

The Global mRNA Expression Profiles of Inhibiting PHGDH Induced Cisplatin Resistance in Gastric Cancer

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Abstract

Objective: Drug resistance is the main hindrance to improve the prognosis of patients with gastric cancer. Amino acid metabolic reprogramming is essential to satisfy the different requirements of cancer cells during drug resistance, of which serine deprivation could promote resistance to cisplatin in gastric cancer. As the key enzyme in the de novo biosynthesis of serine, phosphoglycerate dehydrogenase (PHGDH) inhibition could also induce cisplatin resistance in gastric cancer. This study aims to reveal the potential mechanisms of drug resistance induced by PHGDH inhibition via exploring the global mRNA expression profiles.

Materials and Methods: In this experimental study, the viability and the apoptotic rate of gastric cancer cells were evaluated by using Cell Counting Kit-8 (CCK-8) analysis and flow cytometric determination, respectively. The identification of differentially expressed genes (DEGs) was tested by mRNA-sequencing (mRNA-Seq) analysis. The confirmation of sequencing results was verified using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR).

Results: The inhibition of PHGDH significantly increased the viability and decreased the apoptotic rate induced by cisplatin in gastric cancer cells. mRNA-Seq analysis revealed that the combined treatment of NCT503 reduced the number of DEGs induced by cisplatin. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Enrichment Analysis (GSEA) showed that unfolded protein response, ECM receptor interaction and cell cycle signaling pathways were modulated by NCT503 treatment. Hub genes were identified by using protein-protein interaction network modeling, of which E1A binding protein p300 (EP300) and heat shock protein family A (Hsp70) member 8 (HSPA8) act as the vital genes in cisplatin resistance induced by the inhibition of PHGDH.

Conclusion: These findings suggested that the inhibition of PHGDH promoted cisplatin resistance in gastric cancer through various intercellular mechanisms. And appropriate serine supplementation or the modulation of EP300 and HSPA8 may be of great help in overcoming cisplatin resistance in gastric cancer.

Keywords: Cisplatin, Drug Resistance, Gastric Cancer, Phosphoglycerate Dehydrogenase

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Introduction

Gastric cancer is the leading cause of cancer death worldwide, with the fifth-highest incidence and third-highest mortality (1). Due to its late detection, aggressive characteristic and poor response to available therapies, gastric cancer displays a poor prognosis. Chemotherapy is the standard first-line treatment for patients with advanced gastric cancer (2). And cisplatin is one of the most widely used chemotherapeutic drugs inducing DNA double-strand break and finally leads to cell apoptosis (3). However, the development of drug resistance in gastric cancer is still a hindrance of cisplatin to effective cancer treatment (4). Therefore, understanding the molecular mechanisms that control cisplatin resistance in gastric cancer is imperative and important for the establishment of new strategies in its clinical treatment.

The mechanisms of drug resistance in gastric cancer involves decreased drug uptake, increased drug efflux, enhanced DNA damage repair, epigenetic modifications changes, autophagy and metabolic reprogramming (5-

8). Metabolic reprogramming, which is essential to satisfy the different requirements of cancer cells during tumorigenesis, invasion and resistance to drug therapy, is one of the significant characteristics of cancers (9, 10). Recently, serine/glycine metabolism has attracted more attention. Our previous study demonstrated that the deprivation of serine could promote resistance to cisplatin in gastric cancer. And the inhibition of phosphoglycerate dehydrogenase (PHGDH), the first and only rate-limiting enzyme of serine biosynthesis pathway (SSP), could promote cisplatin resistance and attenuate DNA damage induced by cisplatin in gastric cancer cells (11). However, the specific mechanisms of inhibiting PHGDH promoted cisplatin resistance in gastric cancer remain unclear.

Transcriptomics analysis can reflect the gene expression information of cells in different conditions quickly and comprehensively (12). Due to the advantages such as low background signals, the accurate quantification of expression levels, and RNA sequencing can provide us deep insight into the

regulation mechanisms of specific factors on cells. To clarify the intracellular mechanism changes induced by PHGDH inhibition, we used RNA-Seq analysis to reveal gene expression patterns.

On the basis of our previous study that serine deprivation promotes cisplatin resistance in gastric cancer, here, through RNA-Seq analysis combined with cell functional validation, we gained a comprehensive and detailed understanding of the mechanisms of PHGDH inhibition-induced cisplatin resistance in gastric cancer. Our data provided possible mechanisms for understanding the cisplatin resistance in gastric cancer.

Materials and Methods

Reagents

In this experimental study, human gastric cancer cell lines SGC7901 and MGC803 was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The following reagents were used in this research: RPMI-1640 medium (31800-105) from Gibco (Grand Island, New York, USA). Cell Counting Kit-8 agent (CCK-8, C0042) from Beyotime (Shanghai, China). Penicillin streptomycin solution (GNM15140) and trypsin (GNM25200) from Genome (Hangzhou, Zhejiang, China). Fetal bovine serum (11011-8611) from Every Green (Hangzhou, China). NCT-503 (HY-101966) from MedChemExpression (Shanghai, China). TRIzol reagent (CW0580) from Cwbio (Beijing, China).

