

Exploration of the sequential gene changes in epithelial ovarian cancer induced by carboplatin via microarray analysis

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Abstract. The purpose of the current study was to explore the carboplatin-induced sequential changes in gene expression and screen out key genes, which were associated with effects of carboplatin on epithelial ovarian cancer (EOC). The microarray dataset GSE13525 was downloaded from the Gene Expression Omnibus database, including 6 EOC cell samples separately treated with carboplatin at 24, 30 and 36 h (case group), and 6 samples treated with phosphate-buffered saline at the same time points (control group). A total of 3 sets of differentially expressed genes (DEGs) were respectively identified in case samples at 24, 30 and 36 h compared with the control group via the Limma package, and separately recorded as DEG-24, DEG-30 and DEG-36. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the overlapped DEGs were performed via the Database for Annotation, Visualization and Integrated Discovery. The protein-protein interaction (PPI) network was constructed and analyzed by Cytoscape software. In addition, the survival curves were drawn to illustrate the association between the expression levels of certain critical genes and the prognosis of EOC. A total of 170, 605 and 1043 DEGs were separately obtained in DEG-24 DEG-30 and DEG-36, and 110 overlaps were identified. The overlaps were enriched in 77 GO terms and 3 KEGG pathways. A total of 152 pairs were involved in the PPI network, and the abnormal expression levels (high or low) of *c-Jun* and *cyclin B1* (*CCNB1*) would reduce the survival time of patients with EOC. The study indicated that *c-Jun* and *CCNB1* may be the prognostic biomarkers of EOC treated with carboplatin, and certain pathways (such as p53 signaling pathway, cell cycle and mitogen-activation

protein kinase signaling pathway) may be involved in carboplatin-resistant EOC.

Introduction

Ovarian cancer is a malignancy with one of the poorest prognoses, which was reported to rank as the fifth leading cause of cancer in women, with ~140,200 deaths annually worldwide (1). Epithelial ovarian cancer (EOC) is one of the most frequently observed types of gynecological cancer, accounting for 85-90% of cases of ovarian cancer (2). EOC is commonly diagnosed at an advanced stage, resulting in a poor 5-year overall survival rate of 25-30% (3). The main reasons for the poor prognosis lie in the difficult to identify clinical features, early lymph metastasis and common recurrence. In addition, EOC presents with a variety of clinical manifestations, genetic mutations and tumor morphologies, which add further difficulty to the diagnosis and treatment (4).

Carboplatin [diammine (1,1-cyclobutanedicarboxylato) platinum (II)] is one of the most promising second generation platinum compounds. In clinical trials, carboplatin has been demonstrated to be as active, however exhibits less nephrotoxicity and neurotoxicity than cisplatin in previously untreated patients with advanced ovarian cancer (5). Despite the initially high response rate to carboplatin, the relapse rate in ovarian cancer is high and numerous patients will experience recurrence within 6 months, which leads to no improvement in the long-term survival rate (6). Platinum resistance, which predominantly includes carboplatin resistance and cisplatin resistance, is considered as the main reason for the unsatisfactory curative effect, and has led to widespread concerns in EOC (7,8). Peters *et al* (9) identified that carboplatin-resistant vs. -sensitive ovarian cancer cells differentially expressed genes (DEGs) were associated with apoptosis, cell-cell communication, cell adhesion, DNA repair and cell proliferation. However, fewer biomarkers were identified of carboplatin resistance and the specific mechanism remains unclear. Therefore, further potential key genes associated with effects of carboplatin on EOS are urgently required in order to confirm, and further explore the mechanisms of carboplatin resistance. In the present study, carboplatin-induced sequential gene expression changes in EOS were identified and analyzed via microarray analysis, in order to screen out certain biomarkers or pathways of EOS that may be involved in the mechanism of carboplatin resistance.

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Materials and methods

Microarray data. The expression profile of GSE13525 (10) was downloaded from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/). There were 12 EOC cell samples in this profile, including 6 samples treated with carboplatin at 24, 30 and 36 h, with 2 samples at every time point (case group), and 6 samples treated with phosphate-buffered saline at the same time points (control group). Here, EOC cell samples were 36M2 cell lines, which were sensitive to carboplatin. Detection of this profile was performed based on the platform of GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array (Affymetrix, Inc., Santa Clara, CA, USA).

