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Genetic polymorphisms of GSTP1 related to response to 5-FU-oxaliplatin-based chemotherapy and clinical outcome in advanced colorectal cancer patients

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Summary

Objective: To determine whether genetic polymorphisms of GSTP1 Ile105Val (A→G) predict chemosensitivity and clinical outcome in patients with advanced colorectal cancer, treated by 5-FU/oxaliplatin-based chemotherapy.

Methods: In this retrospective study, the population consisted of 122 advanced colorectal cancer patients (III stage 51, IV stage 71). Patients were treated with 5-FU-oxaliplatin-based chemotherapy, and their response was evaluated after at least two cycles of treatments; all patients (122) were evaluated for median survival time (MST). GSTP1 genotypes were detected by TaqMan-MGB probe methods.

Results: 75 patients (61.47%) were Ile/Ile genotype, 10 (8.2%) were Val/Val genotype, and 37 (30.33%) were Ile/Val genotype. Patients possessing the glutathione S-transferase P1-105 Val/Val genotype showed a response rate of 60.0% compared to 25.89% in patients harboring at least one GSTP1-105 Ile allele (p = 0.032). GSTP1-105 Val/Val patients demonstrated a significant superior median survival time of 20.4 months (95% CI: 11.85 to 28.95) compared to 6.5 months (95% CI: 4.26 to 8.74) in patients with 105 Ile/Ile genotype and 10.3 months (95% CI: 7.05 to 13.55; p <0.01) in patients with GSTP1 105 Ile/Val genotype.

Conclusion: The GSTP1 105Val/105Val genotype is associated with a higher clinical response rate to oxaliplatin-based chemotherapy and with increased survival of patients with advanced colorectal cancer, receiving 5-FU/oxaliplatin chemotherapy.

Key words: colorectal neoplasms/genetics; polymorphisms; alleles; oxaliplatin/drug therapy; Glutathione S-transferase P1/genetics

Introduction

Pharmacogenetics is becoming an increasingly important field in the study of cancer chemotherapy. Genetic factors affecting the metabolism and transport of drugs partly explain inter-individual variability in drug response, both in terms of efficacy and safety. Polymorphisms in genes encoding specific drug-metabolizing enzymes can result in individuals in the general population being characterized as low, rapid, or even ultrarapid metabolizers. One of the remaining challenges is to identify markers that dramatically influence clinical outcome to specific chemotherapeutic agents.

The glutathione S-transferase (GST) family consists of a group of important drug-metabolizing enzymes that catalyze the conjugation of reduced glutathione with a variety of electrophilic compounds. Growing evidence indicates that GST enzymes determine the cytotoxicity of a variety of chemotherapeutic drugs. The mammalian cytosolic GSTs are divided into five subfamilies (Alpha, Mu, Pi, Theta and Zeta) on the basis of similarity in primary structure [1]. The subclass GSTP1 is widely expressed in normal human epithelial tissues and has been shown to be highly over-expressed in colon cancer [2]. Drug-resistant tumors were found to contain increased levels of GSTP1. GSTP1 directly participates in the detoxification of platinum compounds and is an important mediator of both intrinsic and acquired resistance to platinum [3]. A single nucleotide substitution (A→G) at position 313 of the GSTP1 gene, which results in replacing isoleucine with valine, substantially diminishes GSTP1 enzyme activity [4].

Oxaliplatin is an innovative, third-generation, platinum compound with powerful antineoplastic

No conflict of interest in relation to this article.

competence, a lack of cross drug resistance with cisplatin, a synergistic effect with 5-FU, and a satisfactory safety profile [5, 6]. Oxaliplatin is an alkylating agent that inhibits DNA replication by forming adducts between adjacent guanines or guanine and adenine. Moreover, these oxaliplatin adducts appear to be more effective than those of cisplatin with regard to inhibiting DNA synthesis. In addition, oxaliplatin has a more favorable toxicity profile than cisplatin. The oxaliplatin/5-FU combination has proven to be an effective first- or second-line treatment for advanced colorectal cancer [7, 8], and the preliminary results of several recent studies indicate that various combinations of oxaliplatin and 5-FU may be as effective in gastric cancer [9–11]. Given the biochemical evidence that GST mediates inactivation of platinum drugs, this study retrospectively analyzed the common polymorphisms of GSTP1 gene in 122 previously treated patients with metastatic colorectal cancer, to determine whether the presence of polymorphisms is associated with the clinical outcome to 5-FU/oxaliplatin chemotherapy.