Cell culture

SGC7901 and MGC803 were cultured at 37°C in an atmosphere of 5% CO₂. All cells were kept in RPMI-1640 medium, supplemented with 100 mg/mL streptomycin, 100 U/mL penicillin and 10% fetal bovine serum.

Cell viability

Cells were plated at a density of 1×10⁴ cells per well onto 96-well plates. These cells were divided into 2 groups (control group and cisplatin treatment group). Then, each group was subdivided into 4 groups (treated with 0, 1, 10 or 50 μM NCT503, respectively) and each concentration has 3 repeats. After 24 hours, cells were treated with cisplatin combined with different concentrations of NCT503 for 24 h. Then 100 μL medium containing 10 μL CCK-8 was added after discarding the supernatant and reacted at 37°C for 1 hour. The optical density values at 450 nm which reflect the viability of cells were determined using a Microplate Reader (Synergy HTZX-22; Bio-Tek Instruments, USA).

Cell apoptosis

Cells were plated onto 6-well plates at a density of 70-80% for 24 hours. Cells were divided into four groups:

Control (CN), Cisplatin (DDP), NCT503 (NCT) and Cisplatin combined with NCT503 (CB). The cells were treated with vehicles, 1.2 μg/mL cisplatin, 50 μM NCT503 and 1.2 μg/mL cisplatin combined with 50 μM NCT503 respectively. Among which each group was set to three biological replicates. After 24 hours, cells including floating and adherent were collected. Then 300 μL Annexin V-FITC binding reagent containing 6 μL propidium iodide (PI) and 3 μL Annexin V-FITC (C1062L, Beyotime Biotechnology, China) was added and reacted at 37°C for 15 minutes. The apoptotic rate was measured using a Flow cytometer (EasyCell 204A1/206A1, Wellgrow, China).

Real-time quantitative reverse transcription polymerase chain reaction

The total cellular RNA was extracted using TRIzol reagent and cDNAs were synthesized using PrimeScript™ RT Master Mix (RR036A, Takara). Real-time PCR was performed using SYBR Green Realtime PCR Master Mix (RR430, Takara) and Cobas z 480 (Roche, Basel, Switzerland). The primers for HSPA8, EP300, ras homolog family member A (*RHOA*), RNA polymerase II subunit C (*POLR2C*) and β -ACTIN were synthesized by TSINGKE Biological Technology (Beijing, China). β -actin was served as an internal reference of RNA integrity. The sequences are as following:

HSPA8-

F: 5'-ATGGTGCAGCTGTCCAG-3'
R: 5'-ACAGTCATGACTCCACCAG-3'

EP300-

F: 5'-ATGGCACAGATTTTGGCTCTC-3'
R: 5'-TACCAGATCGCAGCAATTCTGAC-3'

RHOA-

F: 5'-ATGGAAAGCAGGTAGAGTTGGC-3'
R: 5'-ACTATCAGGGCTGTTCGATGG-3'

POLR2C-

F: 5'-GCTCACAGGCTTGGATTAATTCC-3'
R: 5'-ATGTGCAGTCCCGAGAGTACTG-3'

β -ACTIN-

F: 5'-ACTCTTCCAGCCTTCCTTCC-3'
R: 5'-CGTCATACTCCTGCTTGCTG-3'

Total RNA extraction and transcriptome sequencing

Total RNA was enriched by Oligo (dT) and then fractured into short pieces using Fragmentation buffer. The double-stranded cDNA was synthesized and purified. The cDNA was then repaired, added with base A and a sequencing adapter. The target size fragments are recovered by agarose gel electrophoresis and PCR amplification is performed to construct the sample library. Finally, the constructed library was detected on the Illumina platform. Sequencing analysis was conducted

by JiGuang Gene (Nanjing, China).

Bioinformatics analyses

Transcripts Per Million (TPM) was used to represent gene expression levels (13). Limma was used to normalize the data and the DEGs were visualized by ggplot2 and pheatmap packages (14). The threshold of DEGs was set to $P < 0.05$ and $|\log FC| > 1$. ClusterProfiler package was used to perform GO (<http://www.geneontology.org/>) and KEGG (<http://www.genome.jp/kegg/>) pathway analyses based on DEGs. And the results were visualized by GPlot and ggplot2 packages (15). GSEA analysis was performed using GSEA software (v. 4.1.0). The PPI analysis was produced in STRING website (<https://string-db.org/>), and the results were subjected into Cytoscape (<https://www.cytoscape.org>). Then MCODE, a Cytoscape plugin, was used to find clusters in the network. The clusters, whose score > 8 , were subjected to ClueGo for GO and KEGG analysis. Finally, hub genes were predicted by cytoHubba. The prognostic values of these hub genes were assessed with the Kaplan-Meier Plotter tool (<http://kmplot.com>).

Statistical analysis

All data were processed by GraphPad Prism 9 for Mac (GraphPad Prism Software Inc., San Diego, CA, USA). Differences between two groups were evaluated by the unpaired two-tailed student's *t* test. One-way analysis of variance (ANOVA) was used to assess differences between multiple groups. Data are presented in the form of mean \pm standard deviation (SD). $P < 0.05$ denotes statistical significance.

