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Original Research

Label free quantitative proteomics analysis on the cisplatin resistance in ovarian cancer cells

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Abstract: Quantitative proteomics has been made great progress in recent years. Label free quantitative proteomics analysis based on the mass spectrometry is widely used. Using this technique, we determined the differentially expressed proteins in the cisplatin-sensitive ovarian cancer cells COC1 and cisplatin-resistant cells COC1/DDP before and after the application of cisplatin. Using the GO analysis, we classified those proteins into different subgroups bases on their cellular component, biological process, and molecular function. We also used KEGG pathway analysis to determine the key signal pathways that those proteins were involved in. There are 710 differential proteins between COC1 and COC1/DDP cells, 783 between COC1 and COC1/DDP cells treated with cisplatin, 917 between the COC1/DDP cells and COC1/DDP cells treated with LaCl3, 775 between COC1/DDP cells treated with cisplatin and COC1/DDP cells treated with cisplatin and LaCl3. Among the same 411 differentially expressed proteins in cisplatin-sensitive COC1 cells and cisplain-resistant COC1/DDP cells before and after cisplatin treatment, 14% of them were localized on the cell membrane. According to the KEGG results, differentially expressed proteins were classified into 21 groups. The most abundant proteins were involved in spliceosome. This study lays a foundation for deciphering the mechanism for drug resistance in ovarian tumor.

Key words: COC1 cell line; COC1/DDP cell line; cisplatin, Maxquant; GO analysis; KEGG analysis.

Introduction

Ovarian cancer (OC) is the 9th most common cancer in the female population, the 2nd most common gynecological cancer after cancer of corpus uteri and the most lethal cause of death among gynecological malignancies (1). In the recent years, survival rate of ovarian cancer patients are increased due to the surgical techniques and the platinum-based treatment (2). However, the prognosis of ovarian cancer patients remains poor. Approximately 60% of patients with advanced disease at primary diagnosis will experience recurrent disease within 5 years from diagnosis, and a majority of patients relapse and eventually succumb to this disease (3). For the platinum-based chemotherapy, the development of cisplatin resistance has long been a focus of ovarian cancer research because many patients will ultimately develop disease that is unresponsive to therapy despite the clinical effect of initial platinum-based therapy is apparent (4-7). Therefore, it is necessary and urgent to elucidate the mechanism of resistance to platinum-based treatment of ovarian cancer.

Mass spectrometry (MS)-based proteomics can identify and quantify thousands of proteins in complex samples (8-10). In label-free quantitative proteomics analysis, peptide intensities measured during individual liquid chromatography (LC) runs are compared across runs (11, 12). Label-free quantitation is attractive because it can be applied to any proteomic sample without the need of introducing isotopes for quantitation. In this study, we used this technique to determine the differentially expressed proteins in the cisplatin sensitive and resistant ovarian cells before and after the treatment of cisplatin. Furthermore, we used GO and KEGG analysis to classified those proteins and found the potentially involved critical signaling pathway. Through this study, new insights were provided into disclosing the possible mechanism of resistance to cisplatin in the treatment of ovarian cancer.

Materials and Methods

Protein sample preparation

Proteins were extracted from four different cell groups, including COC1 cells, COC1/DPP cells, COC1 cells with cisplatin, and COC1/DDP cells with cisplatin. Then, proteins were mixed with STD buffer and incubated in boiled water bath for 5 min. The protein was broken by ultrasonication. The supernatant was kept by centrifuge. The protein concentrations were measured by BCA assay.

FASP enzymolysis

Proteins with 100mM DTT were boiled in water bath for 5min. Then, UA buffer (8M Urea,150mM TrisHCl pH8.0) was added to collect the precipitation by centrifuge. The precipitation was sequentially mixed with IAA, NH_4HCO_3 and Trypsin to collect the supernatant by centrifuge. OD280 value was measured to quantify the protein.

LCMS/MS analysis

2μg protein after enzymolysis was analyzed by LCMS/MS. Analysis was conducted under optimized conditions for each component. Chromatography was performed on EASY-nLC1000. A gradient chromatographic system was utilized using A aqueous formic acid (0.1%): 2% methyl cyanide and B aqueous formic acid (0.1%): 84% methyl cyanide. A simple linear gradient was run at a flow rate of 300 μL/minute over 105 minutes from 0% B to 45% B, 5 minutes from 45% B to 100% B, and maintained at 100% B for 10 minutes. A 10-minute period of equilibration to initial conditions was performed between each run. Positive mode mass spectroscopic detection was performed by Q-Exactive.

