GSTP1 determines cis-platinum cytotoxicity in gastric adenocarcinoma MGC803 cells: regulation by promoter methylation and extracellular regulated kinase signaling

Ye Zhang[a], Xiujuan Qu[a], Wei Jing[a], Xuejun Hu[b], Xianghong Yang[c], Kezuo Hou[a], Yuee Teng[a], Jingdong Zhang[a] and Yunpeng Liu[a]

Detoxification mechanisms can play a pivotal role in determining tumor cell responses to platinum-based chemotherapy. Glutathione *S*-transferase-pi (GSTP1) belongs to a supergene family of detoxifying enzymes involved in the prevention of DNA damage and subsequent platinum resistance in numerous cancers. The role of GSTP1 in gastric cancer sensitivity to chemotherapy is, however, not known. In this study, we found that the human gastric cancer cell line MGC803 was significantly more sensitive to cis-platinum (CDDP) than the other gastric cancer lines examined (BGC823 and SGC7901). To explore the potential role of GSTP1 in drug resistance, we measured GSTP1 expression in these cells. GSTP1 mRNA and protein were not detectable in MGC803 cells; both were present in BGC823 and SGC7901 cells. GSTP1 CpG island DNA methylation was examined. We report that promoter hypermethylation was associated with the absence of GSTP1 expression in MGC803 cells. Treatment of these cells with 5-aza-2′-deoxycytidine, a DNA methyltransferase inhibitor, restored GSTP1 expression and suppressed sensitivity to CDDP. The selective mitogen-activated protein kinase/extracellular regulated kinase (ERK) pathway inhibitor PD98059 decreased GSTP1 expression in 5-aza-2′-deoxycytidine-treated cells. A similar decrease was observed in the BGC823 and SGC7901 cell lines, suggesting that mitogen-activated protein kinase/ERK signaling stimulates GSTP1 expression. CDDP sensitivity was also enhanced by PD98059. These observations indicate that somatic promoter hypermethylation and impaired ERK signaling are associated with decreased GSTP1 expression and CDDP sensitivity in gastric cancer cell lines. Evaluation of promoter methylation and ERK activity may be useful for predicting tumor sensitivity to platinum-based chemotherapeutics. *Anti-Cancer Drugs* 20:208–214

Anti-Cancer Drugs 2009, **20:**208–214

Keywords: cisplatin, DNA methylation, extracellular regulated kinase, gastric cancer, glutathione *S*-transferases

Department of [a]Medical Oncology, [b]Medical Respiratory, The First Hospital and [c]Department of Pathology, Shengjing Hospital, China Medical University, Shenyang, China

Correspondence to Yunpeng Liu, MD, PhD, Department of Medical Oncology, The First Hospital, China Medical University, NO.155, North Nanjing Street, Heping District, Shenyang 110001, China
Tel: +86 24 8328 2312; fax: +86 24 8328 2543;
e-mail: cmuliuyunpeng@yahoo.cn

Ye Zhang and Xiujuan Qu contributed equally to this study

Received 1 September 2008 **Revised form accepted** 19 November 2008

Introduction

Gastric cancer is one of the most common malignancies worldwide. Global estimates in the year 2008 reveal that the incidence of gastric cancer is very high in East Asia, and particularly in China (36/100 000) [1]. Numerous therapies for advanced gastric cancer have been explored, including different chemotherapeutic modalities, and these have significantly prolonged survival. Drug resistance is, however, a major problem that limits the effectiveness of chemotherapies used to treat gastric cancer.

Cisplatin (cis-platinum, CDDP) is among the most widely used and broadly active cytotoxic anticancer drugs. Treatment leads to the intracellular formation of highly reactive platinum complexes. These inhibit DNA synthesis through covalent binding to DNA molecules, forming intrastrand and interstrand DNA cross-links. Earlier study has evaluated candidate predictive markers for responses to platinum-based chemotherapy including Na^+/K^+-ATPase, multidrug resistance protein 1, the glutathione *S*-transferase (GST) family, excision repair cross-complementing 1 and 2, and p53 [2–5]. The multifunctional detoxifying GST enzymes have been specifically implicated in the metabolism of platinum drugs [6]. Glutathione *S*-transferase-pi (GSTP1) is an important member of the GST family and detoxifies electrophilic carcinogens by catalyzing their conjugation to glutathione. Cells with higher levels of GSTP1 would thus be expected to be more resistant to platinum-based chemotherapy. Conversely, lower detoxification capacity might be associated with drug sensitivity. Singal *et al.* [7] reported that high GSTP1 levels are associated with poor prognosis in prostate cancer. We reported previously that GSTP1 is widely overexpressed in different gastric cancer

0959-4973 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e328322fbaa

classes ranging from dysplasia to advanced carcinoma [8,9]. Similar results have been reported in other recent studies [10,11]. There has therefore been considerable clinical interest in evaluating GSTP1 as a candidate predictive marker for platinum-based chemotherapy of gastric cancers [12].