Statement of ethics

The design and the protocols of the experiments have been reviewed and approved by the Medical Ethics Committee of Jinhua Central Hospital (No. 2020-205-001).

Results

The inhibition of PHGDH promoted cisplatin resistance in gastric cancer

To verify the effect of PHGDH in cisplatin resistance in gastric cancer. We treated SGC7901 and MGC803 cells with differential concentrations of NCT503, an inhibitor of PHGDH, combined cisplatin. The results showed that the inhibition of PHGDH slightly decreased the viability of MGC803 cells. And the treatment of cisplatin impaired the viability of both SGC7901 and MGC803 cells. However, the cell viability was significantly increased by the combined treatment of NCT503 and cisplatin compared to cisplatin-treated cells, especially in SGC7901 cells. And a high concentration of NCT503 correlated with a high cell viability (Fig.1A, B). We further examined the apoptosis rate in SGC7901 cells due to the significant response of SGC7901 to NCT503. The results showed that the treatment of cisplatin significantly promoted cell apoptosis, and the inhibition

of PHGDH partially reversed the process of cell apoptosis (Fig.1C, D). Together, our results demonstrated that the inhibition of PHGDH promoted cisplatin resistance in gastric cancer.

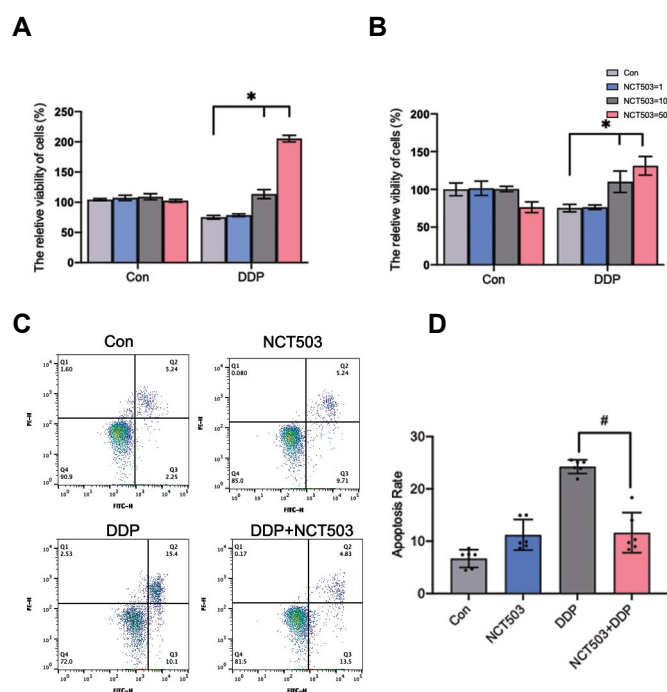


Fig.1: The inhibition of PHGDH promoted cisplatin resistance in gastric cancer. **A.** Cell viability in SGC7901 cells with vehicles or 1.2 $\mu\text{g/ml}$ cisplatin under the treatment of different concentration of NCT503 (0, 1, 10, 50 μM , $n=3$). **B.** Cell viability in MGC803 cells with vehicles or 3.0 $\mu\text{g/ml}$ cisplatin under the treatment of different concentration of NCT503 (0, 1, 10, 50 μM , $n=3$). **C, D.** Flow cytometric determination of cell apoptosis rate in SGC7901 cells with vehicles, 50 μM NCT503, 1.2 $\mu\text{g/ml}$ cisplatin or the combination of NCT503 with cisplatin ($n=3$). *; $P < 0.05$, compared to control group and #; $P < 0.05$, compared to DDP group, PHGDH; Phosphoglycerate dehydrogenase, and DDP; Cisplatin.

Identification of DEGs in NCT503-induced cisplatin resistance

To fully understand the potential molecular mechanisms of NCT503-induced cisplatin resistance, we divided SGC7901 cells into four groups: control group (CN), cisplatin-treated group (DDP), NCT503-treated group (NCT) and cisplatin combined with NCT503 treated group (CB), and carried out a series evaluation using RNA-Seq.

The DEGs was selected according to the criteria of $P < 0.05$ and $|\log FC| > 1$. The integral gene expression variation between each group were visualized by volcano plots. The results showed that after treatment with cisplatin, a total of 3240 genes displayed differential expression, including 1467 upregulated genes and 1773 downregulated genes (Fig.2Aa). The inhibition of PHGDH led to 468 DEGs, within 179 upregulated genes and 289 downregulated genes (Fig.2Ab). In addition, the combination treatment of NCT503 significantly reduced the number of DEGs caused by cisplatin and just 2031

genes showed significantly difference (Fig.2Ac). A comparative analysis between CB group and DDP group showed that 411 genes were significantly different, of which 196 genes upregulated and 215 one down regulated (Fig.2Ad). Heatmaps were used to reflect the differences and clustering between different groups (Fig.2B).

Since the combination use of NCT503 significantly reduced the number of DEGs caused by cisplatin, these genes that no longer have significant differences may be the key genes that promote cisplatin resistance in gastric cancer. Therefore, we analyzed the DEGs between CB group and DDP group. The result showed that 1582 genes were no longer significantly different after NCT503 combined treatment, and 373 genes significantly changed on the basis of cisplatin treatment (Fig.2C).