Data pre-processing. For the expression profile, the original data were converted into a recognizable format with the affy package (11). The method of Robust Multi-array Average (12) was used for normalization and logarithmic conversion. If multi-probes corresponded to a gene symbol, the average value was regarded as the gene expression value.

Identification and comparison of DEGs. Subsequent to the data pre-processing, DEGs were selected out using Limma (13) package according to the criteria: $P < 0.05$, \log_2 (fold-change) > 0.05 . In the current study, 3 sets of DEGs were obtained, including DEGs in EOC cell samples treated with carboplatin compared with the control group at 24, 30 and 36 h, respectively, which were separately recorded as DEG-24, DEG-30 and DEG-36. The 3-set DEGs were compared and the overlapped DEGs were screened out. In addition, the cluster analysis of the overlapped genes was conducted.

Functional and pathway enrichment analysis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the overlapped DEGs were performed via the Database for Annotation, Visualization and Integrated Discovery (<http://david.abcc.ncifcrf.gov/>) (14). The GO terms and the KEGG pathways were screened out with the criteria $P < 0.05$.

Construction of the protein-protein interaction network and the survival curve. The interactions among the overlapped genes were explored with the Search Tool for the Retrieval of Interacting Genes/Proteins database (string-db.org) (15). Subsequently, the protein-protein interaction (PPI) network was constructed by Cytoscape software (16). Certain critical nodes with higher degrees were analyzed, and the 'degree' represented the connections with other nodes. In addition, the interactions between the expression values of the critical nodes and the survival period were evaluated with the KMplot software version 4.7.2 (ChinaUnix; www.chinaunix.net), and the survival curves were plotted. In addition, correlation analysis between some important nodes and the outcome of EOC was performed.

Results

DEGs and overlaps. A total of 170,605 and 1,043 DEGs were obtained in DEG-24 DEG-30 and DEG-36, and the Venn diagram is presented in Fig. 1. It was clearly identified that there were 110 overlaps in the 3-set DEGs, and 40 out of the

Table I. 40 out of the 110 overlapping DEGs.

Gene	logFC-24	logFC-30	logFC-36
c-Jun	0.597575	1.328237	1.18567
ATF3	1.23664	2.04034	2.048266
MYC	0.897095	1.506238	1.185579
SMYD3	-0.79469	-0.98629	-0.9974
SUGCT	-0.7813	-1.11645	-0.83396
CCNB1	-0.7313	-0.56078	-0.51498
H3F3A	-0.68925	-0.68752	-0.86869
ZFH4-AS1	-0.68093	-0.60716	-0.61631
SLIT2	-0.67813	-0.85581	-0.76238
TENM2	-0.65069	-0.82711	-1.25469
EID2B	-0.64698	-0.52262	-0.61981
GPC6	-0.6393	-0.95796	-1.33614
TYRP1	-0.63771	-0.85061	-1.41218
COX7B2	-0.60476	-0.96575	-1.20234
CCDC102B	-0.59926	-0.99286	-0.83222
SLC39A11	-0.58811	-0.80546	-0.8992
FAM155A	-0.57958	-0.5765	-0.61568
DIAPH2	-0.57649	-0.60056	-0.74229
ARL15	-0.57305	-0.57539	-0.95364
ZNF804A	-0.57214	-0.73618	-1.06175
RBMS3	-0.56804	-0.72509	-0.66383
COLEC12	-0.5643	-0.56611	-0.92667
FAM172A	-0.54727	-0.73332	-0.70188
FRMPD4	-0.54134	-0.65706	-0.60094
MROH2A	-0.53617	-0.53163	-0.79999
CSN3	-0.53327	-0.55616	-0.52556
TMEM117	-0.52405	-0.78864	-1.25632
NRXN3	-0.5218	-0.62797	-0.83785
ALG14	-0.51912	-0.69366	-0.92383
LINC01279	-0.51733	-0.85673	-1.16606
SPA17	-0.50974	-0.62461	-0.80034
RNASE4	-0.50843	-0.63322	-1.26577
ROBO1	-0.50546	-0.82298	-1.17568
DLGAP5	-0.50254	-0.62455	-0.62672
CDH13	-0.50095	-0.80013	-1.29452
SLC25A25	0.501864	0.517077	0.791164
RELB	0.504759	1.161993	1.440501
E2F8	0.508001	0.684447	0.689356
FAM53C	0.509583	0.939359	0.974481
NFKBIE	0.525352	1.418745	1.240866

DEGs, differentially expressed genes; logFC-24, the log (fold-change) in DEG-24; logFC-30, the log (fold-change) in DEG-30; logFC-36, the log (fold-change) in DEG-36.

