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Confluence analysis of multiple omics on platinum resistance of ovarian cancer

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Summary

Objective: The study aimed to provide novel insight into the mechanism of platinum resistance of ovarian cancer. *Materials and Methods*: RNA-seq data ERP000710 were obtained from Gene Expression Omnibus database, including specimens from six platinum sensitive samples and six platinum tolerance samples. The author analyzed the data of the 12 samples as a whole because of the low flux sequencing. Single nucleotide polymorphisms (SNPs) were identified between platinum-sensitive and platinum-tolerant samples using VARSCAN, followed by functional prediction of the SNPs. After processed by Btrim software, the data were subjected to Cuffdiff for the identification of differentially expressed genes (DEGs), followed by function and pathway enrichment analysis. In addition, VARSCAN software was used to detect the specific mutations in platinum tolerance samples, combined with functional prediction of mutations. *Results*: The author obtained 38 new SNPs after excluding 22 SNP from dbSNP database and 1000 Genomes Project and found ESRP1, LDHA, DDX5, and HEXA were associated with platinum resistance of ovarian cancer. Totally, 290 upregulated and 157 downregulated genes were selected. Biological processes such as immune response, inflammatory response, and response to wounding and pathways such as cell adhesion molecules, calcium signaling, and NOD-like receptor signaling pathways were enriched with upregulated genes. Cell-cell signaling, cell morphogenesis, and basal cell carcinoma pathway were related to downregulated genes. *Conclusion*: Based on high-throughput RNA-seq data and confluence analysis of multiple omics, the author explored the biological mechanisms on platinum tolerance of ovarian cancer, which may provide new ideas and methods for further research.

Key words: Ovarian cancer; Differentially expressed genes (DEGs); Single nucleotide polymorphisms (SNPs); Function and pathways enrichment.

Introduction

Ovarian cancer is a type of tumor from uncontrolled cell growth in several different parts of ovary. It has a high incidence of morbidity and mortality among all gynecological cancers [1]. Currently, it has been reported that the five-year survival of ovarian cancer is roughly 30%, since a large proportion of cancer was diagnosed at an advanced stage [2]. Previous studies revealed that a number of possible factors are thought to be involved in the cause of ovarian cancer, such as age (especially in the elder infertile women) [3], family history of ovarian cancer or breast cancer [4], and abdominal distension. In addition, as far as we know, hereditary forms of ovarian cancer by far can be caused by mutations in specific genes (most notably BRCA1 and BRCA2) [5]. However, only ten percent of ovarian cancer has a genetic link.

Treatment for ovarian cancer involves surgery, chemotherapy, a combination of surgery with chemotherapy, and sometimes radiotherapy [6]. The kind of treatment depends on many factors, including the type, stage and grade of ovarian cancer, as well as the general health of the patient. Currently, surgery is the first choice for the treatment of ovarian cancer [7] while the developmental combination chemotherapy regimen of MECCA (consisted by mitomycin C, etoposide, cisplatin, and carboplatin) was utilized for the purpose of significantly longer survival after the primary cytoreductive surgery [8]. However, intrinsic or acquired resistance of cell to cisplatin limits the effect of chemotherapy in cancer [9]. Early studies suggest that cisplatin enters the cells (malignant or non-malignant cells from cancer patients) and forms platinum-DNA adduct that reacts with nucleophilic sites in cellular macromolecules [10]. The objective of this adduct is to trigger cell cycle arrest and apoptosis. There are many reasons that may lead to resistance to cisplatin: increased DNA repair ability, decreased intracellular concentration because of decreased drug uptake, and increased reflux or increased inactivation through sulfhydryl molecules [9, 10]. It is reported that about 25% of patients exhibit primary resistance at an early stage of chemotherapy, and for the other 75%, approximately 15% to 20% of them resisted to chemotherapeutic cisplatin adducts after relapse [11]. Consequently, platinum resistance is a core problem in treatment.