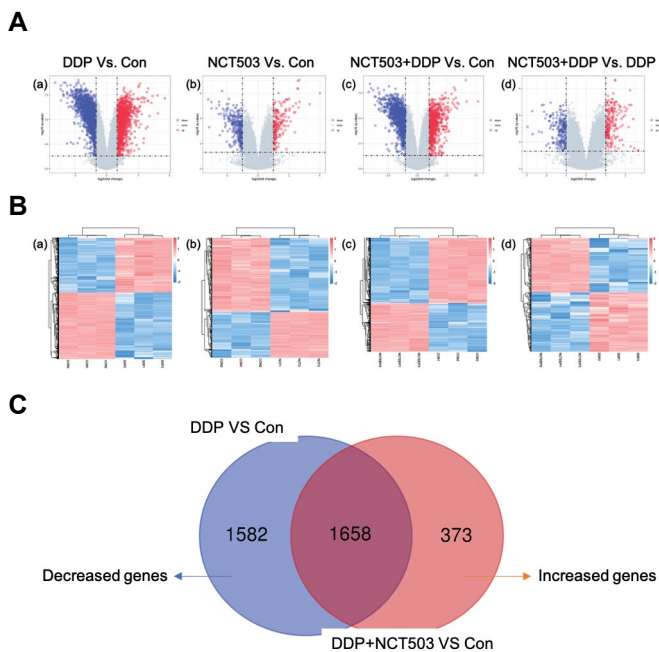


Fig.2: Identification of DEGs in NCT503-induced cisplatin resistance. **A.** Volcano plots showing the gene expression alterations between different drug treatment groups. Blue dots and red dots represent downregulated genes and upregulated genes, respectively. **B.** Heat maps of significantly differentially expressed genes between each group. **C.** Venn diagram showing common regulated genes shared between cisplatin induced DEGs and the combination of cisplatin with NCT503 induced DEGs; DEGs; Differentially expressed genes, DDP; Cisplatin, and Con; Control.

GO analysis revealed the related biological processes

To further demonstrate the mechanisms induced by the inhibition of PHGDH, GO enrichment analysis was conducted. GO analysis includes cellular component (CC), biological process (BP) and molecular function (MF), of which BP analysis can reveal the BP that the DEGs participated in. Circle plot was used to visualize the GO-BP analysis, in which upregulated genes were red and downregulated genes were blue. In DDP group, we found that the genes related with cell-substrate adhesion and extracellular matrix organization were downregulated, and the genes related to cell cycle arrest were upregulated

(Fig.3A). After the inhibition of PHGDH, the genes related to cellular response to unfolded protein (UPR) and response to endoplasmic reticulum stress (ERS) upregulated (Fig.3B). Also the treatment of NCT503 combined with cisplatin downregulated extracellular structure organization related genes, and upregulated *UPR* related genes (Fig.3C). In addition, compared with DDP group, the CB group upregulated *UPR* related genes (Fig.3D). Our results indicated that the BP of *UPR* may be the key of NCT503-induced cisplatin resistance in gastric cancer cells.

To confirm the effect of *UPR* in gastric cisplatin resistance, we performed GO-BP analysis on the reduced DEGs in CB group compared to DDP group. The result showed that these genes were related to protein folding and ribosome biogenesis, which promote protein expression (Fig.3E). And the increased DEGs in CB group were related to *UPR* (Fig.3F). Under the treatment of drugs, misfolded and unfolded proteins in cells accumulate in the endoplasmic reticulum, thereby inducing ERS. Cells can initiate *UPR* to restore cell homeostasis by reducing the rate of protein synthesis, activating ER-related degradation and other mechanisms (16). Therefore, we hypothesized that cisplatin promoted cell apoptosis through the accumulation of misfolded proteins and NCT503 promote cisplatin resistance through activating the BP of *UPR* to balance cell homeostasis.

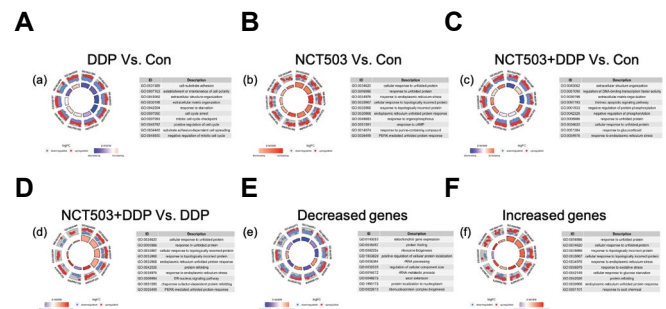


Fig.3: GO analysis revealed the related biological processes. The top 10 GO-BP annotations of the DEGs are shown. **A.** GO analysis of DEGs between DDP group and CN group, **B.** GO analysis of DEGs between NCT group and CN group, **C.** GO analysis of DEGs between CB group and CN group, **D.** GO analysis of DEGs between CB group and DDP group, **E.** GO analysis of the decreased DEGs between CB group and DDP group, **F.** GO analysis of the increased DEGs between CB group and DDP group. Red dots represent upregulated genes; blue dots represent downregulated genes. The gradual size and color of rectangles in the middle represents the adjust P value and the regulation of genes in the enriched biological process, respectively. GO; Gene Ontology, BP; Biological process, DEGs; Differentially expressed genes, DDP; Cisplatin, NCT; NCT503, CB; Cisplatin combined with NCT503, and CN; Control.