Subjects and methods

Eligible subjects and chemotherapy

134 patients with advanced colorectal cancer enrolled in this study and were treated between January 2006 and March 2008, at the Oncology Department of the Affiliated Hospital, Medical College, Qingdao University. Eligibility criteria for patient recruitment included histological confirmation of advanced colorectal cancer and less than two prior other chemotherapy regimens. The ethnic backgrounds were all Chinese Han nationality. Patients' performance status was classified according to Eastern Cooperative Oncology Group (ECOG) criteria; eligible patients were required to have an ECOG Status 0 to 2 and an estimated life expectation of at least eight weeks. All participants gave their written informed consent prior to entering the study. Patients were required to have bi-dimensionally measurable disease at the time of protocol entry. To evaluate response to therapy, response evaluation was assessed according to Response Evaluation Criteria in Solid Tumours (RECIST) criteria. 95 cases were administered with modified FOLFOX4 regiment and 39 with Xelox regiment. The modified FOLFOX4 chemotherapy consisted of oxaliplatin 130 mg/m^2 as a 2-hour intravenous drip infusion on day 1, followed by infusion of 5-fluorouracil 300 mg/m^2, leucovorin 130 mg/m^2, respectively on day 1 to 5. Xelox regiment consisted of oxaliplatin 130 mg/m^2 as a 2-hour intravenous drip infusion on day 1, plus capecitabine for oral use at a dose of 1250 mg/m^2/day in two divided doses on day 1 to 14 of each 3-week cycle.

Genotyping

Blood samples were obtained from each patient before chemotherapy for DNA isolation and determination of genotypes. DNA was extracted from these samples using Blood Genomic DNA Isolation Kit (ShangHai, Watson). GSTP1 Ile105Val polymorphisms were assessed by nuclease allelic discrimination assay (TaqMan-MGB) using fluorescent temperature cycler (Rotor-Gene 3000A Real Time PCR System, Australia). Briefly, the 25 µl PCR mixture contained DNA 20 ng, 12.5 µl Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 1.25 µl 20 × Taqman SNP Genotyping Assay Mix (Applied Biosystems, Foster City, CA, USA), 9.25 µl ultrapure water. The PCR conditions were as follows: 95 °C for 10 min, followed by 40 cycles at 92 °C for 15s, 60 °C for 1 min, 72 °C for 1 min, followed by 1 cycle at 72 °C for 7 min. Sequences of primers and probes were designed by Applied Biosystems (Lot Number 480524 Assay ID c-622564-10). The DNA extraction and the genotyping success rate were 93.3% (125 cases) and 98.4% (123 cases) respectively. A minimum of 32 randomly selected DNA samples was genotyped at least twice to confirm the results. Discrepancies were seen in one of the samples. Those with discordant results from two analyses were excluded from the final data analysis.

Statistical analysis

The purpose of this analysis was to evaluate the association between the polymorphisms and demographic data, pretreatment characteristics, response to chemotherapy and survival time. The clinical data and specimens were evaluated retrospectively. Survival was calculated as the time from the start of treatment until death from any cause, or until last contact if the patient was known to be alive. Patients who were alive at the last follow-up or who were taken out of the study or who died before progression, were censored at the time that they were taken out of the study. Contingency tables and Fisher's exact test were used for the categorical variables to evaluate the association of the expression of markers and the response to chemotherapy, where appropriate. The log-rank test and Kaplan-Meier plots were used to evaluate the association of genotypes and overall survival. Also the Cox proportional hazards model, after adjustment for patients' clinical characteristics, was performed. Statistical analyses were carried out using SPSS for Windows (Version 13.0). All p values cited were two-sided and p values <0.05 were judged as statistically significant.

Results

Patient characteristics

In the end, 122 of 134 patients registered from January 2006 to March 2008 were eligible for further data analysis in this study. The 12 dropouts were either because of failure with DNA extraction or failure with genotyping. Characteristics of the 122 eligible patients are listed in table 1. These patients included 56 (45.91%) women and 66 (54.09%) men. The median age was 58 years (range 34–80 years).

