DNA to form DDP-DNA binding with covalent bonds, resulting in the formation of covalent bonds between or within DNA chains, preventing DNA replication, and thus destroying tumor cell

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proliferation and division.[7,8] This DNA damage may activate the apoptotic cascade, which eventually starts the cell to repair DNA.[7,8] Activation of these repair pathways leads to the cell cycle stopping in the S and G2 phases, followed by cell death, apoptosis.[7,8] Mechanisms related to platinum resistance include limiting the formation of cytotoxic drug platinum-like DNA adducts and preventing cell death after the formation of platinum-like adducts.[9,10] The former may be due to reduced entry of platinoid drugs into cells or increased drug excretion by changing transporters, or the conversion of platinoid drugs into platinoid mercaptan conjugates to inactivate platinoid drugs in cells. The latter may develop resistance due to activation of DNA repair mechanisms after adduct formation.[11] DNA repair mechanisms include nucleotide excision repair, mismatch repair, homologous recombination, base excision repair, and transdamaged DNA replication. Changes in multiple proteins associated with these repair mechanisms are associated with platinum resistance, such as the high expression of ERCC1 protein,[12] MLH1, MSH2, MSH1 mutation and downregulation,[13] and BRCA1/2 secondary mutation.[14] With the deepening of research and the development of medicine, the five-year survival rate of ovarian cancer has not been significantly improved, indicating that the mechanism is extremely complex and has not been clearly clarified.

The advent of high-throughput technologies has significantly advanced our understanding of cancer biology, particularly in the context of drug resistance.[15,16] Among these technologies, bioinformatics analysis of publicly available datasets, such as those in the Gene Expression Omnibus (GEO), has become a powerful tool for uncovering the molecular mechanisms underlying chemoresistance.[17,18] By systematically analyzing large-scale gene expression data from various studies, researchers can identify differentially expressed genes (DEGs) that are consistently associated with drug resistance across multiple datasets. This approach not only allows for the discovery of potential biomarkers but also provides insights into the complex biological pathways involved in the development of resistance.[19,20] In this study, three GEO datasets, namely GSE98230, GSE149146, and GSE149724, were employed to compare the transcriptomic profiles in ovarian cancer cells with or without DPP resistance. We initially analyzed differentially expressed genes (DEGs) in these datasets, then performed functional enrichment analyses of these DEGs and identified key genes through protein-protein interaction (PPI) networks. After above analyses, we selected a key gene ANXA4 for functional validation.

Materials and Methods

Data Source

Download ovarian cancer cell RNA-sequencing data from GEO database (https://www.ncbi.nlm.nih.gov/geo/). Data sets include human ovarian cancer cells compared with drug-resistant cells in the experiment. For GSE98230, the DEGs between platinum-sensitive A2780 and platinum resistant-cells A2780 were compared. For GSE149146, the DEGs between platinum-sensitive A2780 and platinum resistant-cells CP70 were compared. For GSE149724, the DEGs between platinum-sensitive OVCAR5 and platinum resistant-cells OVCAR5 were compared.

Data Process and DEGs Extraction

Robust multi-array average (RMA) method is used to preprocess all the original chip data, normalize the chip data, and then compare the chip data of normalized ovarian cancer cells compared with drug-resistant cells. The Limma package in R identifies Differentially expressed genes (DEGs) and computes DEGs using a combined linear model. $P<0.05$ and log fold change (FC) absolute value $(\text{logFC})>2$ were selected as thresholds, in order to eliminate background errors caused by different sequencing platforms and samples from different research units. Venny software (https://bioinfogp.cnb.csic.es/tools/venny/) was used to screen the shared differentially expressed genes in three datasets.

Gene Ontology (GO) Functional Enrichment and KEGG Pathway Analysis of Common Differentially Expressed Gene

GO enrichment and KEGG pathway enrichment analysis was performed by EnrichR tool (https://maayanlab.cloud/Enrichr/). Through the GO analysis of the screened common differential genes, GO classification entries of enriched differential genes can be found, and then the differential genes of different samples may be related to the functional

changes of genes. KEGG pathway analysis could determine the relationship between the components of the signaling pathway.

Construction of Protein Interaction Regulatory Network

The online software STRING10 (Search Tool for the Retrieval of Interacting Genes/Proteins) was used to construct PPI networkof DEGs. This paper attempts to explore the core modules of gene interaction regulation that are significantly related to the occurrence of drug resistance, and further explore the biological functions of these modules and their relationship with the occurrence of drug resistance. The PPI network was further visualized by Cytoscope software and the core network of the PPI network was extracted using Cytohubba applications of the Cytoscape software.

Expression Analysis of Hub Gene Using Human Protein Atlas (HPA) Database

HPA (https://www.proteinatlas.org/) database was used to analyze ANXA4 expression in ovarian tissues by using immunohistochemistry analysis.

Survival Analysis of ANXA4 in Ovarian Cancer Patients

HPA database, GEPIA database (http://gepia.cancer-pku.cn) and the Kaplan–Meier plotter (http://kmplot.com/analysis/) were used to analyze the relationship between ANXA4 expression and survival rates in ovarian cancers based on hazard ratios (HR) and log-rank P-values. In HPA database, overall survival (OS) of ovarian cancer patients were extracted for analysis. In GEPIA database, OS and disease-free survival (DFS) of ovarian cancer patients were extracted for analysis. In Kaplan-Meier plotter database, OS, progression-free survival (PFS) and post-progression survival (PPS) of ovarian patients were extracted for analysis.

Cell Lines

Ovarian cancer cell lines SKOV3, A2780 and OVCAR3 were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). SKOV3 cells were cultured in McCoy's 5A (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% P/S. A2780 cells were cultured in RPMI1640 (Gibco, Carlsbad, CA, USA) full medium. OVCAR3 cells were maintained in RPMI-1640 (Gibco)Media supplemented with 20% FBS, 0.01mg/mL insulin and 1% P/S.

