RESEARCH COMMUNICATION

Predictive Role of Glutathione-S-transferase Gene Polymorphisms in the Survival of Gastric Cancer Cases

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Abstract

Aim: We conducted a prospective study in an Chinese population to detect the association between GSTM, GSTT and GSTP gene polymorphisms and survival of gastric cancer. Methods: A prospective follow-up study with 317 gastric cancer patients was conducted between January 2003 and January 2005. GSTM1, GSTT1 and GSTP1 genotyping was performed using ABI TaqMan Gene Expression assays. Results: Of 317 patients, 5 were lost to follow-up due to migration, while the remaining 302 patients completed the study. The median follow-up time was 34.2 months (range: 2 to 60 months), during which a total of 120 (39.1%) died of gastric cancer. The GSTT1-null genotype showed a significant increased risk of death from gastric cancer, with an HR (95% CI) of 1.59 (1.04-3.58). Moreover, we found individuals carrying null-GSTM1 and null-GSTT1 had a moderate higher risk of death from gastric cancer, with an HR of 1.92 (1.05-3.65). Conclusion: This study reported the carriage of null GSTT1 and null GSTM1 might be linked to the higher death risk from gastric cancer in Chinese population.

Keywords: Glulathione-S-transferases - polymorphism - survival - gastric cancer - Chinese patients

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Introduction

Worldwide, about one million new cases of gastric cancer were estimated to have occurred in 2008 (988 000 cases, 7.8% of the total), making it currently the fourth most common malignancy in the world, behind cancers of the lung, breast and colo-rectum. More than 70% of cases (713 000 cases) occur in developing countries (467 000 in men, 246 000 in women), and half the world total occurs in Eastern Asia (mainly in China). Moreover, gastric cancer is the second most frequent cause of death from cancer. The prognosis of gastric cancer is poor. The median survival time of patients with advanced gastric cancer ranges approximately from 7.5 to 12 months (Parkin, 2001). Previous studies suggested the genetic polymorphisms in gene involved in metabolism, signaling, transport, DNA-repair and cellular response pathways, and all of them contribute to inter-patient variability of drug response and toxicity.

Glutathione-S-transferases (GSTs) are an enzyme super-family involved in the Phase II metabolism (Hayes and Pulford, 1995; Hayes and Strange, 2000; Dalhoff et al., 2005). GSTs catalyze the conjugation of glutathionme to electrophilic species, resulting in hydrophilic species that are more easily excreted (Boyer and Strange, 2000). GSTs play an important role in drug metabolism, including many cancer chemotherapeutic agents (Hayes and Pulford et al., 1995). Genotypic and phenotypic variation in GST activity has been noted, and is thought to affect risk and prognosis in several cancers (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 GSTM, GSTT and GSTP genotypes have been hypothesized to affect the risk of gastric cancer (Piao et al., 2009; Chen et al., 2010; Nguyen et al., 2010), gastric survival, 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 GSTM, GSTT and GSTP gene polymorphisms have been widely examined and related to the survival of several cancers, their role in gastric survival in Chinese population has not been established. Therefore, we conducted this prospective study.

Materials and Methods

Patients

This was a prospective follow-up study with 317 gastric cancer patients between January 2003 and January

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2004, including 217 males and 100 females. All the patients were diagnosed by pathological examination and were newly diagnosed within one month.

Data collection

Face to face interview was performed for all subjects. Two interviewers were trained and were not aware of the study hypothesis. A self-designed questionnaire was used to collected the sociodemographic characteristics and clinic characteristics. Information included smoking, drinking, family cancer history of first relative, H.pylori infection, tumor localization and TNM stage. Cancer patients were asked to refer about dietary habit a year before diagnosis.

H. pylori detection

A whole blood sample was drawn and the serum was kept at -20 °C. The anti-H. pylori serum IgG titers were quantified by ELISA(Anti-Helicobacter pylor ELISA (IgG), EuroImmun, Germany). Participants were classified as negative if they had o16RU ml^{-1}, as borderline if their antibody concentration was between 16 and 22RU ml^{-1} and as positive if this was X22RU ml^{-1}, according to the manufacturer's instructions. For analysis, subjects with borderline IgG titres were classified as infected.

