Transcriptomic profiling of cisplatin resistance signet ring gastric carcinoma cell line

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Abstract: (1) Background: Gastric cancer is the fourth most common cancer worldwide and is the second most common cancer related death. Although cisplatin-based chemotherapy is effectively used in treatment of gastric cancer, development of drug resistance is still increasing. Cisplatin resistance is multifactorial: failure in binding of drug to its targets; enhance DNA repair, or suppression of apoptosis that antagonize cisplatin cytotoxicity. So, in this study, next generation sequencing approach and bioinformatics were used to explore the difference in gene expression level between cisplatin resistance and sensitive gastric cancer cell line (2) Methods: Cisplatin resistance gastric cancer cell line KATO/DDP was derived from parental KATOIII signet ring cell gastric carcinoma by stepwise treated with cisplatin and RNA sequencing was performed in both cell line by illumine HWI-ST1276 platform with paired-end sequencing strategy. Bioinformatics analysis was used to identify the differential genes expression and candidate genes. (3) Results: In KATO/DDP cell line, 5966 genes were differentially expressed, of which 2571 genes were upregulated while 3395 were down regulated when compared to drug sensitive cell line KATOIII. Moreover, it showed 13 hub genes UGT1A1, UGT1A10, CYP1A1, CBR3, HSD17B2, AKR1C1, AKR1C3, CXCL8, CXCL11, CXCL13, NMUR2, ADCY7 and SSTR5 that play an important role in development of drug resistance. (4) Conclusions: This study indicated that 13 candidate genes were promoting the drug resistance by increasing the metabolism of the drug, modulating immune system, and enhancing epithelial to mesenchymal transition.

Keywords: Signet ring cell gastric carcinoma; Cisplatin resistance; RNA sequencing

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1. Introduction

Gastric cancer is one of the threatening health burdens worldwide and is the fifth most common type of cancer and is now reaching to third leading cause of death worldwide. The extensive treatment may include surgery, chemotherapy, radiation therapy or immuno-therapy, either alone or in combinations. Among this treatment, cisplatin become drug of choice for chemotherapy. At first it may successfully control the tumor growth but the tumor can eventually evolve to become drug-resistant and regrow rapidly (1).Various mechanisms were proposed for cisplatin resistance including reducing intracellular accumulation of cisplatin either by reducing uptake or increasing efflux by modifying various transporters, increasing sequestration by cytoplasmic scavengers such as glutathione or metallothionines, impairment in DNA repair mechanism and defective in apoptosis signal transduction pathways generated by DNA damage(2). KATOIII gastric cancer cell line represents the signet ring gastric carcinoma which is one of the common types in gastric cancer. So, in this study we aimed to identify

the differentially expressed genes between cisplatin sensitive and resistance signet ring gastric cancer cell lines by using transcriptome sequencing and bioinformatics analysis.

2. Materials and Methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin and trypsin were purchased from Gibco (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Thermo Scientific Company (Waltham, MA, USA). Cisplatin also called cis–diamminedichloroplatinum was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The stock of cisplatin solution was prepared by using normal saline at concentrations of 3000μM.

2.2. Cell line and culture condition

KATOIII gastric cancer cells were cultured in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum and 1% (v/v) penicillin and streptomycin. These culture cells were maintained at 37°C in a 95% humidified atmosphere and 5% CO2 conditions.

2.3. Establishment of cisplatin resistance gastric cancer cell line

In order to establish acquired cisplatin resistance gastric cancer cell line, the parental KATOIII gastric cancer cell lines were stepwise treated with cisplatin concentration from 0.5 to 3 μM over 10 months and this cell line was termed KATO/DDP. Then, the resistance phenotype was maintained by 3 μM concentration of cisplatin and these cells were cultured in drug free media for at least two passages before each experiment.

