Basic Study

Dysregulation of mRNA profile in cisplatin-resistant gastric cancer cell line SGC7901

Xiao-Que Xie, Qi-Hong Zhao, Hua Wang, Kang-Sheng Gu

Abstract

AIM
To explore novel therapeutic target of cisplatin resistance in human gastric cancer.

METHODS
The sensitivity of SGC7901 cells and cisplatin-resistant SGC7901 cells (SGC7901/DDP) for cisplatin were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. High-quality total RNA which isolated from SGC7901/DDP cells and SGC7901 cells were used for mRNA microarray analysis. Results were analyzed bioinformatically to predict their roles in the development of cisplatin resistance and the expression of 13 dysregulated mRNAs we selected were validated by quantitative real-time polymerase chain reaction (qRT-PCR).

RESULTS
SGC7901/DDP cells highly resistant to cisplatin demonstrated by MTT assay. A total of 1308 mRNAs (578 upregulated and 730 downregulated) were
differentially expressed (fold change ≥ 2 and P-value < 0.05) in the SGC7901/DDP cells compared with SGC7901 cells. The expression of mRNAs detected by qRT-PCR were consistent with the microarray results. Gene Ontology, Kyoto Encyclopedia of Genes and Genomes pathway and protein-protein interaction analysis demonstrated that the differentially expressed mRNAs were enriched in PI3K-Akt, Notch, MAPK, ErbB, Jak-STAT, NF-kappaB signaling pathways which may be involved in cisplatin resistance. Several genes such as PDE3B, VEGFC, IGFBP3, TLR4, HIPK2 and EGF may associated with drug resistance of gastric cancer cells to cisplatin.

CONCLUSION
Exploration of those altered mRNAs may provide more promising strategy in diagnosis and therapy for gastric cancer with cisplatin resistance.

Key words: Gastric cancer; Dysregulate; Cisplatin resistance; Microarray; Biology

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: We tested the sensitivity of human gastric cancer cells SGC7901/DDP and SGC7901 for cisplatin and compared their mRNA expression profile using a human mRNA microarray, and then performed bioinformatics analysis to depict comprehensively the properties of the differentially expressed mRNAs. Results demonstrated that the dysregulated mRNA were enriched in functions and pathways that may be involved in cisplatin resistance. Exploration of the dysregulated genes could suggest a promising strategy in diagnosis and therapy of gastric cancer with cisplatin resistance.

Xie XQ, Zhao QH, Wang H, Gu KS. Dysregulation of mRNA profile in cisplatin-resistant gastric cancer cell line SGC7901. World J Gastroenterol 2017; 23(7): 1189-1202 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i7/1189.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i7.1189

INTRODUCTION
Gastric cancer is the fourth most common cancer and the second leading cause of cancer death globally[1], and more than two thirds of patients when diagnosed with unresectable disease[2]. The 5-year overall survival rate of patients with advanced gastric cancer approximately 25%[3]. Currently, platinum-based chemotherapy regimen is the standout chemotherapy frequently used for advanced gastric cancer[4-6], and median overall survival and progression free survival was significantly longer in cisplatin-containing combination therapy compared to non-cisplatin containing regimens[6,7]. However, cisplatin-based chemotherapeutic agents are often limited in chemotherapy due to drug resistance[8,9].

Cisplatin resistance of gastric cancer is multifactorial, accumulating evidence have suggested that the aberrant expression of proteins which associated with decreased cellular accumulation, increased DNA repair capacity, increased drug inactivation[10] play important role in the acquisition of cisplatin resistance. Previous researches have shown that abnormal expression of copper transporter 1 (CTR1) and MRP2 lead to cisplatin resistance by reducing the concentration of cisplatin in cells[11-13]. Moreover, the upregulation of excision repair cross complementing 1 (ERCC1)[14], X-ray repair cross complementing 1 (XRCC1)[15] and breast cancer 1 (BRCA1)[16] have shown to be involved in cisplatin resistance by removal of Pt-DNA adducts[17,18]. Other studies have shown that downregulation of the human epidermal growth factor receptor II (ErbB2) can significantly enhanced the apoptosis-inducing effects of cisplatin in gastric cancer[19,20].

The mechanisms of cisplatin resistance are quite complex and have not been fully revealed till now, so investigation of the molecular mechanisms and biomarkers is urgently needed. This study aims to analyze mRNA expression profiles in SGC7901/DDP cells to explore more chemotherapeutic molecular targets and to guide appropriate chemotherapy for gastric cancer with cisplatin resistance.

MATERIALS AND METHODS

Cell lines and culture
The human cisplatin-resistant gastric cancer cell line SGC7901/DDP and its parental cells SGC7901 were purchased from KeyGEN Biotechnology Company (Nanjing, Jiangsu, China). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, United States) containing 10% fetal calf serum (Gibco, NY, United States) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in a humidified atmosphere with 5% CO2 at 37 ℃. Cisplatin (Sigma, CA, United States) with final concentration of 800 ng/mL was added to the culture media for SGC7901/DDP cells to maintain the cisplatin-resistant phenotype.

