Drug resistance-related gene targets and molecular mechanisms in the A2780/Taxol-resistant epithelial ovarian cancer cell line

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Abstract. Epithelial ovarian cancer (EOC) is a fatal gynecological malignant tumor with a low 5-year survival rate. The use of the first-line chemotherapeutic drug, paclitaxel, for the treatment of EOC is associated with resistance, often leading to treatment failure. The present study investigated the gene targets in an A2780 paclitaxel-resistant EOC cell line (A2780/Taxol), and the potential underlying mechanisms using transcriptome sequencing technology and bioinformatics analysis. The transcriptome of the A2780/Taxol cell line was sequenced, and 498 differentially expressed genes were obtained contained in the Gene Expression Omnibus dataset. Further bioinformatics analysis revealed that matrix metalloproteinase 1 (MMP1), zyxin (ZYX) and Unc-5 netrin receptor C (UNC5C) may be gene targets related to paclitaxel resistance. Moreover, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis indicated that a potential mechanism associated with paclitaxel resistance was related to cell migration. Furthermore, the expression levels of MMP1, ZYX and UNC5C were verified using western blotting, immunofluorescence and immunohistochemistry *in vitro*. The results revealed that the expression levels of MMP1 and ZYX were significantly increased in A2780/Taxol cells, while UNC5C expression was significantly decreased, which was consistent with the results of the transcriptome sequencing. The present study demonstrated that MMP1, ZYX and UNC5C may be the gene targets associated with paclitaxel resistance in EOC. These genes have potential to be used as molecular markers for EOC drug therapy, targeted elimination of drug resistance, and evaluation of treatment efficacy and patient prognosis.

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Abbreviations: EOC, epithelial ovarian cancer; DEGs, differentially expressed genes; RNA-seq, RNA-sequencing; OS, overall survival; paclitaxel, Taxol; MMP1, matrix metalloproteinase 1; ZYX, zyxin; UNC5C, Unc-5 netrin receptor C; GEO, Gene Expression Omnibus; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction

Key words: EOC, paclitaxel, drug resistance, transcriptome sequencing, A2780/Taxol

Introduction

Ovarian cancer is a fatal gynecological malignant tumor. Although ovarian cancer has a lower incidence rate than endometrial cancer, with 313,959 new cases in 2020, it is associated with a high mortality rate of 207,252 cases, ranking it third among all gynecological malignancies (1). Based on histological differences, as demonstrated by the World Health Organization, ovarian cancer is divided into four categories: i) Epithelial; ii) gonadal-mesenchymal; iii) germ cell; and iv) metastatic. Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer. Of note, >70% of patients with EOC are diagnosed at an advanced stage, and the 5-year survival is ~48% (2). With extensive research performed on EOC, several treatment modalities are available, including surgical treatment, chemotherapy, immunotherapy, targeted therapies and others; however, primary debulking surgery and combination chemotherapeutic regimens, including paclitaxel (Taxol), remain the standard of care for patients

with advanced-stage EOC (3-5). Although patients with EOC initially respond to treatment, the majority of them experience relapse within a few years due to chemotherapeutic resistance, which is one of the reasons for the low survival rate of patients with EOC (6,7).

Paclitaxel is one of the first-line drugs approved for the treatment of EOC, which has a unique mechanism of action, and is considered to be one of the most successful natural anti-cancer drugs (8,9). The mechanism of Taxol involves binding with the 31-amino acid N-terminal from the β-microtubule protein subunit to induce microtubule stability and prevent its depolymerization, generating G2/M phase accumulation in tumor cells, thus inhibiting mitosis and cell proliferation, and promoting cell apoptosis (10-12). However, the development of resistance to Taxol severely limits the clinical chemotherapeutic efficacy in patients with EOC (13). It has been reported that the upregulation of cytoplasmic polyadenylation element binding protein 4 promotes Taxol resistance in ovarian cancer via the translational regulation of CSAG family member 2 *in vitro* (14). Feng *et al* (15) demonstrated that glucose-6-phosphate dehydrogenase promoted Taxol resistance in EOC cells by regulating the expression of glutathione S-transferase P1. However, Taxol resistance is a complex process. It is important to identify novel promising gene targets associated with Taxol resistance for EOC drug therapy, targeted elimination of drug resistance, and improvement of treatment efficacy and patient prognosis.

In the present study, transcriptome sequencing technology and the Gene Expression Omnibus (GEO; an international public repository of microarray chips, second-generation sequencing and other forms of high-throughput genomic data uploaded by researchers worldwide) dataset were used to explore the genes related to paclitaxel resistance in an EOC cell line. Through bioinformatics analysis and verification *in vitro*, the present study aimed to find new targets with potential as a molecular marker of EOC resistance, and also to provide a new basis for the clinical prediction of the key molecular mechanisms of Taxol resistance.

Materials and methods

Cell culture. The human EOC A2780 cell line and the A2780-Taxol-resistant cell line were purchased from ImmoCell Biotechnology Co., Ltd. (provided by American Tissue Culture Collection). A2780 and A2780/Taxol cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Zhejiang Tianhang Biotechnology Co., Ltd.) and 1% penicillin-streptomycin solution (Biosharp Life Sciences), at 37˚C in a humidified incubator with 5% CO_2. The medium for the A2780 cell line was additionally supplemented with 1% L-glutamine (Procell Life Science & Technology Co., Ltd.). The medium for the A2780/Taxol cell line was also supplemented with 60 ng/ml Taxol (cat. no. H20203702; Sichuan Huiyu Pharmaceutical Co., Ltd.).

RNA-seq analysis. Total RNA was extracted from ~1x10^6 cells using Trizol (Beyotime Institute of Biotechnology), followed by RNA-seq analysis performed by BGI [Platform: DNBSEQ (Homo sapiens)]. The RNA-seq data were filtered with SOAPnuke (version, 1.5.2). Reads were mapped to the reference genome (version, GCF_000001405.39_GRCh38.p13) and aligned using HISAT2 (version, 2.0.4) and Bowtie2 (version, 2.2.5) for comparison with the human reference genome.

DEG analysis. Gene expression levels were calculated for both the A2780 and the A2780/Taxol cell lines in the present study using RSEM (version 1.2.8) to screen DEGs1 with |log2(Fold Change)|≥1 and FDR≤0.001. The gene expression level file GSE159791 (https://www.ncbi.nlm.nih.gov/geo/) of A2780/Taxol cells was downloaded from the GEO database (16). Combination with DEGs1, common genes were selected and analyzed using the R software package DESeq2 (version 1.42.1) with the A2780 cell as the control. DEGs were screened from common genes with |log2(Fold Change)|≥1 and P_{adj}<0.01. Results were visualized using the R packages pheatmap (version 1.0.12) and ggplot2 (version 3.4.3).