Maxquant label free analysis

All the MS data were analysis by Maxquant software 1.3.0.5. The database is uniprot_human_142483_20150901.fasta. The major parameters are as follows: Main search ppm: 6; Missed cleavage: 2; MS/MS tolerance ppm: 20; De-Isotopic: TRUE; enzyme: Trypsin; database: uniprot_human_142483_20150901.fasta; Fixed modification Carbamidomethyl (C); Variable modification: Oxidation (M), Acetyl (Protein N-term); Decoy database pattern: reverse; Lable free quantification (LFQ): TRUE; LFQ min ratio count: 1; Match between runs: 2min; Peptide FDR: 0.01; Protein FDR: 0.01.

Perseus statistical and bioinformatic analysis

Maxquant data were analyzed by Perseus software 1.3.0.5.

Results

Differential protein expression in different groups

LCMS/MS analysis identified 22046 different peptides in 3537 protein groups. Then, we analyzed the number of differentially expressed proteins in cells under various conditions. There are 710 differential proteins between COC1 and COC1/DDP cells, 783 between COC1 and COC1/DDP cells treated with cisplatin, 917 between the COC1/DDP cells and COC1/DDP cells treated with LaCl3, 775 between COC1/DDP cells treated with cisplatin and COC1/DDP cells treated with cisplatin and LaCl3. We also found 411identical differential expressed proteins between COC1 cells treated with and without cisplatin, and 536 identical differential expressed proteins in COC1/DDP cells.

GO (Gene Ontology) analysis

Among the same 411 differentially expressed proteins in cisplatin-sensitive COC1 cells and cisplain-resistant COC1/DDP cells before and after cisplatin treatment, 14% of them were localized on the cell membrane (Fig. 1A). This indicated proteins involved in transport and metabolism is critical to the mechanism of cisplatin-resistance. Those membrane proteins form the transport channels, playing roles in the uptake and excretion of medicine. This will alter the drug metabolism so as to elicit the primary and acquired drug resistance. Besides the cell membrane, 14% of the differentially expressed proteins are within macromolecular complex, 24% within the cell organelles, 9% in the extracellular region, and 12% in the membrane enclosed lumen (Fig.1A).

Next, we determined the differentially expressed proteins based on the biological process. Proteins involved in metabolic process and single-organism process accounted for 12%, and cellular process is 14% (Fig.1B). Besides, proteins in biological regulation were 10%. We also found that 6 differentially expressed proteins are related with DNA repair, and 9 with cellular response to DNA damage stimulus. Cisplatin-induced DNA damage was recovered in the cisplatin-resistant cells. Thus, the differential expression of those proteins leads to or closely related to the drug resistance.

Thirdly, determined the differentially expressed proteins based on the molecular function. 53% proteins function as binding protein, and 26% are involved in catalytic activity, as well as 5% are molecular function regulators (Fig.1C).

Studies indicated that proteins abundant with mercapto groups play roles in cisplatin-resistance. We detected that 52 differentially expressed proteins are related with sulfur compound biosynthetic process, indicating proteins abundant with mercapto groups could inactivate cisplatin and initiate the drug resistance. We also found 1 protein related to the apoptosis. Since deficiency in cell apoptosis signal pathway caused the resistance to cisplatin, this protein may play vital role in the development of drug resistance.

