RESEARCH ARTICLE

Predictive Role of Glutathione-S-transferase Gene Polymorphisms in Risk and Prognosis of Hepatocellular Carcinoma

Cheng-Gang Li[&], Zhi-Ming Zhao[&], Ming-Geng Hu, Rong Liu*

Abstract

Aim: We conducted a prospective study in an Chinese population to detect associations of GSTM, GSTT and GSTP polymorphisms with hepatocellular carcinoma (HCC), and analyze roles in determining survival outcome. Methods: A prospective follow-up study was conducted with 476 HCC patients and 481 controls collected from May 2005 to May 2007. All patients were followed up until the end of Dec. 2011. GSTM1, GSTT1 and GSTP1 genotyping were performed by PCR-CTPP methods. Results: Null GSTM1 carriers had a 1.64 fold risk of HCC compared with non-null genotype, while GSTP1 Val/Val carriers had a 93% increased risk over the GSTP1 IIe/IIe genotype. The median follow-up time for the 476 patients was 34.2 months (range: 1 to 78 months). Individuals with null GSTM1 genotype had better survival of HCC than non-null genotype carriers (HR=0.71, 95%CI=0.45-0.95). Similarly, GSTP1 Val/Val genotypes had significant better survival than the GSTP1 IIe/IIe genotype (HR=0.34, 95%CI=0.18-0.65). Individuals carrying null GSTM1 and GSTP1 Val/Val who received chemotherapy had lower risk of death from HCC than those without chemotherapy. Conclusion: This study indicated carriage of null GSTM1 and GSTP1 Val/Val genotypes to have roles in susceptibility to and survival from HCC.

Keywords: HCC - glutathione-S-transferases - polymorphisms - risk - survival

Asian Pacific J Cancer Prev, **13**, 3247-3252

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men (523 000 cases, 7.9% of the total) and the seventh in women (226 000 cases, 6.5% of the total), and most of the burden is in developing countries, where almost 85% of the cases occur, and particularly in men. the overall sex ratio of male to female is 2.4. The regions of high incidence are Eastern and South-Eastern Asia, Middle and Western Africa, but also Melanesia and Micronesia/Polynesia (particularly in men) (IARC, 2008). The wide geographic variation at an international levels of EC in terms of incidence and mortality suggested the role of genetic and environmental factors in the pathogenesis of this cancer.

Recently evidence indicated that carcinogen-metabolizing genes and DNA-repair genes may play a critical role in determining individual susceptibility to cancers (Han et al., 2012). Polymorphisms in these genes encoding the enzymes, possibly by altering their expression and function, may increase or decrease carcinogen activation or detoxication and modulate DNA repair.

Xenobiotics can be detoxified by phase II enzymes, such as GSTM1, GSTT1 and GSTP1 which have been suggested to be involved in detoxification of polycyclic aromatic hydrocarbons (PAHs) and benzo(a)pyrene (Schneider et al., 2004), which could detoxify carcinogens and reactive oxygen species (Rebbeck, 1997). Individuals who have homozygous deletions for GSTM1, GSTT1 and GSTP1 gene have reduced enzyme function. Lack of these enzymes may potentially increase cancer susceptibility due to a decreased ability to detoxify carcinogens such as benzo[α]pyrene-7,8-diol epoxide, the activated form of benzo[α]pyrene. The misses substitution Ile105Val results from an A3G base substitution at nucleotide 313. The Val105 form of the GSTP1 enzyme may be 2–3 times less stable than the canonical Ile105 form (Johansson et al., 1998) and may be associated with a higher level of DNA adducts (Ryberg et al., 1997). Number of published studies have focused on GSTM1, GSTTI and GSTP1 genetic variation with respect to HCC, but yielded conflicting results. Whether GSTM1, GSTT1 and GSTP1 polymorphisms are risk factors for HCC remains largely uncertain.