Earlier study has shown that GSTP1 expression is regulated at the transcriptional level [13]. Hypermethylation of the promoter region is often associated with loss of GSTP1 expression, this phenomenon has been observed in solid tumors [14–16]. GST gene expression is also responsive to extracellular stimuli and in HepG2 cells is coordinately regulated by transcription factors [17]. The mitogen-activated protein kinase (MAPK)/extracellular regulated kinase (ERK) signaling pathway plays a central role in transcription activation. It was recently shown that MAPK/ERK signaling could promote resistance to platinum-based compounds in both lung cancer [18–20] and ovarian cancer [21,22]. However, it remains unknown whether the MAPK/ERK pathway regulates GSTP1 gene expression and platinum resistance in gastric cancer.

In this study, we first investigated a potential association between gene methylation and GSTP1 enzyme expression in gastric cancer cell lines. We also investigated the role of MAPK/ERK signaling in GSTP1 expression. Finally, we examined whether GSTP1 expression modulates chemotherapeutic sensitivity to CDDP.

Materials and methods

Reagents and antibodies

3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide, and 5-aza-2′-deoxycytidine (5-aza-dC) were purchased from Sigma (St. Louis, Missouri, USA). The specific ERK1/2 inhibitor PD98059 was purchased from Promega (Madison, Wisconsin, USA). Anti-ERK1/2 and phospho-ERK1/2 were purchased from Cell Signal Technology (Danvers, Massachusetts, USA). Anti-GST-P1 and anti-tubulin monoclonal antibodies were from Santa Cruz Biotechnology (Santa Cruz, California, USA).

Cells and cell culture

The human gastric cancer cell lines MGC803, BGC823, and SGC7901 were cultured in RPMI1640 medium (Gibco BRL, Grand Ysland, New York, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Culture media were changed every 2–4 days.

Cell viability assay

Cell viability was determined using the MTT-uptake colorimetric assay. Cells were seeded at 2×10^3 cells/well in 96-well plates and incubated in culture medium overnight. Separate experimental groups were treated with different concentrations of anticancer agents, including CDDP, 5-fluorouracil, epirubicin, or paclitaxel. Cells were treated with 10 μmol/l 5-aza-dC (72 h) before the addition of anticancer agents (or saline in controls) and incubation for a further 48 h. Twenty microliters of MTT solution (5 mg/ml) was added to each well and incubation was continued for 4 h at 37°C. The cell culture medium was removed, cells were lysed in 150 μl of dimethylsulfoxide, and the optical density (OD) was measured at 570 nm using a microplate reader (Bio-Rad, Hercules, California, USA). The following formula was used: cell viability = (OD of the experimental sample/OD of the control group) × 100%.

RNA extraction, reverse transcription, and amplification

Cell lines MGC803, BGC823, and SGC7901 were collected and washed twice with ice-cold phosphate-buffered saline and total RNA extracted with the RNeasy mini kit (Qiagen, Carlsbad, California, USA) as described by the manufacturer. RNA was reverse transcribed as follows. One microgram of RNA was mixed with 1 μl of random hexamer primer (0.5 μg/μl) and incubated for 10 min at 70°C. To each sample was added 4 μl of first strand buffer, 2 μl of 0.1 mol/l dithiothreitol, 1 μl of deoxyribonucleotide triphosphate mix, and 1 μl of Superscript II (Invitrogen, Carlsbad, California USA) and incubated for 1 h at 42°C and 15 min at 70°C. Fifty nanograms of cDNA were used for PCR amplification; GSTP1 gene expression was determined as described [23].

Immunoblotting

Cells were divided into two groups. One group was exposed to 1, 5 or 10 μmol/l 5-aza-dC for 72 h, the other was exposed to 20 μmol/l PD98059 for 1 h before adding 10 μmol/l 5-aza-dC and incubating for a further 72 h. Immunoblotting was performed as described previously [24]. Briefly, cells were collected and lysed in cell lysis buffer. After incubation on ice for 30 min and centrifugation (12 000 rpm, 10 min) supernatant total protein content was determined by the Bradford colorimetric method. After denaturation, 50 μg of protein from each sample was separated by 10% SDS–polyacrylamide gel electrophoresis and electroblotted to nitrocellulose. Membranes were blocked with 5% nonfat milk in Tris-buffered saline Tween (TBST) for 2 h at room temperature before overnight incubation at 4°C in 5% nonfat milk in TBST containing either tubulin, phosphorylated ERK1/2, ERK1/2, or GSTP1 antibody, followed by 1 h incubation with a peroxidase-conjugated secondary antibody. After extensive washing with TBST, proteins were visualized using an enhanced chemiluminescence reagent (SuperSignal Western Pico Chemiluminescent Substrate; Pierce, Rockford, Illinois, USA).

Detection of GSTP1 hypermethylation by methylation-specific PCR

Bisulfite treatment

Genomic DNA was isolated from gastric cancer cells. Sodium bisulfite conversion of 2 μg of genomic DNA was performed by a modification of a previously described method [23]. Briefly, NaOH was added to denature DNA (final concentration, 0.2 mol/l) and incubated for 20 min at 50°C. A volume of 500 μl freshly made bisulfite solution (2.5 mol/l sodium metabisulfite and 125 mmol/l hydroquinone, pH 5.0) was added to each sample, incubation was continued at 50°C for 3 h in dark. Modified DNA was purified using the Wizard DNA purification resin according to the manufacturer's instructions (Promega) and was eluted with 45 μl of water at 80°C. After treatment with NaOH (final concentration, 0.3 mol/l; 10 min, 37°C), 75 μl of 7.5 mol/l ammonium acetate was added and incubated for 5 min at room temperature. Finally, modified DNA was precipitated with 2.5 vol 100% ethanol and 2 μl of glycogen (5 mg/ml). The pellet was washed with 70% ethanol, dried, and eluted in 30 μl of 5 mmol/l Tris–HCl pH 8.0.