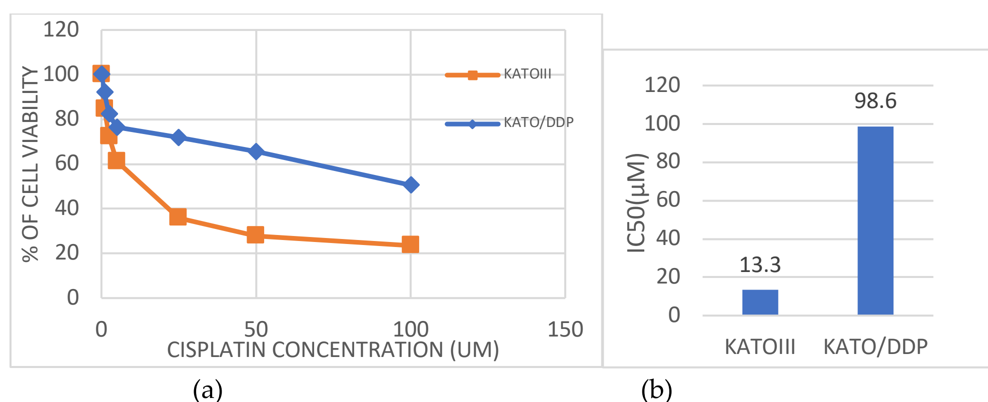
2.4.RNA isolation and transcriptomic sequencing

Nucleozol RT reagent was used to extract the total RNA.The purity and integrity of total RNA were checked by nanodrop and 1% clorox agarose gel elcetrophoresis respectively. RNA sequencing was conducted by Novogene Co., Ltd using illumina HWI-ST1276 platform with paired-end sequencing strategy. The total RNA extracted from three indepentent replicates from each cell line were sent for transcriptomic sequencing. The raw data FASTQ file were analyzed by CLC genomic work bench version 10.0 that include mapping to reference genome and counting maps to achieve the gene expression level.

3. Results

3.1. Assessment of resistance property

After 10 months stepwise treated with cisplatin, cell viability was assessed by using typhan blue cell viability assay. The cells were treated with cisplatin 0-100μM concentration and cell viability was assessed after 48hrs. IC 50 of KATOIII was 13.3±1.15μM and that of KATO/DDP was 98.6±1.52μM. The resistance ratio was determined by dividing the IC50 of resistance cell line to IC 50 of sensitive one and that was 7.4 Fig1.

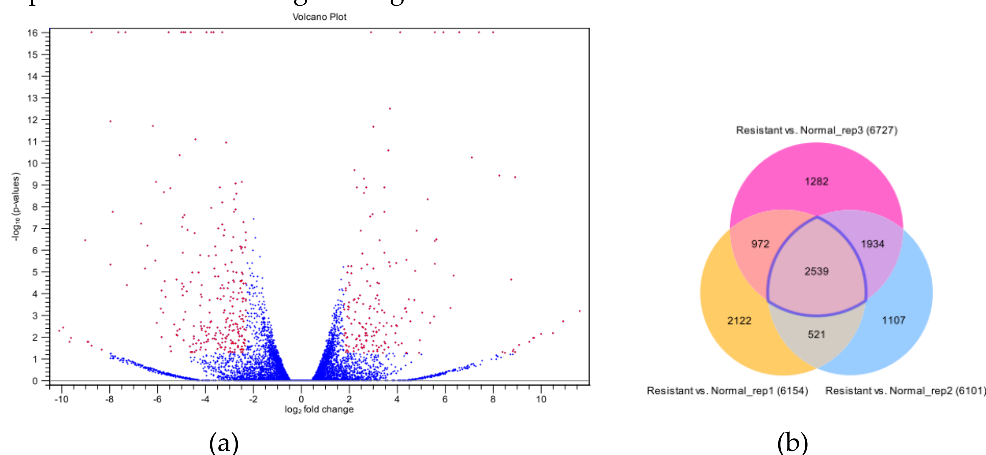


(a) (b)

Figure 1. Assessment of cell viability in gastric cancer cell line. (a) KATOIII and KATO/DDP were treated with 0-100 µM concentration of cisplatin; (b) IC50 of KATOIII and KATO/DDP cell line.