The OVCAR3/DDP cisplatin-resistant cell line was developed by gradually increasing the cisplatin concentration from 2.5 µM to 20 µM over a span of six months, following a previously established protocol.[21] In summary, OVCAR3 cells were first exposed to 2.5 µM cisplatin for three days, followed by a three-day recovery period. This cycle was repeated twice at the 2.5 µM cisplatin concentration. Subsequently, the cells were subjected to 5 µM cisplatin for the next two cycles. This stepwise process continued, with the cisplatin concentration being incrementally raised to a maximum of 20 µM. Throughout the induction of cisplatin resistance, the IC50 values of cells from every third passage were evaluated and compared with those of the original, non-resistant cells until the IC50 value stabilized. The resulting cisplatin-resistant cell lines were then maintained in growth media containing 10 µM cisplatin. The A2780/DDP cell line was obtained from Sigma-Aldrich, and the A2780/DDP cell line was cultured under the same conditions as the A2780 cells.

Plasmids, siRNAs Construction and Transfections

Three siRNAs targeting ANXA4 (si#1, si#2 and si#3) and nonsense siRNA control (siNC) were designed using the online siRNA Design tool and synthesized by RiboBio (Guangzhou, China). The pcDNA-ANXA4 plasmid was an ANXA4-overexpressing vector, while the empty plasmid served as the control. siRNAs and plasmid were transfected into the cancer cells with Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions.

Quantitative Real-Time PCR (qRT-PCR)

RNA extraction from cancer cells was carried out using TRIZOL reagent (Invitrogen). cDNA was synthesized through reverse transcription utilizing the PrimeScript RT Reagent kit (Takara). Real-time PCR was then performed with the SYBR Green PCR kit (Takara) on an Applied Biosystems 7900 system (Life Technologies), following the manufacturer's

protocols. GAPDH served as the internal control for normalization. Gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell Proliferation Assay

Cell viability of the cancer cells was evaluated using a Cell Counting Kit-8 (CCK-8) assay kit. Twenty-four hours after subjecting to different treatments, the cancer cells were incubated with CCK-8 solution for 4 hours at room temperature. Subsequently, cell viability was determined by measuring 450 nm OD value.

Caspase-3 and Caspase-9 Activities

Caspase-3 and Caspase-9 were evaluated using Caspase-3 Assay Kit (Fluorometric) (ab39383) and Caspase 9 Assay Kit (Fluorometric) (ab65607), according to the manufacturer's instructions.

Statistical Analysis

All the data were analyzed using GraphPad Prism software. Significant differences between/among different groups were assessed tested by using the *t*-test or one-way or two-way ANOVA, followed by Bonferroni's test or unpaired Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results

DEGs Extraction

DEGs were extracted from three GEO datasets. Scatterplots of the mean–variance relationship for three RNA-seq datasets were shown in Supplemental Figure S1. In GSE98230, there are 5828 DEGs (2974 up-regulated, 2854 down-regulated) identified. In GSE149146, there are 3411 DEGs (1783 up-regulated, 1628 down-regulated) observed. In GSE149724, there are 525 DEGs (124 up-regulated, 401 down-regulated). Heatmaps and volcano plots in Figure 1 showed the DEGs from these datasets. Venn diagram shows a total of 33 common DEGs in the three data sets. (Figure 2).

Functional Enrichment

Bioplanet enrichment showed that the 33 DEGs were primarily clustered in pathways such as EGFR1 pathway, Interferon alpha/beta signaling, Folate-alcohol and cancer pathway, Integrin cell surface interactions, BDNF signaling pathway, Cell surface interactions at the vascular wall and others (Supplemental Figure S2A). In KEGG enrichment, these DEGs were enriched in pathways like Human immunodeficiency virus 1 infection, Citrate cycle (TCA cycle), Pathways in cancer, Allograft rejection, Glycine, serine and threonine metabolism, and others (Supplemental Figure S2B). In Reactome enrichment, these DEGs were clustered into pathways including Integrin cell surface interactions R-HAS-216083, Cytokine signaling immune system R-HAS-1280215, Interferon alpha/beta signaling R-HAS-909733, Fructose catabolism R-HAS-70350, Interferon gamma signaling r-has-877300, and others (Supplemental Figure S2C).

The results of GO analysis showed that their biological processes (BP) suggest the 33 DEGs are mainly involved in Negative regulation of cytokine production, Autonomic nervous system development, Positive regulation of Wnt signaling pathway, Peptide cross-linking, Heterotypic cell-cell adhesion, Endoderm formation, and others (Supplemental Fig. 3SA). In GO cellular component terms, the DEGs are involved in Cell-cell contact zone, ER to golgi transport vesicle membrane, Coated vesicle membrane, Transport vesicle membrane, and others (Supplemental Figure S3B). In GO_Molecular function terms, they included Actin monomer binding, RNA polymerase II-specific DNA-binding transcription factor binding and others (Supplemental Figure S3C).

PPI Network Analysis

In constructed PPI network, 28 nodes and 98 edges were identified (Figure 3A). We then use Cytohubba tool from Cytoscape for further subnetwork extraction to extract a subnetwork. In this subnetwork, 10 nodes and 18 edges were identified, and ANXA4 showed high degree of connections with other genes (Figure 3B).