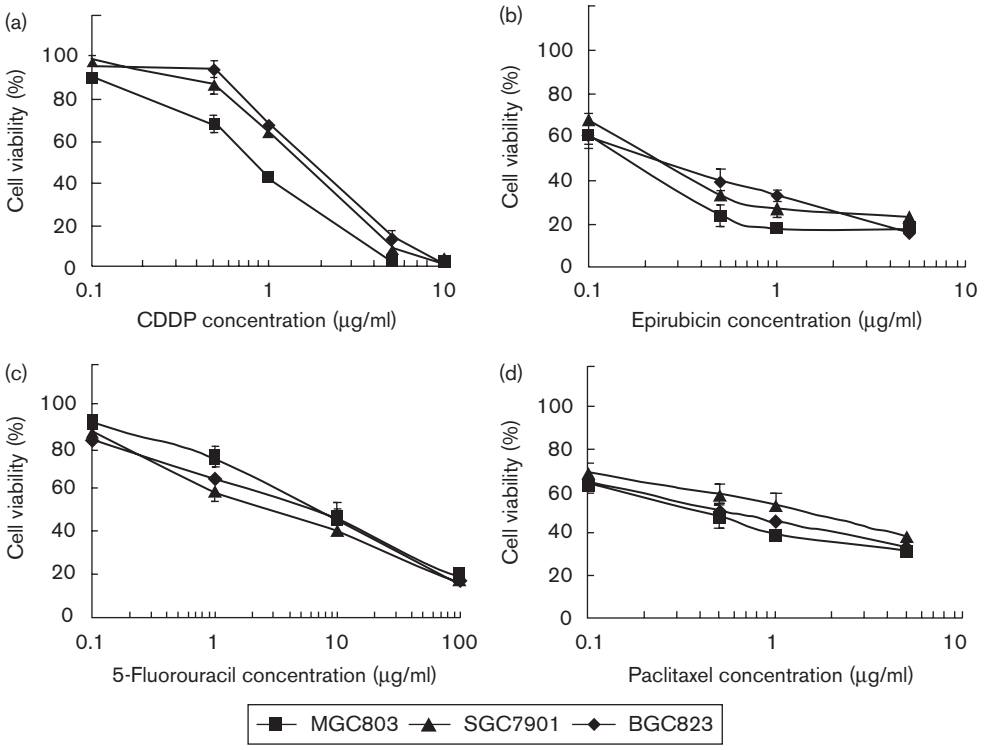
Methylation-specific PCR analysis

For PCR amplification, 2 μl of bisulfite-modified DNA was incorporated into a final volume of 25 μl of PCR mix containing 1 μl PCR buffer (16.6 mmol/l ammonium sulfate, 67 mmol/l Tris–HCl pH 8.8, 6.7 mmol/l $MgCl_2$, 10 mmol/l 2-mercaptoethanol), deoxyribonucleotide triphosphates (each at 1.25 mmol/l), 1 U Platinum Taq DNA polymerase (Life Technologies, Rockville, Maryland, USA) and primers (each 300 ng per reaction). Primer sequences for methylated or modified unmethylated GSTP1 were as described [23]. Methylation-specific PCR was carried out as follows: 1 cycle at 95°C for 1 min; 35 cycles of 1 min at 95°C, then 1 min at 62°C, 1 min at 72°C, with final extension for 10 min at 70°C. A negative control (water without DNA) was included in each amplification. PCR products (8 μl) from each of the samples were separated on a 2.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Statistical analysis

Data are presented as means ± standard deviations. The significance of the difference between groups

Fig. 1



Sensitivies of gastric cancer cell lines MGC803, BGC823, and SGC7901 to different anticancer drugs. Cell viability was determined by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide uptake colorimetry after 48 h exposure to anticancer drugs at the specified concentrations. (a) Cell viability after exposure to cis-platinum (CDDP); MGC803 cells were significantly more sensitive to CDDP than either BGC823 or SGC7901cells. (b) Cell viability after exposure to epirubicin. (c) Cell viability after exposure to 5-fluorouracil. (d) Cell viability after exposure to paclitaxel.

was assessed by the χ^2 test. A probability value of less than 0.05 was considered significant. All means were calculated from at least three independent experiments.

Results

Sensitivity of human gastric cancer cell lines to different anticancer agents

We used cytotoxicity assays to compare the sensitivity of gastric cancer cell lines MGC803, BGC823, and SGC7901 to different anticancer agents including CDDP, 5-fluorouracil, epirubicin, and paclitaxel. The dose–response curves show that MGC803, BGC823, and SGC7901 cell lines are sensitive to anticancer agents, but the MGC803 cell line was markedly more sensitive to CDDP than the other lines tested (Fig. 1).

