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Original Article

SOD2 rs4880 CT/CC genotype predicts poor survival for Chinese gastric cancer patients received platinum and fluorouracil based adjuvant chemotherapy

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Abstract: Adjuvant chemotherapy is a standard therapy for gastric cancer patients, however, treatment response is quite heterogeneous. Molecular biomarkers will be highly valuable to guide the therapy and predict the response and prognosis in these patients. The antioxidant enzymes superoxide dismutase 2 (SOD2) and glutathione S-transferase pi 1 (GSTP1) are involved in oxidative stress and drug detoxification, which modulate the efficacy of anticancer drugs. Here, we investigated the clinical associations of two functional single nucleotide polymorphisms of *SOD2* and *GSTP1* in stage II-III postoperative gastric cancer patients. *SOD2* rs4880 and *GSTP1* rs1695 were genotyped in 207 patients received postoperative platinum and fluorouracil based chemotherapy and 304 patients who did not. *SOD2* rs4880 CT/CC significantly associated with decreased median overall survival time of 23 months when compared to the TT genotype (mean overall survival time of 65.2 months, P=0.002) only for patients received adjuvant chemotherapy. Stratification analysis showed *SOD2* rs4880 CT/CC affected most significantly the clinical outcome for patients with tumor arising at gastric body (HR, 5.707, P=0.002), well to moderately differentiated adenocarcinoma (HR, 4.900, P<0.001), tumor of intestinal type (HR, 4.398, P<0.001), or tumor size less or equal to 5 cm (HR, 2.490, P=0.004); while *GSTP1* rs1695 GA/GG was significant decreased overall survival time among patients with tumor arising at fundus or cardia (HR, 3.001, P=0.004), or mucinous or signet-ring cell carcinoma (HR, 4.750, P=0.042). The present study suggested the two polymorphisms would affect the adjuvant chemotherapy outcome in specific subtype of gastric cancer. *SOD2* rs4880 could be used as a biomarker to predict the prognosis and response to therapy.

Keywords: Adjuvant chemotherapy, gastric cancer, single nucleotide polymorphism, superoxide dismutase 2, glutathione s-transferase PI 1, prognosis

Introduction

Although the death rate of cancer patients was gradually declining in the last decade, gastric cancer still represents a devastating disease in Asia [1, 2]. Surgery remains the mainstay treatment, and the incorporation of perioperative chemoradiation or chemotherapy could improve patients' surgical outcome. In general, comparing to surgery alone, the adjuvant chemotherapy increased 5-year survival from 49.6% to 55.3% in the meta-analysis [3]. To date, platinum and fluorouracil (PF) based adjuvant chemotherapy has been widely accepted and shows significant improved local control rate and survival benefit for postoperative stage II-III gastric cancer [4]. However, there is still a proportion of stage II-III gastric cancer patients have early disease progression despite sufficient period of adjuvant treatment in current pr-

Table 1. Characteristics of the two cohorts of the gastric cancer patients

Clinicopathologic features	Chemotherapy (n=207)	No chemotherapy (n=304)	*P*
Age (years, mean±SD)	59±9.48	60±10.74	0.404
Sex			
Male	166	130	
Female	41	74	0.237
Tumor size[a]			
≤5 cm	118	165	
>5 cm	89	139	0.587
Tumor location[b]			
Antrum	42	59	
Fundus or cardia	78	104	
Body	51	103	
Multiple locations	7	21	0.116
Invaded depth of tumor[c]			
T1	0	4	
T2	18	29	
T3	1	6	
T4	188	261	0.163
Regional lymph node[c]			
N0	47	67	
N1	55	71	
N2	55	103	
N3	50	59	0.268
Tumor stage[c]			
II	61	97	
III	146	207	0.558
Tumor differentiation[b,d]			
Well to moderately	61	84	
Poorly	130	196	
Mucinous	15	23	0.897
Lauren classification[b]			
Intestinal type	64	85	
Diffuse type	142	219	0.449
SOD2 rs4880			
TT	151	223	
CT	47	68	
CC	3	8	0.673
GSTP1 rs1695			
AA	126	185	
GA	69	99	
GG	5	12	0.642