For an improved understanding of platinum resistance mechanisms, in the present study, the author screened specific mutations in platinum resistance samples by analyzing

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the mRNA expression profile data of ovarian cancer from Expression Omnibus (GEO) database. Also, the author predicted specific mutation functions of platinum resistance samples and discussed the biology functions and pathways of the screened differentially expressed genes (DEGs). This research has a potential to explore platinum resistance mechanism of ovarian cancer, which will provide a theoretical guidance for effective therapies, or for approaches that can improve therapeutic responses to the treatment of ovarian cancer.

Materials and Methods

RNA-seq data of ovarian cancer

The mRNA expression profile data of ovarian cancer were obtained from NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) Gene Expression Omnibus (GEO) database (access number: ERP000710) [12, 13], and the Platform was Illumina Genome Analyzer IIx. A total of 12 cell specimens from two cases of high-grade serous ovarian carcinoma were acquired before and after clinical platinum resistance developed in patients, including six platinum-sensitive samples and six-platinum tolerance samples. Due to the data deficiency in each sample, the six platinum-sensitive samples were regarded as one group, while the six platinum-tolerant samples were the other group. The author analyzed the information of the 12 samples in the two groups.

Data processing and genome mapping

Btrim [14] software was used to preprocess the raw data. A number of five consecutive bases were treated as a window before the author calculated the average weight. If the average weight was less than 20, the author trimmed the end of the sequence as well as the sequence whose reads length was less than 35 bp. Afterwards, Ensembl GRCh37 was used for reference sequence and tophat2 [15] software was utilized for genome mapping. Only the reads which mapped to specific genome locations were retained.

Screening for new single nucleotide polymorphisms (SNPs)

Samtools [16] was applied for the purpose of processing the mapped data into mpileup format. Then, through software VARSCAN [17], the author analyzed the mutations. The mutation selection criteria is zero frequency in the platinum-sensitive samples whereas > 0.2 frequency in the samples of platinum resistance. Moreover, SNPs annotation was obtained after the screening by Seattle [18], followed by removing SNPs from dbSNP database and the 1000 Genomes Project. Retained SNPs were new mutations namely, the specific mutations of platinum-resistant samples.

Functional prediction of specific mutations

In terms of SNPs located on genes, the author queried the functions of SNP located genes in NCBI, and forecasted the possible influence of new SNPs on gene function. As for the SNPs located between genes, according to ENCODE data, were analyzed for the influence they made at genome regulation level.

Differentially expressed genes (DEGs) analysis

Cuffdiff [19] was utilized to identify DEGs. To ensure the results reliability, genes only with the |fold change| value > 2, p-value < 0.05 and FPKM (reads per kilobase of exon model per million mapped reads) ≥ 1 at least in one sample were selected as DEGs.

Table 1. — *Function distributions of new SNPs.*

Function	Number
Intron	10
Intergenic	5
Coding-synonymous	5
Missense	9
Missense-near-splice	5
Utr-3	4
Total	38

Table 2. — *Missense SNP loci.*

Chromosome	Position	GeneList
5	176764384	LMAN2
7	134851617	C7orf49
8	95677177	ESRP1
10	135123755	ZNF511
11	18421080	LDHA
12	6484002	SCNN1A
15	72645480	HEXA
17	62498131	DDX5
19	6677984	C3

Function and pathway enrichment analysis

Gene Ontology (GO) [20] analysis was used for functional annotation of upregulated and downregulated genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool [21]. Through the gene-annotation enrichment analysis, it is possible to reduce the dimension of the data and increase the likelihood for researchers to identify the most relevant biological processes they need [20]. A p-value < 0.05 and count value > 2 was chosen as the cut-off criterion. Moreover, DAVID online tool was utilized to analyze Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in which DEGs were enriched [22]. The count number > 2 and p-value < 0.05 were chosen as cut- off criteria.

Results

Specific mutations screening of drug resistance samples

Based on VARSCAN, the author selected 59 specific mutations of platinum resistance samples. Afterwards, annotation of the mutation sites was obtained, and 22 SNPs from dbSNP database and 1000 Genomes Project were excluded. The author finally obtained 38 new SNPs. The function distributions of the 38 new SNPs are shown in Table 1.