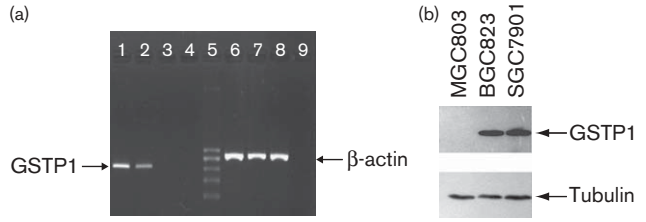
GSTP1 mRNA and protein expression in cell lines MGC803, BGC823, and SGC7901

To determine whether changes in GSTP1 expression might be responsible for the increased CDDP sensitivity of the MGC803 cell line, GSTP1 mRNA and protein expression levels were analyzed. As shown in Fig. 2a, GSTP1 mRNA was readily detected in BGC823 and SGC7901 cells, but was not detectable in MGC803 cells. Protein expression levels largely paralleled the different mRNA levels (Fig. 2b), demonstrating that GSTP1 enzyme is not expressed significantly in the MGC803 cell line.

DNA hypermethylation and GSTP1 gene expression in human gastric cancer cell lines

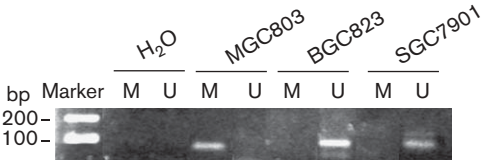
To determine whether the lack of GSTP1 expression in the MGC803 cell line might be associated with GSTP1 gene methylation, methylation-specific PCR analysis was used to explore the methylation status of the CpG island located upstream of the GSTP1 transcription start site. Abnormal GSTP1 CpG island hypermethylation was detected in MGC803, but not in BGC823 or SGC7901 (Fig. 3). To determine whether hypermethylation is associated with GSTP1 transcriptional silencing, MGC803 cells were exposed for 72 h to 5-aza-dC, an inhibitor of DNA methyltransferase. GSTP1 expression was restored after 5-aza-dC treatment (Fig. 4). These data indicate that CpG island hypermethylation may be responsible for the lack of GSTP1 expression in the MGC803 cell line. We then investigated whether restoration of GSTP1 expression with 5-aza-dC might affect the sensitivity of these cells to different anticancer agents. Figure 5 shows that CDDP cytotoxicity was significantly diminished by 5-aza-dC, whereas the cytotoxicities of the other drugs tested (5-fluorouracil, epirubicin, and paclitaxel) were not affected by 5-aza-dC treatment.

Fig. 2



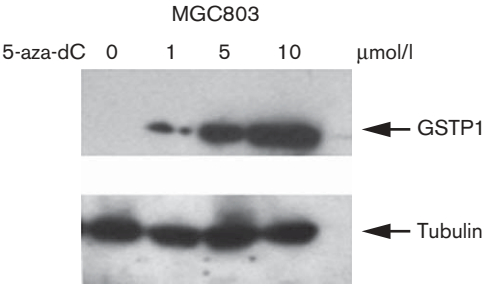
Glutathione *S*-transferase-pi (GSTP1) mRNA and protein expression in gastric cancer cell lines. (a) GSTP1 mRNA levels determined by reverse transcription PCR and agarose gel electrophoresis are (1) SGC7901, (2) BGC-823, (3) MGC-803, (4) control, (5) size markers, (6–9) β-actin internal controls, respectively, from SGC7901, BGC823, and MGC-803. (b) GSTP1 protein detected by immunoblotting; the internal control was tubulin.

Fig. 3



Methylation status of the glutathione *S*-transferase-pi (GSTP1) CpG island in gastric cancer cell lines as revealed by methylation-specific PCR. The presence of a strong PCR product in lanes M (93 bp) indicates that the GSTP1 gene is methylated, whereas the product in lanes U (97 bp) indicates that the promoter region is unmethylated. The GSTP1 promoter region is hypermethylated in MGC803, whereas in BGC823 and SGC7901 this remains unmethylated. Water was used as a negative control. M, methylated; U, unmethylated.