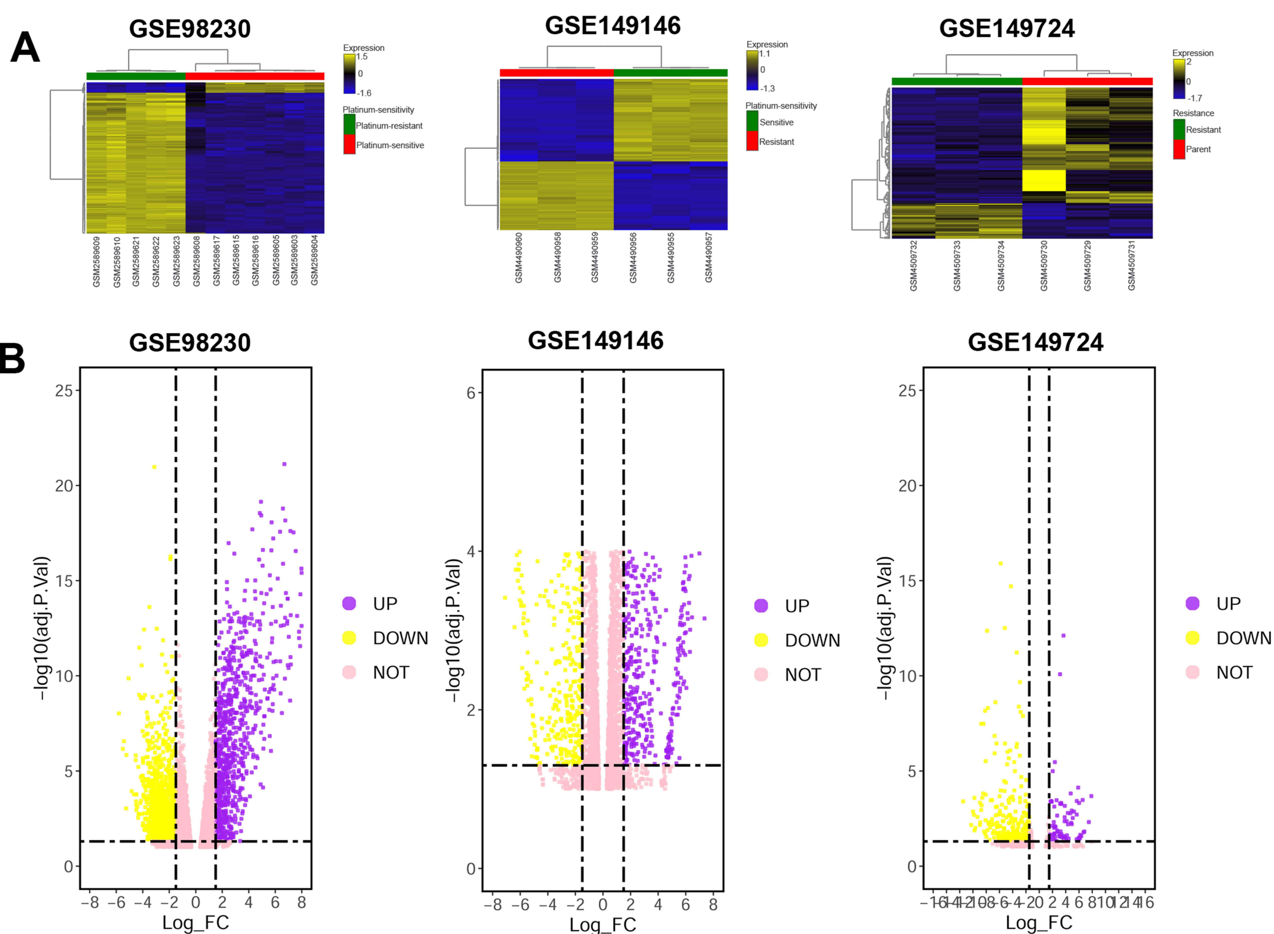

Figure 1 Extraction of DEGs from GSE98230, GSE149146, and GSE149724. (**A**) Heatmap of DEGs for GSE98230, GSE149146, and GSE149724. (**B**) Volcano plot of DEGs for GSE98230, GSE149146, and GSE149724.

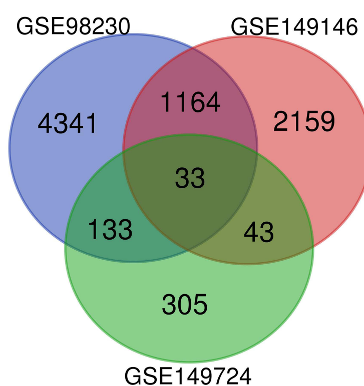

Figure 2 Venn plot shows the common DEGs among GSE98230, GSE149146, and GSE149724.

ANXA4 Expression and Survival Analysis in Ovarian Cancer Patients

Through a literature search of these genes on the network, we found that ANXA4 may be associated with tumor resistance. Moreover, ANXA4's role in the mechanism of resistance in ovarian cancer has not been fully elucidated.

ANXA4 protein staining of normal ovarian and ovarian cancer tissues was shown on the Human protein atlas website, and the expression level of ANXA4 in ovarian cancer tissues is upregulated when compared with normal tissues

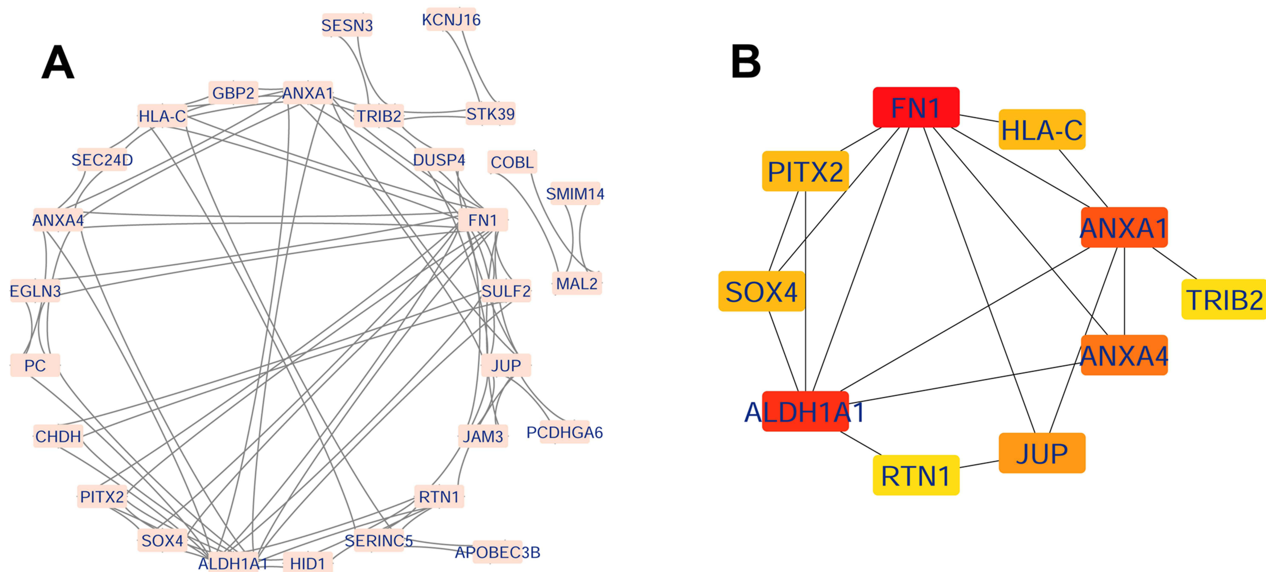

Figure 3 PPI network analysis of DEGs. (**A**) PPI network as constructed by STRING tool. (**B**) Subnetwork of PPI network as constructed by Cytohubba tool.