MTT method assay for SGC7901/DDP and SGC7901 cells viability
SGC7901/DDP and SGC7901 cells were suspended at a density of 1 × 105 cells/mL and planted into 96-well culture plate. After 24 hours, the cells were treated with freshly prepared DDP. The final concentrations were 133.34 μmol/L, 66.67 μmol/L, 6.67 μmol/L, 0.67 μmol/L and 0.067 μmol/L, because the human peak plasma concentration for DDP has been reported...
as 6.67 μmol/L\textsuperscript{[21]}. Cell viability was examined after 48 h and was determined by adding 20 μL MTT (5 mg/mL) to each well and incubated for a further 4 h. The resulting formazan crystal was dissolved by addition of 150 μL dimethyl sulfoxide (DMSO) (sigma, Germany) each well, and then plates were shaken for 10 minutes. The absorbance at 490 nm was measured by spectrophotometer (ELx 800; BioTek; Winooski, VT, United States). The inhibition of growth (IC50) for DDP was calculated by the cells relative viability. Each experiment was performed in triplicate.

**Total RNA extraction and mRNA microarray**

Cells were harvested when they had grown to 80%-90% confluency and were still in logarithmic phase. Total RNA was extracted from the three matched pairs of SGC7901/DDP and SGC7901 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's instructions. The quality of total RNA was measured by NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Waltham, MA, United States). Total RNA from three paired samples were amplified and transcribed into fluorescent cDNA, and then the fluorescent labeled samples were hybridized to the Agilent LncRNA-mRNA Human Gene Expression Microarray V4.0 (Capital Bio Corp, Beijing, China) which contains 25069 human mRNA according to the manufacturer's recommendations. The microarray was scanned by an Agilent Microarray Scanner. Image processing was conducted using Agilent Feature Extraction software and raw microarray signals normalized using Agilent Gene-Spring software. The normalized mRNA expression profiles data output was received in Excel spreadsheets. The two group of samples data were analyzed by t-test to get the P-values. FC values representing the differently expressed mRNAs between SGC7901/DDP and their parental cells. Cluster 3.0 software was performed to show differential expression patterns of mRNAs.

**Bioinformatics analysis**

Bioinformatics analysis were generated using KOBAS software and STRING 9.1 software. KOBAS software was used to analyze Ontology, Disease and pathways of the dysregulated mRNAs. KOBAS associated with 1 ontology database (Gene Ontology), 5 disease databases (OMIM, KEGG DISEASE, PID Reactome, FunDO, GAD, NHGRI) and 7 pathway databases (KEGG PATHWAY, PID Curated, PID BioCarta, BioCyc, eactome, Panther). The entire analysis process includes two steps: first, bring the input gene ID map to the gene in the databases, and then annotate pathways, disease and function of these genes involved in. Second step, compare the first step results with background (usually the entire genome of the gene, or the entire probe on the chip), and unearth statistically significant enrichment pathways, disease or function. Fisher’s exact test and χ\textsuperscript{2} test were used as statistical tests and the FDR was performed to correct the P-value\textsuperscript{[22]}. Additionally, we used STRING 9.1 software to decipher the protein-protein interaction (PPI) network of the differentially expressed proteins. The PPI network may help in understanding the molecular mechanism of cisplatin resistance. All mRNA microarray data were given by Capital Bio Corp.

**Quantitative real-time PCR validation of microarray results**

To validate the reliability of microarray analysis, we performed quantitative real-time PCR (qRT-PCR). The reverse transcription production cDNA was synthesized using oligo-dT primers and Superscript II reverse transcriptase. PCR was performed with SYBR\textsuperscript{R} Premix Ex Taq\textsuperscript{TM} (TakaRa Bio; Japan) by a Light Cycler PCR system (Agilent Technologies, Palo Alto, CA, United States) according to the manufacturer's instructions. After amplification, melting curves were analyzed. Beta-actin snRNA used as endogenous control, each sample was done in triplicate. The relative expression levels of target mRNAs were calculated using the 2^\textsuperscript{ΔΔCt} method (where ΔΔCt is the difference in threshold cycles for the ΔCt of SGC7901/DDP sample and SGC7901 sample, and ΔCt is the difference between the target gene and endogenous control beta-actin). Sequences of primers for qRT-PCR are provided in supporting Table 1.

**Statistical analysis**

MTT test and qRT-PCR statistical analysis was performed using GraphPad Prism software (v. 5.0a; GraphPad Software, La Jolla, CA, United States). We used one-way analysis of variance (ANOVA) followed by Student’s t-test to assess the statistical significance of differences between different cell groups. The threshold for statistical significance was P-values < 0.05. Fold changes of mRNAs validated by qRT-PCR in SGC7901/DDP cells compared with SGC7901 cells are shown as mean ± SD.

**RESULTS**

**Sensitivity of SGC7901/DDP and SGC7901 cells to DDP**

To determine the chemotherapy sensitivity of SGC7901/DDP and SGC7901 cell line to cisplatin, varying concentrations of cisplatin were added into the 96-well plates and incubated for 48 h. From these data, half maximal inhibitory concentration (IC50) cisplatin dose was calculated. IC50 cisplatin doses for SGC7901/DDP and SGC7901 (after 48 h in DDP-containing media) were 43.47 ± 0.21 μmol/L and 1.24 ± 0.02 μmol/L, respectively, and the resistance index for SGC7901/DDP cell lines was 35.12, confirming that these cells are refractory to cisplatin. Cell viability was checked by MTT assay (Figure 1).
Validation of microarray results by qRT-PCR of 13 mRNAs

First, we concentrated on validating the microarray results. From the abnormally expressed (P < 0.05) mRNAs obtained from the microarray analyses, we selected 8 upregulated (HIPK2, PDE3B, FGF2, TWIST1, ZEB2, VEGFC, SPHK1, BAX) and 5 downregulated (PTEN, HTRA1, CCL5, TGM2, TLR4) mRNAs for qRT-PCR validation. The relative fold-changes (SGC7901/DDP vs SGC7901) detected by qRT-PCR were consistent with the microarray results (Figure 3), indicating the dependability of our microarray platform.