Furthermore, GSTs play an important role in drug metabolism, including many cancer chemotherapeutic agents (Hayes and Pulford, 1995). Genotypic and phenotypic variation in GST activity has been noted, and is thought to affect risk and prognosis in several cancers

*Department of Surgical Oncology, Chinese PLA General Hospital, Beijing, China [&]Equal contributors *For correspondence: liangjun_1999@yahoo.cn, liurong30101@126.com*

(Wiencke et al., 1990; Pemble et al., 1994; Hayes and Pulford, 1995; Dalhoff et al., 2005; Yang et al., 2005; Goekkurt et al., 2006; Reszka et al., 2006; Shiga et al., 2006). The Single nucleotide polymorphisms (SNPs) of GST genes induced the different expression of the gene product, and GSTM1, GSTT1 and GSTP1 genotypes have been hypothesized to affect the risk of HCC (Zhang et al., 2005; White et al., 2008; Giera et al., 2010), and response to chemotherapy (Ott et al., 2008; Tahara et al., 2011). GSTM1 and GSTT1 polymorphisms result in the absence of the gene product, while the GSTP1 IIe105Val polymorphism results in the substitution of valine for isoleucine at codon 105. The non-symonymous substitution results in altered catalytic activity of the gene product (Watson et al., 1998; Srivastava et al., 1999). Despite the fact that GSTM1, GSTT1 and GSTP1 gene polymorphisms have been widely examined and related to the survival of several cancer, their role in the survival of HCC in Chinese population has not been established.

Therefore, based on the literature and current understanding, this study was designed to investigate the association of GSTM, GSTT and GSTP polymorphisms with HCC, and analyze their roles in determining survival outcome in Chinese populaiton.

Materials and Methods

Patients

498 HCC patients were consecutively collected from May 2005 to May 2007. All HCC patients with newly diagnosed primary HCC in the hospital were invited for face-to-face interviews within one months after diagnosis. All cases recruited in our study were histologically confirmed. Among a total of 498 eligible cases, 476 were interviewed with a participation rate of 95.6%, including 297 males and 179 females. 513 controls were randomly selected from people who requested general health examinations in the same hospital during the same period, and 481 controls approved to participate our study with a participation rate of 93.8%. The controls were confirmed to have no malignancy, digestive diseases, chronic diseases and also no prior history of malignancy. The controls were matched with cases by ages within five years age. All the patients were followed up until the end of Dec. 2011.

Genomic DNA Exaction

For DNA extraction, 5 ml blood were provided by each collected subjects. Blood samples were stored at -20 °C. DNA was extracted from whole blood or lymphoblastoid cell lines usingthe Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's instructions. More than 80% of the genotypes were determined from DNA directly extracted from whole blood.

Genotyping of GSTM1, GSTTI and GSTP1

Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pair primer (PCR-CTPP) method (Chen et al., 2010; Kao et al., 2010). Briefly, the sequences of primers used for polymorphism of GSTM1, GSTTI and GSTP1 were amplified by using the following primers. The primers of GSTM1 were 5'-GAACTCCCTGAAAAGCTAAGC-3' and 5'-GTTGGGGCTCAAATATACGGTGG-3'. The primers of GSTT1 were 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCAGGCCAGCA-3'. The primers of GSTP1 were 5'-ACCCCAGGGCTCTATGGGAA-3' and 5'-TGAGGGCACAAGAAGCCCCT-3'. Each 30 μL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L Mg^{2+}, 2.4 μL dNTPs, 8 primers, 15 pmol of each primer and 5-8Ml template. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min. After transient centrifugation, agarose electrophoresis was conducted.

Statistical analysis

All analysis was performed by using the STATA statistical package (version 9, STATA, College Station, TX). Hardy-Weinberg equilibrium of alleles at controls was assessed by using exact tests. Categorical variables were compared with use of the chi-square test or Fisher's exact test (when one expected value was < 5). Unconditional logistic regression was undertaken to estimate odds ratios (ORs), and their 95% confidence intervals (95% CIs) after adjusting for potential confounding factors was described in previous studies. The primary death of HCC was defined as the failure event, and time of survival was defined as the time between diagnosis and death. The cause of death was reported by the hospital or cancer registration. If the patient died of other causes rather than HCC, he/she was censored at the date of death. The outcome for the study was overall survival, which was estimated using the Kaplan-Meir method. A univariate Cox's regression analysis was used to assess the association of GSTM1, GSTTI and GSTP1 gene polymorphism and survival. The relative risk [hazard ratio (HR)] and 95% CI were calculated from the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). All statistical tests were two sided, and differences were taken as significant when the P value was less than 0.05.

