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 respectively identified in DEG-24, DEG-30 and DEG-36. 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.

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, llog2 (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 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)
signal 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

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 2. Heatmap of the overlapping differentially expressed genes.

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

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.

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

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