Fig. 4

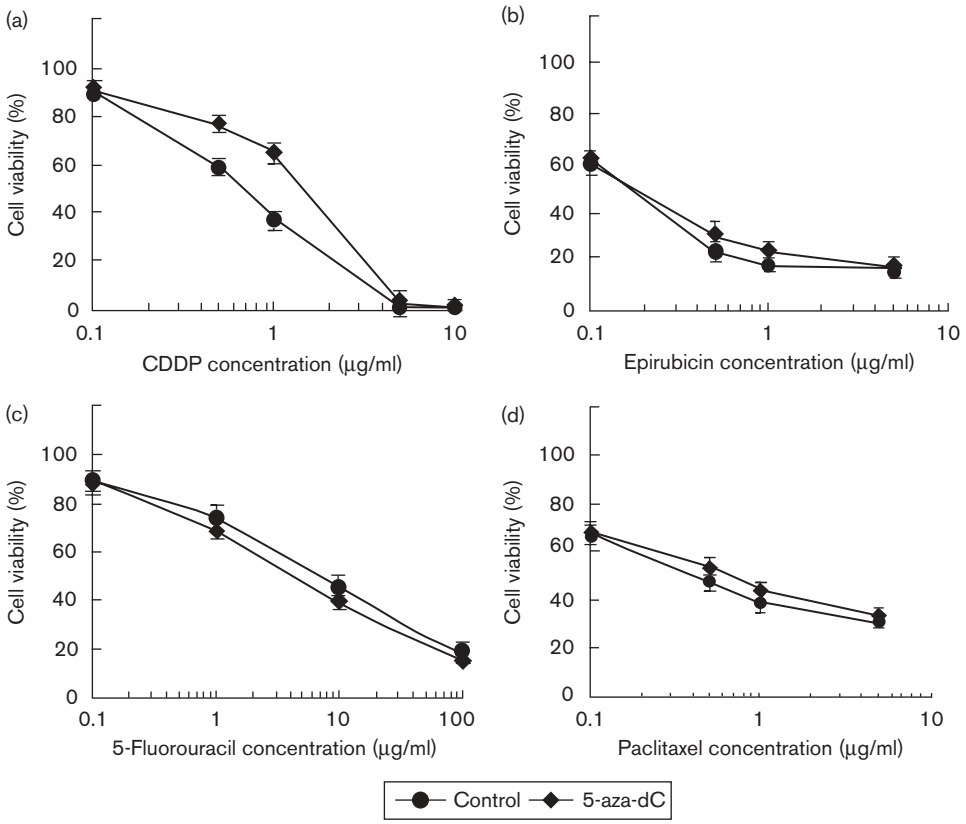


Glutathione *S*-transferase-pi (GSTP1) expression in MGC803 cells treated with 5-aza-2′-deoxycytidine (5-aza-dC). MGC803 cells were exposed to 5-aza-dC at the indicated concentrations for 72 h. The GSTP1 protein was then detected by western blotting; the arrow indicates the position of the 27 kDa polypeptide. Tubulin provided the loading control.

Inhibition of the mitogen-activated protein kinase/extracellular regulated kinase signaling pathway and GSTP1 expression

We then addressed whether MAPK/ERK signaling also regulates GSTP1 expression in gastric cancer cells. We examined the effects of the selective MAPK/ERK pathway inhibitor PD98059 on GSTP1 expression and drug resistance. PD98059 (20 μmol/l) completely or

Fig. 5



Effect of 5-aza-2′-deoxycytidine (5-aza-dC) treatment on the sensitivity of MGC803 to anticancer drugs. Cells were pretreated with 5-aza-dC for 3 days before exposure (48 h) to different concentrations of anticancer drugs (a) cis-platinum (CDDP), (b) epirubicin, (c) 5-fluorouracil, or (d) paclitaxel. The cytotoxicity of CDDP was significantly reduced by pretreatment with 5-aza-dC.

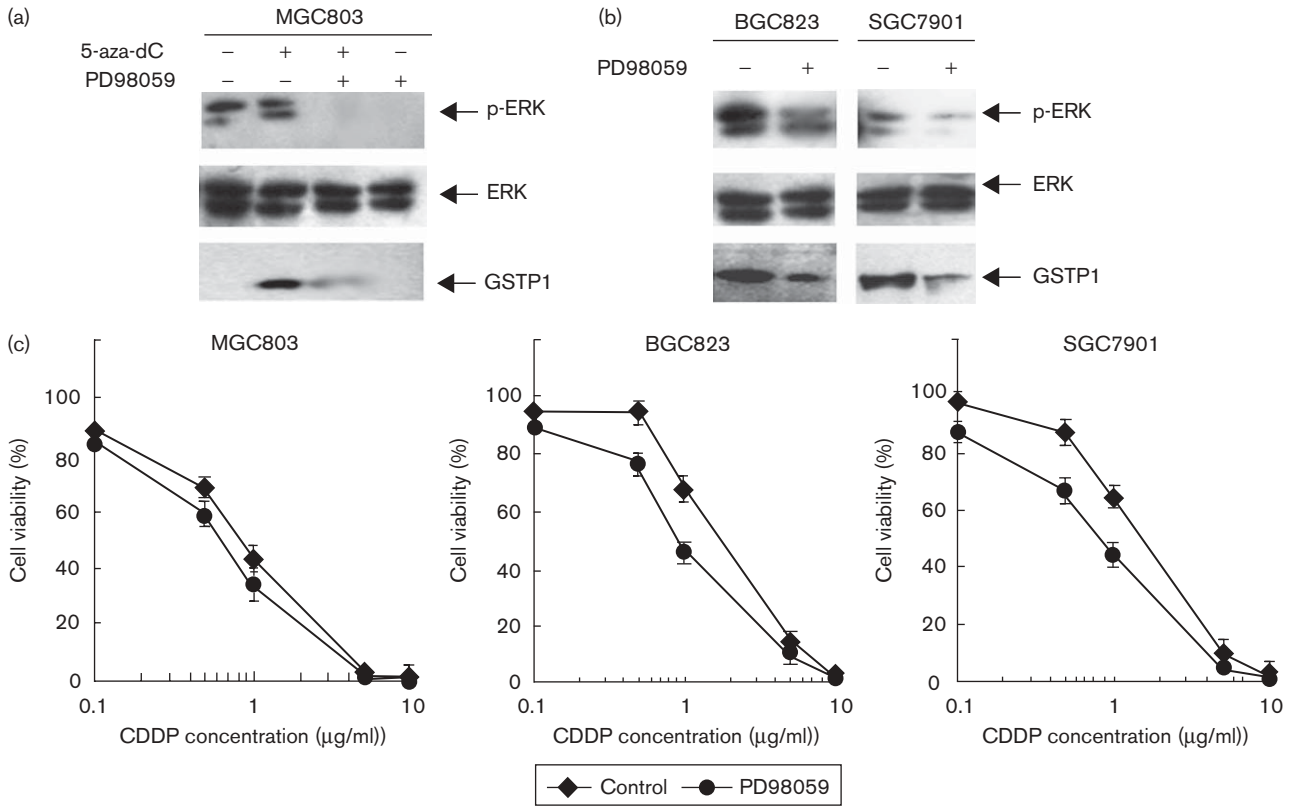
partly suppressed the phosphorylation of ERK1/2 in gastric cancer cells. Moreover, PD98059 not only suppressed the upregulation of GSTP1 protein induced by 5-aza-dC in MGC803 (Fig. 6a), but also suppressed the normal expression of GSTP1 in BGC823 and SGC7901 cells (Fig. 6b). We then investigated whether MAPK/ERK inhibition might affect the CDDP sensitivity of gastric cancer cells. Cytotoxicity was significantly enhanced in the BGC823 and SGC7901 cell lines. In contrast, MAPK/ERK inhibition did not significantly affect the sensitivity of MGC803 cells that do not express GSTP1 (Fig. 6c).