(Figure 4A). The high ANXA4 expression showed poor prognosis with lower OS in ovarian cancer patients (Figure 4B). Furthermore, we used GEPIA and Kaplan-Meier plotter databases to analyze survivals of ovarian cancer patients with high and low expression of ANXA4. In the GEPIA database, high expression of ANXA4 was related to shorter OS but

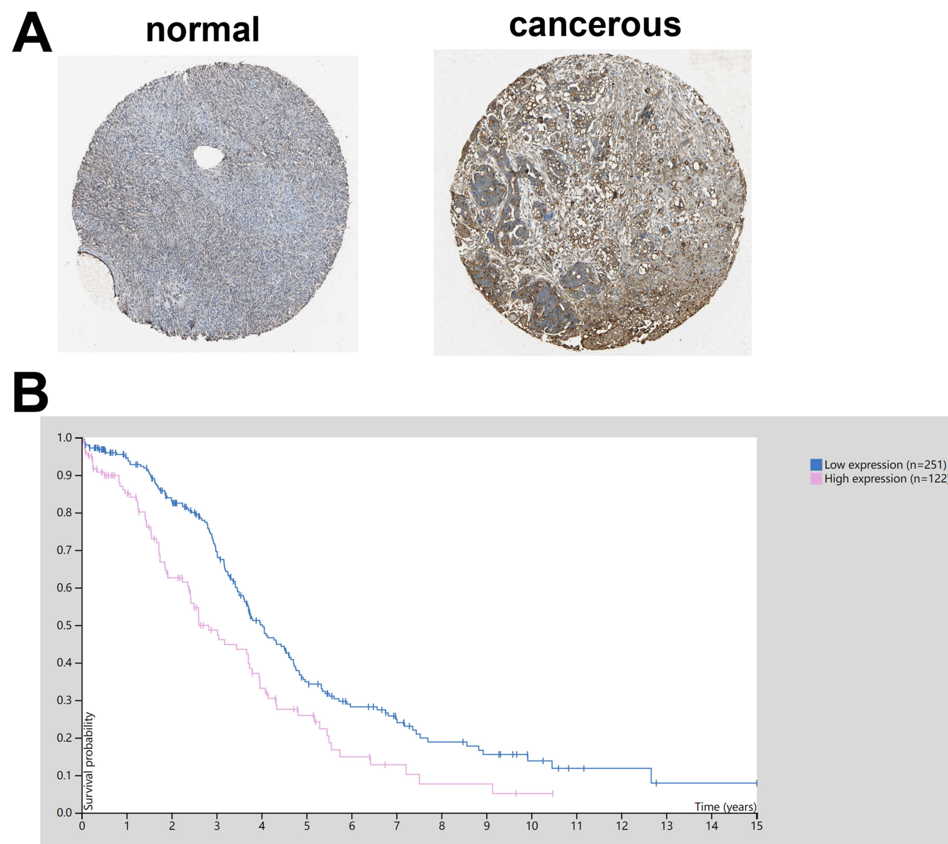

Figure 4 Expression analysis of hub gene using HPA database. (**A**) Expression of ANXA4 protein as determined by IHC in normal ovarian tissues and ovarian cancer tissues. (**B**) OS analysis of patients with ovarian cancer using HPA database.

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not shorter PFS of patients with ovarian cancer (Figure 5A and B). In the KM-Plotter database, high expression of ANXA4 was related to lower OS and PPS but not shorter PFS of patients with ovarian cancer (Figure 5C–E).

Effects of ANXA4 Silence on Ovarian Cancer Cell Progression

The expression of ANXA4 was detected in three ovarian cancer cell lines (SKOV3, A2780 and OVCAR3) and normal ovarian epithelial cells, and the results showed that the expression of ANXA4 in ovarian cancer cells was significantly higher than that in normal ovarian epithelial cells. We designed three siRNAs targeting ANXA4 and found that these sequences significantly down-regulated ANXA4 mRNA expression after transfection in A2780 and OVCAR3 cell lines. At the same time, the inhibition rate of si-ANXA4 2# on mRNA is more than 50%, showing the highest efficiency. Therefore, this siRNA sequence was selected for subsequent experiments (Figure 6A–C).

Moreover, we investigated whether ANXA4 silencing could impact cell proliferation, apoptosis and EMT. As shown in Figures 6D–I, ANXA4 silencing inhibited cell proliferation and increased caspase-3 and caspase-9 activities in both A2780 and OVCAR3 cells (Figure 6D–G). Furthermore, ANXA4 silencing resulted in down-regulation of N-cadherin, vimentin, slug, snail and ZEB1, while upregulation of E-cadherin expression in both A2780 and OVCAR3 cells (Figure 6H and I).

ANXA4 Silencing Attenuated DDP Resistance in A2780/DDP and OVCAR3/DDP

Figure 7A and B showed the cell viability of A2780 cells and A2780-DDP-resistant cells, as well as OVCAR3 cells and the corresponding DDP-resistant cells treated with different concentrations of cisplatin (Figure 7A and B). In the DDP-resistant cells, the cell viability was less sensitive to DDP compared to parental cells (Figure 7A and B). ANXA4 siRNA also down-regulated ANXA4 expression in both DDP-resistant cell lines (Figure 7C). Furthermore, we explored whether silencing ANXA4 could restore DDP sensitivity in both A2780/DDP and OVCAR3/DDP cells. Viability of both resistant