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis. GO terms and pathways were obtained from the GO (ftp://ftp.ncbi.nih.gov/gene/DATA/gene2go.gz) and KEGG databases (version 101.0). The P-value was calculated using hypergeometric test in the function phyper of R, and the package qvalue (version 2.4.2) was used to perform a multiple positive test on the P-value. Q-value (corrected P-value) of <0.05 as the threshold to select the significantly enriched GO term and pathway, visualized with R language package GOplot (version 1.0.2) and ggplot2. The files of protein-protein interaction (PPI) analysis for DEGs in the pathway were downloaded from the STRING database (https://cn.string-db.org/), and visualized with Cytoscape (version 3.8.0).

MTT cell viability assay. A2780 and A2780/Taxol cells (6x10^3 cells/well) were cultured into 96-well plates, treated with varying concentrations of Taxol solution (0, 30, 60, 120, 240, or 480 ng/ml), with five replication wells per group. After 48 h, 10 μl MTT (Biofroxx; NeoFroxx) was added to each well and incubated at 37˚C for 4 h. The supernatant was discarded, and 150 μl DMSO solution was added to measure absorbance at 490 nm and calculate the IC_{50}.

Western blotting. The A2780 and A2780/Taxol cells were lysed to extract total protein with RIPA and PMSF solution (100:1 ratio; Beyotime Institute of Biotechnology). Total protein concentration was quantified with the BCA Protein Concentration Assay Kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. The protein samples (20 μg per lane) were separated on 10% gels using SDS-PAGE. PVDF membranes (Beyotime Institute of Biotechnology) were blocked with non-fat powder milk for 2 h at room temperature and incubated overnight at 4˚C with rabbit anti-MMP1 (cat. no. AF0231; Beyotime Institute of Biotechnology), rabbit anti-ZYX (cat. no. 38377; SAB Biotherapeutics, Inc.) and rabbit anti-UNC5C (cat. no. 44671; SAB Biotherapeutics, Inc.) diluted at a ratio of 1:1,000. Membranes were washed with TBST (contain 0.1% Tween) and incubated with HRP-conjugated affinipure goat anti-rabbit IgG (H+L) (1:1,000 dilution; cat. no. A0208; Beyotime Institute of Biotechnology) at room temperature for 2 h. Bands were

visualized using a chemiluminescence kit (Beyotime Institute of Biotechnology). Protein hybridization results were observed using an automated chemiluminescence instrument (Tanon-5200; Ewell Biotechnology), and abundance was processed using Fiji (-win64; ImageJ, National Institutes of Health).

Immunohistochemistry. A total of $4x10^4$ cells/ml were cultured in 12-well plates overnight. Cells were fixed with 4% paraformaldehyde (Wuhan Servicebio Technology Co., Ltd.) for 1 h at room temperature. Cells were then washed with PBS, and 50-100 μl film breaking solution was added to the plate for incubation at room temperature for 20 min. A total of 3% BSA was added for blocking for 30 min at room temperature, followed by incubation in a wet box at 4˚C overnight with the primary antibody (anti-MMP1: 1:100 dilution; cat. no. AF0231; Beyotime Institute of Biotechnology; anti-ZYX: 1:100 dilution; cat. no. 38377; SAB Biotherapeutics, Inc; and anti-UNC5C: 1:100 dilution; cat. no. bs-11493R; Beijing Bioss Biotechnology Co., Ltd.). The plate was incubated for 50 min with HRP-labeled secondary antibody (1:100 dilution; cat. no. GB23303; Wuhan Servicebio Technology Co., Ltd.), and freshly prepared DAB color developing solution was added controlling the color developing time. Hematoxylin was used as a counterstain at room temperature for 3 min, and the hematoxylin fractionation solution fractionated for a few sec. Hematoxylin re-blueing solution (Wuhan Servicebio Technology Co., Ltd.) was used for staining and rinsed. The slivers were dehydrated and sealed with neutral gum. After light microscopic examination, images were collected for analysis.

Cellular immunofluorescence (IF) assay. For the IF assay, the medium was aspirated, and the cell crawls were washed three times with cold PBS. After being fixed with 4% paraformaldehyde for 30 min, the cells were penetrated with membrane breaking working solution for 20 min. Subsequently, the cells were blocked with 3% BSA for 30 min (Wuhan Servicebio Technology Co., Ltd.), washed three times with PBS and incubated with the primary antibody (same as aforementioned immunohistochemistry antibodies) overnight. After washing three times with PBS, the cells were incubated with the secondary antibody (1:100 dilution; cat. no. E032420; EarthOx Life Sciences) coupled with DyLight 594-TFP ester for 2 h. After washing three times with PBS, the cells were stained with DAPI dye solution, and incubated for 10 min in the dark. Finally, the cell crawls were washed three times with PBS, and blocked with an anti-fluorescence quencher. Images were captured using a fluorescence microscope (BX51-32FL; Olympus Corporation). The average fluorescence density was calculated using Fiji.

Analysis on clinical information and RNA-seq data from the cancer genome atlas (TCGA). Clinical information of patients with EOC and other cancers was obtained from TCGA database using the R package TCGAbiolinks (version 3.14). RNA-seq data and survival-related files of patients from TCGA database were downloaded using the UCSC Xena online tool (https://xenabrowser.net/datapages/) for subsequent analysis.

Statistical analysis. Kaplan-Meier survival curves were created and visualized using the packages survminer (version 0.4.9), survival (version 3.5-7) and TSHRC (version 0.1-6; https://cran.r-project.org/web/packages/TSHRC/TSHRC.pdf) in R (version 4.3.1). Statistical analysis was carried out using the log-rank test for Kaplan-Meier survival curves. One-way ANOVA and least significant difference tests were used for the statistical analysis and were performed using SPSS (version 26.0; IBM Corp.); visualization was carried out using GraphPad Prism (version 5; Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference. A total of three biologically independent repeats were carried out, and the data are presented as mean ± SD.

Results

DEGs in A2780/Taxol cells. The drug resistance index (RI) of the A2780/Taxol cell line in the present study was 33.62, exhibiting highly resistant characteristics (Fig. S1B). The common gene expression profile was similar to that of other Taxol-resistant cell lines in the GEO database, but different from that of the A2780 cell line [Figs. 1A and S1B; other RI values from the data in the study by Szenajch *et al* (16)]. Through differential expression analysis, 6,226 DEGs (DEGs1) were identified in the transcriptome sequencing data (Fig. 1B). The A2780 cells were used in the present study as control to reprocess the drug resistance data of the GEO database; 498 DEGs (DEGs2) were finally obtained based on DEGs1 (Fig. 1C; screening method, Fig. S1C and D; gene names, Table SI).