Results

Characteristics of HCC cases

The demographic characteristics of subjects included and clinical features of HCC patients are shown in Table 1. The average age is 47.2±6.4 years in HCC cases, and is 47.6±6.9 years in controls. There was no significant difference for gender, age and smoking (P>0.05). The lower annual income and lower BMI were associated with a higher risk of HCC (P<0.05), and HCC patients have higher proportion of positive HBsAg and positive Anti-HCV than controls (P<0.05). The HCC patients had higher consumption of alcohol drinking than controls (P<0.05). Moreover, relatives have a history of HCC would increase the risk of HCC (P<0.05).

Distribution of GSTs genotypes in HCC patients and controls

The frequency distributions of GSTs (GSTM1, GSTTI

Table 1. Demographic Characteristics of the Study Population

Parameter	Cases N=476(%)	Controls N=481(%)	P value
Gender			
Male	316(66.4)	309(64.2)	0.486
Female	160(33.6)	172(35.8)	
Age (mean±SD)	47.2±6.4	47.6±6.9	0.82
Annual income (RM)			
<5000	231(48.5)	167(34.7)	<0.001
5000-10000	193(40.6)	214(44.4)	
>10000	52(10.9)	101(20.9)	
BMI(Kg/m2) (one year before)			
<18.5	170(35.7)	156(32.5)	<0.05
18.5-23.9	191(40.2)	174(36.1)	
≥24	115(24.1)	151(31.4)	
Smoking			
Ever	178(37.4)	157(32.6)	0.12
No	298(62.6)	324(67.4)	
Drinking			
Ever	278 (58.3)	165(34.2)	<0.001
No	198 (41.7)	316(65.8)	
HBsAg			
+	260 (54.7)	210(43.6)	<0.001
-	216 (45.3)	271(56.4)	
Anti-HCV			
+	64 (13.4)	12(2.5)	<0.001
-	412 (86.6)	469(97.5)	
First-degree history of HCC			
Yes	41 (8.7)	6(1.3)	<0.001
No	435 (91.3)	475(98.7)	

Table 2. The Gene Frequencies of GSTs Genotypes in Cases and Controls

Gene polymorphisms	Cases N=476(%)	Controls N=481(%)	OR	Adjusted OR[1]
GSTM1				
Non-null	231(48.7)	270(56.1)	1.0(Reference)	1.0(Reference)
Null	244(51.3)	211(43.9)	1.35(1.04-1.76)	1.64(1.23-2.04)
GSTT1				
Non-null	355(74.8)	387(80.4)	1.0(Reference)	1.0(Reference)
Null	120(25.2)	94(19.6)	1.13(0.79-1.54)	1.33(0.86-1.77)
GSTP1				
IIe/ IIe	192(40.5)	211(43.9)	1.0(Reference)	1.0(Reference)
IIe /Val	205(43.1)	220(45.7)	1.14(0.79-1.46)	1.36(0.84-1.66)
Val/Val	78(16.4)	50(10.4)	1.71(1.12-2.63)	1.93(1.32-2.95)

and GSTP1) genotypes are shown in Table 2. Among controls, the frequencies of GSTM1, GSTTI and GSTP1 Val allele are 43.9%, 19.6% and 56.1%, respectively, and they did not deviate from Hardy-Weinberg equilibrium (P = 0.22, 0.13 and 0.08, respectively). The null GSTM1 genotype is detected in 51.3% of the HCC cases, and the proportion of them was higher than controls (P<0.05). The frequencies of GSTP1 1a/1a, 1a/1b and 1b/1b in cases (40.5%, 43.1% and 16.4%) showed significant difference compared with those in controls (43.9%, 45.7% and 10.4%) (p<0.05). Null GSTM1 carriers had a 1.64 fold risk of HCC compared with non-null genotype. GSTP1 1b/1b carriers had the OR (95% CI) of 1.93 (1.32-2.95) for HCC risk. No significant difference was found in the GSTM1 genotypes between cases and controls (P>0.05).