Statistical analysis

To depict comprehensively the properties of the differentially expressed mRNA in SGC7901/DDP cells, GO annotation and enrichment analysis was performed to evaluate which cellular components, molecular functions and biological processes may be are affected by this dysregulation. The GO enrichment analysis showed that the differentially expressed genes were involved in a variety of functions, including locomotion, chemotaxis, cell adhesion, regulation of cell migration, extracellular matrix disassembly, response to xenobiotic chemotaxis, localization of cell adhesion and blood vessel morphogenesis (Figure 4A).

Additionally, 59 human diseases were significant enriched (P < 0.05) in five human disease databases

Validation of microarray results by qRT-PCR of 13 mRNAs

First, we concentrated on validating the microarray results. From the abnormally expressed (P < 0.05) mRNAs obtained from the microarray analyses, we selected 8 upregulated mRNAs (HIPK2, PDE3B, FGF2, TWIST1, ZEB2, VEGFC, SPHK1, BAX) and 5 downregulated (PTEN, HTRA1, CCL5, TGM2, TLR4) mRNAs for qRT-PCR validation. The relative fold-changes (SGC7901/DDP vs SGC7901) detected by qRT-PCR were consistent with the microarray results (Figure 3), indicating the dependability of our microarray platform.

Expression profile of mRNAs in SGC7901/DDP cells

To show mRNA expression profile in cisplatin-resistant SGC7901/DDP cells, we used a stringency cutoff to identify significantly differently mRNAs (P < 0.05, FC ≥ 2) and two-dimensional hierarchical clustering 3.0 to represent expression profiles between samples (Figure 2). The results indicated that 1308 mRNAs were significantly differentially expressed in SGC7901/DDP cells compared with SGC7901 cells. Among these transcripts, 578 mRNAs were upregulated, and 730 mRNAs were downregulated.
Figure 2 mRNA expression levels from microarray. A: The volcano plot image showed the mRNA expression levels of microarray in SGC7901/DDP cells compared with SGC7901 cells. Black dots: equally expressed mRNAs between SGC7901/DDP cells and SGC7901 cells (FC ≤ 2); red dots: mRNAs were over-expressed in SGC7901/DDP cells compared with SGC7901 cells (FC ≥ 2); green dots: mRNAs in SGC7901/DDP cells were down-expressed compared to SGC7901 cells (P-values < 0.05, FC ≥ 2). Fold changes of these mRNAs in SGC7901/DDP cells compared with SGC7901 cells are shown as mean ± SD; B: Two-dimensional hierarchical clustering image of the 1308 dysregulated mRNAs in the SGC7901/DDP cells compared with the SGC7901 cells, each row represents an mRNA, each column represents a sample. 7901-1, 7901-2 and 7901-3 represent the three samples of SGC7901 cells, DDP-1, DDP-2 and DDP-3 represent the three samples of SGC7901/DDP cells. Red: Higher expression levels; green: Lower expression levels.

Figure 3 Quantitative real-time polymerase chain reaction validation of the microarray results of the 13 mRNAs. Relative fold changes in expression between SGC7901/DDP cells and SGC7901 cells were in agreement with microarray.

Interaction network analysis
The STRING 9.1 software (Search Tool for the Retrieval of Interacting Genes) was used to perceive functional relations and generate networks of differential expression of proteins (Figure 6). For all of the 1002 differentially expressed proteins, we extracted a network containing 443 upregulated and 559 downregulated proteins which functionally associated with each other. We found that interacting proteins which participate in angiogenesis, toll-like receptor signaling pathway and cell adhesion had a high level of co-expression.

DISCUSSION
Cisplatin is widely used against a variety of solid neoplasms, including testicular, ovarian, colorectal, bladder, head and neck cancers and gastric cancer[23]. However, the repeated clinical expose to cisplatin often results in the tumor cells evading the apoptosis program initiated by cisplatin. Therefore, there is a need to explore the molecular mechanisms of cisplatin resistance, in order to overcome drug resistance in tumor therapy. Recently, several studies have indicated that many proteins are involved in the recognition of Pt-DNA adducts and cisplatin-induced apoptosis program[24,25]. In this study, we used microarray, GO, KEGG pathway and protein-protein interaction (PPI) analysis to explore the roles of differentially expressed mRNAs in cisplatin resistance and to support other studies.

Many genes which shown differentially expression in the microarray analysis have been demonstrated to be associated with cisplatin resistance in human cancer
Table 4, such as PDE3B, which was substantially upregulated ($P$ value = 0.00029, Fold Chang (FC) = 10.45) in SGC7901/DDP cells. Treatment with a combination of a PDE3B inhibitor and DDP can significantly increase the number of apoptotic and cell growth-suppressive cancer cells in cisplatin resistant
squamous cell carcinoma (SCC) and Hela cells\(^{[26]}\). Research shows that VEGFC, which is upregulated in our data (\(P\) value = 0.00013 FC = 2.93), enhanced cell invasion and cisplatin resistance in gastric cancer\(^{[27]}\). In non-small cell lung cancer, loss of IGFBP-3 expression may activate the PI3K/AKT pathway and induce resistance to cisplatin\(^{[28]}\). In support of this association, our results showed that this mRNA is downregulated (\(P = 0.00007\), FC = 2.93) in SGC7901/DDP cells.