Discovering DEGs significantly associated with the overall survival (OS) of patients with EOC and Taxol resistance. As chemoresistance is associated with a decreased survival rate of patients, genes in DEGs2 that were both up or down-regulated in the A2780/Taxol and drug resistance datasets were selected for survival curve analysis. A total of 27 genes were found to be significantly associated with the OS of patients with EOC (Figs. 1D, S2 and S3). Considering that the log-rank test might lose power when the survival curves crossed at a later stage (17), a two-stage test (TS) weighted analysis was carried out for POLR3GL, ZNF239, FLRT3 and ZFHX4. The TS P-values of POLR3GL, ZNF239, FLRT3 and ZFHX4 were 0.66, 0.35, 0.35 and 0.28, respectively (Figs. S2 and S3). Since these values are >0.05, they were excluded from subsequent analysis. Of these survival-related genes, increased levels of GDF15 were found to be associated with enzalutamide and EPI-001 resistance in prostate cancer cells (18). NR1D2 was shown to be associated with enzalutamide resistance in neuroendocrine prostate cancer, and PHLDA1 was found to be associated with Lewis(y) highly expressing chemoresistant ovarian cancer cell (19,20). Imiquimod facilitates chemoresistance via the upregulation of MMP1, and RNA interference targeting ZYX reduces tumor cell HN12 resistance to cisplatin (DDP) (21,22). The downregulation of NCAM2 was shown to be associated with resistance to the monoclonal antibody drug trastuzumab in $HER2^+$ breast cancer, and FBXL7 knockdown affects DDP resistance in nasopharyngeal carcinoma (23,24). In summary, these 23 genes were intimately associated with resistance to

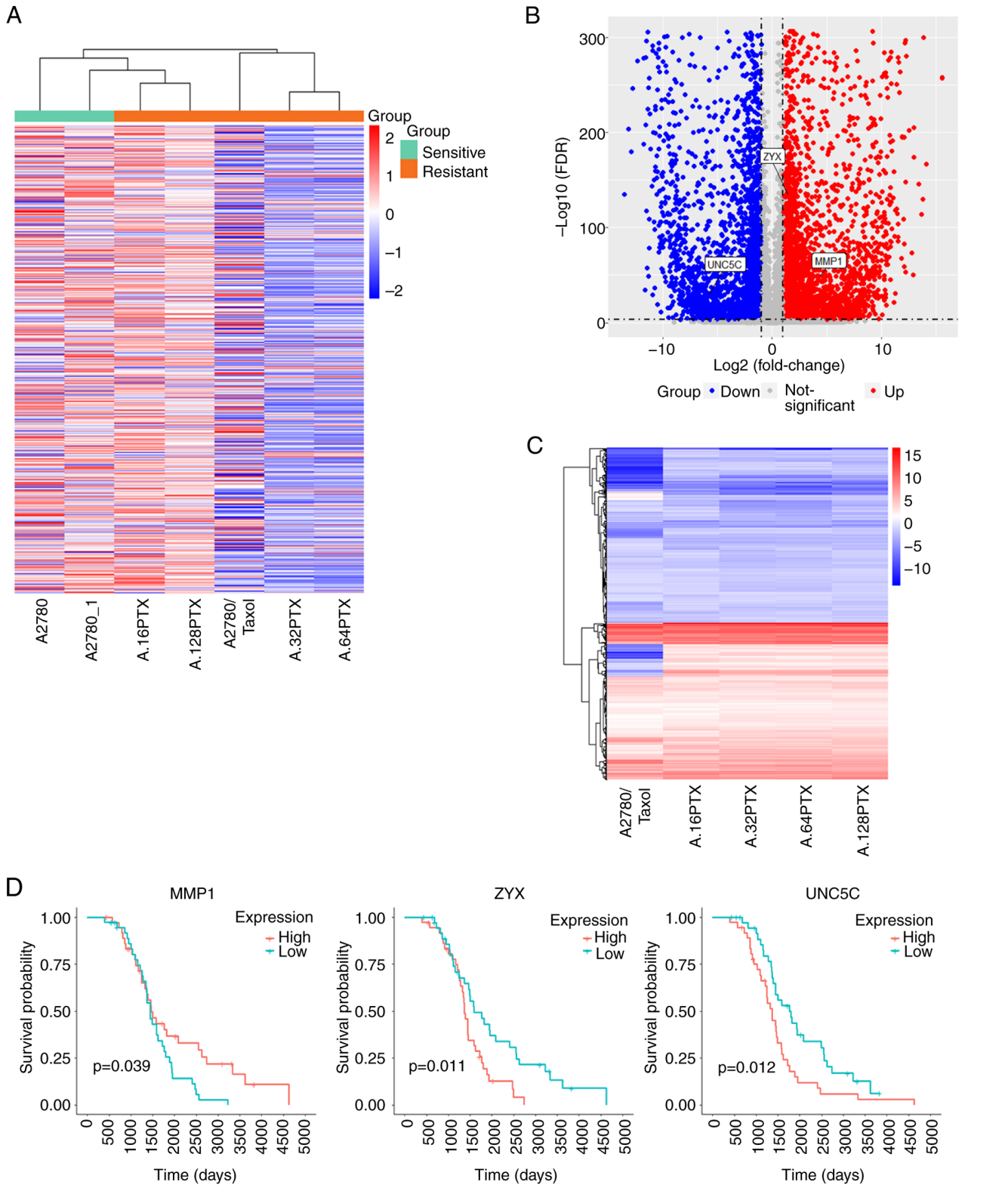

Figure 1. Analysis of DEGs in Taxol-resistant cells. (A) Common gene expression profile of Taxol-sensitive and -resistant cells. A2780 and A2780/Taxol cells: transcriptome sequencing data from the present study; A2780_1, A.16PTX, A.32PTX, A.64PTX and A.128PTX cells, Gene Expression Omnibus data. (B) Volcano plot of DEGs between A2780 and A2780/Taxol cells. (C) Heatmap of 498 DEGs; color indicates log2FoldChange. (D) The differential expression of MMP1, ZYX and UNC5C was associated with a poor prognosis of patients with epithelial ovarian cancer treated with Taxol. DEGs, differentially expressed genes; ZYX, zyxin; MMP, matrix metalloprotease; UNC5C, Unc-5 netrin receptor C.

chemotherapy in various types of cancer, demonstrating the feasibility and accuracy of the screening performed in the present study for these Taxol resistance-associated genes that were closely associated with the OS of patients with EOC.