3.2. Identification of differentially expressed genes between drug resistance and sensitive cell lines.

After counting maps to achieve the genes expression level, the differential expression analysis was done by CLC plug in program. The results showed that 5966 genes were differentially expressed, of which 2571 genes were upregulated while 3395 were down regulated in KATO/DDP when $\log_2$ fold change ≥1.8 or ≤-1.8 and FDR p value <0.05 were set as a significant level Fig 2a. The Venn diagram revealed the intersection between 3 sample sets included 2539 genes Fig 2b.



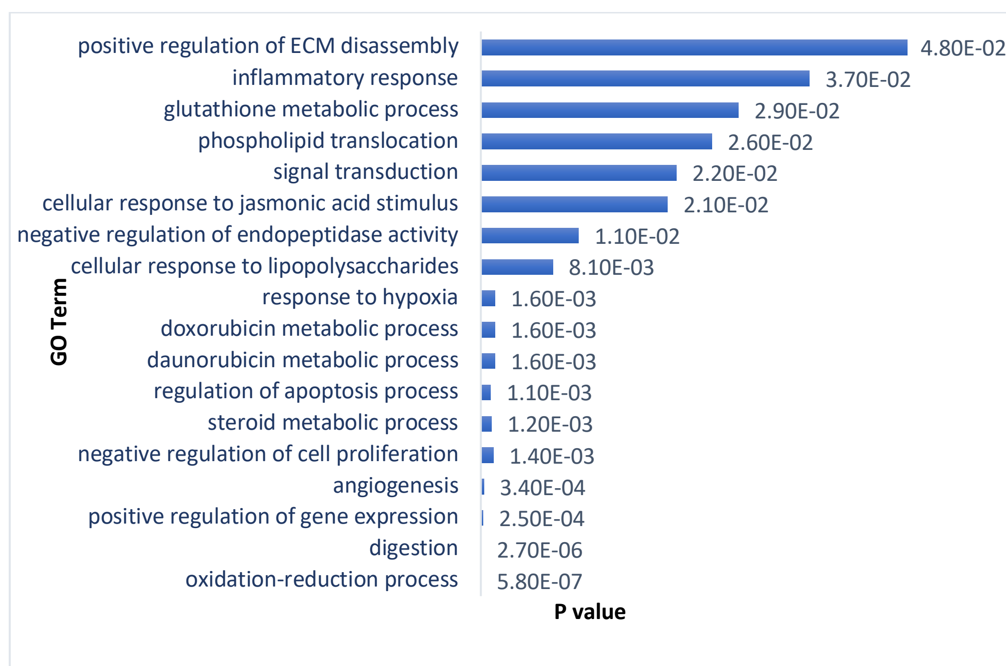
(a) (b)

Fig 2. Identification of differentially expressed genes. (a) Volcano plot of differentially expressed genes between KATO/DDP and KATOIII gastric cancer cell lines; (b) Venn diagram of differentially expressed genes between 3 sample sets.

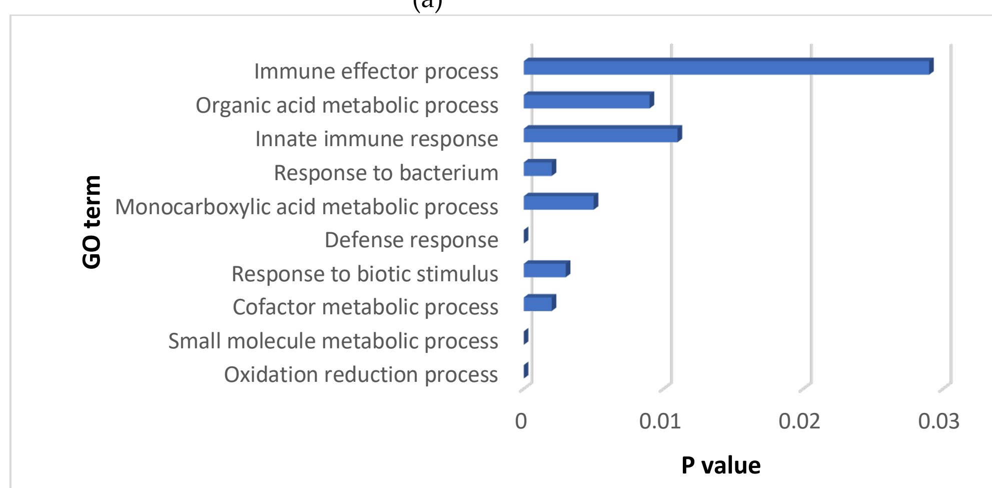
3.3. Functional annotation of differentially expressed genes.