GST genotyping

The Genomic DNA was extract from whole blood samples by using the Qiagen Blood Kit (Qiagen, Chatsworth, CA). The GSTM1, GSTT1 and GSTP1 genotyping was performed by using the ABI PRISM® 7900HT Sequence Detection System(Applied Biosystems, Carlsbad, CA). The primers and probes for the TaqMan assay were designed using Primer Express software and are available from the authors on request. For each SNP, a minimum of 10% randomly selected DNA samples were genotyped at least twice to confirm the results.

Statistical analysis

The primary death of gastric cancer 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 gastric cancer, he/she was censored at the date of death. Association between the different genotypes and clinical and histopathological features were tested by χ^2 analysis or Fisher's exact test where appropriate. Survival rates were estimated according to Kaplan-Meier. Relative risks were estimated by calculating hazard ratio from Cox proportional hazard models. All statistical testing was two sided and conducted at the 0.05 level. SPSS software (16.0, SPSS, Chicago, IL, USA) was used.

Table 1. Demographic and Clinic Characteristics of Gastric Cancer Patients

Characteristics	Cases N=317(%)	%
Age (years)	46.4±5.2	
Sex		
Male	217	68.3
Female	100	31.7
Smoking		
Ever	131	41.4
No	186	58.6
Drinking		
Ever	146	45.9
No	171	54.1
Family cancer history of first relative		
Yes	40	12.5
No	277	87.5
H.pylori infection		
Positive	212	66.8
Negative	105	33.2
Tumor localization		
Proximal third	183	57.7
Middle third	104	32.7
Distal third	30	9.6
Lauren classification		
Intestinal	139	43.8
Non-intestinal	178	56.2
TNM stage		
I	40	12.5
II	79	24.9
III	148	46.7
IV	50	15.9
Postoperative characteristics		
Resection	240	75.6

Results

Characteristics of gastric cancer cases

The demographic characteristics of patients are shown in Table 1. The average age of gastric cancer cases was 46.4±5.2 years. Patients were followed from diagnosis until the end of Dec. 2011. Of 317 patients, 5 patients were lost to follow-up due to migration, while the remaining 302 patients completed the study. The median follow-up time was 34.2 months (range: 2 to 60 months). A total of 120 patients (39.1%) died of gastric cancer during the follow-up period.

GST genotypes and prognosis of gastric cancer

The GSTM1-null genotype showed a non-significant increased risk of death from gastric cancer (HR=1.42, 95% CI=0.90-2.28) (Table 2). We found a significant association between null GSTT1 and gastric cancer

Table 2. Kaplan-Meier Survival Estimation of 5-years Survival and HRs with GSTM1, GSTTI and GSTP1 and Gastric Cancer Risk

Genetic polymorphisms	Cases N=317(%)	Death, n(%) N=120(%)	5-years survival(%)	HR (95% CI)[1]
GSTM1				
Non-null	132(41.5%)	40(33.6)	69.40%	1.0(reference)
Null	185(58.5%)	80(66.4)	57.00%	1.42(0.90-2.28)
GSTT1				
Non-null	144(45.3%)	42(34.7)	71.00%	1.0(reference)
Null	173(54.7%)	78(65.3)	54.80%	1.59(1.04-3.58)
GSTP1				
1a/1a	176(55.5%)	66(54.6)	62.80%	1.0(reference)
1a/1b	92(28.9%)	31(25.7)	66.30%	0.93(0.54-1.76)
1b/1b	49(15.6%)	24(19.7)	52.20%	1.38(0.81-2.65)

[1]Adjusted for age, sex, smoking, drinking, family cancer history of first relative, H.pylori infection, tumor localization and TNM stage