KEGG and GO enrichment analysis of the DEGs, MMP1, ZYX and UNC5C. To investigate the role of DEGs on the basis of cell integral changes, the DEGs1 genes were subjected to GO and KEGG enrichment analysis. A Q value of <0.05 was

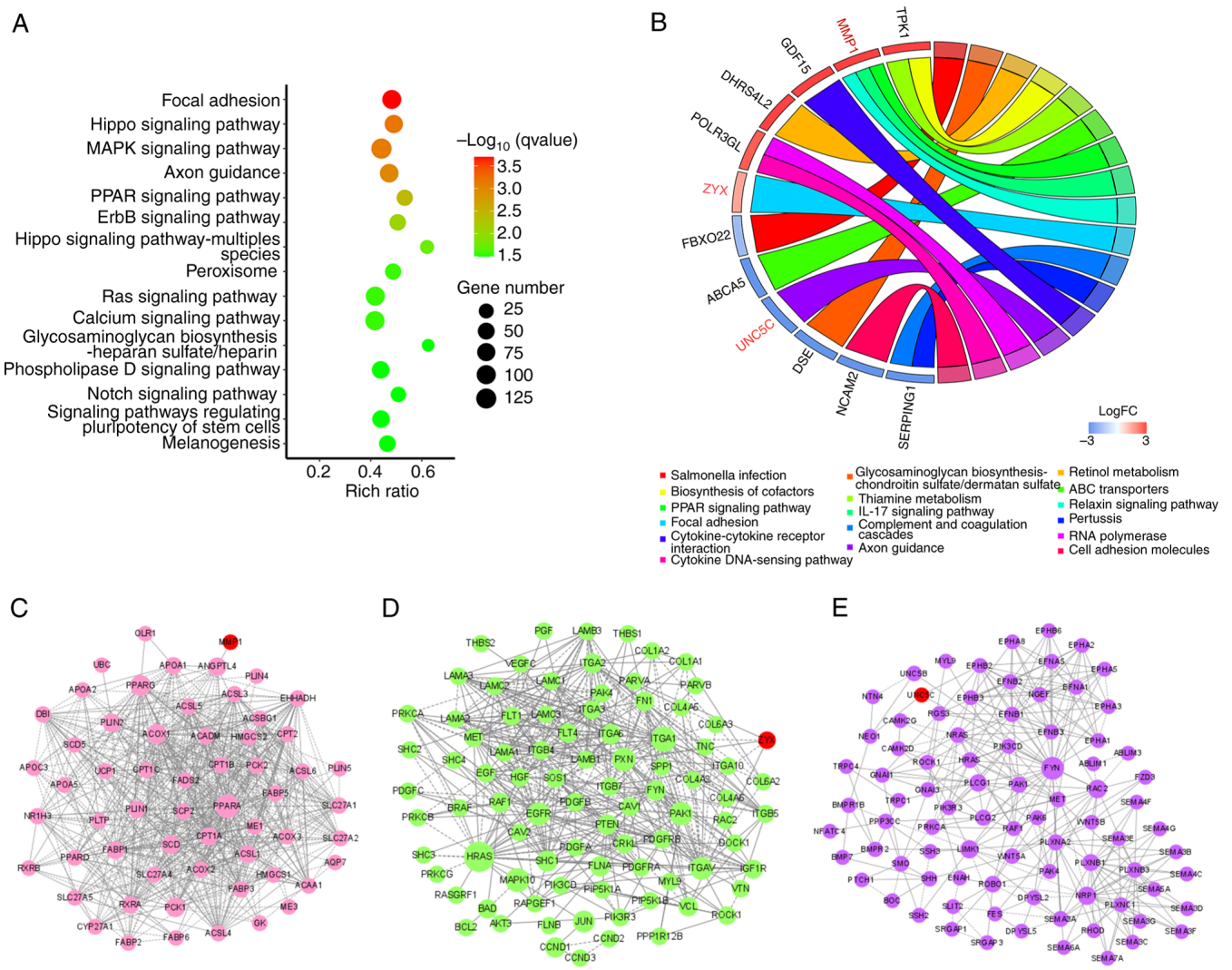

Figure 2. KEGG enrichment and protein-protein interaction network analysis. (A) Bubble chart of the top 15 significant enrichment KEGG pathways. (B) Chord chart of survival-related genes and KEGG pathways. (C) Interaction between MMP1 and DEGs enriched in the 'PPAR signaling' pathway. (D) Interaction between ZYX and DEGs enriched in the 'focal adhesion' pathway. (E) Interaction between UNC5C and DEGs enriched in the 'axon guidance' pathway. ZYX, zyxin; KEGG, Kyoto Encyclopedia of Genes and Genomes; MMP, matrix metalloprotease; DEGs, differentially expressed genes; UNC5C, Unc-5 netrin receptor C.

considered to indicate significant enrichment. As shown by the KEGG enrichment analysis, the most significant pathway was 'focal adhesion', while in cellular component and biological process of GO, 'cell junction' and 'cell adhesion' were the significant pathways, indicating that they were associated with Taxol resistance (Figs. 2A and S4A-C). In addition, the results of the enrichment analysis indicated the involvement of the cell migration process (25-27), suggesting that cell migration affects the occurrence of Taxol resistance in A2780 cells. The 23 survival-related genes were mapped into the significant enrichment pathways, and only MMP1, ZYX and UNC5C were enriched in the 'PPAR signaling', 'focal adhesion' and 'axon guidance' pathways, respectively (Fig. 2B). Therefore, these three genes were used as gene targets for follow-up experiments.