KEGG and GSEA enrichment revealed the related signaling pathways

To further reveal the potential signaling pathway of NCT503-mediated cisplatin resistance, we performed KEGG pathway enrichment analysis. The results showed that cisplatin is mainly involved in the regulation of P53 signaling pathway, adherens junction and Apoptosis

(Fig.4Aa). NCT503 affects the P53 signaling pathway, IL-17 signaling pathway and Protein processing in ER (Fig.4Ab). The combination treatment of NCT503 and cisplatin regulated P53 signaling pathway, Adherens junction and TNF signaling pathway (Fig.4Ac). In addition, NCT503 combination mainly influenced Glycine, serine and threonine metabolism, Biosynthesis of amino acid and Protein processing in ER compared to cisplatin-treated group (Fig.4Ad). And after the combination of NCT503, the genes no longer have significant differences were related to Proteasome and Cell cycle pathway, and the genes changed were related to TNF signaling pathway (Fig.4A-e, f). Together, our results showed that both cisplatin and NCT503 could modulate P53 signaling pathway, and the combination of NCT503 could restore the expression of cell cycle related proteins, at the same time affect TNF signaling pathway and Protein processing in endoplasmic reticulum.

The intracellular signaling pathway is often amplified by signal cascades, and slight changes in upstream signal molecules that can also cause significant changes in downstream genes. Therefore, pathway enrichment analysis based on DEGs often fails to capture the overall signaling pathways changed in the cell. GSEA, which bases on the whole genes obtained by RNA-Seq, overcome this shortcoming perfectly. We used GSEA to analyze the effect of PHGDH inhibition on cisplatin resistance. The results showed that, consistent with GO and KEGG analysis, cisplatin treatment resulted in the downregulation of Adherens junction and ECM receptor interaction (Fig.4Ba, b). In addition, compared with DDP group, the combination of NCT503 upregulated genes related to Adherens junction and ECM receptor interaction (Fig.4Ca, b). And NCT503 combination also upregulated Mismatch repair and VEGF signaling pathway (Fig.4Cc, d). Altogether, our results showed that NCT503 promoted cisplatin resistance in gastric cancer cells by restoring Adherens junction and ECM receptor interaction and promoting Mismatch repair and VEGF signaling pathway.

Protein-protein interactions networks analysis

Proteins function often based on the protein-protein interactions which is essential for almost all biochemical activities. For deeply understanding the regulatory mechanisms in NCT503-induced cisplatin resistance in gastric cancer cells, we performed PPI analysis based on DEGs between DDP group and CB group. STRING was used to construct the PPI network, in which the network with the highest confidence score (0.9) was selected and imported into Cytoscape for functional enrichment analysis (Fig.5A). MCODE plugin was used to find clusters in the network (Fig.5B). Then the top highly interconnected cluster was dissected using ClueGo. The result of GO-BP analysis showed that the cluster predominantly involved UPR and Negative regulation of inclusion body assembly (Fig.5C). And pathway enrichment analysis showed that this cluster was related to Antigen processing and presentation and Protein processing in ER (Fig.5D).

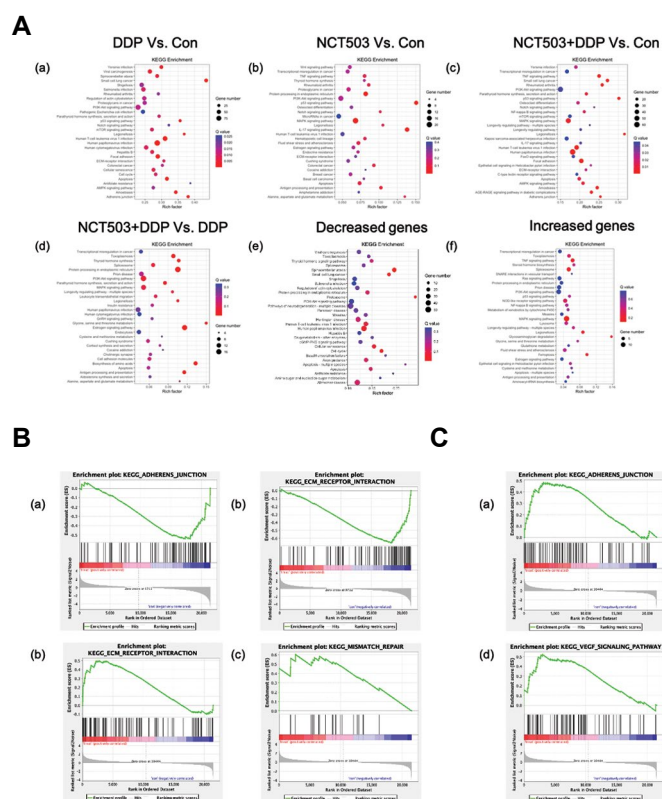


Fig.4: KEGG and GSEA enrichment revealed the related signaling pathways. **A.** KEGG pathway enrichment analysis of DEGs. The gradual color represents the Q value, and the size of the black spots represents the gene number enriched in this pathway. **B.** GSEA analysis of detected genes between DDP group and CN group. **C.** GSEA analysis of detected genes between CB group and DDP group. KEGG; Kyoto Encyclopedia of Genes and Genomes, GSEA; Gene Set Enrichment Analysis, DEGs; Differentially expressed genes, CN; Control, CB; Cisplatin combined with NCT503, and DDP; Cisplatin.