Kaplan-Meier survival analysis of HCC patients and the effect of GSTM1 and GSTP1 on survival outcome

Patients were followed from diagnosis until the end

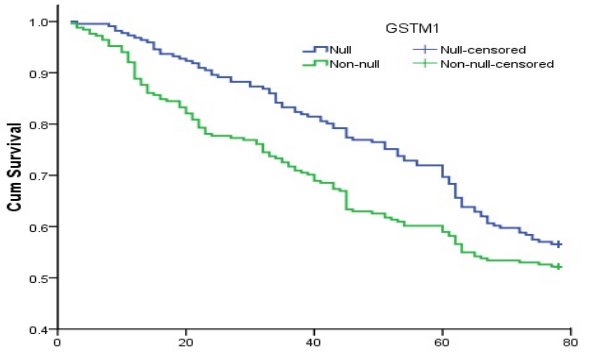

Figure 1. Kaplan-Meier Survival Analysis on the Effect of GSTM1 Polymorphism and Survival Outcome of HCC

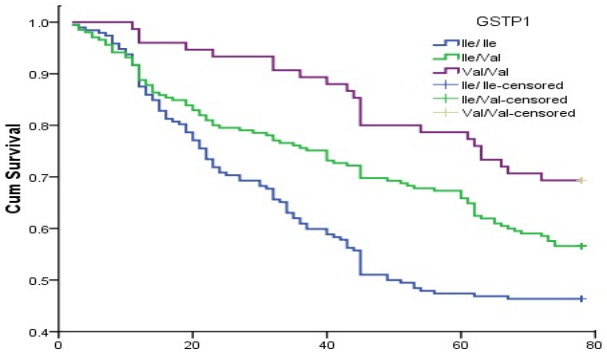

Figure 2. Kaplan-Meier Survival Analysis on the Effect of GSTP1 Polymorphism and Survival Outcome of HCC

Table 4. Association Between GSTs Genotype and Survival by Chemotherapy

Genotype	Chemotherapy		No chemotherapy	
	Deaths, n(%)	HR (95% CI)	Deaths, n(%)	HR (95% CI)
GSTM1				
Non-null	102(47.3)	1.0(Reference)	88(40.6)	1.0(Reference)
Null	114(52.7)	0.66(0.41-0.88)	128(59.4)	0.78(0.46-1.07)
GSTP1				
IIe/ IIe	86(39.9)	1.0(Reference)	94(43.5)	1.0(Reference)
IIe/Val	82(38.1)	0.63(0.46-1.06)	92(42.6)	0.76(0.62-1.23)
Val/Val	48(22.0)	0.30(0.12-0.62)	30(13.9)	0.44(0.26-0.78)

of Dec. 2011. Of 476 patients, 5 patients were lost to follow-up due to migration, while the remaining 472 patients completed the study. The median follow-up time was 34.2 months (range: 1 to 78 months). A total of 216 patients (39.1%) died of HCC during the follow-up period. Individuals with null GSTM1 genotype had better survival of HCC than Non-null genotype carriers, and the HR(95% CI) was 0.71 (0.45-0.95) (Table 3). Similarly, GSTP1 IIe/ Val or GSTP1 Val/Val genotypes had better survival than the GSTP1 IIe/IIe genotype, and the HR (95% CI) was 0.34 (0.18-0.65).

Interaction of GSTT1 and GSTP1 with chemotherapy

Results stratified by chemotherapy, present in Table 4, provide further evidence of interaction between GSTs genotypes and chemotherapy. Among those who did not receive chemotherapy, the HR (95% CI) associated with null GSTM1 genotype was approximately 0.66 (0.41-0.88), with 95% confidence interval consistently including 1.0. Among subjects who received chemotherapy, the HR (95% CI) associated with null GSTM1 was statistically

Table 3. Kaplan-Meier Estimation of the 5-years Survival and HRs(95% CI) with Gene Polymorphism

Gene polymorphisms		Cases, N=472(%)	Death, N=216(%)	Median survival (in months)	5-years survival rate	Adjusted GSTM1 HR(95% CI)
GSTM1	Non-null	230(48.7)	113(52.3)	56.1(52.7-59.4)	50.90%	1.0(Reference)
	Null	242(51.3)	103(47.7)	63.1(60.2-65.9)	57.40%	0.71(0.45-0.95)
GSTP1	IIe/ IIe	192(40.5)	103(47.9)	50.4(46.6-54.4)	45.90%	1.0(Reference)
	IIe/Val	205(43.1)	89(41.3)	58.8(55.1-62.4)	56.10%	0.69(0.51-1.12)
	Val/Val	78(16.4)	23(10.8)	67.3(62.9-71.6)	69.90%	0.34(0.18-0.65)

significant (HR=0.66, 95% CI=0.41-0.88). Moreover, those with GSTP1 Val/Val genotype showed a better survival compared with IIe/IIe genotype (HR=0.30, 95% CI=0.12-0.62). The P value for interaction between GSTT1 and GSTP1 genotype and chemotherapy was 0.04 and 0.012, respectively. Some evidence of dose-dependent effect was found in GSTP1 genotypes, but no significant difference was found.