Role of MMP1, ZYX and UNC5C in KEGG pathways through PPI network analysis. Through KEGG pathway analysis, the enrichment pathways of MMP1, ZYX and UNC5C was identified. However, the mechanisms through which these genes play a role in the pathway remain unknown. Therefore, PPI network analysis was performed on the DEGs enriched in 'PPAR signaling', 'focal adhesion' and 'axon guidance' pathways. MMP1 interacted with angiopoietin-like 4 (ANGPTL4; Fig. 2C). It was hypothesized that MMP1 may affect cell migration through ANGPTL4, leading to the generation of drug resistance. The results from the study by Liao *et al* (28) confirmed that the downregulation of MMP1 hindered the migration and invasion of head and neck squamous cell carcinoma cells enhanced by EGF and recombinant ANGPTL4. ZYX interacted with paxillin (PXN) and vinculin (VCL) (Fig. 2D). It was hypothesized that ZYX may affect cell adhesion through PXN and VCL, thus mediating the generation of drug resistance. The study by Legerstee *et al* (29) on protein pairs related to the function of 'focal adhesion' demonstrated that the binding of ZYX and PXN, and that of VCL and vasodilator stimulated phosphoprotein affected cell adhesion and migration. In the PPI network of the 'axon guidance pathway'

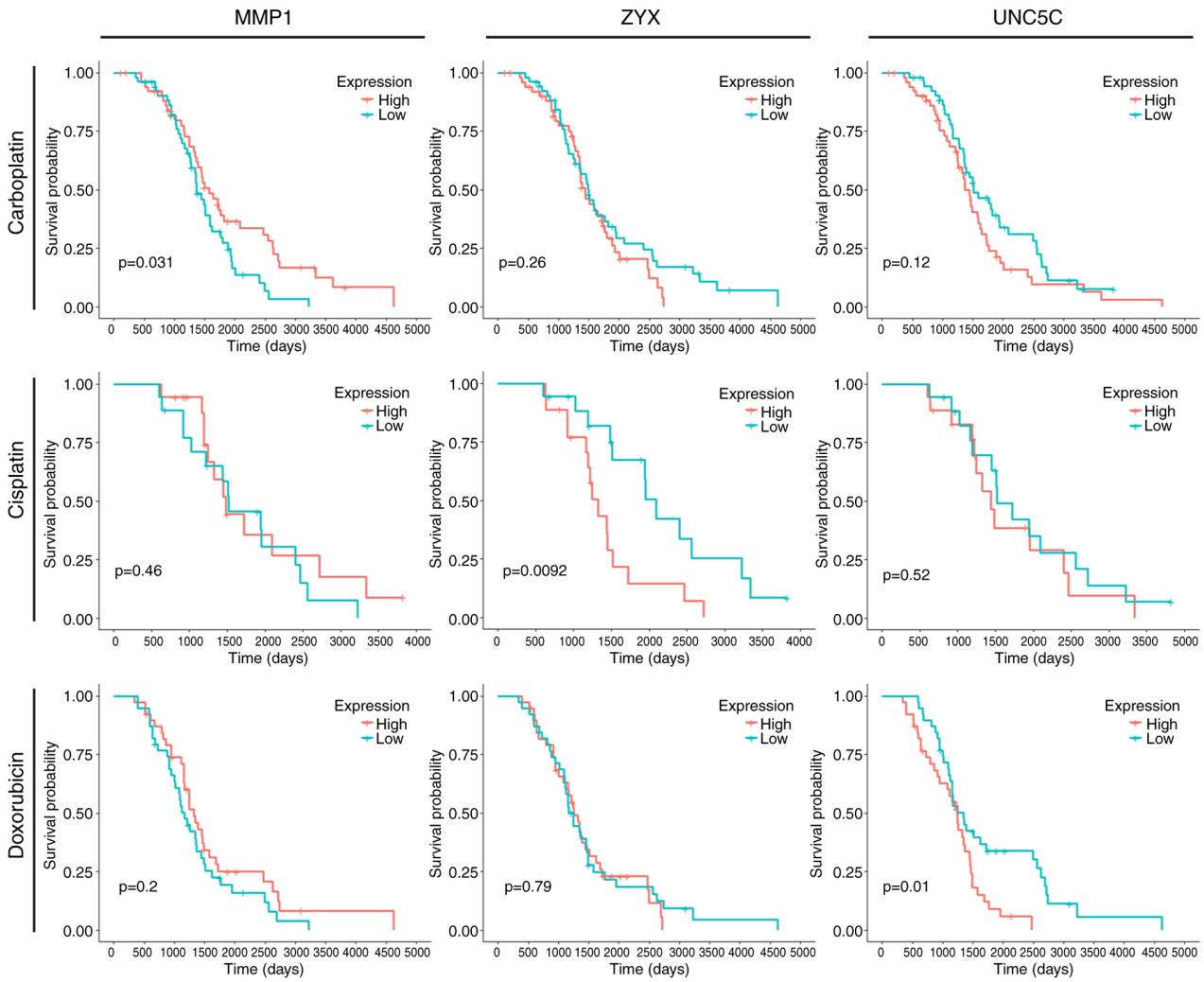

Figure 3. Survival curves of MMP1, ZYX and UNC5C in patients with epithelial ovarian cancer treated with carboplatin, cisplatin or doxorubicin. ZYX, zyxin; MMP, matrix metalloprotease; UNC5C, Unc-5 netrin receptor C.

shown in Fig. 2E, FYN, the largest node, was the core of the network and interacted with UNC5C. According to literature, the knockdown of UNC5C enhances the phosphorylation of FAK and SRC (30), and FYN is a specific member of the SRC kinase family (31). Therefore, it was hypothesized that UNC5C mediated the generation of drug resistance by affecting Src kinase activity through FYN. Furthermore, these results confirmed the reliability of PPI network analysis to examine the interactions between proteins, and provided molecular information that the three targets may participate in the drug resistance mechanisms of EOC.

At the same time, in order to investigate the association of Taxol resistance of EOC with disease stage, the associations between MMP1, ZYX and UNC5C, and the disease stage of EOC (stage II, III and IV) were examined. The results indicated that MMP1, ZYX and UNC5C were not associated with the disease stage of patients with EOC (P>0.05, not statistically significant; Fig. S4D-F).

MMP1, ZYX and UNC5C were significantly associated with carboplatin (CBP), DDP and doxorubicin (DOX) resistance in patients with EOC, respectively. Chemotherapeutic drugs for EOC, in addition to Taxol, include CBP, DDP and DOX. CBP, DDP and DOX limit DNA replication and transcription via various mechanisms. Although DNA replication affected by CBP, DDP and DOX occurs during the S phase, and Taxol induces G2/M phase accumulation, these drugs ultimately lead to apoptosis, and they may share common signaling pathways and networks in the final stage (32-34). Therefore, it was hypothesized that Taxol resistance may cause resistance to other chemotherapeutic drugs as well. In the present study, CBP, DDP and DOX, as well as Taxol, were selected to analyze the association between MMP1, ZYX and UNC5C and the OS of patients with EOC in TCGA. The results revealed that MMP1, ZYX and UNC5C were significantly associated with the survival of patients treated with CBP, DDP and DOX, respectively, and this trend was consistent with Taxol, indicating that Taxol may share key resistance-related targets with CBP, DDP and DOX (Fig. 3). This finding preliminarily confirmed one of the ways that Taxol resistance causes resistance to other chemotherapeutic drugs.