KEGG Pathway analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of databases dealing with genomes, biological pathways, diseases, drugs, and chemical substances. According to the KEGG results, differentially expressed proteins were classified into 21 groups. The most abundant proteins were involved in spliceosome (Fig.2). The other important pathways included RNA transport, oxidative phosphorylation, endocytosis, pathway in cancer, PI3K-Akt signaling pathway, etc. Then, we further analyzed the proteins involved in the pathway in cancer. As shown in Fig.3, all the differen-

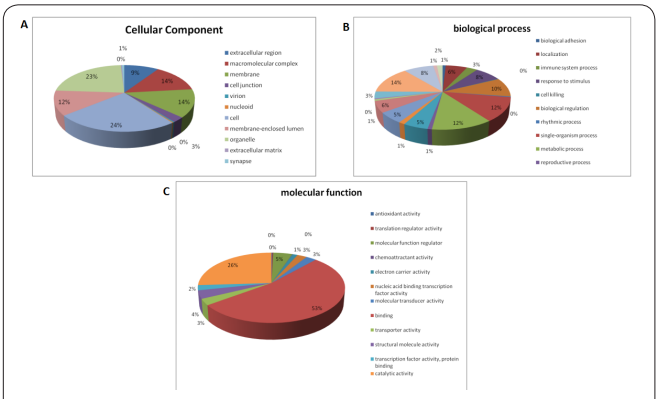

Figure 1. A. Differentially expressed proteins in the cisplatin-sensitive ovarian cancer cells COC1 and cisplatin-resistant cells COC1/DDP before and after the cisplatin treatment were classified under the category of cellular component using the GO analysis; B. Differentially expressed proteins in the cisplatin-sensitive ovarian cancer cells COC1 and cisplatin-resistant cells COC1/DDP before and after the cisplatin treatment were classified under the category of biological proces; C.Differentially expressed proteins in the cisplatin-sensitive ovarian cancer cells COC1 and cisplatin-resistant cells COC1/DDP before and after the cisplatin treatment were classified under the category of molecular function.

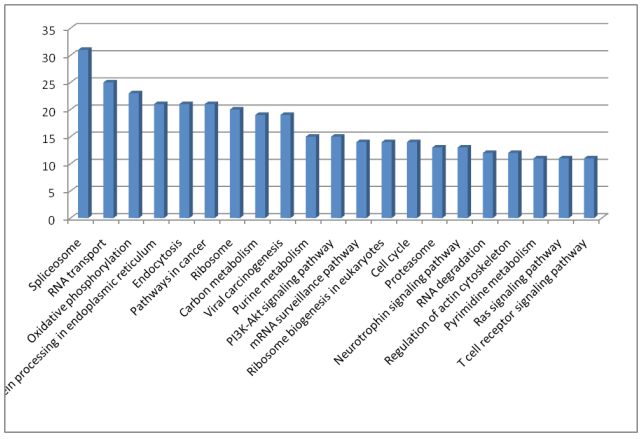

Figure 2. KEGG analysis showed the key signal pathways those identified differentially expressed proteins were involved.

tially expressed proteins were upregulated and were labeled in green, including HSP, CBL, TCP/LEF, CDK, STAT3, Ras, TCF, etc.

Discussion

Resistance to cisplatin in the treatment of ovarian cancer is a very complex process. The reported factors resulting in the development of cisplatin resistance includes increased DNA repair, decreased accumulation of the drug within the cells, and post-translational modification (13). However, the concrete mechanism is still elusive. The specific proteins responsible for the drug resistance were worthy investigation. In this study, we determined those proteins with differential expression level between the sensitive and resistant ovarian cancer cells with and without the application of cisplatin. Those proteins were also classified into different groups and their related signaling pathways were also dug. We anticipated that this study could facilitate disclosing the mechanism in the drug resistance.

In the past few years, scientists have developed and established several methods for qualitative and quantitative membrane proteomics analyses (14, 15). Among them, MS-based proteomics technology has been shown to be a powerful tool for large-scale protein identification and quantitation and been widely applied. Recent established MS-based label-free quantitative proteomics studies are more reliable, versatile, and cost-effective than the labelled quantitation (16). Furthermore, combining label-free quantitation combined with the MaxQuant approach can improve the accuracy of quantitative proteomics (17). Therefore, we used label free quantitative proteomics to analyze cisplatin resistance in the ovarian cancer. Through the GO analysis, we found the most abundant differentially expressed proteins were located on the cell membrane. Those membrane proteins could form the transport channels, playing roles in the uptake and excretion of cisplatin. Change in these proteins expression led to altering the drug metabolism so as to elicit the primary and acquired drug resistance. Some proteins were related with cellular response to DNA damage stimulus. Alteration in those proteins could cause the recovery of cisplatin-induced DNA damage, which gradually formed the drug resistance in cancer cells.