DAVID (https://david.ncifcrf.gov/home.jsp; version 6.8), an online tool, was used to identify gene ontology molecular function, biological process and cellular component. Gene ontology biological process showed that oxidation reduction, digestion, positive regulation of gene expression, angiogenesis, negative regulation of cell proliferation and steroid metabolic process were significantly enriched Fig 3a. In gene ontology molecular function, oxidation reduction process, small molecule metabolic process, cofactor metabolic process, response to biotic stimulus and defense response were significantly enriched Fig 3b. Intracellular, extracellular space, perinuclear region of cytoplasm and basolateral plasma membrane were significantly enriched in gene ontology cellular component Fig 3c. Gene Set Enrichment Analysis (GSEA) version 4.1.0. was used to

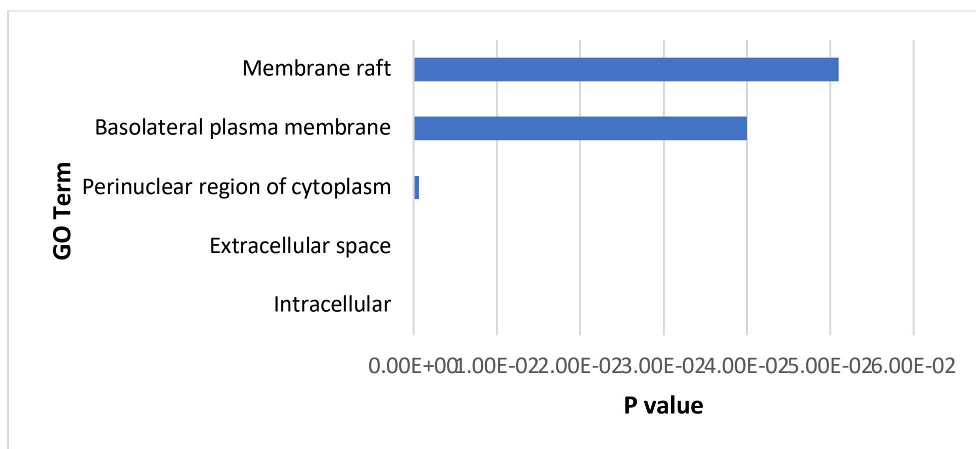
identify which process were enriched in drug resistance cell line KATO/DDP, and it confirmed that oxidation reduction process, small molecule metabolic process, cofactor metabolic process, response to biotic stimulus, defense response and monocarboxylic acid metabolic process were significantly enriched Fig 4. Cytoscape version 3.8.2. was used to construct the protein-protein interaction network with 371 nodes and 483 edges Fig 5a. The significant module was identified by using Cytoscape plug-in MCODE. It revealed 13 nodes and 36 edges with score 6 in the most significant module fig 5b, which can be considered as hub genes. These included UGT1A1, UGT1A10, CYP1A1, CBR3, HSD17B2, AKR1C1, AKR1C3, CXCL8, CXCL11, CXCL13, NMUR2, ADCY7 and SSTR5.



(a)



(b)



(c)

Fig 3. Functional annotation of differentially expressed genes by DAVID. (a) Gene ontology biological process; (b) Gene ontology molecular function; (c) Gene ontology cellular component.