Analysis of the association between MMP1, ZYX and UNC5C and the OS of Taxol-treated patients with head and neck squamous cell carcinoma (HNSC), lung cancer (LUNG) and uterine corpus endometrial cancer (UCEC). Taxol is also used

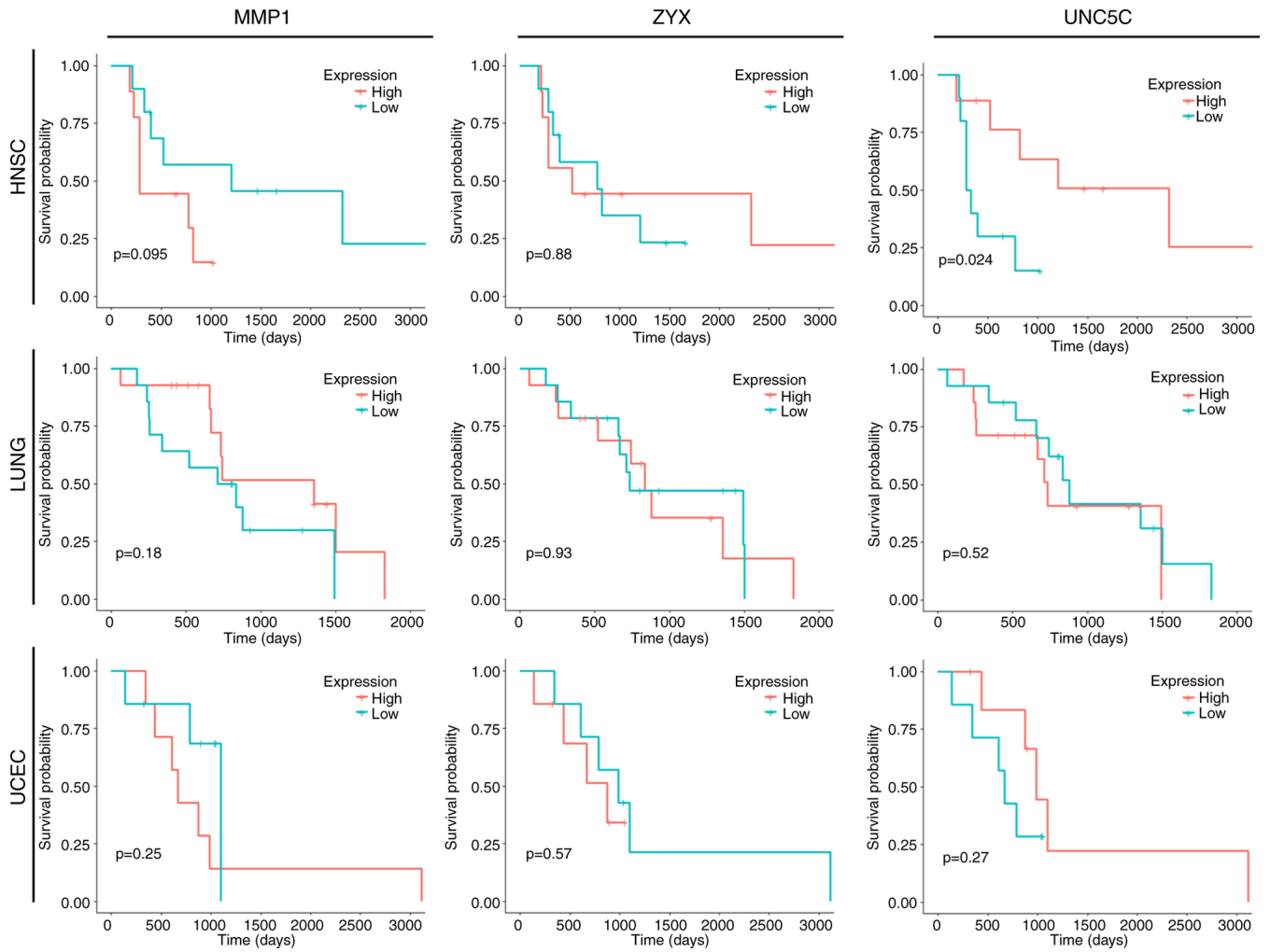

Figure 4. Survival curves of MMP1, ZYX and UNC5C in patients with other types of cancer treated with Taxol. ZYX, zyxin; MMP, matrix metalloprotease; UNC5C, Unc-5 netrin receptor C; HNSC, head and neck squamous cell carcinoma; LUNG, lung cancer; UCEC, uterine corpus endometrial cancer.

clinically in the treatment of patients with LUNG, HSSC and UCEC, as well as other types of cancer (35-37). Regarding HNSC, LUNG and UCEC, patients with a history of treatment with Taxol in TCGA, excluding patients with free tumor tissue, were selected for Kaplan-Meier survival curve analysis to investigate the association between MMP1, ZYX and UNC5C, and the OS of other patients with cancer treated with Taxol. The results revealed that only UNC5C was significantly associated with the survival of patients with HNSC treated with Taxol. MMP1, ZYX and UNC5C were not significantly associated with the survival of patients with LUNG and UCEC treated with Taxol (Fig. 4). The results indicated that MMP1 and ZYX may be specific in Taxol resistance in EOC, while the common resistance occurrence and mechanisms of UNC5C in EOC and HNSC remain to be explored.

Expression of MMP1 and ZYX was increased in A2780/Taxol cells, while UNC5C expression was decreased. The protein expression of MMP1 and ZYX was significantly increased in A2780/Taxol cells ($P<0.05$ and $P<0.01$, respectively), while the expression of UNC5C was significantly decreased ($P<0.05$; Fig. 5A). The same results were obtained by immunofluorescence and immunohistochemistry (Fig. 5B and C), and were consistent with the RNA-seq data. This demonstrated the reliability of the selection of Taxol resistance-associated gene targets through RNA-seq, and the stability and accuracy of the multiple validation work. These *in vitro* cellular results confirmed that the expression of MMP1 and ZYX was significantly upregulated, and the expression of UNC5C was significantly downregulated in A2780/Taxol cells, demonstrating that the three gene targets are potential and promising molecular markers of Taxol resistance in EOC.