GO enrichment analysis exhibits many functions which the differently expressed mRNAs are involved in, including locomotion, chemotaxis, cell adhesion,
regulation of cell migration, extracellular matrix disassembly, response to xenobiotic chemotaxis, localization of cell adhesion and blood vessel morphogenesis. Functional annotation showed that the differently expressed mRNAs mainly regulate cellular biological behaviors in the progress of regulation of transcription. How the underlying targets of each GO term are implicated in the cisplatin resistance needs further investigation in the future.

Our KEGG pathway analysis showed that the differently expressed mRNAs are enriched in pathways of ECM-receptor interaction, PI3K-Akt, Rap1, MAPK, Notch1, ErbB, ABC transporters, Jak-STAT, NF-κB, HIF-1 and TGF-β. All of those pathways have been confirmed to be involved in cisplatin resistance in different experiments described previously. For example, the inhibition of PI3K-Akt signaling pathway may increase the sensitivity of gastric cancer cells to cisplatin chemotherapy[29]. Another study found that Janus kinase 2 (JAK2) signal transducer and activator of transcription 3 (STAT3) signaling pathways were activated by overexpressed AKT in cisplatin resistant human gastric cancer cells[30]. A study revealed that the canonical NF-κB signaling pathway was involved in APRIL-mediated cisplatin resistance in gastric cancer[31]. Our data are consistent with these previous

Figure 6 Interaction network analyses of differentially express proteins. In the network, nodes represents proteins, lines as functional associations between the abnormal expressed proteins and the thickness of the lines indicates the level of confidence in association reported.