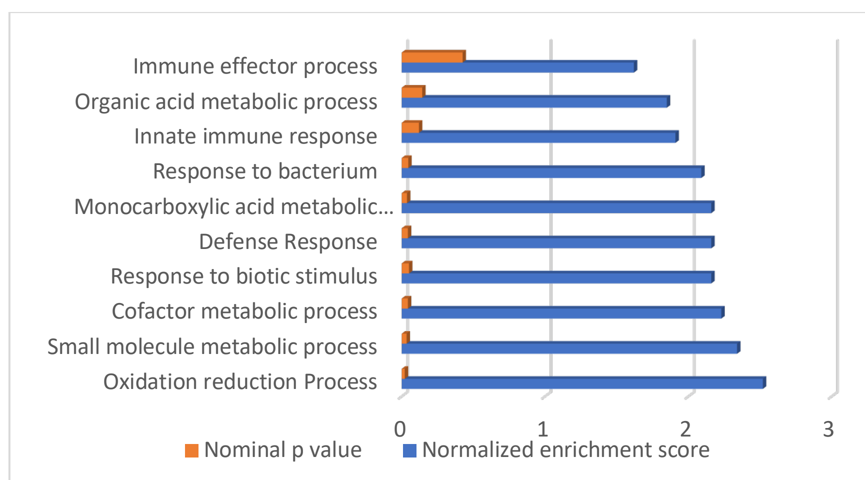
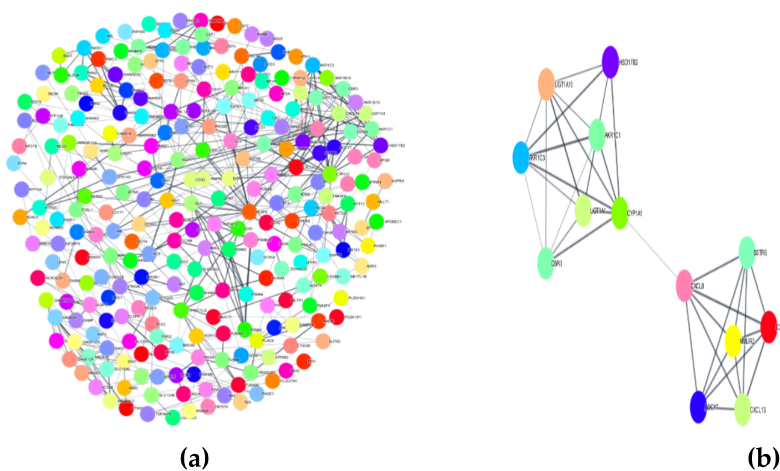


Fig 4. Gene set enrichment analysis of differentially expressed genes by GSEA version 4.1.0. *FDR q <0.25.



(a) **(b)**

Fig 5. Protein-protein interaction network construction by using cytoscape. (a) PPI network of differentially expressed genes (371 nodes and 483 edge); (b) Most significant module obtained by MCODE (13 nodes and 36 edges , score 6).

4. Discussion

In clinical management of gastric cancer, chemotherapy, has been becoming very effective against advanced stage gastric cancer. It mainly uses platinum based drugs such as cisplatin, carboplatin and oxaliplatin (3).Even with the best treatment, there is still tumor recurrence and development of drug resistance. The mechanisms of cisplatin resistance involved multiple protein as well as multiple signaling pathway. So, this study was aimed to figure out the most overwhelming pathway promoting cisplatin resistance in gastric cancer by using transcriptomic sequencing approach. Previous study stated that in non small cell lung cancer (A549 cells), periostin promotes cisplatin resistance via the activation of Stat3 and Akt and upregulation of survivin and sliencing of periostin show greater reduction of tumor volume after cisplatin treatment in xenograft tumors(4). In ovarian cancer cells (A2780/DDP and SKOV3/DDP), knockdown of NEAT1 inhibited cisplatin resistance by up-regulating miR 770-5p and down-regulating PARP1, and interference of NEAT1 decreased xenograft tumor growth by regulating miR-770-5p and PARP1 in previous study(5). So, in our in vitro study, we can achieve the possible mechanism for cisplatin which we further need to prove by in vivo study. We hope that this combined study can be apply for real clinical situation.