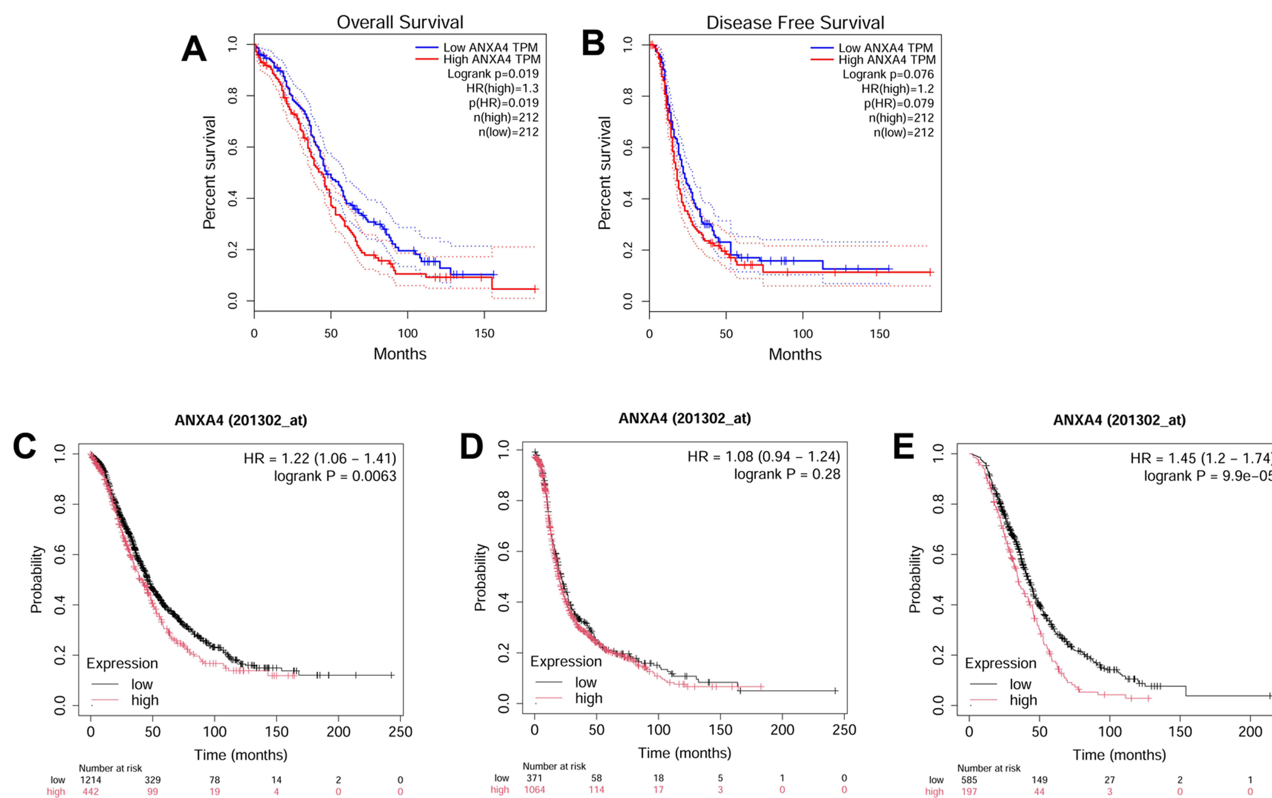

Figure 5 Survival analysis of patients with ovarian cancer using GEPIA and KM-Plotter tool. (**A**) OS and (**B**) DFS analysis of patients with ovarian cancer using GEPIA database. (**C**) OS, (**D**) PFS and (**E**) PPS analysis of patients with ovarian cancer using KM-Plotter database.