Discussion

This study firstly analyzed the influence of GSTM1, GSTT1 and GSTP1 gene polymorphisms on the susceptibility to HCC. Individuals carrying null GSTM1 or GSTP1 Val/Val genotype had a significant increased risk of HCC, while GSTM1 gene did not show significant association with risk of HCC. Moreover, association was found between the different genotypes of GSTTM1 and GSTP1 and survival of HCC. Null GSTM1 and GSTP1 val/val carriers had a better survival among HCC patients with chemotherapy (HR=0.66, 95% CI=0.41-0.88 and HR=0.30, 95% CI=0.12-0.62, respectively). A significant interaction was found between GSTM1 and GSTP1 genotype and chemotherapy.

GSTs belong to a super-family of detoxification enzymes, which play a role in resisting a large variety of chemical carcinogens and environmental toxicants. The null GSTM1, null GSTT1 and GSTP Val/Val reduced function genotypes, one of the phase II enzymes, are known to link with increasing incidence of certain cancers, most likely due to inefficient carcinogen detoxification and therefore a higher risk of developing cancer. Previous meta-analysis studies indicated that null GSTM1, null GSTT1 and GSTP1 Val/Val genotypes might have a significant association with increased risks of breast cancer, lung cancer, gastric cancer and HCC in Chinese population (Sull et al., 2005; Saadat, 2006; Hosgood et al., 2007; Shi et al., 2008). In our study, we did not findthe association of null GSTM1 and GSTT1 with HCC, and the GSTP1 Val/Val genotype frequency was associated with a 1.93 times higher risk of HCC than GSTP IIe/IIe genotype. In a recent meta-analysis, it was observed that null GSTT1 conferred a light increased risk (White et al., 2008). Previous epidemiological studies showed GSTP1 Val/Val genotype was considered as a factor increasing the susceptibility to and risk of HCC in Taiwanese (Chen et al., 2010), and null GSTT1 and GSTM1 genotypes did not show association with increased risk of HCC (Kao et al., 2010). However, the results of GSTs and risk of HCC are conflicting in studies conducted in other ethnicities (Abd et al., 2008; Kiran et al., 2008; Asim et al., 2010). The reason might be ethnic difference in the gene-susceptibility.

Increased evidence has suggested an important role for drug-metabolizing enzymes in determining interindividual variations in therapeutic response. GSTs are enzymes which detoxify a variety of electrophilic compounds. Few studies reported the genetic polymorphisms in GSTs gene influencing the efficacy of detoxifying cytotoxins generated by chemotherapeutics. Our results showed patients carrying null GSTM1 and GSTP1 Val/Val genotypes had a significant decreased risk of death from HCC. Previous studies showed the GSTM1 gene polymorphism might be a predictor factors for the prognosis of certain cancer among Asian population, including breast cancer, lymphoblastic leukemia, advanced oral cancer, non-small cell lung cancer and gastric cancer (Mahimkar et al., 2011; Saip et al., 2011; Suneetha et al., 2011; Borst et al., 2012; Zha et al., 2012). There still no report on the role the GSTs polymorphisms on the survival of HCC. We found increased survival in null GSTM1 and GSTP1 Val/Val genotypes which is obvious because the two genotypes carriers have reduced enzyme function and thus, may have decreased DNA repair function and enhance the effect of chemotherapy.

Overall, this study reported the carriage of null GSTM1 and GSTP1 Val/Val genotype have a role in susceptibility and survival of HCC. However, the gene susceptibility showed variable in different ethnicities and a lower number of patients with survival data. Therefore, further large sample studies are needed to confirm the genetic role of GSTs on HCC.

References

Abd El-Moneim E, Younis FA, Allam N, Gameel K, Osman M (2008). Gene deletion of glutathione S-transferase M1 and T1 and risk factors of hepatocellular carcinoma in Egyptian patients. *Egypt J Immunol*, **15**, 125-34.

Ambrosone CB, Sweeney C, Coles BF, et al (2001). Polymorphisms in glutathione S-transferases (GSTM1 and GSTT1) and survival after treatment for breast cancer. *Cancer Res*, **61**, 7130-5.