Discussion

A significant proportion of gastric cancers fail to respond to platinum-based chemotherapy. The identification of predictive markers has therefore been an important research priority [25,26]. GSTP1 has emerged as a prognostic marker in human prostate cancer [27–29]. Expression of GSTP1 can diminish the cytotoxicity of platinum drugs and GSTP1 overexpression has been implicated in resistance to platinum-based chemotherapeutics. We reported earlier that approximately 70% (91/124) of human gastric cancers ubiquitously express GSTP1, raising the possibility that GSTP1 expression might modulate platinum drug sensitivity in gastric cancer.

We report here that the MGC803 human gastric cancer cell line is significantly more sensitive to CDDP than either the BGC823 or SGC7901 gastric cancer lines. Expression of GSTP1 mRNA and protein was not detected in MGC803 cells, but both GSTP1 transcripts and protein were readily detected in BGC823 and SGC7901. We conclude that MGC803 cells do not express detectable levels of GSTP1. We then examined DNA methylation status in the vicinity of the GSTP1 promoter. In the nonexpressing cell line MGC803, the GSTP1 promoter region was fully methylated. This contrasted with the GSTP1-positive lines BGC823 and SGC7901 where promoter methylation was not detected. Importantly, treatment of MGC803 cells with the demethylating agent 5-aza-dC restored GSTP1 expression. We also report that CDDP cytotoxicity was significantly reduced in the presence of 5-aza-dC. The same result was obtained when CDDP was replaced by oxaliplatin (data not shown). These data suggest that

Fig. 6



Mitogen-activated protein kinase (MAPK)/extracellular regulated kinase (ERK) inhibition, glutathione *S*-transferase-pi (GSTP1) expression, and cis-platinum (CDDP) cytotoxicity in gastric cancer cell lines. (a) MGC803 cells treated with 5-aza-2′-deoxycytidine (5-aza-dC) for 72 h in the presence or absence of the selective MAPK/ERK inhibitor PD98059 (20 μmol/l); lysates were analyzed by western blotting using antibodies specific for phosphorylated (p-ERK), ERK, or GSTP1. (b) BGC823 and SGC7901 cells were treated with 20 μmol/l PD98059 for 24 h; lysates were analyzed by western blotting using the antibodies described in (a). (c) MGC803, BGC823, and SGC7901 cells were treated with cis-platinum (CDDP) in the presence or absence of PD98059 for 48 h. PD98059 significantly enhanced CDDP cytotoxicity in the MGC803 cell line but not in the BGC823 or SGC7901 cell lines.

abnormal promoter methylation may play a causative role in downregulating GSTP1 gene expression in MGC803 cells. GSTP1 hypermethylation has been reported in cancers of prostate [30], liver [31,32], breast [33,34], and lung [35,36].

The MAPK/ERK signaling pathway is vital mediator of cell fates including growth, proliferation, and survival [37]. Constitutively activated ERK signaling has been reported to mediate CDDP resistance in human nonsmall cell lung cancer cells [19,20]. Activation of ERK signaling is also associated with decreased CDDP sensitivity in ovarian cancer cell lines [21]. No studies have, however, so far addressed the role of MAPK/ERK signaling in gastric cancer cell resistance to CDDP. In this study, treatment of human gastric cancer lines with the selective MAPK/ERK pathway inhibitor PD98059 suppressed ERK1/2 phosphorylation. We report that PD98059 inhibited GSTP1 expression in BGC823 and SGC7901 cells. Importantly, MAPK/ERK inhibition also blocked the upregulation of GSTP1 protein in MGC803 cells brought about by the demethylating agent 5-aza-dC. In the GSTP1-expressing cell lines, BGC823 and SGC7901, CDDP cytotoxicity was also significantly increased by MAPK/ERK inhibition. These data suggest that the MAP/ERK signaling pathway activates GSTP1 expression; inhibition of this pathway with PD98059 leads to downregulation of GSTP1 expression and increased CDDP sensitivity. CDDP and PD98059 may therefore act synergistically; the possibility that a combination of the two drugs could be of utility in a clinical setting deserves attention.