Abbreviations: A, adenine; C, cytosine; G, guanine; rs1695, A→G substitution at codon 105 of the GSTP1 gene; rs4880, C→T transition at codon 16 of the SOD2 gene; T, thymine. [a]Tumor size was measured by the length of the tumor. [b]Partial data were not available and statistics were based on available data. [c]Data were defined according to the TNM classification (AJCC 7th, seven edition of the American Joint Commission on Cancer Staging Manual) for gastric cancer. [d]Classification is based on the predominant pattern of tumor as tubular adenocarcinoma (well to moderately differentiated), poorly differentiated adenocarcinoma (poorly differentiated), mucinous carcinoma, and Signet-ring cell carcinoma is included as poorly differentiated.

actice. Since there is no clear evidence that any conventional clinicopathological factors that could distinguish patients who are likely to benefit from adjuvant chemotherapy or not [5], identifying biomarker that can help to individualize adjuvant therapy would allow tailored chemotherapy regimens and avoid unnecessary toxicities and financial burdens.

One mechanism of anticancer drugs is capable of increasing the intracellular reactive oxygen species (ROS) levels to induce apoptosis [6]. Excessive ROS induces oxidative stress that triggered hemostatic imbalance, leading to DNA damage in cells or on contrary to malignant transformation. Cancer cells survive under low hypoxic stress that ultimately contributes to malignant progression and chemoresistance whereas prolonged stress triggers cell death [7]. However, there have been arguments that the oxidative stress produced during treatments with antineoplastic agents such as cisplatin and doxorubicin could interfere with cell cycle, hence reduce the treatment efficacy of certain cell cycle-dependent regimens [8]. Superoxide dismutase 2 (SOD2) and glutathione S-transferase pi 1 (GSTP1) are two antioxidant enzymes, which are involved in regulation of oxi-

dative stress and drug detoxification. SOD2 is one of the major superoxide scavengers in mitochondria, converses endogenously produced superoxide into hydrogen peroxide by protecting cells from ROS- and lipid peroxidation-related oxidative damage [9]. *SOD2* rs4880 (Val16Ala) is a C to T substitution in its mitochondrion targeting sequence, resulting in a substitution of valine (Val) by alanine (Ala). Compared with *SOD2* Val variant, which is localized in the mitochondrial membrane, the Ala variant presents in the mitochondrial matrix, shows increased enzymatic activity [10]. Up-regulation of SOD2 was observed in an oral squamous cell line that had been genetically engineered to be resistant to cisplatin [11], a widely used anticancer drug and an adjuvant chemotherapy regimen for gastric cancer. GSTP1 is a member of a superfamily of dimeric phase II metabolic enzymes that play an important role in the cell defense system [12]. Alkylating agents such as cisplatin and anthracyclines are substrates of the GSTP1 isozyme [13]. *GSTP1* rs1695 (Ile105Val) is an A to G transition at codon 105 that results in an isoleucine (Ile) to valine (Val) with decreased enzymatic activity and less effective detoxification. This genetic polymorphism is associated with drug resistance in a number of cancers [14, 15], although it remains unclear whether these genetic variations could predict outcome of PF-based adjuvant chemotherapy in gastric cancer.

In the current study, we investigated the clinical implications of the functional polymorphisms of *SOD2* rs4880 and *GSTP1* rs1695 in postoperative Chinese gastric cancer patients received PF based adjuvant chemotherapy and compared analyzed their associations in another cohort of patients who did not receive any adjuvant chemotherapy due to the financial reasons. We also explored the prognostic associations of these SNPs in certain subtypes of gastric cancer.