Discussion

The standard treatment for EOC is surgery and chemotherapy. However, surgery for advanced-stage ovarian cancer often leads to severe post-operative complications, including patient mortality or the impossibility of the administration of subsequent oncological treatments, which can directly affect the survival rate (38). By contrast, treatment with chemotherapeutic drugs is safer for patients. However, Taxol is clinically ineffective as it often induces drug resistance, leading to multidrug resistance. Therefore, in the present study, Taxol resistance-related gene targets in patients with EOC were selected to break through the reversal of drug resistance,

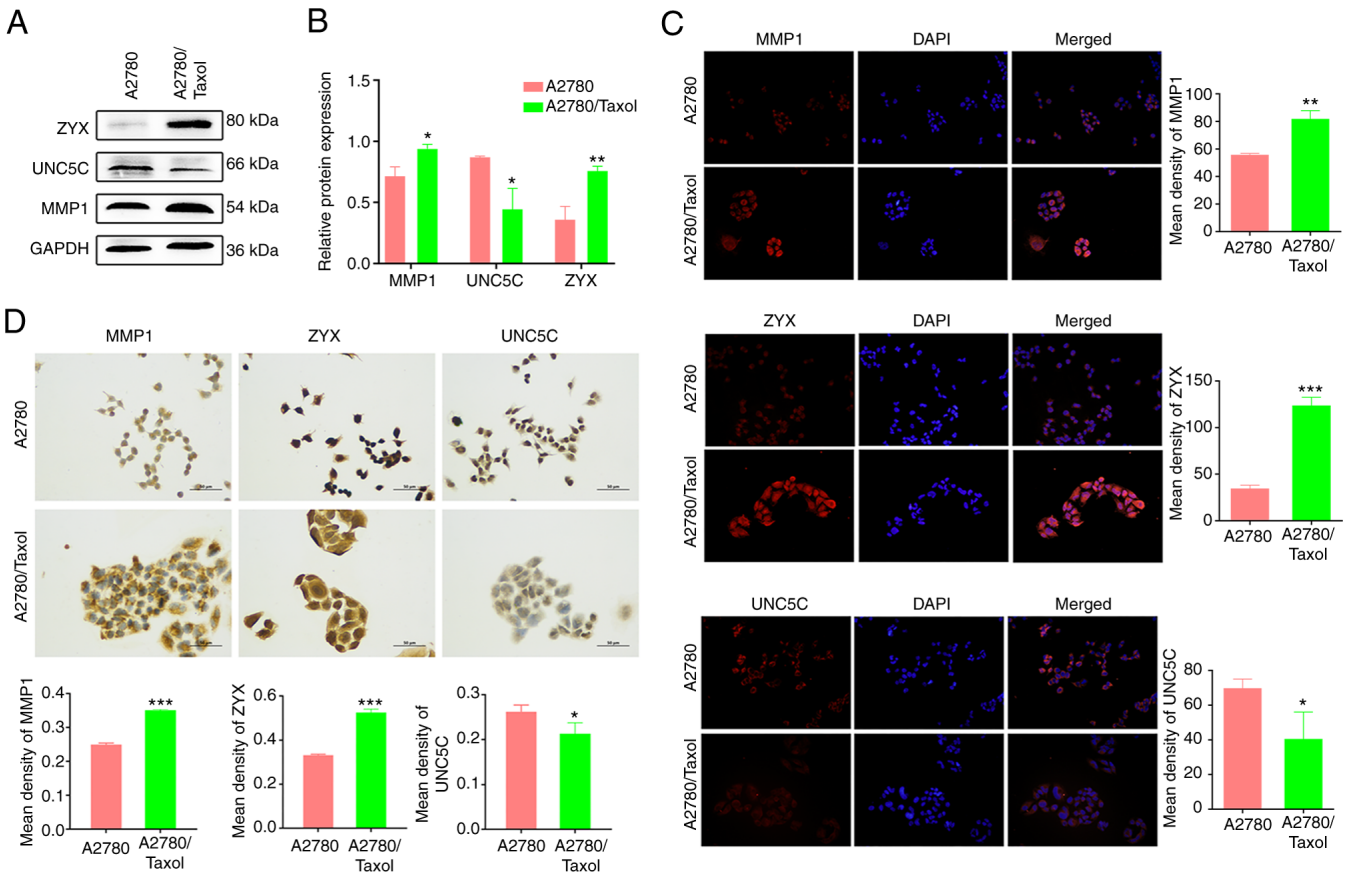

Figure 5. MMP1, ZYX and UNC5C expression was verified at the cell level *in vitro*. (A) Western blotting expression bands. (B) Western blotting gray scale statistical analysis. (C) Immunofluorescence hybridization and statistical analysis of fluorescence intensity. Magnification, x400. (D) Immunohistochemical staining and statistical analysis of the staining intensity. Magnification, x400. The results were repeated three times and the average was calculated. *P<0.05, **P<0.01, ***P<0.001 vs. A2780. ZYX, zyxin; MMP, matrix metalloprotease; UNC5C, Unc-5 netrin receptor C.

promote the clinical efficacy of Taxol and ultimately improve the survival rate of patients with EOC.

In the present study, it was found that DEGs were most significantly enriched in the focal adhesion pathway. Focal adhesions are subcellular structures that provide strong adhesion to the extracellular matrix (ECM) and serve as scaffolds for a number of signaling pathways involving integrins or mechanical forces applied to cells. Currently, focal adhesions have been revealed to be a key determinant of cell migration and play a critical role in promoting tumor cell invasion (39). The most significant enrichment results in the GO analysis of DEGs were also associated with cell connection or migration, indicating that DEGs may contribute to Taxol resistance in EOC through cell migration.

Through Kaplan-Meier survival analysis and KEGG pathway enrichment analyses of the DEGs, three Taxol resistance-related gene targets were finally obtained: MMP1, ZYX and UNC5C. MMP1 can affect ECM and basement membrane degradation or increase AKT phosphorylation to activate the AKT pathway, leading to cell migration (40,41). ZYX is an adhesion protein that affects cell adhesion and cytoskeletal rearrangement, leading to cell proliferation and migration (42,43). UNC5C affects FAK and FYN activity, and mediates cell migration by promoting integrin-dependent cell adhesion and increasing skeletal rearrangements. The downregulation of UNC5C may also activate the PI3K/AKT pathway and MMP9 expression, leading to cell migration and proliferation (30,44). Moreover, FYN interacts with PXN, both of which affect cell migration (45). Combined with the results of PPI network analysis in the present study, the potential mechanism by which MMP1, ZYX and UNC5C induce the development of EOC Taxol resistance by promoting cell migration was obtained (Fig. 6). In addition, MMP1 was upregulated in A2780/Taxol cells; however, MMP1 overexpression increased the OS of Taxol-treated patients with EOC. It was hypothesized that MMP1 upregulation activated the AKT pathway to induce mitophagy, promoting cell apoptosis, thus producing a potent self-protective effect on the organism (46). This eventually revealed the development of Taxol resistance in patients with EOC, but an increase in survival. The downregulation of UNC5C resulted in Taxol resistance; however, the increase in the OS of patients with EOC may be due to the activation of AKT, similar to MMP1. The present study analyzed the association between the upregulation of ZYX expression and the OS of patients with EOC treated with Taxol; the results revealed a significant reduction in OS which was anticipated considering that ZYX upregulation could initiate the cell migration pathway that induced Taxol resistance and thus hindered the chemotherapeutic effect. These results indicated that ZYX may be used as a potential marker of Taxol resistance.