110 overlaps (arbitrarily selected) were presented in Table I. In addition, the heatmap of the overlaps was presented in Fig. 2.

GO terms and KEGG pathways. The overlaps were enriched in 77 GO terms and 3 KEGG pathways [p53 signaling pathway, cell cycle and mitogen-activated protein kinase (MAPK)

Table II. The top 10 most significant GO terms of the overlapping differentially expressed genes.

Category	Term	Count	P-value
GOTERM_CC_5	GO:0005634~nucleus	45	4.27E-05
GOTERM_BP_5	GO:0051173~positive regulation of nitrogen compound metabolic process	14	1.24E-04
GOTERM_BP_5	GO:0048660~regulation of smooth muscle cell proliferation	5	1.72E-04
GOTERM_BP_5	GO:0031328~positive regulation of cellular biosynthetic process	14	2.29E-04
GOTERM_BP_5	GO:0009891~positive regulation of biosynthetic process	14	2.64E-04
GOTERM_BP_5	GO:0043065~positive regulation of apoptosis	11	2.68E-04
GOTERM_BP_5	GO:0043068~positive regulation of programmed cell death	11	2.83E-04
GOTERM_BP_5	GO:0010942~positive regulation of cell death	11	2.94E-04
GOTERM_CC_5	GO:0043231~intracellular membrane-bounded organelle	58	3.03E-04
GOTERM_BP_5	GO:0006355~regulation of transcription, DNA-dependent	23	6.52E-04

GO, Gene Ontology.

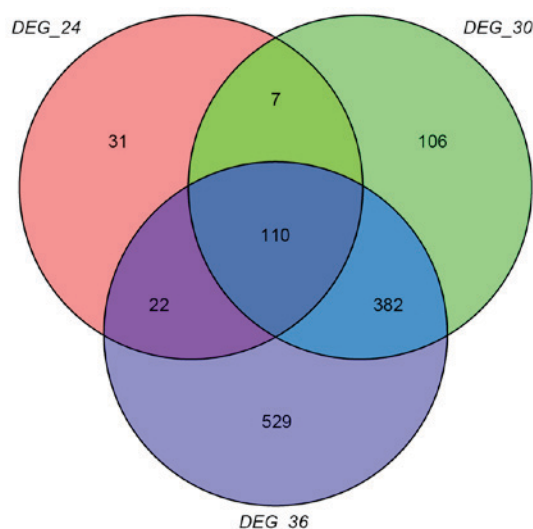


Figure 1. Venn diagram of DEG-24, DEG-30 and DEG-36. DEG, differentially expressed gene.

signaling pathway], and the top 10 most significant GO terms were exhibited in Table II.

The PPI network and survival curves. The PPI network of the overlaps was established and exhibited in Fig. 3, including 152 interaction pairs. The top 30 nodes with high degrees were presented in Table III (e.g. *c-Jun* and *CCNB1*). In addition, the survival curves were drawn to demonstrate the associations between the gene expression levels and the prognosis of EOC for *c-Jun* and *CCNB1*, respectively. The two survival curves were presented in Figs. 4 and 5. It was clear that the high or low expression probability of *c-Jun* and *CCNB1* was negatively associated with the survival time of patients, that is, the abnormal expression probability of *c-Jun* and *CCNB1* was positively correlated with a poor outcome of EOC.