Materials and methods

Ethics statement

The study protocol was approved by the Institutional Review Board of Nanjing Medical University (Nanjing, China). All patients have given written informed consents on the use of clinical specimens for medical research.

Study population

All patients had curative surgery and confirmed of stage II-III disease through pathological examinations and imaging studies at the Yixing People's Hospital (Yixing, Jiangsu Province, China) between 1999 and 2006 were recruited for retrospective analysis [16]. None of them had perioperative chemoradiation or neoadjuvant chemotherapy. Two hundred and seven patients had PF-based adjuvant chemotherapy within one month after surgery, and the other 384 patients had not due to the financial reasons. Overall survival was determined from the date of surgery to the date of death or last follow-up (March 31, 2009, ranging from 3-118 months). The demographic features and clinico-pathologic data are summarized in **Table 1**. Surgical specimens were processed immediately after the operation by fixing in buffered paraformaldehyde before embedding in paraffin. The samples used for genotyping were reviewed and classified by 2 independent pathologists.

Treatment plan

The adjuvant chemotherapy consisted of at least 4 cycles of PF-based regimens, including combinations of cisplatin and fluorouracil, or oxaliplatin and fluorouracil. Chemotherapy was given only if the patient had neutrophil count of 1.5×10^9/L, platelet count of 100×10^9/L, a hemoglobin level of ≥8 g/dl and no sign of organ toxicity. Antiemetics and mannitol diuresis were given according to institutional protocols.

Genotyping

Genomic DNA was extracted from tumor specimens by proteinase K digestion, isopropanol extraction, and ethanol precipitation [17]. The *SOD2* (rs4880) and *GSTP1* (rs1695) SNPs were examined by multiplex SNaPshot technology using an ABI fluorescence-based assay allelic discrimination method (Applied Biosystems, Foster City, CA) as described previously [18]. The primers and extension primers for each SNP had previously been reported [16]. The SNPs were analyzed using an ABI 3130 Genetic Analyzer, and the genotypes were determined by using GeneMapper 4.0 software (Applied Biosystems). Genotyping was validated in randomly selected 10% of samples by Sanger sequencing, and the results were 100% concordant.

Table 2. Associations of *SOD2* rs4880 and *GSTP1* rs1695 with gastric cancer-specific overall survival in both cohorts

Genetic Models	Genotype	Adjuvant chemotherapy (n=207)			No chemotherapy (n=304)		
		MST (month)	*P*[a]	HR (95% CI)[a]	MST (month)	*P*[a]	HR (95% CI)[a]
SOD2 rs4880							
Codominant model	TT	65.2[b]	*0.008*	1	65.0	0.498	1
	CT	30.0		2.015 (1.267-3.204)	46.0		1.089 (0.753-1.575)
	CC	14.0[b]		2.497 (0.599-10.406)	67.0[b]		0.292 (0.171-1.700)
Dominant model	TT	65.2[b]	*0.002*	1	65.0	0.949	1
	CT/CC	23.0		2.042 (1.298-3.212)	46.0		1.012 (0.707-1.448)
Recessive model	TT/CT	62.0[b]	0.310	1	66.7	0.276	1
	CC	14.0		2.086 (0.505-8.620)	67.3[b]		0.529 (0.168-1.662)
GSTP1 rs1695							
Codominant model	AA	62.7[b]	0.379	1	58.0	0.568	1
	GA	43.0		1.325 (0.852-2.060)	48.0		1.163 (0.835-1.620)
	GG	40.8[b]		0.597 (0.081-4.413)	62.0		0.840 (0.388-1.819)
Dominant model	AA	62.7[b]	0.340	1	58.0	0.506	1
	GA/GG	52.1[b]		1.281 (0.827-1.984)	50.0		1.114 (0.810-1.533)
Recessive model	AA/GA	61.2[b]	0.542	1	50.0	0.565	1
	GG	40.8[b]		0.538 (0.073-3.951)	62.0		0.799 (0.372-1.716)

Abbreviations: A, adenine; C, cytosine; CI, confidence interval; G, guanine; HR, hazard ratio; MST, median survival time; T, thymine. [a]Calculated in Cox regression and adjusted for age, sex and tumor stage. [b]Mean survival time was presented when the median survival time could not be measured.