GSTP1 genotyping

GSTP1 genotype was assessed for 122 patients, of which 10 (8.20%) were homozygous for the 105Val/105Val GSTP1 genotype, 37 (30.33%) were heterozygous (105Ile/105Val), and 75 (61.47%) were homozygous for the 105Ile/105Ile GSTP1 genotype. The genotype frequencies of the GSTP1 105 Ile→Val variation were in Hardy-Weinberg equilibrium. The current observed allele frequency for the GSTP1 105 Val allele was 0.23 (57/244) and was similar to previous findings which report that frequency in healthy Chinese and in colorectal cancer patients. No association was observed between the demographic characteristics of the study participants (age, gender) and the GSTP1 genotypes. Pathologic (differentiation, number of metastases, site of metastasis) characteristics were not statistically associated with GSTP1 genotypes.

Table 1

Characteristics of 122 colorectal cancer patients.

Characteristics	Cases (%)
Gender	
Male	66 (54.09%)
Female	56 (45.91%)
Median age (range)	58 (34–80)
≤50	24 (19.67%)
51–69	68 (55.72%)
≥70	30 (24.51%)
Original tumor site	
Colon	52 (42.62%)
Rectum	70 (57.18%)
Differentiation	
Well-differentiated	12 (9.82%)
Moderately differentiated	68 (55.72%)
Poorly differentiated	42 (34.46%)
Stage	
IIIA	32 (26.24%)
IIIB	19 (15.57%)
IV	71 (58.19%)
PS score	
0~1	79 (64.75%)
2	43 (34.25%)
Chemotherapy regimens	
L-OHP+CF+5-Fu	89 (72.96%)
L-OHP+Xeloda	33 (27.04%)
Number of organs involved	
1	48 (39.34%)
2	31 (25.41%)
3	40 (32.79%)
4	3 (2.46%)

Association among GSTP1-105 polymorphisms, chemotherapy response and survival

For association analysis of genotype and response to chemotherapy, patients with complete disappearance of the disease (CR) and at least 50% reduction in tumor load of the lesions (PR) were determined "responders". Patients with stable disease (≤25% progression, <50% shrinkage, SD) and cancer progression (size enlargement >25% or appearance of new lesions, PD) were referred to as 'nonresponders'. Patients were also divided into a favorable (homozygous GSTP1-105Val) and an unfavorable genotype group (heterozygous and homozygous GSTP1-105Ile) according to their genotypes. Of 122 patients, those possessing the GSTP1-105 Val/Val genotype showed a significantly superior response rate of 60.0% (6/10) compared to only 25.89% (29/112) in patients harboring at least one GSTP1-105 Ile allele (P = 0.032, Fisher's exact test) (table 2). Compared with patients with one or two 105 Ile alleles, patients with a homozygous 105Val/105Val GSTP1 genotype had an increased survival time, with a median survival time of 20.4 months (95% confidence interval (CI): 11.85 to 28.95). Meanwhile, the median survival time for patients with a homozygous 105Ile/105Ile GSTP1 genotype was 6.5 months (95% CI: 4.26 to 8.74), whereas for patients with a heterozygous 105Ile/105Val GSTP1 genotype was 10.3 months (95% CI: 7.05 to 13.55). The log-rank test resulted in significant *p* values of 0.008 (χ^2 = 9.56, with Ile/Ile and Ile/Val as separated group, fig. 1) and 0.007 (χ^2 = 7.38, with Ile/Ile and Ile/Val as a combined group, fig. 2), respectively. In the Cox proportional hazards model, adjusted for stage,

Figure 1

Association between GSTP1-105 genotypes (Ile/Ile and Ile/Val as separated group) and overall survival in patients with metastatic colorectal cancer receiving 5-FU/oxaliplatin chemotherapy. The vertical hash marks denote the time of last follow-up for those patients who were still alive at the time of the data analysis.

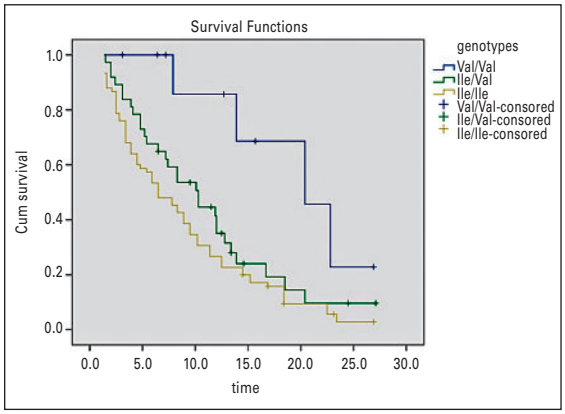

Table 2

Response to chemotherapy according to GSTP1 genetic polymorphisms.