Hub genes play vital roles in BP that the other genes often affected by these genes in related pathways. Thus, we screened the hub genes, that modulated NCT503-mediated cisplatin resistance in gastric cancer cells, using cytoHubba. The 10 highest scoring genes were shown in Figure 5E, of which the genes associated with UPR including heat shock protein family A (Hsp70) member 5 (HSPA5), HSPA8 and heat shock protein family A (Hsp70) member 1A (HSPA1A) were screened.

Considering the combination of NCT503 reduced the number of DEGs induced by cisplatin. We also performed PPI analysis using these reduced DEGs. The PPI network and the highest clusters were shown in Figure 5F, G and S1 (See Supplementary Online Information at www.celljournal.org). Figure 1A-C. For GO-BP analysis, these clusters were mainly focused on mRNA export from nucleus and positive regulation of viral transcription (Fig.5H). For KEGG analysis, there were mainly related to Spliceosome, Ubiquitin mediated proteolysis and mRNA surveillance pathway (Fig.5I). In addition, the hub genes including RHOA, EP300 and POLR2C were screened by using cytoHubba (Fig.5J).

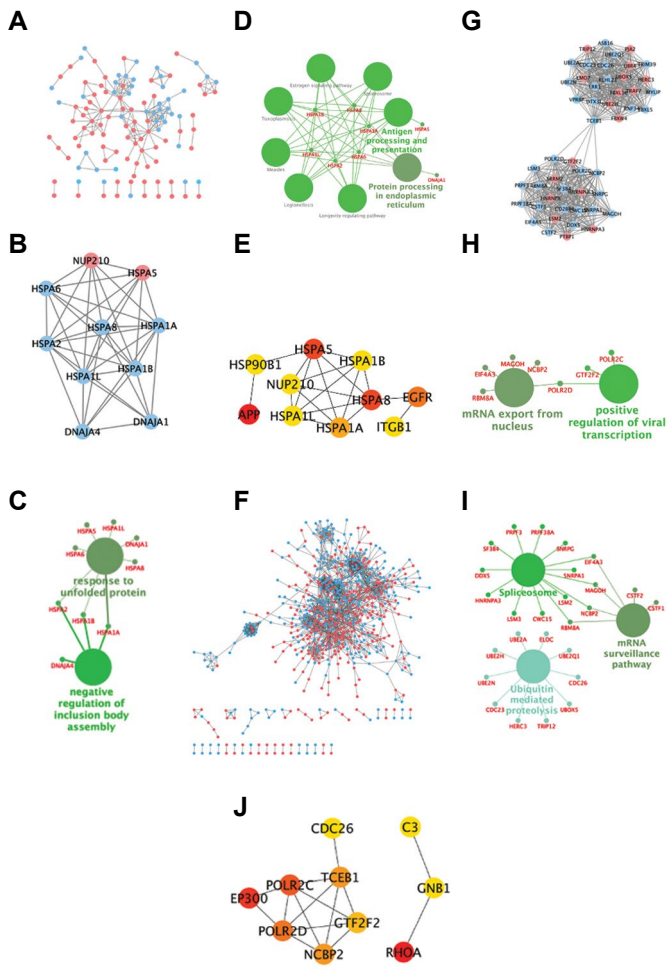


Fig.5: PPI networks analysis. **A.** PPI network of DEGs between CB group and DDP group. Red nodes represent upregulated genes; blue nodes represent downregulated genes. **B.** The most highly connected module identified by MCODE. **C-D.** GO and KEGG analysis of the module by ClueGo. **E.** The top 10 hub genes of DEGs identified by cytoHubba. **F.** PPI network of DEGs decreased by the combination of NCT503 compared to the cisplatin induced DEGs. **G.** The most highly connected module. **H-I.** GO and KEGG analysis of the module. **J.** The top 10 hub genes of DEGs. PPI; Protein-protein interactions, DEGs; Differentially expressed genes, CB; Cisplatin combined with NCT503, DDP; Cisplatin, GO; Gene Ontology, and KEGG; Kyoto Encyclopedia of Genes and Genomes.

Validation of hub genes

The prognostic significance of the 20 hub genes in gastric cancer patients was investigated using the Kaplan-Meier Plotter tool. All of these genes were found to be significantly associated with both overall survival (OS) and first progression (FP) that 19 genes were significantly associated with post progression survival (PPS). As show in Figure 6A-C, 11 of them were protective factors for prognosis (HR<1), whereas 9 were risk factors for prognosis (HR>1).