Statistical analysis

The SPSS Statistical Package for Windows (version 16; SPSS Inc. Chicago, IL) was used for data analysis. All statistical tests were two-sided, and an association was considered statistically significant with a *P* value of <0.05. Kaplan-Meier survival curves and the log-rank test were used for survival analysis. Chi-squared test was used to assess differences in the frequency of characteristics between patient subgroups. Multivariate Cox regression analysis was used to determine the hazards ratios (HRs) and the independence of effects.

Results

Study population characteristics and survival

A total of 511 patients were recruited in this study. Two hundred and seven of them had PF-based adjuvant chemotherapy and 304 patients did not have chemotherapy after curative surgery. There are no significant differences with respect to clinical and -pathological factors in the two cohorts. The clinical characteristics and genotype information of the patients were summarized in **Table 1**. Eleven patients and fifteen patients were excluded for further *SOD2* and *GSTP1* analysis, respectively, because of missing genotype information. The genotype frequency distribution for *SOD2* rs4880 in all of the patients was 74.8% (374 patients) for the TT variant, 23% (115 patients) for the CT variant, 2.2% (11 patients) for the CC variant; for *GSTP1* rs1695 was 62.7% (311 patients) for the AA variant, 33.9% (168 patients) for the GA variant, 3.4% (17 patients) for the GG variant. The genotype distributions of the two SNPs were found to be indifferent between both cohorts.

In all patients, the median overall survival time (OS) was 62 months (95% CI, 46.4-77.6). Except for the patients with stage III was associated with a poorer median OS of 43 months than those with stage II (mean OS of 75 months, *P*<0.001). The mean OS of patients who had adjuvant chemotherapy was 61.5 months, which was longer but not significantly different from the median OS of patients had not adjuvant chemotherapy (54 months, *P*=0.307). In the adjuvant chemotherapy cohort, 119 patients received cisplatin and fluorouracil (CF) regimen and 88 of them received oxaliplatin and fluorouracil. The OS of oxaliplatin and fluorouracil treated patients was not significant different from that of CF treated patients (*P*=0.12).

SOD2 rs4880 polymorphisms predicted overall survival in gastric cancer patients receiving PF-based adjuvant chemotherapy

To determine the effects of the two polymorphisms of predicting clinical outcome, Cox regression analyses were used to assess associations of *SOD2* rs4880 and *GSTP1* rs1695 genotypes with overall survival in different