Ile 105 Val Genotypes	Case [n (%)]		Chi-Square Test		Cox regression analysis	
	Responder	Non responder	χ^2	p	HR	95%CI
Val/Val	6 (60.0%)	4 (40.0%)	5.22	0.032	3.42	1.076–10.85
Ile/Ile+Ile/Val	29 (25.89%)	83 (74.11%)				
Total	35 (28.69%)	87 (71.31%)				

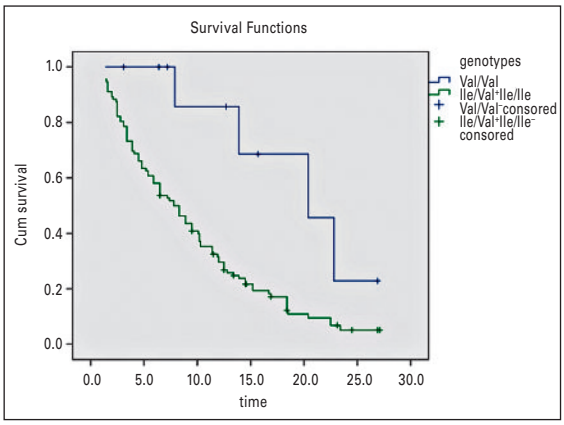

Figure 2

Association between GSTP1-105 genotypes (Ile/Ile and Ile/Val as a combined group) and overall survival in patients with metastatic colorectal cancer receiving 5-fluorouracil/oxaliplatin chemotherapy. The vertical hash marks denote the time of last follow-up for those patients who were still alive at the time of the analysis of the data.

performance status, and chemotherapy regimen, GSTP1 Ile/Ile and Ile/Val genotypes were both genetic factors significantly associated with an increased risk of dying (HR = 4.89; 95% CI, 1.51~15.84; and HR = 2.28; 95% CI, 0.69~7.52, respectively).

Discussion

In this retrospective study, it was observed that among colorectal cancer patients who received F-FU/oxaliplatin-based chemotherapy, those possessing the GSTP1 105 Val variant allele showed statistically significant increased sensitivity of cancer to 5-FU/oxaliplatin-based chemotherapy and reduction in the risk of dying, and the trends increased with the number of GSTP1 105 Val alleles. These results were independent of other clinical or pathologic prognostic markers such as tumor differentiation, performance status, and side of tumor location. Thus the authors may postulate that GSTP1 may be a key player in the metabolism of 5-FU/oxaliplatin-based regimen.

The GSTP1 enzyme can mediate the detoxification of numerous chemicals including chemotherapy agents. Recent experiments from some laboratories [12] suggest that the 105Val allele was associated with lower GSTP1 enzyme activity in colorectal cancer tissue samples. Increased expression of GSTP1 in tumors has been hypothesized to play a role in the drug resistance seen in many cancers, and this phenomenon has been observed in cancers of the breast, head and neck, and skin and in acute leukemia [13]. Stoehlmacher et al. [14] demonstrated that the GSTP1 Ile→Val polymorphisms was associated in a dose-dependent manner with increased survival of patients with advanced colorectal cancers receiving 5-FU/oxaliplatin chemotherapy. They suggest that the effect of certain chemotherapeutic drugs might be altered when enzymes that could enhance the elimination of these drugs show a reduced activity.

In contrast, other studies found that patients with GSTP1 Val-type had a worse prognosis than the patients with GSTP1 Ile-type, even after adjustment for gender, age, tumor location, Dukes' stage and differentiation. A study in breast cancer found that a significantly higher proportion of breast cancer patients with a GSTP1 Val-type had more frequency of p53 mutations and loss of heterozygosity at the TP p53 gene locus, compared with GSTP1 Ile-type [15]. It has been widely accepted that altered p53 predicts a poor prognosis in breast cancer patients, although there is no direct evidence of GSTP1 in relation to survival, in their study. It seems that GSTP1, through the Ile→Val" polymorphisms, may reduce its effect on the inactivation of toxic and carcinogenic electrophones. The opposite results related to survival in colorectal cancer patients may be due to the different characteristics of patients included in the two studies.