Xie XQ et al. Dysregulated mRNAs in SGC7901/DDP cells
| Term                                           | Database                | P value | Input gene symbols                      |
|------------------------------------------------|-------------------------|---------|-----------------------------------------|
| Gastric cancer                                 | KEGG DISEASE            | 0.0016  | DCC, CD44, CDH1, VEGFC, EGF, TGFA       |
| Skin diseases                                  | KEGG DISEASE            | 0.0078  | DSP, TGM1, CCL5, IL13RA, SPINK5, HLA, FERM1, KRT14, CTSC, COL17A1, LAMA3, REEP1, RIN2, ALOX3, ABCC6, WNT10A, FBLN5 |
| Skin and soft tissue diseases                  | KEGG DISEASE            | 0.0078  | DSP, TGM1, CCL5, IL13RA, SPINK5, HLA, FERM1, KRT14, CTSC, COL17A1, LAMA3, REEP1, RIN2, ALOX3, ABCC6, WNT10A, FBLN5 |
| Macular degeneration                           | KEGG DISEASE            | 0.0140  | C3, FBLN5, CFH, TLR4                    |
| Cancers of the digestive system                | KEGG DISEASE            | 0.0439  | DCC, CD44, CDH1, VEGFC, EGF, TGFA       |
| Familial thoracic aortic aneurysm and dissection (TAAAD) | KEGG DISEASE            | 0.0459  | MYLK, TGFBR1                             |
| Hypomagnesemia                                 | KEGG DISEASE            | 0.0459  | TRPM6, EGF                               |
| Multiple epiphyseal dysplasia (MED)            | KEGG DISEASE            | 0.0459  | COL9A3, MATN3                            |
| Transient neonatal diabetes mellitus (TNDM)    | KEGG DISEASE            | 0.0459  | PLAGL1, ZFSP5                            |
| Non-syndromic autosomal dominant mental retardation | KEGG DISEASE            | 0.0461  | EPB41L1, Dock8, PACS1, SMARCA4           |
| Cardiac hypertrophy                            | NHGRI GWAS Catalog      | 0.0028  | PLXNA2, GRIK2, COL17A1, JAG1, SNAP25, BTRD3, SLX4IP |
| Response to fenofibrate (adiponectin levels)   | NHGRI GWAS Catalog      | 0.0046  | OAS2, PMEPA1, SHANK2, SCLUBE1, SCLC0A4, PCK1 |
| Complement C3 and C4 levels                    | NHGRI GWAS Catalog      | 0.0094  | HLA, CFHR3, CFH, C3                     |
| Neutrophil count                               | NHGRI GWAS Catalog      | 0.0119  | PLC84, TGFA, FGGY, PDDFD, PDDF3          |
| Neoplasia (idiopathic membranous)              | NHGRI GWAS Catalog      | 0.0137  | HLA, ITGB6, PLX2R                       |
| Sleep duration                                 | NHGRI GWAS Catalog      | 0.0195  | PLP2, TM5, ADAMTS14                     |
| Airflow obstruction                            | NHGRI GWAS Catalog      | 0.0259  | HYYK, LEF1, SERPINB4, GPRI2, MAPIK13, PTTRPD |
| Cystic fibrosis                                | NHGRI GWAS Catalog      | 0.0265  | HLA, EHF, AHRR                           |
| Metabolic levels (5-HIAA/MHPG Ratio)           | NHGRI GWAS Catalog      | 0.0265  | PIEZ2, ROBO2, ADAM212                   |
| Bronchopulmonary dysplasia                     | NHGRI GWAS Catalog      | 0.0296  | PLXCDC2, ZNF770, SPCOK1, TRPS1, RASGFI1, HIVEP3 |
| Major depressive disorder                      | NHGRI GWAS Catalog      | 0.0346  | PCLO, SLC6A15, ENOX1, SLY2F, IGF1B1, IGF1BP3, C12orf5, ATXN1, PIEZO2, TRPS1, RASGFI1, FGER2, KCN5H |
| IgA nephropathy                                | NHGRI GWAS Catalog      | 0.0346  | HLA, ACOX1, TNFSF13                     |
| Pelvic floor function decline                  | NHGRI GWAS Catalog      | 0.0368  | MUSK, CSM1, RORA, FRTK2                  |
| Palmatic acid (16:0) plasma levels             | NHGRI GWAS Catalog      | 0.0368  | SCN, CNDK3, GRIK2, PTTRPD                |
| Male-pattern baldness                          | NHGRI GWAS Catalog      | 0.0439  | AUTS2, EDAR2, AR                        |
| Response to citralopram treatment              | NHGRI GWAS Catalog      | 0.0439  | LAMA1, RORA, EGFALAM                    |
| Hyperlipidemia                                 | FunDO                   | 0.0050  | IRS1, CCL5, C3, PAPPA, TXNIP, APOC1, F3, SCD |
| Thrombocytopenia                               | FunDO                   | 0.0068  | GATA1, CCL5, ITGB3, IL1, CXCL8, MPL      |
| Fibromyalgia                                   | FunDO                   | 0.0126  | MAO6B, CXCL8, BDNF, IGF1B3              |
| Cirrhosis                                      | FunDO                   | 0.0209  | RB5P, KRT18, IGF1B5, RTRG, EGF, F3, FGF2CGBP1 |
| Hepatitis C                                    | FunDO                   | 0.0221  | CD274, CCL5, RB5P, MK167, CXCL8, KRT18, TLR4, KRT8, FGF2 |
| Thalassemia                                    | FunDO                   | 0.0345  | LCN2, CXCL8, ANK2, KIR3DL1, MUC1        |
| Gingival overgrowth                            | FunDO                   | 0.0417  | EDN1, IL1, FGFP                        |
| Pulmonary fibrosis                             | FunDO                   | 0.0474  | CSF1, BDNF, MMP7, EDN1, CCL5, ERBB3     |
| Ovary cancer                                   | FunDO                   | 0.0477  | LCN2, IL1, CXCL8, FGFP, CASP1           |
| Esophageal tumor                               | FunDO                   | 0.0477  | CD274, TSPAN8, FRAT1, PCDCHLD2, FGFP2   |
| Hyperlipidemia                                 | GAD                     | 0.0093  | CCL5, HLA, CXCL8, CD22, TNFSF18, CDY9   |
| Thrombocytopenia                               | GAD                     | 0.0114  | CSM1D1, PTK4X, GALNT16, SOBP, PLXCD2, SESCNS3, ADAMTS5, EHF, TMCS, LPL, CD109, FAM1178, PDE1C, TACGN1, PTN, FGD4, DYNCH11, GNG4, MUSK, FBLN5, CCD5C4, T9C9, PMEPA1, TL4A, AN6K, EDAR2R, APOCI, BMP2, TOX3, NRK1, ITPK1, PTTRPD, KLFS, PAM, PTPRN, LEPR, KKIF2, LHX5, MCTP2, ANKR5D0, SEMA6D, PLXNA2, DPDY, GRK3, SGAP, ACOX1, TDKRH, FAM135B, VEGFC, CHST2 |
| Fibromyalgia                                   | GAD                     | 0.0136  | IRS1, CCL5, ITGCS, NFRP1, NFRP3, APOC1, LPL |
| Cirrhosis                                      | GAD                     | 0.0204  | DPDY, CELF4, CELF2, FAM1178, TDRKRH, LPCAT4, FBLN5, SOBP, PMEPA1, CSM1D1, STOX1, CACNB2, CADMI, VEGFC, SCLC0A4, PCL1, CD109, MCTP2, SCLC0A4, PTTRPD, ITPK1 |
| Hepatitis C                                    | GAD                     | 0.0258  | DPDY, CELF4, CELF2, FAM1178, TDRKRH, LPCAT4, FBLN5, SOBP, PMEPA1, CSM1D1, STOX1, CACNB2, CADMI, VEGFC, SCLC0A4, PCL1, CD109, MCTP2, SCLC0A4, PTTRPD, ITPK1 |
| Thalassemia                                    | GAD                     | 0.0362  | MCTP2, PSDF, CCDC54, ROBO2, ELOV16       |
| Gingival overgrowth                            | GAD                     | 0.0419  | PLXNA2, ATXN1, IGF2B2, ABCA13, FNI1, FG5T0, NCOA7, SCIN, TNS1, FAM135B, MUC16, ADAM19, ATXN1, MTL1B2, NNX12, KCNQ3, ANPEP, CDH2 |
| Pulmonary fibrosis                             | GAD                     | 0.0420  | CREG2, GALNT16, LINOC1550, KIF168, SHIRGR, TRPS1, PDE1C, NCKAP5, TNFSR21, RYR3, MAGE2, EDIL3, CXCL16, MCF2, DTD1, GPC5, KLF6, IKZF2, KCHN5, AJP1, BTRD3, PHACTR2, ITPK1, IGF5T0, SGAP, C12orf5, AB13BP, FOS, SCLUBE1 |
**Table 3  Cisplatin resistance pathway and input gene (P < 0.05, FC > 2.0)**