In summary, our data suggest that GSTP1 expression modulates the platinum resistance of gastric cancer cells. GSTP1 gene expression seems to be coordinately regulated by methylation of the gene promoter and by the MAPK/ERK signaling pathway. Evaluation of ERK activity may therefore provide a further marker of utility in predicting which gastric cancers are likely to respond

to platinum therapy. These results extend our understanding of the mechanisms of platinum resistance in gastric cancer and suggest new approaches to reversing platinum resistance.

Acknowledgements

This work was supported by a grant from National Natural Science Foundation of China (No. 30770993).

References

1 Yamaoka Y, Kato M, Asaka M. Geographic differences in gastric cancer incidence can be explained by differences between Helicobacter pylori strains. *Intern Med* 2008; **47**:1077–1083.
2 Leung EL, Fraser M, Fiscus RR, Tsang BK. Cisplatin alters nitric oxide synthase levels in human ovarian cancer cells: involvement in p53 regulation and cisplatin resistance. *Br J Cancer* 2008; **98**:1803–1809.
3 Wei J, Zou Z, Qian X, Ding Y, Xie L, Sanchez JJ, *et al*. ERCC1 mRNA levels and survival of advanced gastric cancer patients treated with a modified FOLFOX regimen. *Br J Cancer* 2008; **98**:1398–1402.
4 Valentini A, Pucci D, Crispini A, Federici G, Bernardini S. Acridine Orange based platinum (II) complexes inducing cytotoxicity and cell cycle perturbation in spite of GSTP1 up-regulation. *Chem Biol Interact* 2006; **161**:241–250.
5 Liu B, Wei J, Zou Z, Qian X, Nakamura T, Zhang W, *et al*. Polymorphism of XRCC1 predicts overall survival of gastric cancer patients receiving oxaliplatin-based chemotherapy in Chinese population. *Eur J Hum Genet* 2007; **15**:1049–1053.
6 Frankmoelle WP, Medina JC, Shan B, Narbut MR, Beckmann H. Glutathione S-transferase metabolism of the antineoplastic pentafluorophenyl-sulfonamide in tissue culture and mice. *Drug Metab Dispos* 2000; **28**:951–958.
7 Singal R, Ferdinand L, Reis IM, Schlesselman JJ. Methylation of multiple genes in prostate cancer and the relationship with clinicopathological features of disease. *Oncol Rep* 2004; **12**:631–637.
8 Wang X, Wang L, Yuan Y. Expression of pi glutathione S-transferase in intestinal metaplasia and its relationship with *Helicobacter pylori* infection. *Zhonghua Yi Xue Za Zhi* 2002; **82**:1033–1036.
9 Zhang Y, Sun LP, Chen W, Bai XL, Liu J, Wang XG, *et al*. A molecular epidemiological study on the relationship between the polymorphism of GSTP1 and susceptibility to gastric cancer in northern Chinese. *Yi Chuan* 2007; **29**:293–300.
10 Shi H, Lu D, Shu Y, Shi W, Lu S, Wang K. Expression of multidrug-resistance-related proteins P-glycoprotein, glutathione-S-transferases, topoisomerase-II and lung resistance protein in primary gastric cardiac adenocarcinoma. *Cancer Invest* 2008; **26**:344–351.
11 Chen WY, Mao WM, Zhao L, Chen GP, Shu Y, Shen YF, *et al*. Expression of P-gp, GST-pi and Topo II alpha in gastric and colorectal cancers and their clinical significance. *Zhonghua Zhong Liu Za Zhi* 2005; **27**:738–740.
12 Eguchi-Nakajima T, Yamada Y. Predictive markers for platinum-based chemotherapy. *Gan To Kagaku Ryoho* 2008; **35**:185–189.
13 Doğru-Abbasoğlu S, Mutlu-Türkoğlu U, Türkoğlu S, Erbil Y, Barbaros U, Uysal M, *et al*. Glutathione S-transferase-pi in malignant tissues and plasma of human colorectal and gastric cancers. *J Cancer Res Clin Oncol* 2002; **128**:91–95.
14 Ellinger J, Bastian PJ, Jurgan T, Biermann K, Kahl P, Heukamp LC, *et al*. CpG island hypermethylation at multiple gene sites in diagnosis and prognosis of prostate cancer. *Urology* 2008; **71**:161–167.
15 Arai T, Miyoshi Y, Kim SJ, Taguchi T, Tamaki Y, Noguchi S. Association of GSTP1 CpG islands hypermethylation with poor prognosis in human breast cancers. *Breast Cancer Res Treat* 2006; **100**:169–176.
16 Dang DT, Chen F, Kohli M, Rago C, Cummins JM, Dang LH. Glutathione S-transferase pi1 promotes tumorigenicity in HCT116 human colon cancer cells. *Cancer Res* 2005; **65**:9485–9494.
17 Kim SG, Lee SJ. PI3 K, RSK, and mTOR signal networks for the GST gene regulation. *Toxicol Sci* 2007; **96**:206–213.
18 Judde JG, Rebucci M, Vogt N, de Cremoux P, Livartowski A, Chapelier A, *et al*. Gefitinib and chemotherapy combination studies in five novel human non small cell lung cancer xenografts. Evidence linking EGFR signaling to gefitinib antitumor response. *Int J Cancer* 2007; **120**:1579–1590.