Functional prediction of specific mutations

Initially, the author conducted mutation functional prediction of nine missense SNP loci (Table 2), and found epithelial splicing regulatory protein 1 (ESRP1), lactate dehydrogenase A (LDHA), hexosaminidase A (HEXA), and (DEAD (Asp-Glu-Ala-Asp) box helicase 5) (DDX5) were closely related to platinum resistance. Next, the author analyzed the other 29 new SNPs, and found that these loci did not cause protein structure changes while they were likely to

Table 3. — *Top ten ranked biological processes enriched of upregulated DEGs.*

Term	Count	Genes	*p*-value
GO:0007610~behavior	23	EGR1, CXCL1, S100P, CCL2, IL8, S100A9, OBP2B, CX3CL1, CCL28, DDO, PLAUR, CXCL10, SAA2, CCL20, SAA1, CXCL16, GRIN2D, GRPR, ADRA1B, CHRNB4, IL1B, CHRNB2, PLAU	5.99E-09
GO:0007626~locomotory behavior	18	CXCL1, CCL2, IL8, S100A9, CX3CL1, CCL28, PLAUR, CXCL10, SAA2, CCL20, SAA1, CXCL16, GRIN2D, ADRA1B, CHRNB4, IL1B, CHRNB2, PLAU	6.33E-09
GO:0042330~taxis	14	CXCL1, CCL2, IL8, S100A9, CX3CL1, CCL28, CXCL10, PLAUR, SAA2, CCL20, SAA1, CXCL16, IL1B, PLAU	1.77E-08
GO:0006935~chemotaxis	14	CXCL1, CCL2, IL8, S100A9, CX3CL1, CCL28, CXCL10, PLAUR, SAA2, CCL20, SAA1, CXCL16, IL1B, PLAU	1.77E-08
GO:0006955~immune response	26	CXCL1, IFIH1, CCL2, VTCN1, SUSD2, RSAD2, TNFSF14, IL32, CX3CL1, CCL28, TNFSF18, IFI35, CXCL10, IL23A, CCL20, PGLYRP2, IL1B, IL1A, EBI3, ICAM1, IL8, IL1RN, OASL, UNC13D, CXCL16, IFI6	8.57E-08
GO:0030595~leukocyte chemotaxis	8	CCL2, SAA2, IL8, SAA1, CXCL16, S100A9, IL1B, CX3CL1	1.16E-07
GO:0050900~leukocyte migration	9	ICAM1, CCL2, SAA2, IL8, SAA1, CXCL16, S100A9, IL1B, CX3CL1	1.61E-07
GO:0060326~cell chemotaxis	8	CCL2, SAA2, IL8, SAA1, CXCL16, S100A9, IL1B, CX3CL1	1.70E-07
GO:0006952~defense response	24	CXCL1, IFIH1, CCL2, NMI, IL8, S100A8, IL1RN, S100A9, RSAD2, IL32, CX3CL1, CXCL10, LGALS3BP, UNC13D, IL23A, SAA2, CCL20, SAA1, CXCL16, PGLYRP2, SERPINA3, IL1B, SERPINA1, IL1A	1.72E-07
GO:0006954~inflammatory response	17	CXCL1, CCL2, NMI, IL8, S100A8, S100A9, IL1RN, CXCL10, UNC13D, IL23A, SAA2, CCL20, SAA1, SERPINA3, IL1B, SERPINA1, IL1A	4.39E-07
GO:0009611~response to wounding	20	CXCL1, CCL2, NMI, IL8, S100A8, IL1RN, S100A9, PLAUR, CXCL10, UNC13D, IL23A, SAA2, CCL20, SAA1, GRIN2C, SERPINA3, IL1B, SERPINA1, PLAU, IL1A	4.21E-06

DEGs: differentially expressed genes; GO: Gene Ontology; Count: gene numbers; BP: biological process; FDR: false discovery rate.