Among the 20 genes, four genes showed the most significant prognostic value for OS, FP or PPS (HSPA8 and POLR2C for OS, RHOA and POLR2C for FP, HSPA8 and EP300 for PPS). Therefore, we examined the expression level of the four hub genes (HSPA8, RHOA, POLR2C and EP300) by RT-qPCR in SGC7901 cells.

The results showed that cisplatin treatment upregulated POLR2C expression, downregulated HSPA8 and EP300 expressions, the combined usage of NCT503 reversed the changes of HSPA8 and EP300 induced by cisplatin (Fig.6D). Our results showed that the inhibition of PHGDH could reverse the expression model of HSPA8 and EP300 to promote cisplatin resistance in gastric cancer cells.

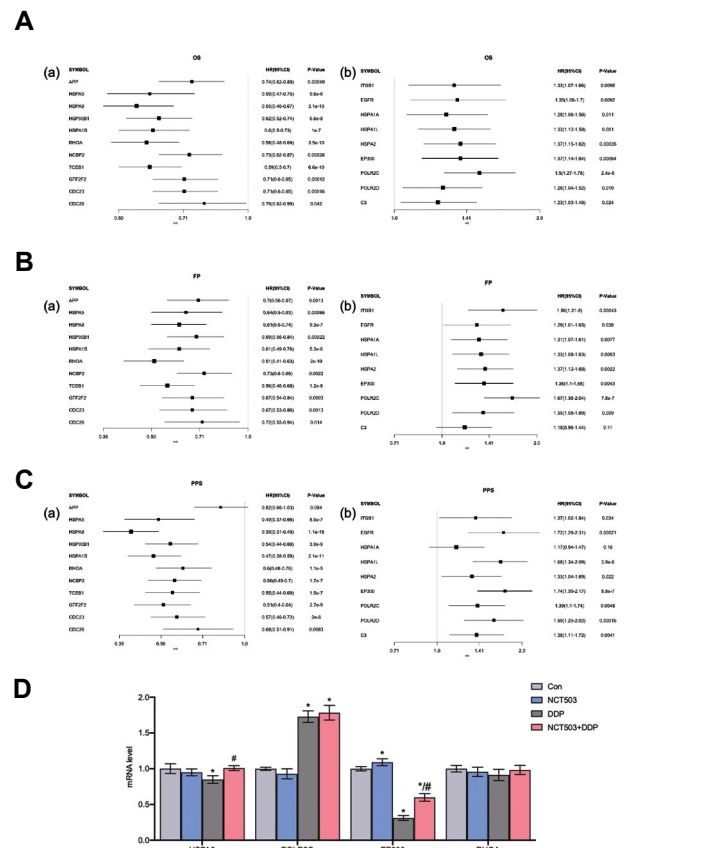


Fig.6: Validation of hub genes. **A-C.** OS, FP and PPS of hub genes, among which the genes on the left forest plots represents prognostic protective genes and the right represents prognostic risk genes. **D.** The mRNA levels of HSPA8, POLR2D, RHOA and EP300 in different groups. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression levels (n=6). *, P<0.05. Compared to control group, #, P<0.05. Compared to DDP group. OS; Overall survival, FP; First progression, PPS; Post progression survival, and DDP; Cisplatin.

Discussion

Emerging evidence suggests that SSP is closely associated to cancer progression. Tumor needs numerous nutrients to sustain its rapid proliferation. Serine provides the necessary precursor for the biosynthesis of other amino acids such as glycine and cysteine and the production of phospholipids such as sphingolipids and phosphatidylserine (17). Serine is synthesized by the glycolytic intermediate 3-phosphoglycerate through a three-step enzymatic transformation. As the first and only rate-limiting enzyme of SSP, PHGDH is overexpressed in various types of cancers including breast (18), glioma (19), gastric (20) and so on. And genetic silencing or

inhibition of PHGDH can disrupt the serine synthesis pathway and suppress tumor growth (21). In addition, studies showed that the inhibition of PHGDH could promote drug sensitivity in various cancers [eg, breast (22), hepatocellular carcinoma (23), lung adenocarcinoma (24), melanoma (25), renal cell carcinoma (26) and multiple myeloma (27)]. However, our previous results showed that the combination of PHGDH inhibitors with cisplatin failed to increase drug sensitivity in gastric cancer. On the contrary, the inhibition of PHGDH significantly reduced the cytotoxicity of cisplatin and the addition of extra serine increased the efficacy of cisplatin in gastric cancer cells (11). Therefore, the mechanisms of serine metabolism on the chemotherapy sensitivity of gastric cancer may be different from other cancers. Gaining a good command of the mechanisms of cisplatin resistance induced by PHGDH inhibitors is of importance to overcome the obstacle of drug resistance in gastric cancer.

In this study, high-throughput sequencing was used to detect DEGs in cells treated with different drugs. Compared to control group, the treatment of cisplatin dysregulated the expression levels of 3240 genes. And the combination of NCT503 significantly reduced the number of DEGs to only 2031 dysregulated genes. In order to investigate the key genes and potential mechanisms involved in cisplatin resistance, DEGs were proved to integrative bioinformatic analyses. GO, KEGG and GSEA analyses were used to discover the potential functions of these DEGs.