In conclusion, the resistance to cisplatin is a very complicated process, involved by multiple genes, factors and steps. This research provided the theoretical basis for the mechanism of this resistance, and more mechanism related proteins will be explored for further investigation.

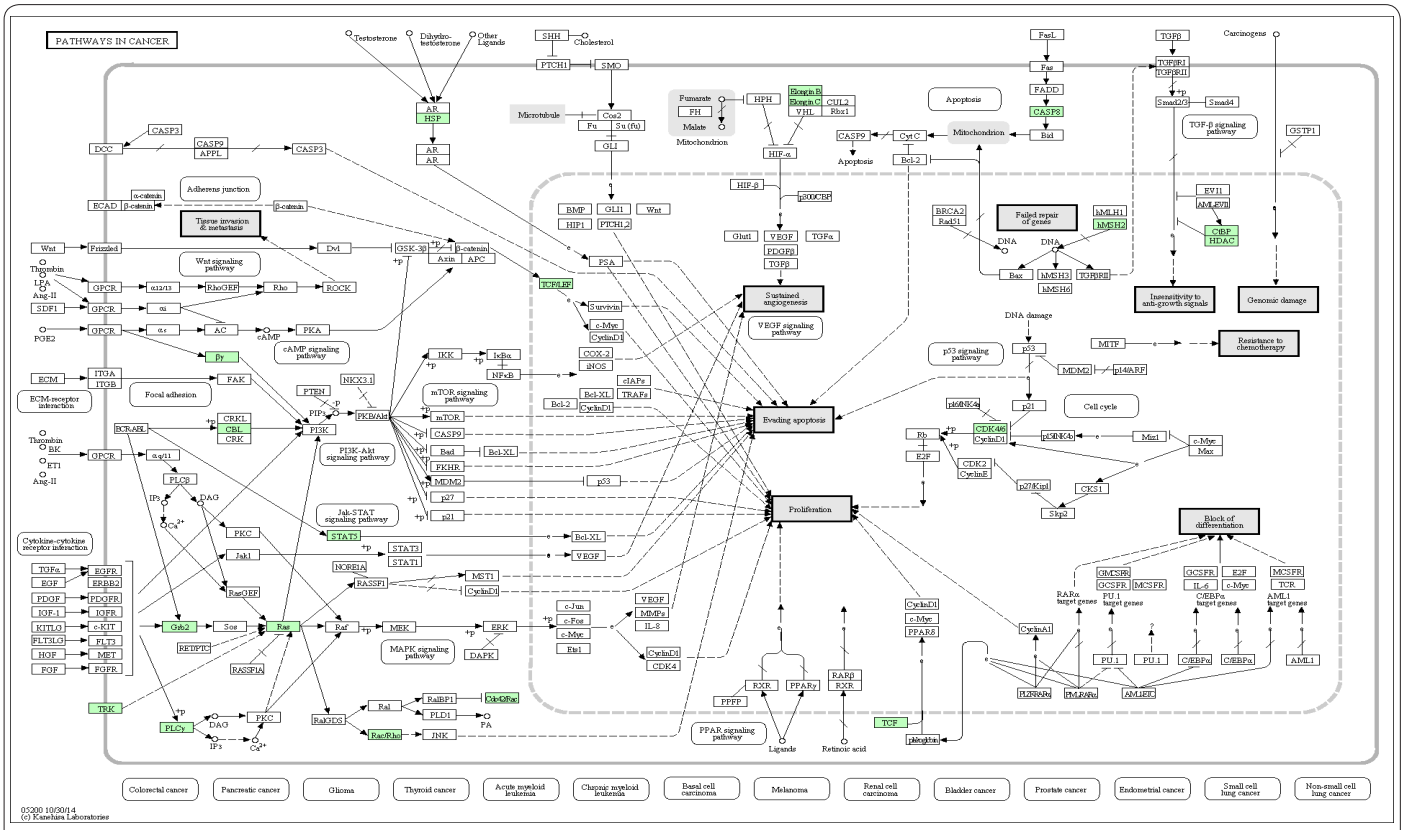

Figure 3. Differentially expressed proteins in the cancer-related pathways were identified through KEGG pathway analysis, highlighted in green frame.

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