Table 4. — *Biological processes enriched of downregulated DEGs.*

Term	Count	*p*-value	Genes
GO:0007267~cell-cell signaling	10	0.003291	FGF5, CBLN1, BMP2, CXCL14, APOE, LPAR3, SLC22A3, GJA1, KCNQ2, WNT6
GO:0006928~cell motion	7	0.03385	NDN, FOXJ1, TUBBP1, TNR, VIM, SCNN1G, ISL1
GO:0000902~cell morphogenesis	6	0.035021	BMP2, NDN, FOXJ1, TNR, GJA1, ISL1
GO:0048858~cell projection morphogenesis	5	0.036334	NDN, FOXJ1, TNR, GJA1, ISL1
GO:0008285~negative regulation of cell proliferation	6	0.036849	BMP2, GPC3, NDN, FOXJ1, APOE, GJA1
GO:0030030~cell projection organization	6	0.039506	NDN, FOXJ1, TNR, LPAR3, GJA1, ISL1
GO:0032990~cell part morphogenesis	5	0.041612	NDN, FOXJ1, TNR, GJA1, ISL1
GO:0048754~branching morphogenesis of a tube	3	0.043402	BMP2, GPC3, MYCN
GO:0042063~gliogenesis	3	0.043402	FGF5, NDN, SOX11

DEGs: differentially expressed genes; GO: Gene Ontology; Count: gene numbers; BP: biological process.

influence genome regulation level. However, sites with regulatory function were not found in these 29 SNPs through ENCODE data.

Identification of DEGs

Based on the Cuffdiff, the author obtained 447 DEGs of ovarian cancer, of which 290 were upregulated DEGs and 157 were downregulated DEGs.

Function and pathway annotation of DEGs

To explore the roles of DEGs in platinum resistance in ovarian cancer, the author adopted both GO biological process enrichment analysis and KEGG pathway enrichment analysis to analyze upregulated and downregulated DEGs. The enriched GO categories of up-regulated genes, such as behavior, chemotaxis, immune response, leukocyte chemotaxis, and leukocyte migration are listed in Table 3, while the biological processes with a highly significant correlation with downregulated genes, such as cell-cell signaling, cell motion, cell morphogenesis, and negative regulation of cell proliferation are listed in Table 4. Through KEGG pathway enrichment analysis, the author found four significant pathways that were enriched by upregulated genes, including chemokine signaling pathway, ribosome, cell adhesion molecules (CAMs), calcium signaling pathway, and NOD-like receptor signal-

Table 5. — *Enriched pathways of upregulated DEGs.*

Term	Count	*p*-value	Genes
hsa04062: chemokine signaling pathway	9	2.41E-03	CXCL1, CCL2, IL8, CCL20, ADCY9, CXCL16, CX3CL1, CCL28, CXCL10
hsa03010: ribosome	6	4.82E-03	RPS27, RPL41, RPS29, RPL34, RPL39, RPL13AP5
hsa04514: cell adhesion molecules (CAMs)	7	6.43E-03	CLDN8, ICAM1, CLDN9, CLDN3, ITGB8, CLDN6, SDC4
hsa04020: calcium signaling pathway	7	2.41E-02	ADCY9, TNNC1, GRIN2C, GRIN2D, GRPR, ADRA1B, ITPR3
hsa04621: NOD-like receptor signaling pathway	4	4.42E-02	CXCL1, CCL2, IL8, IL1B

DEGs: differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; Count: gene numbers.

Table 6. — *Pathway enriched of downregulated DEGs.*

Term	Count	P-value	Genes
hsa05217: basal cell carcinoma	3	2.94E-02	BMP2, WNT6, FZD7

DEGs: differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; Count: gene number.

ing pathway (Table 5). Furthermore, downregulated genes were significantly enriched in basal cell carcinoma pathway (Table 6).

Discussion

At present, with the development of high-throughput sequencing technologies and large-scale clinical trials being carried out, pharmacogenetics has played an increasingly important role in the field of cancer chemotherapy. Due to the better understanding of clinical drug resistance-related SNP, researchers have been exploring the reality of the growing list of genetic mechanisms of cancer and chemoresistance [23]. Nowadays, RNA-Seq can carry on the gene expression difference studies in the whole-genome level [24]. Research used this method to show that quantitative analysis is more accurate and reliable, because the analysis testing range is wider of higher repeatability [25]. In addition to analysis of gene expression, RNA-Seq can also detect new SNP, transcripts, splice variants, and provide an allele-specific gene expression [26]. The wider dynamic range and a smaller false positive of RNA-Seq mean that the reproducibility of RNA-Seq data should be higher than the chip [25]. In this study, RNA-Seq data were used to detect DEGs and SNPs in platinum resistance of ovarian cancer.