Table 3. Combined Genotype Analysis of GSTM1 and GSTT1 Genotypes on Gastric Cancer Risk

Genetic polymorphisms	Cases N=317(%)	Death, n(%) N=120(%)	5-years survival(%)	HR (95% CI)[1]
GSTM1/GSTT1				
Non-null/ Non-null	43	21	51.2	1.0(reference)
Non-null/Null	46	19	58.7	0.95(0.42-2.04)
Null/ Non-null	37	21	43.2	1.32(0.67-2.83)
Null/Null	68	57	16.2	1.92(1.05-3.65)

[1]Adjusted for age, sex, smoking, drinking, family cancer history of first relative, H.pylori infection, tumor localization and TNM stage

prognosis, with a average survival time of 30.3 months for individuals with null GSTT1 genotype, and 36.1 months for those with the non-null genotype (HR=1.59, 95% CI=1.04-3.58). Moreover, we found a non-significant increased risk of death from gastric cancer (HR=1.38, 95%CI=0.81-2.65).

We further analyzed the association between the combination of GSTM1 and GSTT1 polymorphisms and gastric cancer (Table 3). When compared with non-null GSTM1 and non-null GSTT1 genotypes, we found individuals carrying null-GSTM1 and null-GSTT1 had a moderate higher risk of death from gastric cancer, with a HR of 1.92 (1.05-3.65).

Discussion

This study firstly analyzing the influence of GSTM1, GSTT1 and GSTP1 gene polymorphisms on the prognosis in gastric cancer. In our study, no association was found between the different genotypes of GSTM1 and GSTP1 and survival of gastric cancer, and only null GSTT1 was related with higher risk of gastric cancer. The combination of null GSTM1 and null GSTT1 was associated with higher risk of gastric cancer progression.

The increasing 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 such as platinum agents. However, the results of those studies have been conflicting. Due to impairment of the GSTM1 capacity, patients with null variant allele may be less capable of detoxifying oxaliplatin when compared with patients carrying non-null genotype. Our results showed patients carrying null GSTM1 genotype had a non-significant light risk of death risk from gastric cancer. A previous meta-analysis study showed the GSTM1 gene polymorphism might be a risk factor for gastric cancer among Asian population (Chen et al., 2010). Another study conducted in German showed non-null GSTM1 genotype was associated with a better prognosis in gastric cancer patients. Our study results also supported that the deficiency GSTM1 genotype may increase risk of death from gastric cancer. Moreover, our study showed a association between null GSTT1 genotype and gastric cancer prognosis. A previous conducted in Sweden showed a borderline inverse association between carriage of the GSTT1 null genotype and gastric cancer prognosis. However, this was not observed in the our study in this particular relationship, and also not observed in previous studies (Roth et al., 2004; Wideroff et al., 2007). The reason might be that there is ethnic difference in the gene-susceptibility.

Our study showed the combination of GSTT1- and GSTM1- null was association with gastric cancer prognosis. Previous studies suggested the glutathione S-transferase isoforms exhibiting overlapping substrate specificity with different combinations of various unfavorable deletion genotypes may increase the risk for head and neck cancers (Hashibe et al., 2003). Both GSTT1- and GSTM1- null were reported a higher risk for ovarian cancer (Howells et al., 2004) and gastric cancer (Ott et al., 2008). However, previous studies showed the inactive genotypes GSTT1-null and GSTM1-null were associated with improved survival in breast cancer, breast cancer, colorectal cancer and advanced gastric cancer prognosis (Ambrosone et al., 2001; Stoehlmacher et al., 2002; Goekkurt et al., 2006). The contradiction results may be due to the different risk factors of gastric cancer in different ethnicities. Therefore, further studies for these two genotypes are needed.

In conclusion, this study reported the carriage of null GSTT1 and null GSTM1 might be linked to the death risk from gastric cancer in Chinese population. However, the gene susceptibility showed variable in different ethnicities. Therefore, further large sample studies are needed to confirm the genetic role of GSTs on the prognosis of gastric cancer.

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