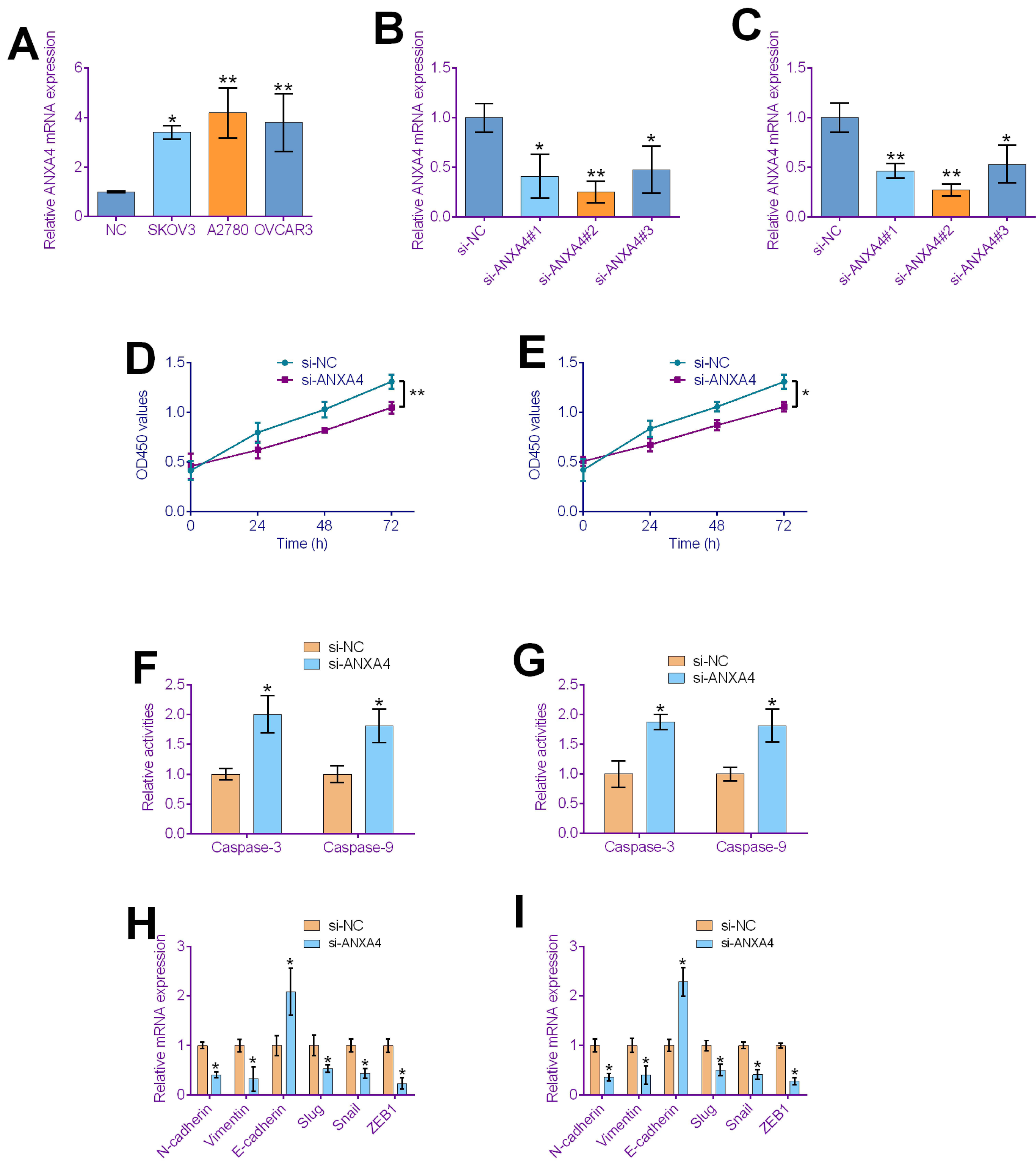

Figure 6 Effects of ANXA4 silence on ovarian cancer cell proliferation, caspase-3/-9 activities and EMT in A2780 and. (**A**) Expression of ANXA4 in normal ovarian epithelial cells and ovarian cancer cells. (**B** and **C**) Expression of ANXA4 mRNA in (**B**) A2780 cell and (**C**) OVCAR3 cells after being transfected with different siRNAs for ANXA4 or scrambled siRNA. (**D** and **E**) Cell viability of A2780 (**D**) and OVCAR3 cells (**E**) after being transfected with ANXA4 siRNA or scrambled siRNA. (**F** and **G**) Caspase-3 and −9 activities of A2780 (**F**) and OVCAR3 cells (**G**) after being transfected with ANXA4 siRNA or scrambled siRNA. (**H** and **I**) Expression of N-cadherin, vimentin, E-cadherin, Slug, Snail and ZEB1 in A2780 (**H**) and OVCAR3 cells (**I**) after being transfected with ANXA4 siRNA or scrambled siRNA. N = 3. *P<0.05 and **P<0.01.

cells were measured after transfected with ANXA4 siRNA or nonsense siRNA and exposed to DDP. Results showed that the viability was lower in the ANXA4 siRNA treated group than control ones (Figure 7D and E). Moreover, we investigated whether ANXA4 silencing affect cell apoptosis and EMT. As expected, ANXA4 silencing increased

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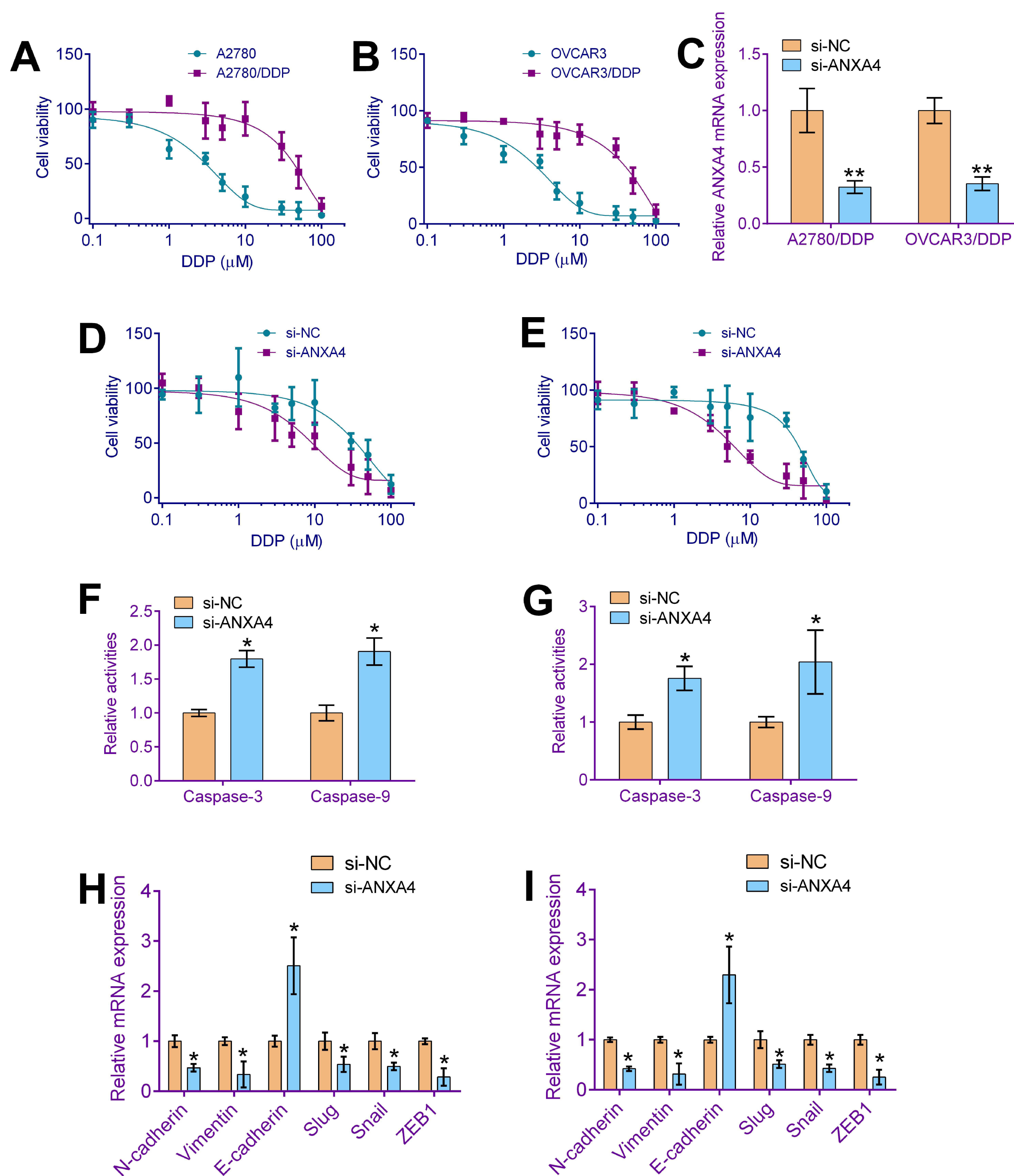

Figure 7 Effects of ANXA4 silence on ovarian cancer cell proliferation, caspase-3/-9 activities and EMT in A2780/DDP and OVCAR3/DDP cells. (**A**) Cell viability of A2780 cells and A2780/DDP cells after being treated with DDP. (**B**) Cell viability of OVCAR3 cells and OVCAR3/DDP cells after being treated with DDP. (**C**) Expression of ANXA4 mRNA in A2780, A2780/DDP, OVCAR3 and OVCAR3/DDP cells. (**D** and **E**) Cell viability of A2780/DDP (**D**) and OVCAR3/DDP cells (**E**) after being transfected with ANXA4 siRNA or scrambled siRNA and treated with different concentrations of DDP. (**F** and **G**) Caspase-3 and −9 activities of A2780/DDP (**F**) and OVCAR3/DDP cells (**G**) after being transfected with ANXA4 siRNA or scrambled siRNA. (**H** and **I**) Expression of N-cadherin, vimentin, E-cadherin, Slug, Snail and ZEB1 in A2780/DDP (**H**) and OVCAR3/DDP cells (**I**) after being transfected with ANXA4 siRNA or scrambled siRNA. N = 3. *P<0.05 and **P<0.01.

caspase-3 and caspase-9 activities in both A2780/DDP and OVCAR3/DDP cells (Figure 7F–G). Furthermore, ANXA4 silencing induced down-regulation of N-cadherin, vimentin, slug, snail and ZEB1, and up-regulating of E-cadherin in both resistant cell lines (Figure 7H and I).