In conclusion, the present study demonstrated that Taxol resistance in A2780/Taxol cells originated from the activation of molecular mechanisms related to cell migration.

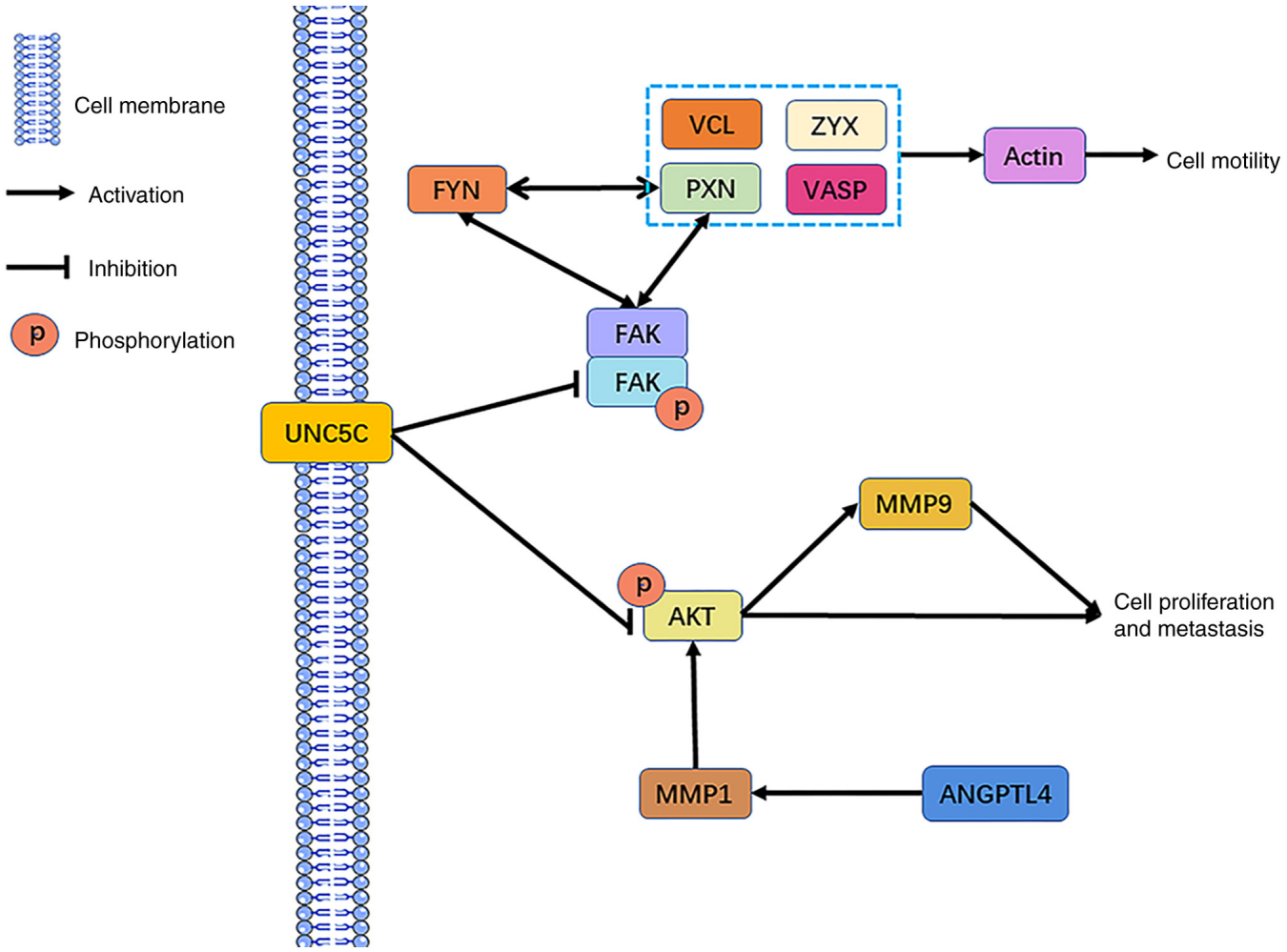

Figure 6. Potential mechanisms of MMP1, ZYX and UNC5C in Taxol resistance. ZYX, zyxin; MMP, matrix metalloprotease; UNC5C, Unc-5 netrin receptor C; p, phosphorylated; VCL, vinculin; PXN, paxillin; VASP, vasodilator stimulated phosphoprotein; FAK, protein tyrosine kinase 2; ANGPTL4, angiopoietin like 4.

Epithelial-mesenchymal transformation (EMT) is considered a promoter of metastasis, during which cancer cells acquire mobility and the ability to migrate from the primary site (47). Simultaneously, EMT mediates the generation of chemical resistance in cancer (48). In the process of enhanced cell migration, there is an inevitable generation of an EOC cell population in the EMT transition state (49), which leads to the development of Taxol resistance. On the other hand, it has been shown that physical confinement during cancer cell migration triggers therapeutic resistance (50). These factors confirm the credibility of the results of the present study.

In the present study, the three Taxol resistance-related gene targets MMP1, ZYX and UNC5C in EOC A2780/Taxol cells were selected using RNA-seq and bioinformatics analysis, and validated *in vitro* in cellular experiments. The present study provides novel drug resistance molecular targets and insights for their clinical application in patients with EOC with Taxol-induced multidrug resistance. These targets may enhance the efficacy of treatment and improve the prognosis of patients.

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Availability of data and materials

The RNA-seq data are available in the GEO database (accession no. GSE230667; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE230667).

Authors' contributions

RY, HZ, ZC, TZ, PW, HL, YH, CZ, XW and YZ contributed to the study conception and design. Experimental design, manuscript writing and experimental cost management were performed by YZ, XW, RY and HZ. RY, ZC, TZ and PW conducted the experiments and collected the data. Statistical

analysis was performed by RY, HL, YH and CZ. RY and YZ confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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