Discussion

Platinum drugs, such as cisplatin and carboplatin, have been most frequently used for treatment of ovarian cancer. However,

platinum resistance has severely limited its efficacy, which is a major clinical problem requiring a solution. In the present study, the carboplatin-induced sequential genes expression changes of EOC were analyzed, and 3 KEGG pathways of overlaps were obtained, including the p53 signaling pathway, cell cycle and MAPK signaling pathway. Certain studies have indicated that these pathways were involved in the platinum resistance of ovarian cancer. One study reported that chaetoglobosin K induced G₂ cell cycle arrest through a p53-dependent pathway in cisplatin-resistant ovarian cancer cells (17). An additional study drew a similar conclusion that theaflavin-3, 3'-digalate induced G₂ cell cycle arrest through the protein kinase B/MDM2/p53 pathway in cisplatin-resistant ovarian cancer cells (18). Meng *et al* (19) hypothesized that ovarian cancer cells expressing *aldehyde dehydrogenase 1 family member A1* may maintain platinum resistance by altered regulation of cell cycle checkpoints and DNA repair network signaling. MAPKs regulate diverse cellular programs including embryogenesis, proliferation, differentiation and apoptosis based on cues derived from the cell surface and the metabolic state and environment of the cell (20). They are activated by dual phosphorylation of threonine and tyrosine in response to a wide array of extracellular stimuli (21). Results of a previous study indicated that cisplatin activated p38 MAPK in all of the cell lines tested, and carboplatin could induce activation of p38 MAPK (22,23). The p38 MAPK pathway was considered as a specific target for cisplatin-based therapy with clinical implications. In addition, MEK inhibition could overcome cisplatin resistance conferred the son of sevenless/MAPK pathway activation in squamous cell carcinoma (24). In the present study, the overlapped DEGs were enriched in p53 signaling pathway, cell cycle and the MAPK signaling pathway. Therefore, it was suspected that these three pathways may be involved in the carboplatin resistance of EOC, although further research and clinical verifications were necessary to confirm it.

The PPI network of the overlaps was analyzed, and *c-Jun* and *CCNB1* were the top 4 nodes with the highest degrees (Table III). In human ovarian cancer, the overexpression of *fucosyltransferase 1 (FUT1)* was associated with advanced pathological stages and involved in cell proliferation, migration and invasion (25-27). Gao *et al* (28) reported that *c-Jun* could

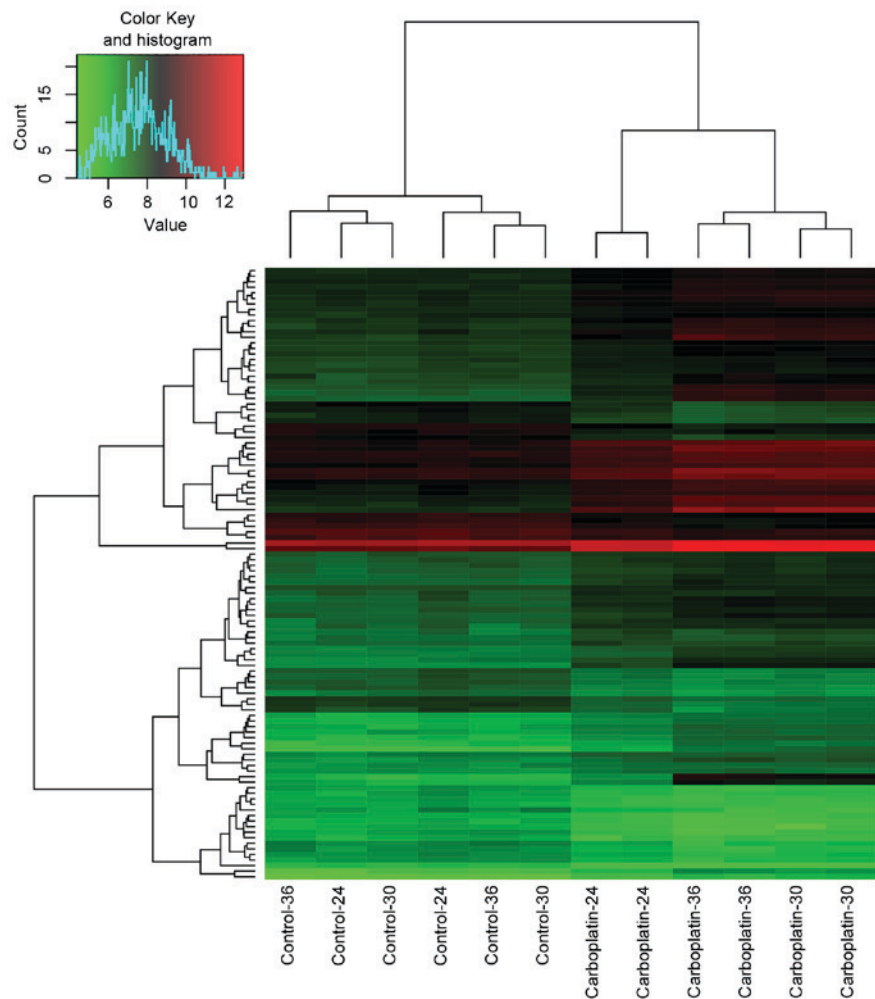


Figure 2. Heatmap of the overlapping differentially expressed genes.