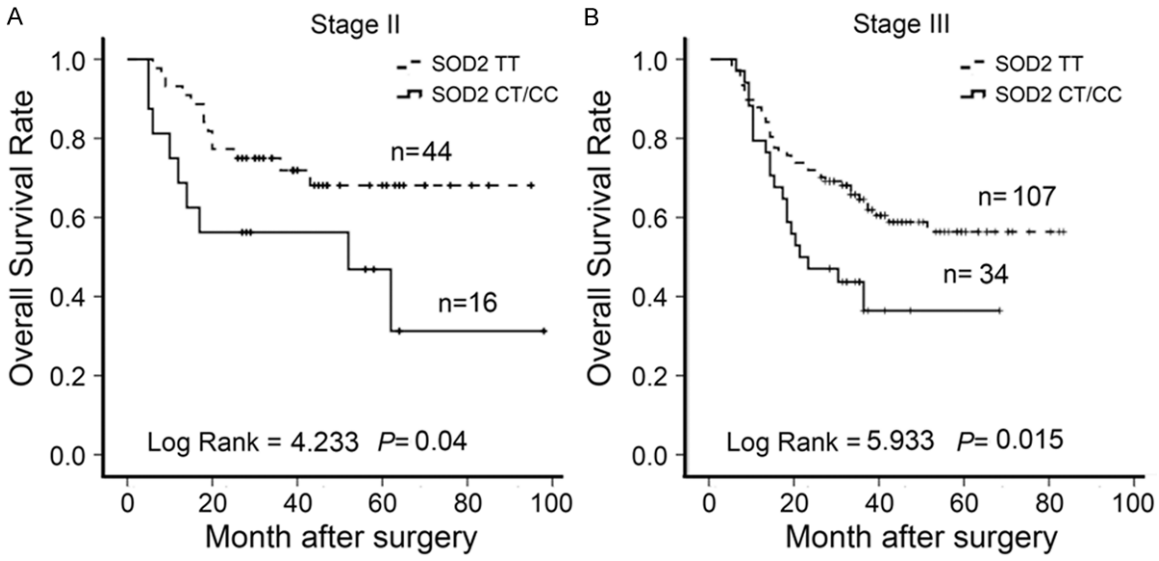

Figure 1. Kaplan-Meier survival curves of *SOD2* rs4880 for overall survival in gastric cancer patients received adjuvant chemotherapy. A. *SOD2* rs4880 CT/CC associated with poor overall survival in stage II patients. B. *SOD2* rs4880 CT/CC associated with poor overall survival in stage III patients.

genetic models for the two cohorts (**Table 2**). When adjusted by age, sex and tumor stage, neither of the two SNPs was associated with overall survival in the patients without adjuvant chemotherapy. However, for those had adjuvant chemotherapy, there was a significant association between *SOD2* rs4880 genotypes and overall survival time in the dominant model (*P*=0.002), where *SOD2* rs4880 CT/CC variant genotypes increased the risk of death (HR, 2.042, 95% CI: 1.298-3.212) when compared to the TT genotype (**Table 2**). The mean overall survival time of patients who carried a *SOD2* rs4880 TT genotype was 65.2 months, whereas the median OS of patients who carried the CT/CC genotypes was only 23 months. Further analysis stratified by tumor stage revealed that *SOD2* CT/CC genotypes significantly correlated with poor outcome independent of tumor stage. The mean survival times for patients who carried a *SOD2* rs4880 TT or CT/CC genotypes were 71.3 and 52 months in stage II (*P*=0.04), 56 and 21 months in stage III (*P*=0.015), respectively (**Figure 1**). However, *GTSP1* rs1695 was not associated with overall survival regardless of adjuvant chemotherapy in different genetic models (**Table 2**).

SOD2 and GSTP1 polymorphisms were associated with specific subtype of gastric cancer

Recent investigations indicated that the distinct pathology of gastric cancer may represent different malignancies arising in the same organ, which could be due to the unique molecular events involved in the different cell types and different initiating pathologic processes [19], and associated with different therapeutic response [20]. Hence, the associations between SOD2 rs4880, GSTP1 rs1695 and survival of post-adjuvant chemotherapy patients were further explored by stratified analysis in tumors with different histology and anatomical sites and adjusted by age, sex and tumor stage (**Table 3**). Compared to the TT genotype, the SOD2 rs4880 CT/CC genotypes was significantly associated with poor survival in gastric cancer patients with tumor arising at gastric body (HR, 5.707; 95% CI: 1.880-17.328, P= 0.002), tumor size less or equal to 5 cm (HR, 2.490; 95% CI: 1.346-4.603, P=0.004), well to moderately differentiated tumor (HR, 4.900; 95% CI: 2.134-11.255, P<0.001) or tumor of intestinal type (HR, 4.398; 95% CI: 2.035-9.502, P<0.001). Stratification analysis for GSTP1 rs1695 showed GA/GG genotype had a significant association with inferior survival with tumor arising at fundus or cardia (HR, 3.001; 95% CI: 1.431-6.294, P=0.004) or mucinous or signet-ring cell tumor (HR, 4.750; 95% CI: 1.056-21.363, P=0.042).