Cisplatin, which is one of the most commonly used drugs for the treatment of cancers, exerts anticancer activity via the generation of DNA lesion by interacting with purine bases on DNA followed by cell cycle arrest and finally lead to cell apoptosis (3). GO enrichment analysis showed that the top-ranking BP modulated by cisplatin in gastric cancer cells comprised cell-substrate adhesion, extracellular matrix organization and cell cycle arrest, which were in accordance with previous studies (28). Compared to cisplatin treated cells, the combination of NCT503 increased the expression levels of genes related to UPR and ERS and decreased the expression levels of genes related to protein folding and rRNA processing. ER is the pivotal organelle responsible for protein folding. Various intrinsic and extrinsic stresses including hypoxia, dysregulation of calcium metabolism and drugs often disturbed the homeostasis of ER, resulting in accumulation of misfolded protein (29). Cancer cells adapt to this homeostasis imbalance by activating UPR which reduces RNA translation and promote the degradation of misfolded proteins (30). Studies also showed that the activation of UPR could promote tumor growth and drug resistance (31). Here, our results showed that the inhibition of PHGDH could reduce sensitivity to cisplatin in gastric cancer cells and increase the expression levels of UPR-related genes suggesting that the drug resistance induced by PHGDH inhibition may related to the activation of UPR.

KEGG enrichment analysis revealed that NCT503

induced Glycine, serine and threonine metabolism and biosynthesis of amino acid based on the treatment of cisplatin which were consistent with the role of PHGDH in regulating serine biosynthesis. Besides, genes participated in the regulation of proteasome and cell cycle signaling pathways were no longer have significant changes compared to cisplatin group after the combination treatment of NCT503. This indicated that PHGDH inhibition dysregulate the metabolism of amino acid and return the expression levels of genes regulating proteasome and cell cycle to a normal line. GSEA analysis showed that cisplatin decreased the gene expression of adherens junction and ECM receptor interaction but NCT503 reversed the downregulation of these genes. As an important component of tumor microenvironment, ECM is involved in almost all development processes of tumors including tumor growth, apoptosis, drug resistance and invasion (32). The downregulation of adherens junction and ECM receptor interaction could promote cell apoptosis and the inhibition of PHGDH promote drug resistance by restoring the expression levels of the genes related to these two signaling pathways.

The construction of PPI network and further module analysis also verified the role of PHGDH inhibition in promoting ERS and UPR. Finally, hub genes were calculated by using the degree algorithm of cytoHubba, of which HSPA8, RHOA, POLR2C and EP300 were significantly associated to the survival analysis. RT-qPCR analysis showed that the cisplatin-induced downregulation of HSPA8 and EP300 could be reversed by the inhibition of PHGDH. As the pivotal protein of the protein quality control system, HSPA8 participated in a wide variety of cellular processes, including folding and transport of newly synthesized polypeptides, protection of the proteome from stress and activation of proteolysis of misfolded proteins (33-35). Our results suggested that the inhibition of PHGDH maintain cell homeostasis by upregulating the expression of HSPA8. EP300 is a histone acetyltransferase, which regulates transcription via chromatin remodeling that is important in the process of cell proliferation and differentiation (36, 37). It has also been identified as an activator of VEGF (38) explaining the upregulation of VEGF signaling pathway in the CB group revealed by the GSEA analysis. Therefore, the high expression of EP300 may serve as a key regulatory molecule for NCT503-mediated cisplatin resistance in gastric cancer and promote the survival of gastric cancer cells through chromatin remodeling and activation of VEGF signaling pathways.

In summary, our studies showed that the PHGDH inhibition promoted cisplatin resistance in gastric cancer cells and the addition of serine could promote the sensitivity of gastric cancer cells to cisplatin (11). These indicated that the combination of PHGDH inhibitors with cisplatin may not be a good strategy in the treatment of gastric cancer. On the contrary, the supplementation of serine combined with cisplatin may be a candidate therapy. But the effectiveness of the therapy still needs

the support of clinical data.

Mechanically, we found that the inhibition of PHGDH mainly activated *ERS* and *UPR*. And it also involved in restoring the expression levels of genes related to ECM receptor interaction and cell cycle signaling pathways to normal levels. These play important roles in maintaining cell homeostasis and cell survival. In addition, we also found that HSPA and EP300 may be the vital regulatory genes involved in intracellular activities after PHGDH inhibition, respectively regulating UPR and chromatin remodeling. The relationship of PHGDH with HSPA8 and EP300 will be further studied in the follow-up studies.

Conclusion

Via transcriptomics analysis, we found the crucial intercellular mechanisms regulating the cisplatin resistance induced by PHGDH inhibition. PHGDH is the key enzyme for the de novo biosynthesis of serine. Therefore, appropriate serine supplementation or the modulation of EP300 and HSPA8 may be of great help in overcoming cisplatin resistance in gastric cancer.

Acknowledgments

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Authors' Contribution

K.P.; Performed all experiments, analyzed the data and wrote the manuscript. X.Z.; Performed cell experiments. W.X.; Contributed to concept and design, financial support, and final approval of the manuscript. All authors read and approved the final manuscript.

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