| Pathway                             | Input gene | Fold change | Regulation | Genomic coordinates | Cyto band |
|-------------------------------------|------------|-------------|------------|---------------------|-----------|
| PI3K-Akt signaling pathway          | LAMA1      | 2.68026     | Up         | Chr18:6958512-6956742 | hs        |
|                                    | LAMA1      | 2.75269     | Up         | Chr18:69492035-6949176 | hs        |
|                                    | GNG4       | 2.08935     | Up         | Chr11:23571443-23574384 | hs        |
|                                    | ITCG3      | 2.96629     | Up         | Chr17:45380027-45389886 | hs        |
|                                    | ITCG6      | 7.72783     | Up         | Chr2:160964233-160958330 | hs        |
|                                    | VEGFC      | 2.92538     | Up         | Chr4:177604882-177604823 | hs        |
|                                    | PDGF      | 2.42861     | Up         | Chr4:177296677-177296618 | hs        |
|                                    | JRSI       | 2.00967     | Up         | Chr2:93536149-93540155 | hs        |
|                                    | GNGT1      | 2.04479     | Up         | Chr4:110952689-110952748 | hs        |
|                                    | CSF1       | 2.28628     | Up         | Chr5:123819301-123819309 | hs        |
|                                    | EGF        | 4.74437     | Up         | Chr8:123819301-123819307 | hs        |
|                                    | FG2        | 3.02437     | Up         | Chr11:216288985-216288927 | hs        |
|                                    | FG2        | 2.99240     | Up         | Chr12:64508971-64508912 | hs        |
|                                    | FNI        | 2.31254     | Up         | Chr1:59246570-59246511 | hs        |
| Ovarian cancer                     | COLA6      | 2.08497     | Up         | Chr10:137399019-137399050 | hs        |
|                                    | FG12       | 10.99211    | Up         | Chr19:1860574-1860515 | hs        |
|                                    | GNG11      | 2.01984     | Up         | Chr11:9355564-93555823 | hs        |
|                                    | FG7        | 2.19252     | Up         | Chr12:157269889-157269869 | hs        |
|                                    | LAMA3      | 2.56116     | Down       | Chr18:21534735-21534794 | hs        |
|                                    | IFNAJ1     | 2.30808     | Down       | Chr9:21166331-21166272 | hs        |
|                                    | CREB3L3    | 2.40183     | Down       | Chr19:4172219-4172278 | hs        |
|                                    | TLR4       | 2.13271     | Down       | Chr12:90476856-90476915 | hs        |
|                                    | COL6A2     | 2.89458     | Down       | Chr21:47540686-4754145 | hs        |
|                                    | CD19       | 2.09022     | Down       | Chr16:28056060-2805659 | hs        |
|                                    | COLA4      | 3.83177     | Down       | Chr12:67269794-67269735 | hs        |
|                                    | COLA4      | 2.11177     | Down       | Chr12:22786723-227867464 | hs        |
|                                    | PCK1       | 4.49558     | Down       | Chr20:56141030-56141089 | hs        |
|                                    | VTN        | 3.82567     | Down       | Chr17:26694806-26694747 | hs        |
|                                    | IL2RG      | 2.68365     | Down       | Chr17:472784034-47283975 | hs        |
|                                    | COL5A3     | 7.53410     | Down       | Chr19:70254554-70254495 | hs        |
|                                    | FG13       | 17.98866    | Down       | Chr21:37713947-37713888 | hs        |
|                                    | FN1C       | 4.57879     | Down       | Chr7:128498036-128498597 | hs        |
|                                    | FN1C       | 4.81302     | Down       | Chr7:128498476-128498535 | hs        |
|                                    | CACNB2     | 7.83203     | Down       | Chr10:18787305-18787364 | hs        |
|                                    | RASGRF1    | 4.87152     | Down       | Chr15:79253554-79254495 | hs        |
|                                    | FO5        | 2.17501     | Down       | Chr14:75748214-75748273 | hs        |
|                                    | JUN        | 2.04000     | Down       | Chr15:59246570-59246511 | hs        |
|                                    | RASGRF2    | 3.10358     | Down       | Chr16:64508971-64508912 | hs        |
| MAPK signaling pathway             | FG13       | 17.88866    | Up         | Chr13:37713947-37713888 | hs        |
|                                    | TGFBR1     | 2.93035     | Up         | Chr9:101961322-101961381 | hs        |
|                                    | TGFBR1     | 4.76437     | Up         | Chr9:110952689-110952748 | hs        |
|                                    | EGF        | 4.76437     | Up         | Chr9:110952689-110952748 | hs        |
|                                    | FG12       | 10.99211    | Up         | Chr19:1860574-1860515 | hs        |
|                                    | MAP3K13    | 2.25019     | Up         | Chr3:18561379-18561590 | hs        |
|                                    | FG2        | 5.02437     | Up         | Chr12:63819300-123819300 | hs        |
|                                    | FG2        | 2.99240     | Up         | Chr12:63819317-123819376 | hs        |
|                                    | MAP2K7     | 2.08267     | Up         | Chr19:79795032-79795061 | hs        |
|                                    | FG7        | 2.19252     | Up         | Chr15:49776810-49776869 | hs        |
|                                    | CACNG4     | 8.83585     | Up         | Chr17:65028139-65028198 | hs        |
|                                    | CACNG4     | 2.94145     | Up         | Chr17:65028115-65028174 | hs        |
|                                    | CACNB4     | 2.14311     | Up         | Chr15:215269439-215269480 | hs        |
|                                    | GADD45A    | 2.56699     | Up         | Chr6:68153371-68153340 | hs        |
|                                    | BDNF       | 2.32411     | Up         | Chr12:6769591-27697900 | hs        |
|                                    | CACNA2D1   | 2.09452     | Up         | Chr7:81579504-81579445 | hs        |