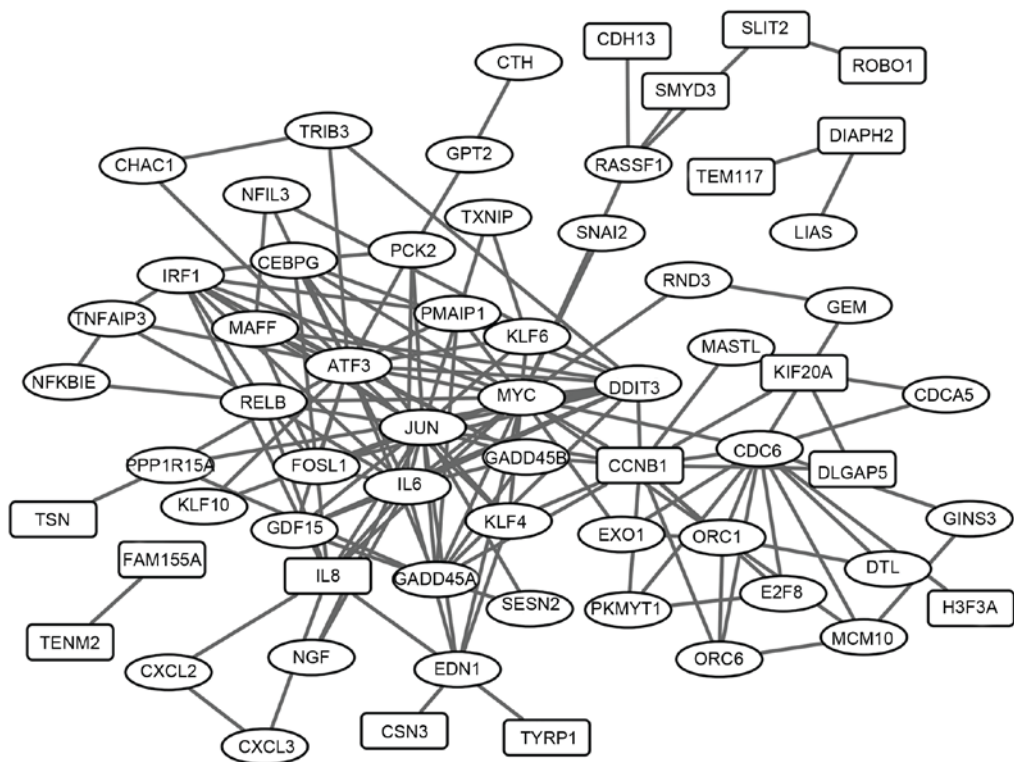


Figure 3. The protein-protein interaction network of the overlapping differentially expressed genes.

Table III. The top 30 nodes with higher degrees in the PPI network.

Gene	Degree
c-Jun	22
ATF3	21
MYC	20
CCNB1	14
CDC6	13
DDIT3	13
IL6	13
GADD45A	10
IRF1	10
FOSL1	9
IL8	9
RELB	8
CEBPG	7
EDN1	7
ORC1	7
GADD45B	6
KLF4	6
GDF15	5
MAFF	5
PCK2	5
KLF6	4
MCM10	4
ORC6	4
PMAIP1	4
PPP1R15A	4
RASSF1	4
TNFAIP3	4
E2F8	3
EXO1	3
KIF20A	3

PPI, protein-protein interaction.