In this study, it showed 5966 differentially expressed genes, of which 2571 genes were upregulated**,** and 3395 genes were downregulated in drug resistance cell line, KATO/DDP when compared to parental sensitive cell line KATOIII. Then we annotated these differentially expressed genes into different pathways, it indicated that oxidation reduction, regulation of apoptosis and steroid metabolic process and drug metabolism were significantly enriched. To validate the result from DAVID gene function annotation, gene set enrichment analysis was used**,** and it revealed that oxidation reduction process, small molecule metabolic process, cofactor metabolic process and pathway concerned with immune function were identified as significant pathways. We also constructed the protein-protein interaction network for the differentially expressed genes by using Cytoscape and identified the hub genes by MCODE. This result showed 13 genes, UGT1A1, UGT1A10, CYP1A1, CBR3, HSD17B2, AKR1C1, AKR1C3, CXCL8, CXCL11, CXCL13, NMUR2, ADCY7 and SSTR5, as candidates.

UGT 1A1 is a family of UDP glucoronyl transferase which participate in phase II biotransformation reactions where substrates are conjugated with glucuronic acid to form inactive metabolites. Previous study stated that in non-small cell lung cancer patients with higher UGT1A1 expression showed lower tumor response, shorter progression free survival and overall survival in irinotecan and cisplatin combination therapy **(6)** and these genes are strongly linked to each other among their family. Nrf2, the major regulator in response to oxidative stress, regulates the expression of CBR3,carbonyl reductase 3, as an antioxidant enzyme via the activation of Nrf2/ARE signaling in colon and hepatocellular carcinoma**(7)**. Aldoketoreductase 1c subfamily members, are NADPH hydroxysteroid dehydrogenase, are also target for Nrf2 **(8)** and play a role in development of cisplatin resistance in colon cancer by neutralizing the reactive oxygen species**(9)**. This may suggest

that a group of these genes acts as a role in neutralizing the activity of chemotherapeutic drug.

Furthermore, CXCL2,11 and 13 were upregulated in GEO dataset of platinum resistance epithelial ovarian cancer although CXCL2 is a key regulator of chemokines family which enhances platinum drug resistance (10). It may consider that these genes alter the sensitivity of platinum drug by modulating the immune function. NMUR2 is a major type of neuromedin receptor which shows coexpression in endometrial cancer. Activation of neuromedin signaling pathway not only promotes EGFR driven and TGFβ receptor driven epithelial to mesenchymal transition but also favors these cells to attract niche growth factors in tumor microenvironment (11). ADCY7 has been recently described as one of the novel biomarkers in docetaxel resistance prostate cancer although the mechanism has not been explored yet (12).

5. Conclusions

Transcriptomic profiling of the cisplatin resistance gastric cancer line showed that cisplatin resistance was not only promoted by increasing metabolizing and neutralizing the drug but also modulating the immune system. It also suggested that epithelial to mesenchymal transition should also be considered as one of the mechanisms promoting drug resistance. So, further experiments are needed to prove for which can be the most overwhelming biomarkers and mechanism in the signet ring gastric cancer cell line.

Author Contributions: Conceptualization, S.Y. and P.L.(D).; methodology, N.P. and S.Y.; software, N.P.; validation, S.Y.; formal analysis, S.Y. and N.P.; investigation, N.P.; resources, P.L.(D). and S.Y.; data curation, N.P.; writing—original draft preparation, N.P.; writing—review and editing, S.Y.; visualization, S.Y.; supervision, S.Y.; project administration, S.Y.; funding acquisition, S.Y.

Funding: This research was funded by Faculty of Medicine Research Fund, Chiang Mai University.

Acknowledgments: This research study was supported by Chiang Mai University, Faculty of Medicine Research Fund.

Conflicts of Interest: The authors declare no conflict of interest.

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