It is clear that the mechanism of ovarian cancer resistant to platinum drug is quite complex, and it has a great many unresolved problems that need to be assessed. Studies have demonstrated that DNA repair undoubtedly affects resistance to platinum-based DNA-damaging agents, and processes involved DNA repair that contribute to more than one drug resistance phenotype [10, 27]. ESRP1 belongs to the RNA-binding protein family which is critical in post-trascriptional control of RNAs, such as RNA splicing, mRNA stabilization, and mRNA localization [28]. In this paper, in querying gene functions of SNPs located, the author found that it is not easy for ESRP1 to regulate RNA splicing, and the mutation loci of N260Y was on the RNA recognized motif of ESRP1. It has also been reported that ESRP1 is associated with the cancer metastasis of lung and is considered as a potential therapeutic target for the prevention of metastasis [29]. Thus, we could hypothesize that mutation of N260Y loci in ESRP1 might lead to the destruction of RNA recognition function, and some changes in the form of gene splicing, and then result in the occurrence of drug resistance. In addition, LDHA, DDX5, and HEXA gene were also found to have mutated in platinum resistance of ovarian cancer. Specifically, LDHA catalyzes L-lactic acid and NAD to generate anaerobic pyruvate and NADH; moreover, recent studies have shown that LDHA inhibition contributes to increased apoptosis through production of reactive oxygen in certain cells, and LDHA might play an role in the metastasis of tumors [12, 30-32]. LDHA may mediate platinum resistance by influencing tumor growth and metastasis. DDX5 is a kind of RNA helicase which plays various roles in cell proliferation, and several studies have revealed that it is associated with tumor initiation and progression [33, 34].

The present author obtained a total of 447 significant DEGs, including 290 upregulated DEGs and 157 downregulated DEGs. In the meantime, by GO biological process enrichment analysis and KEGG pathway enrichment analysis, they screened several processes and pathways which may involve in mechanism of ovarian cancer platinum resistance. Of all biological progress that upregulated DEGs enriched in, immune response, inflammatory response and response to wounding appeared to play a crucial role in drug resistance in ovarian cancer cells. Consistently, Interferon regulatory factor 1(IRF1), an important transcription factor (TF) in the regulation of immune, is linked to platinum sensitivity in high-grade serous ovarian cancer [35]. The involvement of IL-6 in platinum resist-

ance in ovarian cancer has also been validated, and is mediated by upregulation of cellular inhibitor of apoptosis 2 (cIAP-2) expression [36]. In addition, it has been revealed that cell adhesion pathways may contribute to the tolerance in ovarian cancer cells [37]. Appropriate calcium intake is believed to reduce the risk of ovarian cancer [38]. Increasing studies have suggested that NOD-like receptors are associated with reproductive diseases, such as endometrial cancer [39, 40]. The study also suggested that upregulated calcium signaling pathway and NOD-like receptor signaling pathway might be related to the platinum resistance in ovarian cancer.

With the evaluation of detection technology and standardized test results, scientists will be able to detect more DEGs and pathways which are platinum-resistant related in peripheral blood of tumor patients, make a preliminary estimate of the sensitivity to chemotherapeutic drugs, and then design the best treatment plan, which will enable possible individualized chemotherapy with a real significance.

Conclusion

In the present study, based on high-throughput second generation sequencing data, the author used dynamic gene expression profile combined with different levels of biological information. The author identified important mutations such as ESRP1, LDHA, DDX5, and HEXA in this study, might be potential molecular targets to develop novel therapeutic approaches for alleviating platinum resistance. Significant biological functions such as immune response, inflammatory response, and response to wounding and critical pathways which included cell adhesion molecules, calcium signaling pathway and NOD-like receptor signaling pathways might be involved in platinum resistance in ovarian cancer. The more multiple factors that contribute to platinum resistance that are analyzed, the more reasonable therapeutic options for patients with recurrent ovarian cancer are expected to be found.

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