Oxaliplatin, like cisplatin, is inactivated by being reacted with glutathione, a reaction catalyzed by GST. Few biochemical studies and clinical reports provide strong evidence of the direct involvement of GSTP1 in resistance to platinum compounds. GSTP1, however, is directly involved in the detoxification of cisplatin via the formation of cisplatin-glutathione adducts, which indicates that GSTP1 plays a role in the acquisition of resistance to this platinum compound [16]. Clinical reports on head and neck cancers also reflect the important role of GSTP1 enzymes in the metabolism of platinum drugs [17]. The majority of patients who showed low GST protein expression levels in tumor tissues responded to a platinum-based treatment and showed better survival than patients with high GSTP1expression levels.

Due to GSTP1's potential role in detoxifying carcinogenic compounds, it is plausible that individuals with GSTP1 105 Val alleles may be at increased risk of cancer from exposure to chemicals detoxified by the GSTP1 enzyme. Harries et al. [18] found an association between GSTP1 105 Val homozygosity and a risk of bladder and testicular cancers, but they observed no statistically significant association with breast cancer risk. The study by Harries et al. compared a cancer case patient series with an infirmary-based control group and provided no information on basic characteristics of the control groups such as age and sex. In a study of men with lung cancer, hydrophobic DNA adduct levels were higher among the patients with the GSTM1 null genotype who were heterozygous or homozygous for the 105 Val GSTP1 allele [19]. Le Morvan et al. and Kweekel et al. reported that GSTP1 codon 105 polymorphism is not associated with ox-

aliplatin efficacy or toxicity, in advanced colorectal cancer patients [20, 21]. Thus, future investigations would benefit from stronger study designs.

Clinical studies have implicated GSTP1 as a predictive marker for clinical outcome in cancer patients treated with platinum-based chemotherapy. Results from the current study support the predictive value of GSTP1 in platinum-based chemotherapy, but biochemical studies are needed to definitively demonstrate that GSTP1 is directly involved in the detoxification of oxaliplatin. Moreover, the patients in the current study received combination chemotherapy of 5-FU and oxaliplatin. Although strong biochemical evidence is lacking for a detoxification of 5-FU by GSTP1, from the current study we cannot attribute the beneficial effect of reduced GSTP1 function to an alteration of the activity of the platinum compound alone.

In recent years, a growing number of novel anticancer agents for the treatment of colorectal cancer have been developed. Oxaliplatin is one example. Coupled with the variety of options, the ability to identify patients who will be more sensitive or resistant to a specific chemotherapeutic agent carries important clinical implications. Genetic profiles of individual cancer patients have the potential to aid in making treatment decisions. A recent report also points to the importance of GSTP1 in the metabolism of TLK286, a promising new anticancer agent [22]. The current study suggests that GSTP1 genotyping of individual colorectal cancer patients might contribute to improving therapy planning. However, only a relatively small number of patients were evaluated, who all received an identical platinum-based chemotherapy. Furthermore, an association between the GSTP1 genotype and the response to chemotherapy could not be determined conclusively. Additionally, we did not evaluate the toxicity data for the difficulties of extracting the data directly from patients' files. Therefore, this data should be considered as preliminary results, and randomized clinical trials with different treatment arms are needed to confirm a survival benefit for patients who possess the 105Val/105Val GSTP1 genotype and who are being treated with platinum drugs.