Discussion

Adjuvant chemotherapy improved the overall survival for gastric cancer patients, however,

Table 3. Stratified analysis of two polymorphisms with gastric cancer overall survival in patients received adjuvant chemotherapy (n=207)

Clinicopathologic Features	*SOD2* (deaths/patients)				*GSTP1* (deaths/patients)			
	TT	CT/CC	*P*[a]	HR (95% CI)[a]	AA	GA/GG	*P*[a]	HR (95% CI)[a]
Tumor location[b]								
Antrum	14/31	7/10	0.287	1.665 (0.651-4.261)	17/28	3/12	0.054	0.295 (0.085-1.022)
Fundus or cardia	22/55	9/20	0.535	1.284 (0.583-2.830)	12/42	20/34	*0.004*	3.001 (1.431-6.294)
Body	11/42	5/7	*0.002*	5.707 (1.880-17.328)	9/30	7/18	0.599	1.318 (0.471-3.686)
Tumor size								
≤5 cm	29/86	17/29	*0.004*	2.490 (1.346-4.603)	27/69	20/46	0.765	1.094 (0.607-1.970)
>5 cm	26/65	12/21	0.097	1.803 (0.898-3.621)	22/57	15/28	0.214	1.532 (0.782-3.003)
Tumor differentiation[b]								
Well to moderately	18/47	11/13	*<0.001*	4.900 (2.134-11.255)	19/40	11/21	0.574	0.879 (0.464-2.390)
Poorly	32/95	13/31	0.220	1.501 (0.784-2.875)	26/78	18/46	0.583	1.185 (0.646-2.172)
Mucinous or signet-ring cell	5/9	5/6	0.081	4.256 (0.835-21.693)	4/8	6/7	*0.042*	4.750 (1.056-21.363)
Lauren classification[b]								
Intestinal type	19/51	11/12	*<0.001*	4.398 (2.035-9.502)	20/41	11/23	0.818	1.096 (0.502-2.394)
Diffuse type	36/100	18/38	0.086	1.654 (0.932-2.937)	29/85	24/51	0.166	1.470 (0.852-2.536)

Abbreviations: A, adenine; C, cytosine; CI, confidence interval; G, guanine; HR, hazard ratio; T, thymine. [a]Calculated in multivariate Cox regression and adjusted by age, sex and tumor stage. [b]Partial data were not available and statistics were based on available data.

there is no standard regimen established in the meta-analysis [5] and lack of biomarkers to guide the selections of regimens. In our study, we investigated the clinical significance of genetic polymorphisms of 2 ROS metabolic-related genes, *SOD2* and *GSTP1*, in Chinese gastric patients. *SOD2* rs4880 CT/CC genotype was associated with poor overall survival only for the patients received PF-based adjuvant chemotherapy; additionally, the clinical associations between studied polymorphisms and survival were influenced by certain pathological characteristics.