Asim M, Khan LA, Husain SA, et al (2010). Genetic polymorphism of glutathione S transferases M1 and T1 in Indian patients with hepatocellular carcinoma. *Dis Markers*, **28**, 369-76.

Borst L, Buchard A, Rosthøj S, et al (2012). Gene dose effects of GSTM1, GSTT1 and GSTP1 polymorphisms on outcome in childhood acute lymphoblastic leukemia. *J Pediatr Hematol Oncol*, **34**, 38-42.

Boyer TD, Kenney WC (1985). Preparation, characterization and properties of glutathione S-transferases. In: Zakim D, Vessey D, eds. Biochemical pharmacology and toxicology. New York, NY: John Wiley & Sons.

Chen B, Cao L, Zhou Y, et al (2010). Glutathione S-transferase T1 (GSTT1) gene polymorphism and gastric cancer

susceptibility: a meta-analysis of epidemiologic studies. *Dig Dis Sci*, **55**, 1831-8.

Chen YL, Tseng HS, Kuo WH, et al (2010). Glutathione S-Transferase P1 (GSTP1) gene polymorphism increases age-related susceptibility to hepatocellular carcinoma. *BMC Med Genet*, **11**, 46.

Dalhoff K, Buus Jensen K, Enghusen Poulsen H (2005). Cancer and molecular biomarkers of phase 2. *Methods Enzymol*, **400**, 618-27.

Giera S, Braeuning A, Köhle C, Bursch W, Metzger U, Buchmann A, Schwarz M (2010).Wnt/beta-catenin signaling activates and determines hepatic zonal expression of glutathione S-transferases in mouse liver. *Toxicol Sci*, **115**, 22-33.

Goekkurt E, Hoehn S, Wolschke C, et al (2006). Polymorphisms of glutathione S-transferases (GST) and thymidylate synthase (TS)-novel predictors for response and survival in gastric cancer patients. *Br J Cancer*, **94**, 281-6.

Han X, Xing Q, Li Y, Sun J, Ji H, Huazheng P, Jun L (2012). Study on the DNA Repair Gene XRCC1 and XRCC3 Polymorphism in Prediction and Prognosis of Hepatocellular Carcinoma Risk.Hepatogastroenterology, 59(119). doi: 10.5754/hge12096.

Hayes JD, Pulford DJ (1995). The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol*, **30**, 445-600.

Hayes JD, Strange RC (2000). Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology*, **61**, 154-66.

Hohaus S, Di Ruscio A, Di Febo A, et al (2005). Glutathione S-transferase P1 genotype and prognosis in Hodgkin's lymphoma. *Clin Cancer Res*, **11**, 2175-9.

Kao CC, Chen MK, Kuo WH, et al (2010). Influence of glutathione-S-transferase theta (GSTT1) and micro (GSTM1) gene polymorphisms on the susceptibility of hepatocellular carcinoma in Taiwan. *J Surg Oncol*, **102**, 301-7.

Kiran M, Chawla YK, Kaur J (2008). Glutathione-S-transferase and microsomal epoxide hydrolase polymorphism and viral-related hepatocellular carcinoma risk in India. *DNA Cell Biol*, **27**, 687-94.

IARC. Globocan 2008: Liver Cancer Incidence and Mortality Worldwide in 2008 Summary. AIRC, 2008. http://globocan.iarc.fr/factsheets/cancers/liver.asp.

Mahimkar MB, Samant TA, Kannan S, et al (2011). Polymorphisms in GSTM1 and XPD genes predict clinical outcome in advanced oral cancer patients treated with postoperative radiotherapy. Mol Carcinog, Dec 28.

Moy KA, Yuan JM, Chung FL, et al (2009). YuIsothiocyanates, glutathione S-transferase M1 and T1 polymorphisms and gastric cancer risk: a prospective study of men in Shanghai, China. *Int J Cancer*, **125**, 2652-9.

Nguyen TV, Janssen MJ, van Oijen MG, et al (2010). Genetic polymorphisms in GSTA1, GSTP1, GSTT1, and GSTM1 and gastric cancer risk in a Vietnamese population. *Oncol Res*, **18**, 349-55.

Ott K, Lordick F, Becker K, et al (2008). Glutathione-S-transferase P1, T1 and M1 genetic polymorphisms in neoadjuvant-treated locally advanced gastric cancer: GSTM1-present genotype is associated with better prognosis in completely resected patients. *Int J Colorectal Dis*, **23**, 773-82.