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ANXA4 Overexpression Enhanced DDP Resistance

Finally, we investigated the effect of overexpressing ANXA4 in A2780 and OVCAR3 cells. After transfected with ANXA4-overexpressing vector, the expression level of ANXA4 were markedly increased compared to empty vector transfection in both cell lines (Figure 8A). Furthermore, caspase-3 and caspase-9 activities in both resistant cell lines were assessed after transfection. ANXA4 overexpression led to a decrease in caspase-3 and caspase-9 activities of both A2780 and OVCAR cells (Figure 8B and C). In addition, viability of both cell lines transfected with the ANXA4-overexpressing vector and exposed to DDP was higher than pcDNA group (Figure 8D and E). We also investigated whether ANXA4 overexpression influence EMT. ANXA4 overexpression resulted in the upregulation of N-cadherin, vimentin, slug, snail and ZEB1, and downregulation of E-cadherin in both A2780 and OVCAR3 cell lines (Figure 8F–G).

Discussion

This study aimed to explore the molecular mechanisms underlying DDP resistance in ovarian cancer, with a focus on identifying key genes involved in this process. Using three GEO datasets, we identified 33 common DEGs between DDP-

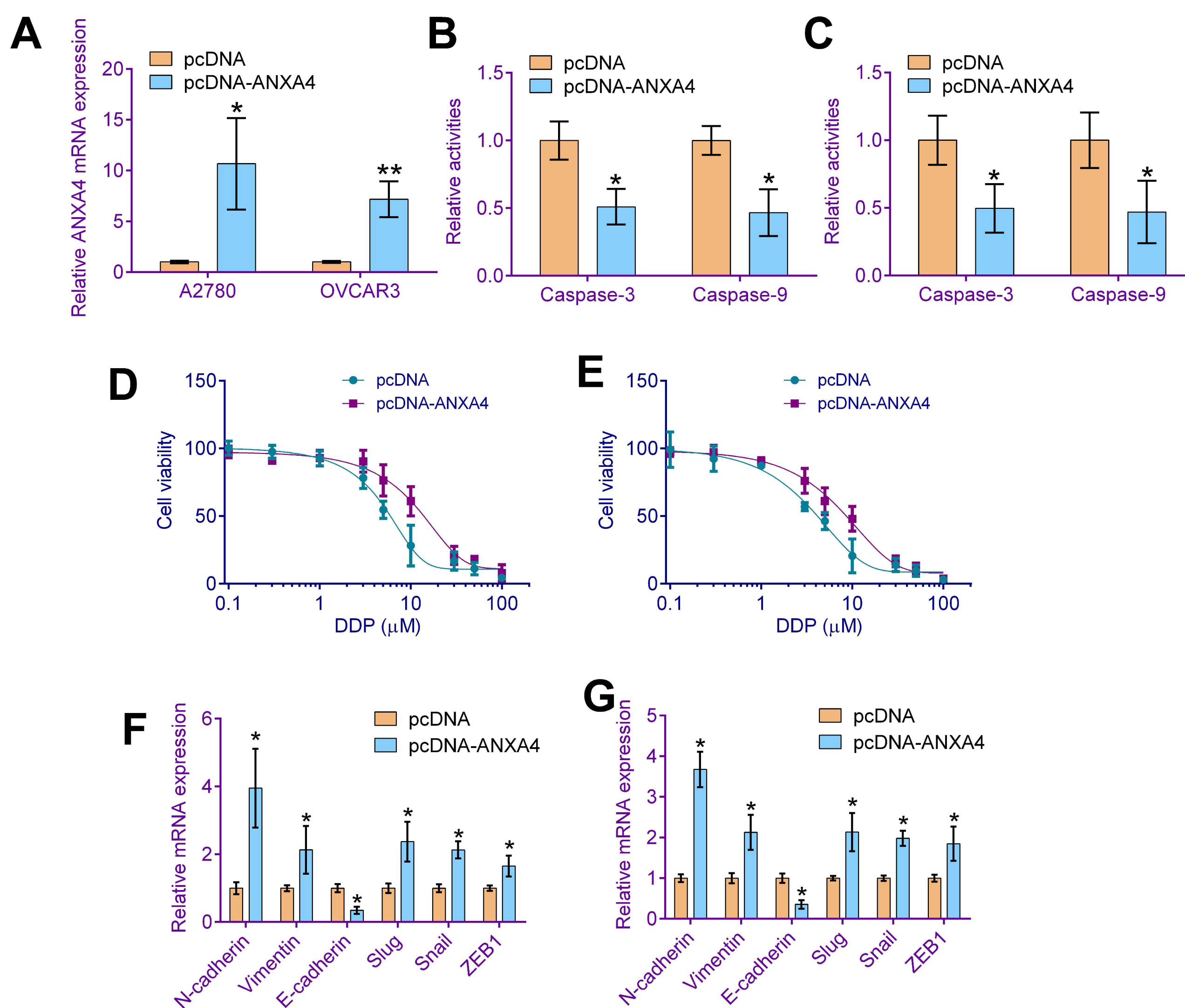

Figure 8 Effects of ANXA4 overexpression ovarian on ovarian cancer cell proliferation, caspase-3/-9 activities and EMT in A2780 and OVCAR3 cells. (**A**) Expression of ANXA4 mRNA in A2780 and OVCAR3 cells after being transfected with pcDNA-ANXA4 or pcDNA. (**B** and **C**) Caspase-3 and −9 activities of A2780 (**B**) and OVCAR3 cells (**C**) after being transfected with pcDNA-ANXA4 or pcDNA. (**D** and **E**) Cell viability of A2780 (**D**) and OVCAR3 cells (**E**) after being transfected with pcDNA-ANXA4 or pcDNA and treated with different concentrations of DDP. (**F** and **G**) Expression of N-cadherin, vimentin, E-cadherin, Slug, Snail and ZEB1 in A2780 (**F**) and OVCAR3 cells (**G**) after being transfected with pcDNA-ANXA4 or pcDNA. N = 3. *P<0.05 and **P<0.01.