transcriptionally modulate *FUT1* expression in ovarian cancer, implicating the potential application of *c-Jun* inhibitors for human ovarian cancer therapy. Echevarría-Vargas *et al* (29) reported that the c-Jun N-terminal kinase 1/c-Jun/microRNA-21 pathway contributed to the cisplatin resistance of ovarian cancer cells, and the activation of *c-Jun* was closely associated with the prognosis. In addition, the present study identified that abnormal expression of *c-Jun* was positively correlated with a poor outcome of EOC (Fig. 4). Therefore, *c-Jun* may be a potential target for the prognosis of EOC. Similarly, *CCNB1* encoded G₂/mitotic-specific cyclin-B1, a member of the highly conserved cyclin family, whose members were characterized by a marked periodicity in protein abundance through the cell cycle. As abovementioned, cell cycle may contribute to the carboplatin-resistance of EOC. A previous study suggested that sulforaphane induced cell cycle arrest in the G₂/M phase via the blockade of *CCNB1*/cyclin-dependent kinase 1 in human ovarian cancer cells (30). An additional study observed

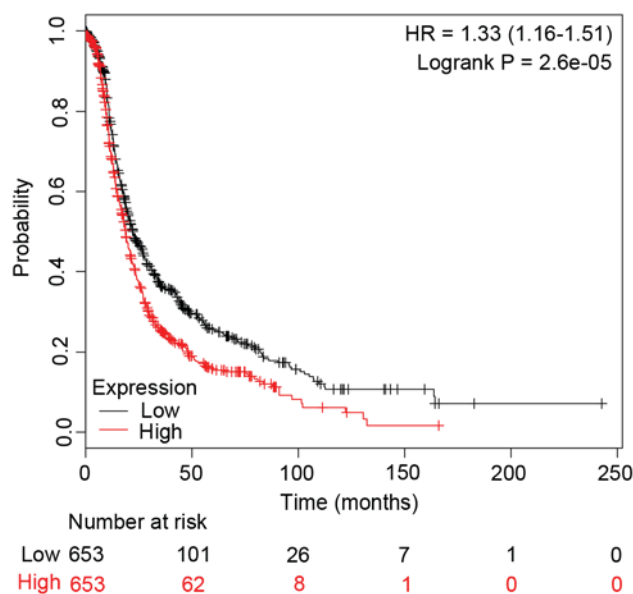


Figure 4. The survival curve between the expression of JUN and the prognosis of epithelial ovarian cancer.

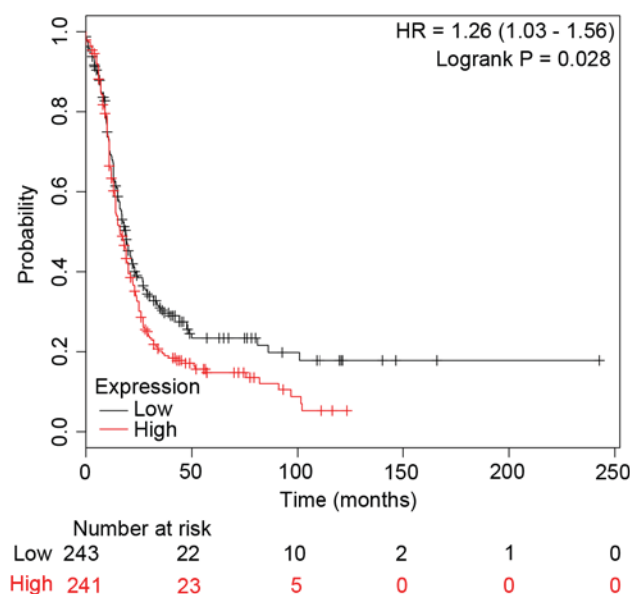


Figure 5. The survival curve between the expression of cyclin B1 and the prognosis of epithelial ovarian cancer.

nuclear *CCNB1* was overexpressed in ovarian tumors and associated with a low potential for malignance, however, this was not the case in EOC. Thus, it is suggested that *CCNB1* may not be suitable targets for EOC treatment (31). The present study indicated that *CCNB1* was differentially expressed in carboplatin-resistant EOC cells, and the differential expression of *CCNB1* was closely associated with the low survival rate (Fig. 5). Therefore, *CCNB1* may be a potential marker for the prognosis of EOC, although further investigation into whether different expression levels or different treatments would affect *CCNB1* expression levels in EOC are required.

In conclusion, the results of the current study suggested that *c-Jun* and *CCNB1* may be prognostic biomarkers of EOC, and

certain pathways (including p53 signaling pathway, cell cycle and MAPK signaling pathway) may contribute to carboplatin resistance of EOC.

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