Anti-neoplastic agents like platinum and fluorouracil, the key components of adjuvant therapy, were shown to generate high level of ROS, as evidenced by increased lipid peroxidation production and marked reduction of tissue glutathione levels [21, 22]. SOD2 is an important regulator involved in the ROS metabolic processes as an antioxidant defender, which may further interfere with the drug-resistance signaling pathways. Significantly higher SOD2 levels have been found in leukemia [23] and ovarian cancer [24], suggesting increased SOD2 activity was required for defense against ROS stress-induced injury and apoptosis [9]. In gastric cancer, elevated levels of SOD2 activity, mRNA and protein have been found to be associated with the aggressiveness of tumor and poor survival [25-29]. The Ala variant of *SOD2* rs4880 is more active than the Val variant, suggesting that the homozygous CC genotype may present higher enzymatic activity than its TT counterpart [30]. Overexpression of SOD2 increases mitochondrion-derived H_2O_2 production and leads to PTEN oxidation and activation of PI3K/Akt pathway activation [31], which is a major drug resistance related signaling pathway. It has been shown that SOD2 antisense oligodeoxynucleotides could enhance the effects of tumor necrosis factor-α and chemotherapy to eliminate highly resistant metastatic melanoma cells [32] and sensitize ovarian cancer cells to doxorubicin and paclitaxel [33]. These findings could be a possible explanation for the association of SOD2 rs4880 CT/CC with poor prognosis was only found in the patients received adjuvant chemotherapy but not in those who had not. In line with these findings, there were several studies verifying the association of SOD2 rs4880 genotypes with therapeutic effect of cyclophosphamide-containing regimens in breast cancer [34, 35] and perioperative chemoradiation for rectal cancer [36]. Although adjuvant chemotherapy generally improved the overall survival for gastric cancer patients [3], our data showed that the carriers of the SOD2 rs4880 CT/CC genotypes had a significantly shortened overall survival time than those of SOD2 rs4880 TT genotype. Stratified analysis on pathological characteristics showed SOD2 rs4880 CT/CC affected most significantly the clinical outcome for post-adjuvant chemotherapy patients with tumor arising

at gastric body, tumor size less or equal to 5 cm, well to poorly differentiated adenocarcinoma, or tumor of intestinal type. These findings suggested that PF based adjuvant chemotherapy might not be given to the patients who had SOD2 rs4880 CT/CC genotypes. Future studies are needed before implementation of personalized treatment strategy for this population.

GSTP1 participates in the intracellular ROS metabolism by catalyzing the reaction of glutathione to conjugate with exogenous and endogenous electrophiles, which yields more water-soluble and less reactive glutathione S-conjugates [37]. Polymorphisms of *GSTP1* rs1695 has been demonstrated with different enzymatic activity [38], and its Val allele is much more protective against cisplatin and carboplatin than the Ile allele in *in vitro* experiment [39]. Studies in gastric cancer, *GSTP1* rs1695 was found to be correlated with the occurrence of adverse drug effect in oxaliplatin-based chemotherapy [40] but not associated with response to cisplatin and 5-fluorouracil chemotherapy in a neoadjuvant setting [41], On the other hand, it was also reported that the *GSTP1* rs1695 homozygote and heterozygote G allele can provide protective effect for patients received platinum or 5-fluorouracil containing chemotherapy with colorectal cancer [42], non-small cell lung cancer [43] and ovarian cancer [44]; while several other studies on esophageal cancer [45] and breast cancer [46] these genotypes are associated with worse chemotherapeutic outcome. Proximal gastric cancer, as tumor at fundus and cardia/gastroesophageal junction, is recognized as a different type of gastric cancer. It has been reported that proximal gastric cancer had distinctive risk factors than distal gastric cancer, [47], unfavorable clinical response [20] and prognosis [48].

Our studies showed that *GSTP1* rs1695 GA/GG genotypes were not associated with overall survival regardless of patients' postoperative treatment when compared to AA genotype, instead, *GSTP1* rs1695 GA/GG genotypes strongly affected patients with cancer arising at gastric fundus or cardia, or mucinous or signet-ring cell carcinoma. To our knowledge, there is the first reported such association between specific markers and cancer type. Further investigations on the roles of ROS-related genes in drug response for selected gastric cancer subtype are needed.

In conclusion, genotyping for *SOD2* rs4880 could distinguish postoperative stage II or III gastric cancer patients who might or might not benefit from PF-based adjuvant chemotherapy. Moreover, *SOD2* rs4880 and *GSTP1* rs1695 showed specific influences on postoperative chemotherapy outcome in specific subtype of gastric cancer. These findings suggested that clinical usage of these SNPs indicator in gastric cancer could be possible, but should be careful, and taken other factors such as pathological characters, chemo regimens and treatment strategies into consideration. Our findings provide a solid foundation for future prospective clinical trials to validate these findings and design for effective regimens for personalized therapy.

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Disclosure of conflict of interest

None to declare.

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