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resistant and non-resistant ovarian cancer cells. Among these, ANXA4 emerged as a potential key player in DDP resistance. Our findings revealed that ANXA4 is significantly upregulated in DDP-resistant ovarian cancer cells compared to non-resistant cells. This observation was consistent across multiple datasets, suggesting a robust association between ANXA4 expression and DDP resistance. Moreover, functional enrichment analysis indicated that the DEGs, including ANXA4, were involved in pathways related to cell survival, proliferation, and apoptosis, which are critical in the development of chemoresistance. The role of ANXA4 in promoting DDP resistance was further supported by our in vitro experiments. Silencing ANXA4 in DDP-resistant ovarian cancer cell lines significantly decreased cell viability and increased apoptosis, as indicated by the elevated activities of caspase-3 and caspase-9. Additionally, ANXA4 silencing led to the reversal EMT markers, a process known to contribute to chemoresistance and metastasis. These results suggest that ANXA4 promotes DDP resistance by enhancing cell survival, inhibiting apoptosis, and maintaining EMT characteristics. Conversely, overexpression of ANXA4 in DDP-resistant cells further enhanced resistance to cisplatin, as evidenced by decreased caspase activities, increased cell viability, and upregulation of EMT markers. This reinforces the notion that ANXA4 plays a critical role in sustaining the resistant phenotype in ovarian cancer cells.

Transcriptomic analysis has been regarded as a very useful tool for high throughput screening. In the GSE98230 dataset, Schott and colleagues carried out a transcriptome analysis, revealing an early increase in the expression of CDKN1A and c-Fos in both platinum-resistant and platinum-sensitive cells after treatment with 5-FdU-ECyd. They also identified the disruption of various cellular pathways related to cell cycle regulation, apoptosis, DNA damage response, and RNA metabolism.[22] Meanwhile, in the GSE149146 dataset, Gallon discovered that chromatin alterations at intergenic regions, which regulate gene expression, were closely linked to drug resistance developed in vivo and the distribution of platinum adducts in patient-derived ovarian cancer models.[23] Furthermore, in the analysis of the GSE149724 dataset, Nair provided new insights into the distinct functional roles of CHK1, suggesting a potential strategy to overcome resistance to CHK1 inhibitors in BRCA wild-type HGSOC through combination therapy.[24] In the present study, we identified 33 common DEGs between DDP-resistant and non-resistant ovarian cancer cells. Among these, ANXA4 emerged as a potential key player in DDP resistance.

ANXA4 is a member of the annexin family of calcium-dependent phospholipid-binding proteins, which play critical roles in various cellular processes, including membrane trafficking, inflammation, and apoptosis.[25] Recent studies have increasingly highlighted the involvement of ANXA4 in the development of chemoresistance across multiple types of cancer. ANXA4 is particularly notable for its role in enhancing the survival and proliferation of cancer cells under the selective pressure of chemotherapy.[26,27] In various malignancies, including ovarian, gastric, and lung cancers, ANXA4 has been found to be upregulated in response to chemotherapeutic agents, contributing to a more aggressive and treatment-resistant phenotype.[28] Mechanistically, ANXA4 is believed to mediate chemoresistance through several pathways. It has been implicated in the regulation of drug efflux, reducing intracellular drug accumulation and thereby diminishing the cytotoxic effects of chemotherapy.[29–31] Furthermore, ANXA4 has been associated with the maintenance of EMT, a process that not only facilitates tumor metastasis but also confers resistance to apoptosis.[29–31] The upregulation of ANXA4 has been correlated with poor clinical outcomes, including reduced sensitivity to chemotherapy and lower overall survival rates.[32–35] In addition, Morimoto et al demonstrated that ANXA4 induces platinum resistance in a calcium- and chloride-dependent manner, where its translocation to the plasma membrane is essential for resistance acquisition.[36] Similarly, Mogami et al confirmed that ANXA4 promotes proliferation, invasion, and carboplatin resistance in ovarian clear cell adenocarcinoma cells,[37] while Zhang et al identified ANXA4 as a survival factor in hepato-pancreatic development, linking it to broader cell viability mechanisms.[38] More recently, studies have revealed higher-order regulatory networks: Tan et al showed that FOXD1-activated ANXA3 enhances cisplatin resistance in lung cancer by upregulating ANXA4,[39] and Liao et al reported that ANXA4 can be transferred via extracellular vesicles to promote platinum resistance in gastric cancer through autophagy.[40] Importantly, therapeutic strategies targeting ANXA4 have begun to emerge—Fhit-derived peptides that delocalize ANXA4 from the membrane to cytosol restore chemosensitivity to paclitaxel in resistant lung cancer cells.[41] Our study aligns with previous reports that have implicated various DNA repair mechanisms and cell survival pathways in

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platinum resistance. However, ANXA4's specific involvement in these processes has not been extensively documented, making our findings particularly novel. The identification of ANXA4 as a contributor to DDP resistance opens new avenues for therapeutic interventions. Targeting ANXA4, either through gene silencing or inhibition, could potentially restore cisplatin sensitivity in resistant ovarian cancer patients, thereby improving treatment outcomes.

Despite the promising results, this study has limitations. The use of cell lines, although a powerful model, may not fully recapitulate the complexity of ovarian cancer in vivo. Future studies should include in vivo models and clinical samples to validate the role of ANXA4 in cisplatin resistance. Additionally, the exact molecular mechanisms by which ANXA4 modulates DDP resistance, particularly its interaction with other signaling pathways, require further investigation.

Conclusions

In conclusion, ANXA4 plays a critical role in promoting DDP resistance in ovarian cancer by enhancing cell survival, inhibiting apoptosis, and maintaining EMT characteristics. Targeting ANXA4 may offer a novel therapeutic strategy to overcome chemoresistance and improve treatment outcomes in patients with ovarian cancer. Future studies should validate these findings in vivo and explore the precise molecular mechanisms by which ANXA4 modulates DDP resistance.

Data Sharing Statement

Data are available upon reasonable request from corresponding author.

Ethical Statement

In accordance with Item 1 and Item 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (China, February 18, 2023), this study is exempt from ethical approval.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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