**Xie XQ et al.**  Dysregulated mRNAs in SGC7901/DDP cells
Table 4  Dysregulated mRNAs ($P < 0.05$, $FC \geq 2.0$) associated with cisplatin resistance

| Gene symbol | $P$ value | FC (abs) | Regulation | Genename                                      | Ref.     |
|-------------|-----------|----------|------------|-----------------------------------------------|----------|
| FGF7        | 0.00035   | 2.19252  | Up         | Fibroblast growth factor 7                    | PMID: 22990560 |
| HIF2K       | 2.63E-06  | 4.06213  | Up         | Homeodomain interacting protein kinase 2      | PMID: 24846322 |
| EDN1        | 9.94E-05  | 2.46437  | Up         | Endothelin 1                                  | PMID: 21220476 |
| CBS         | 0.00108   | 2.39871  | Up         | Cystathionine-beta-synthase                    | PMID: 24326104 |
| PDE3B       | 0.00029   | 10.4498  | Up         | Phosphodiesterase 3B, cgmp-inhibited          | PMID: 2433626 |
| EZF5        | 0.00041   | 2.13295  | Up         | EZF transcription factor 5, p130-binding      | PMID: 22193543 |
| PIN1        | 0.00104   | 2.57958  | Up         | Peptidylprolyl cis/trans isomerase, NIMA-interacting 1 | PMID: 26820938 |
| EGF         | 0.00346   | 2.76437  | Up         | Epidermal growth factor                       | PMID: 2708487 |
| CSF1        | 0.00025   | 2.76200  | Up         | Colony stimulating factor 1 (macrophage)      | PMID: 2205523 |
| PCNA        | 0.00103   | 2.17028  | Up         | Proliferating cell nuclear antigen            | PMID: 24474685 |
| HIPK2       | 2.63E-06  | 4.06213  | Up         | Homeodomain interacting protein kinase 2      | PMID: 24846322 |
| ENTPD6      | 0.00011   | 2.43726  | Up         | Ectonucleoside triphosphate diphosphohydrolase 6 (putative) | PMID: 21519793 |
| AKR1C1      | 0.00097   | 2.29064  | Up         | Aldo-keto reductase family 1, member CI       | PMID: 23165153 |
| ASNS        | 0.00172   | 2.39491  | Up         | Asparagine synthetase (glutamine-hydrolyzing) | PMID: 17266043 |
| BDNF        | 0.00062   | 2.32411  | Up         | Brain-derived neurotrophic factor             | PMID: 23986506 |
| CAYB       | 0.01089   | 2.55664  | Up         | Calcium binding tyrosine-(Y)-phosphorylation regulated | PMID: 24326251 |
| FGF2        | 2.15E-06  | 2.99240  | Up         | Fibroblast growth factor 2 (basic)            | PMID: 12894531 |
| SLC7A1      | 1.95E-05  | 2.93256  | Up         | Solute carrier family 7 member 11             | PMID: 24510643 |
| TLRB3       | 0.00046   | 2.02123  | Up         | Tubulin, beta 3 class III                    | PMID: 25107571 |
| TWIST1      | 0.00180   | 2.96340  | Up         | Twist family bhlh transcription factor 1       | PMID: 22673193 |
| JAG1        | 9.41E-05  | 3.20086  | Up         | Jagged 1                                      | PMID: 24659709 |
| ANXA11      | 0.00031   | 2.36619  | Down       | Annexin A1                                   | PMID: 19484149 |
|             |           |          |            |                                               | PMID: 17982121 |
studies, and these pathways and input genes deserve our attention in gastric cancer cisplatin resistance. Although protein expression is generally stable when organs mature, under various pathological and physiological conditions, gene expression may change and ultimately result in aberrant protein levels. Therefore, research on proteomics is helpful to illustrate some biological mechanisms, including cisplatin resistance. Protein-protein interaction network analysis might uncover previously unknown molecular mechanisms of cisplatin resistance. Hub proteins of subnetworks which interact with many partners might associate with drug resistance. For example, studies have shown that dysregulation of the genes PDE3B, TLR4, and HIPK2 is associated with cisplatin resistance in human SCC cells, ovarian granulosa tumor cells and bladder cancer cells, respectively.[26,32,33] Moreover, hub proteins and their partners may have similar biological functions. Since downregulation of EGF has been shown to substantially overcome resistance to cisplatin in ovarian cancer,[24] we predict that the proteins EDN1 and DCN, whose hub protein is EGF, may contribute to cisplatin resistance in a similar fashion. We also found that ZEB2, which over-expressed in SGC7901/DDP compared with SGC7901 has a similar expression profile to TWIST1, suggesting that ZEB2 may play an important role in cisplatin resistance by regulating the expression of TWIST1. Nevertheless, more evidence and research is needed.

In conclusion, our study identified mRNAs differentially expressed between gastric cancer cell lines SGC7901/DDP and SGC7901. These results provide a global view of the function of the differentially expressed mRNAs. Several molecular and pathway abnormalities detected in our study have previously been reported to be associated with drug resistance in gastric cancer. The dysregulated mRNAs identified participate in cisplatin resistance through diverse mechanisms, and further investigation is required to confirm the role in drug resistance of these transcripts, pathways and the interaction networks of the proteins they code for.