19 Brozovic A, Osmak M. Activation of mitogen-activated protein kinases by cisplatin and their role in cisplatin-resistance. *Cancer Lett* 2007; **251**:1–16.
20 Tsurutani J, West KA, Sayyah J, Gills JJ, Dennis PA. Inhibition of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway but not the MEK/ERK pathway attenuates laminin-mediated small cell lung cancer cellular survival and resistance to imatinib mesylate or chemotherapy. *Cancer Res* 2005; **65**:8423–8432.
21 Lee S, Yoon S, Kim DH. A high nuclear basal level of ERK2 phosphorylation contributes to the resistance of cisplatin-resistant human ovarian cancer cells. *Gynecol Oncol* 2007; **104**:338–344.
22 Villedieu M, Deslandes E, Duval M, Héron JF, Gauduchon P, Poulain L. Acquisition of chemoresistance following discontinuous exposures to cisplatin is associated in ovarian carcinoma cells with progressive alteration of FAK, ERK and p38 activation in response to treatment. *Gynecol Oncol* 2006; **101**:507–519.
23 Esteller M, Corn PG, Urenal JM, Gabrielson E, Baylin SB, Herman JG. Inactivation of glutathione S-transferase P1 gene by promoter hypermethylation in human neoplasia. *Cancer Res* 1999; **58**:4515–4518.
24 Qu X, Liu Y, Ma Y, Zhang Y, Li Y, Hou K. Up-regulation of the Cbl family of ubiquitin ligases is involved in ATRA and bufalin-induced cell adhesion but not cell differentiation. *Biochem Biophys Res Commun* 2008; **367**: 183–189.
25 Anderson C, Nijagal A, Kim J. Molecular markers for gastric adenocarcinoma: an update. *Mol Diagn Ther* 2006; **10**:345–352.
26 Nagashima F, Boku N, Ohtsu A, Yoshida S, Hasebe T, Ochiai A, *et al*. Biological markers as a predictor for response and prognosis of unresectable gastric cancer patients treated with irinotecan and cisplatin. *Jpn J Clin Oncol* 2005; **35**:714–719.
27 Woodson K, O'Reilly KJ, Hanson JC, Nelson D, Walk EL, Tangrea JA. The usefulness of the detection of GSTP1 methylation in urine as a biomarker in the diagnosis of prostate cancer. *J Urol* 2008; **179**:508–512.
28 Kollermann J, Kempkensteffen C, Helpap B, Schrader M, Krause H, Muller M, *et al*. Impact of hormonal therapy on the detection of promoter hypermethylation of the detoxifying glutathione-S-transferase P1 gene (GSTP1) in prostate cancer. *BMC Urol* 2006; **6**:15.
29 Eilers T, Machtens S, Tezval H, Blaue C, Lichtinghagen R, Hagemann J, *et al*. Prospective diagnostic efficiency of biopsy washing DNA GSTP1 island hypermethylation for detection of adenocarcinoma of the prostate. *Prostate* 2007; **67**:757–763.
30 Chuang CK, Chu DC, Tzou RD, Liou SI, Chia JH, Sun CF. Hypermethylation of the CpG islands in the promoter region flanking GSTP1 gene is a potential plasma DNA biomarker for detecting prostate carcinoma. *Cancer Detect* 2007; **31**:59–63.
31 Nomoto S, Kinoshita T, Kato K, Otani S, Kasuya H, Takeda S, *et al*. Hypermethylation of multiple genes as clonal markers in multicentric hepatocellular carcinoma. *Br J Cancer* 2007; **97**:1260–1265.
32 Zhang YJ, Chen Y, Ahsan H, Lunn RM, Chen SY, Lee PH, *et al*. Silencing of glutathione S-transferase P1 by promoter hypermethylation and its relationship to environmental chemical carcinogens in hepatocellular carcinoma. *Cancer Lett* 2005; **221**:135–143.
33 Arai T, Miyoshi Y, Kim SJ, Akazawa K, Maruyama N, Taguchi T, *et al*. Association of GSTP1 expression with resistance to docetaxel and paclitaxel in human breast cancers. *Eur J Surg Oncol* 2008; **34**: 734–738.
34 Lee JS. GSTP1 promoter hypermethylation is an early event in breast carcinogenesis. *Virchows Arch* 2007; **450**:637–642.
35 Kim H, Kwon YM, Kim JS, Han J, Shim YM, Park J, *et al*. Elevated mRNA levels of DNA methyltransferase-1 as an independent prognostic factor in primary nonsmall cell lung cancer. *Cancer* 2006; **107**:1042–1049.
36 Ishii T, Fujishiro M, Masuda M, Teramoto S, Matsuse T. A methylated oligonucleotide induced methylation of GSTP1 promoter and suppressed its expression in A549 lung adenocarcinoma cells. *Cancer Lett* 2004; **212**:211–223.
37 Lee SM, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG. Hypoxia confers protection against apoptosis via PI3K/Akt and ERK pathways in lung cancer cells. *Cancer Lett* 2006; **242**:231–238.