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References

1 Board PG, Baker RT, Chelvanayagam G, Jermiin LS. Zeta, a novel class of glutathione transferases in a range of species from plants to humans. Biochem J. 1997;328:929–35.
2 Moscow JA, Fairchild CR, Madden MJ, Ransom DT, Wieand HS, O'Brien EE, et al. Expression of anionic glutathione S-transferase and P-glycoprotein genes in human tissues and tumors. Cancer Res. 1989;49:1422–8.
3 Peklak-Scott C, Smitherman PK, Townsend AJ, et al. Role of glutathione S-transferase P1-1 in the cellular detoxification of cisplatin. Mol Cancer Ther. 2008;7(10):3247–55.
4 Watson MA, Stewart RK, Smith GB, et al. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis. 1998;19:275–80.
5 Arnould S, Hennebelle I, Canal P, et al. Cellular determinants of oxaliplatin sensitivity in colon cancer cell lines. Eur J Cancer. 2003;39:112–9.
6 Sharma RI, Smith TA. Colorectal tumor cells treated with 5-FU, oxaliplatin, irinotecan, and cetuximab exhibit changes in 18F-FDG incorporation corresponding to hexokinase activity and glucose transport. J Nucl Med. 2008;49(8):1386–94.
7 Sanoff HK, Sargent DJ, Campbell ME, et al. Five-year data and prognostic factor analysis of oxaliplatin and irinotecan combinations for advanced colorectal cancer: N9741. J Clin Oncol. 2008;26(35):5721–7.
8 Rothenberg ML, Oza AM, Bigelow RH, et al. Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III Trial. J Clin Oncol. 2003;21:2059–69.
9 Kim DY, Kim JH, Lee SH, et al. Phase II study of oxaliplatin, 5-fluorouracil and leucovorin in previously platinum-treated patients with advanced gastric cancer. Ann Oncol. 2003;14:383–7.
10 Suh SH, Kwon HC, Jo JH, et al. Oxaliplatin with biweekly low dose leucovorin and bolus and continuous infusion of 5-fluorouracil (Modified FOLFOX 4) as a salvage therapy for patients with advanced gastric cancer. Cancer Res Treat. 2005; 37:279–83.
11 De Vita F, Orditura M, Matano E, et al. A phase II study of biweekly oxaliplatin plus infusional 5-fluorouracil and folinic acid (FOLFOX-4) as first-line treatment of advanced gastric cancer patients. Br J Cancer. 2005;92:1644–9.
12 Kweekel DM, Koopman M, Antonini NF, et al. GSTP1 Ile105Val polymorphism correlates with progression-free survival in MCRC patients treated with or without irinotecan: a study of the Dutch Colorectal Cancer Group. Br J Cancer. 2008;99(8):1316–21.
13 Bennaceur-Griscelli A, Bosq J, Koscielny S, et al. High level of glutathione-S-transferase pi expression in mantle cell lymphomas. Clin Cancer Res. 2004;10(9):3029–34.
14 Stoehlmacher J, Park DJ, Zhang W, et al. Association between glutathione S-transferase P1, T1, and M1 genetic polymorphisms and survival of patients with metastatic colorectal cancer. J Natl Cancer Inst. 2002;94:936–42.
15 Nedelcheva Kristensen V, Andersen TI, Erikstein B, et al. Single tube multiplex polymerase chain reaction genotype analysis of GSTM1, GSTT1 and GSTP1: relation of genotypes to TP53 tumor status and clinicopathological variables in breast cancer patients. Pharmacogenetics. 1998;8:441–7.
16 Peklak-Scott C, Smitherman PK, Townsend AJ, et al. Role of glutathione S-transferase P1-1 in the cellular detoxification of cisplatin. Mol Cancer Ther. 2008;7(10):3247–55.
17 Yasumatsu R, Nakashima T, Uryu H, et al. Correlations between thymidylate synthase expression and chemosensitivity to 5-fluorouracil, cell proliferation and clinical outcome in head and neck squamous cell carcinoma. Chemotherapy. 2009;55(1): 36–41.
18 Harries LW, Stubbins MJ, Forman G, et al. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis. 1997;18:641–4.
19 Ryberg D, Skaug V, Hewer A, et al. Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. Carcinogenesis. 1997;18:1285–9.
20 Le Morvan V, Smith D, Laurand A, et al. Determination of ERCC2 Lys751Gln and GSTP1 Ile105Val gene polymorphisms in colorectal cancer patients: relationships with treatment outcome. Pharmacogenomics. 2007;8(12):1693–703.
21 Kweekel DM, Gelderblom H, Antonini NF, et al. Glutathione-S-transferase pi (GSTP1) codon 105 polymorphism is not associated with oxaliplatin efficacy or toxicity in advanced colorectal cancer patients. Eur J Cancer. 2009;45(4):572–8.
22 Chakrapani H, Kalathur RC, Maciag AE, et al. Synthesis, mechanistic studies, and anti-proliferative activity of glutathione/glutathione S-transferase-activated nitric oxide prodrugs. Bioorg Med Chem. 2008;16(22):9764–71.