### REFERENCES

1. Ferro A, Peleterio B, Malvezzi M, Bosetti C, Bertuccio P, Levi F, Negri E, La Vecchia C, Lunet N. Worldwide trends in gastric cancer mortality (1980-2011), with predictions to 2015, and incidence by subtype. *Eur J Cancer* 2014; 50: 1330-1344 [PMID: 24650579 DOI: 10.1016/j.ejca.2014.01.029]

2. Wöhler RS, Raderer M, Hejna M. Palliative chemotherapy for advanced gastric cancer. *Ann Oncol* 2004; 15: 1585-1595 [PMID: 15520058 DOI: 10.1093/annonc/mdl1422]

3. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; 60: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]

4. Al-Batran SE, Hartmann JT, Probst S, Schmalenberg H, et al. Dysregulated mRNAs in SGC7901/DDP cells. *WJG* 2017; 23(7): 1200-1288 [PMID: 26983899]
Hollerbach S, Hofheinz R, Rethwisch V, Seipel G, Homann N, Wilhelm G, Schuch G, Stoehlmacher J, Derigs HG, Hegewisch-Becker S, Grossmann J, Pauligk C, Atmaca A, Bokemeyer C, Knuth A, Jäger E. Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin in plus either oxaliplatin or cisplatin: a study of the Arbeitsgemeinschaft Internistische Onkologie. *J Clin Oncol* 2008; 26: 1435-1442 [PMID: 18349393 DOI: 10.1200/jco.2007.13.9378]

Kang YK, Kang WK, Shin DB, Chen J, Xiong J, Wang J, Lichintser M, Guan Z, Khasanov R, Zheng L, Philco-Salas M, Suarez T, Santamaria J, Forster G, McClusky PL. Cepacitabin/ cisplatin vs 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial. *Ann Oncol* 2009; 20: 666-673 [PMID: 19153312 DOI: 10.1093/annonc/mdn717]

Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishiizaki T, Kobayashi O, Takiyama W, Toh Y, Nagai T, Takagi S, Yamamura Y, Yanaka K, Orita H, Takeuchi M. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRIT trial): a phase III trial. *Lancet Oncol* 2009; 8: 215-221 [PMID: 18282905 DOI: 10.1016/s1470-4247(08)70035-4]

Wagner AD, Unverzagt S, Groteh J, Kleber G, Grothey A, Metzger R, Rabik CA, Boxem J, Fleig WE. Systemic chemotherapy for advanced gastric cancer. *Cochrane Database Syst Rev* 2010; (3): CD004064 [PMID: 20238327 DOI: 10.1002/14651858.CD004064.pub3]

Rahb CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinum agents. *Cancer Treat Rev* 2007; 33: 9-23 [PMID: 17084534 DOI: 10.1016/j.ctrv.2006.09.006]

García JA, Dreier R. Systemic chemotherapy for advanced bladder cancer: update and controversies. *J Clin Oncol* 2006; 24: 5545-5551 [PMID: 17158540 DOI: 10.1200/jco.2006.08.0564]

Wilson TR, Longley DB, Johnston PG. Chemoresistance in solid tumours. *Ann Oncol* 2006; 17 Suppl 10: x315-x324 [PMID: 17018746 DOI: 10.1093/annonc/mdi280]

Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst* 2000; 92: 1295-1302 [PMID: 10944550]

Hioki M, Gotohda N, Konishi M, Nakagohri T, Takahashi S, Kinoshita T. Predictive factors improving survival after gastrectomy in gastric cancer patients with peritonal carcinomatosis. *World J Surg* 2010; 34: 555-562 [PMID: 20082194 DOI: 10.1007/s00268-010-0396-5]

Yang T, Chen M, Chen T, Thakur A. Expression of the copper transporters hCttr1, ATP7A and ATP7B is associated with the response to chemotherapy and survival in patients with resected non-small cell lung cancer. *Oncol Lett* 2015; 10: 2584-2590 [PMID: 26622894 DOI: 10.3892/ol.2015.3531]

Metzger R, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groschen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Haranda J, Konda B, Leichman L. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 1998; 16: 309-316 [PMID: 9440758]

Xu W, Chen Q, Wang Q, Sun Y, Wang S, Li A, Xu S, Rhee OD, Wang M, Zhang R, Yang L, Zhou J. JWA reverses cisplatin resistance via the CK2-XRCC1 pathway in human gastric cancer cells. *Cell Death Dis* 2014; 5: e1551 [PMID: 25476899 DOI: 10.1038/cddis.2014.517]

Shim HJ, Yun JY, Hwang JH, Bae WK, Cho SH, Lee JH, Kim HN, Shin MH, Kweon SS, Lee JH, Kim HJ, Chung JI. BRCA1 and XRCC1 polymorphisms associated with survival in advanced gastric cancer treated with taxane and cisplatin. *Cancer Sci* 2010; 101: 1247-1254 [PMID: 20331623 DOI: 10.1111/j.1349-7006.2010.01514.x]

Liu J, Deng N, Xu Q, Sun L, Tu H, Wang Z, Xing C, Yuan Y. Polymorphisms of multiple genes involved in NER pathway predict prognosis of gastric cancer. *Oncotarget* 2016; 7: 48130-48142 [PMID: 27348061 DOI: 10.18632/oncotarget.10173]
Woods DC, White YA, Dau C, Johnson AL. TLR4 activates NF-κB in human ovarian granulosa tumor cells. *Biochem Biophys Res Commun* 2011; 409: 675-680 [PMID: 21616060 DOI: 10.1016/j.bbrc.2011.05.063]

Tang XH, Li M, Deng S, Lu MS. Cross-reacting material 197, a heparin-binding EGF-like growth factor inhibitor, reverses the chemo resistance in human cisplatin-resistant ovarian cancer. *Anticancer Drugs* 2014; 25: 1201-1210 [PMID: 25115341 DOI: 10.1097/cad.0000000000000155]