Parkin DM (2001). Global cancer statistics in the year 2000. *Lancet Oncol*, **2**, 533–43.

Pemble S, Schroeder KR, Spencer SR, et al (1994). Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J*, **300**, 271-6.

Piao JM, Shin MH, Kweon SS, et al(2009). Glutathione-S-transferase (GSTM1, GSTT1) and the risk of gastrointestinal cancer in a Korean population. *World J Gastroenterol*, **15**, 5716-21.

Reszka E, Wasowicz W, Gromadzinska J (2006). Genetic polymorphism of xenobiotic metabolising enzymes, diet and cancer susceptibility. *Br J Nutr*, **96**, 609–19.

Roth MJ, Abnet CC, Johnson LL, et al (2004). Polymorphic variation of Cyp1A1 is associated with the risk of gastric cardia cancer: a prospective case cohort study of cytochrome P-450 1A1 and GST enzymes. *Cancer Causes Control*, **15**, 1077-83.

Saip R, Sen F, Vural B, et al (2011). Glutathione S-transferase P1 polymorphisms are associated with time to tumor progression in small cell lung cancer patients. *J BUON*, **16**, 241-6.

Shiga H, Heath EI, Rasmussen AA, et al (1995). Prognostic value of p53, glutathione S-transferase pi, and thymidylate synthase for neoadjuvant cisplatin-based chemotherapy in head and neck cancer. *Clin Cancer Res*, **5**, 4097-104.

Srivastava SK, Singhal SS, Hu X, et al (1999). Differential catalytic efficiency of allelic variants of human glutathione S-transferase Pi in catalyzing the glutathione conjugation of thiotepa. *Arch Biochem Biophys*, **366**, 89-94.

Stoehlmacher J, Park DJ, Zhang W, et al (2002). Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. *J Natl Cancer Inst*, **19**, 936-42.

Suneetha KJ, Nancy KN, Rajalekshmy KR, et al (2011). Role of glutathione-s-transferase and CYP1A1*2A polymorphisms in the therapy outcome of south Indian acute lymphoblastic leukemia patients. *Indian J Med Paediatr Oncol*, **32**, 25-9.

Sweeney C, McClure GY, Fares MY, et al (2000). Association between survival after treatment for breast cancer and glutathione S-transferase P1 Ile105Val polymorphism. *Cancer Res*, **60**, 5621-4.

Tahara T, Shibata T, Nakamura M, et al (2011). Effect of genetic polymorphisms related to DNA repair and the xenobiotic pathway on the prognosis and survival of gastric cancer patients. *Anticancer Res*., **31**, 705-10.

Watson MA, Stewart RK, Smith GB, et al (1998). Human glutathione Stransferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis*, **19**, 275-80.

White DL, Li D, Nurgalieva Z, El-Serag HB (2008). Genetic variants of glutathione S-transferase as possible risk factors for hepatocellular carcinoma: a HuGE systematic review and meta-analysis. *Am J Epidemiol*, **167**, 377-89.

Wideroff L, Vaughan TL, Farin FM, et al (2007). GST, NAT1, CYP1A1 polymorphisms and risk of esophageal and gastric adenocarcinomas. *Cancer Detect Prev*, **31**, 233-6.

Wiencke JK, Kelsey KT, Lamela RA, et al (1990). Human glutathione Stransferase deficiency as a marker of susceptibility to epoxide-induced cytogenetic damage. *Cancer Res*, **50**, 1585-90.

Yang G, Shu XO, Ruan ZX, et al (2005). Genetic polymorphisms in glutathione-S-transferase genes (GSTM1, GSTT1, GSTP1) and survival after chemotherapy for invasive breast carcinoma. *Cancer*, **103**, 52-8.

Zha Y, Cun Y, Zhang Q, Li Y, Tan J (2012). Prognostic value of expression of Kit67, p53, TopoIIα and GSTP1 for curatively resected advanced gastric cancer patients receiving adjuvant paclitaxel plus capecitabine chemotherapy. *Hepatogastroenterology*, **59**, 1327-32.

Zhang YJ, Chen Y, Ahsan H, Lunn RM, Chen SY, Lee PH, Chen CJ, Santella RM (2005).Silencing of glutathione

S-transferase P1 by promoter hypermethylation and its relationship to environmental chemical carcinogens in hepatocellular carcinoma. *Cancer Lett*, **221**, 135-43.