RESEARCH COMMUNICATION

Glutathione S-transferase (GSTM1 and GSTT1) Polymorphisms in Cervical Cancer in Northeastern Thailand

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Abstract

To evaluate the relationships between genetic polymorphisms of the *GSTs* (*GSTM1* and *GSTT1*) and cervical cancer, the null genotype of each gene was studied in squamous cell cervical cancer (SCCA) patients (n=90) and controls (n=94) in Northeast Thailand. The prevalence of the *GSTM1*-null genotype in the controls and SCCA patients was 59.6% and 60.0%, respectively, whereas those of the *GSTT1*-null genotype in the control and SCCA patients was 40.4% and 46.7%, respectively. Neither of the *GST*-null genotypes increased the risk for SCCA (p>0.05); however, the combination of the *GSTM-1* and *GSTT1*-null genotypes showed a non-significant trend to an increased risk for developing cervical cancer with an adjusted OR of 2.7 (95%CI=0.8-9.0, p=0.10). Genetic polymorphisms of *GSTM1* and *GSTT1* were not significant risk factors for cervical cancer in either tobacco-smokers or non-smokers. A different contribution of the *GST* genotype to cancer risk may be attributed to a different, as yet undefined, property of the enzymes.

Key Words: Cervical cancer - *GSTM1, GSTT1* - Northeastern Thailand - genetic susceptibility

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Introduction

Cervical cancer remains a national health problem in Thailand. The principal causative factor is human papillomavirus (HPV) infection and the prevalence of HPV infection in Northeast Thai women is high (Vatanasapt et al., 1995; Settheetham-Ishida et al., 2005). Notwithstanding, only a small proportion of HPV carriers develop cervical cancer, indicating some other factor(s) responsible for the development of cervical cancer. It is widely reported that tobacco smoking increases the risk for many types of cancer including cervical cancer (Simons et al., 1993; Parkin et al., 1994; Prokopczyk et al., 1997). Carcinogens, such as nicotine, cotinine and tobacco-specific nitrosamines, have been detected in the cervical mucus of smokers (McCann et al., 1992). And yet, even though inhaled tobacco-derived components may damage smokers' cervical cellular DNA, not all smokers develop cervical cancer (Ballinger et al., 1996; Prokopczyk et al., 1997). The difference, therefore, in the metabolic efficiency of tobacco smoke pro-carcinogens can influence the individual's susceptibility.

Glutathione S-transferase (GST) is related to human phase II detoxification enzymes. Cytosolic GSTs (GSTM, GSTP and GSTT) play key roles in the detoxification of the carcinogenic electrophiles of aflatoxin and polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke (benzo[a]pyrene and other PAH procarcinogens). The mode of action of GSTs is through the activation and detoxification of tobacco carcinogens; therefore, one might expect to find a relationship between the genetic polymorphisms of *GSTs* and the risk of developing cancer (Heagerty et al., 1994; Nazar-Stewart et al., 1999; Setiawan et al., 2000; Sweeney et al., 2003; Tiwawech et al., 2005).

GSTM1 facilitates the excretion of a wide range of carcinogens, reactive oxygen species and chemotherapeutic agents with a variety of substrate specificities (Rebbeck, 1997). GSTT1 is also involved in the detoxification of environmental carcinogens such as 1,3 butadiene and ethylene oxide in tobacco smoke and ambient air (Landi, 2000). The absence of a homozygous allele in the *GST* (*GST*-null genotype) results in a complete loss of enzyme activity to bind with genotoxic substrates, including epoxides derived from aflatoxin and PAHs (Hayes and Pulford, 1995). Individuals with *GSTM1*-null or *GSTT1*-null genotype have been investigated as to whether they were susceptible to various cancers including lung, bladder, skin, oral, liver, gastric, colorectal, prostrate, breast, ovary, cervix and nasopharynx (Heagerty et al., 1994; Autrup et al., 1999; Gawronska-Szklarz et al., 1999; Nazar-Stewart et al., 1999; Setiawan et al., 2000; Deng et al., 2001; Kietthubthew et al., 2001; Spurdle et al., 2001; Lee et al., 2002; Sweeney et al., 2003; van der Hel et al., 2003; Sierra-Torres et al., 2003), but the interpretation of the results was not consistent.

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In a previous study, we found that smoking was a critical risk factor for the development of cervical cancer (Setteheetham-Ishida et al., 2004) and it is possible that phase II detoxification enzymes play roles. The frequency of the *GST*-null genotype differs by population (Kietthubthew et al., 2001;Tiwawech et al., 2005) and data on *GST* polymorphism in Thai cervical cancer are lacking. We thus designed our study to investigate the *GSTM1*- and *GSTT1*-null genotypes and their susceptibility to cervical cancer among women in Northeast Thailand.

Materials and Methods

Subjects

Women between 27 and 74 years of age attending Srinagarind Hospital, Khon Kaen University, Thailand, were recruited. Cases (n=90) were defined as squamous cell cervical cancer (SCCA) with cytological, colposcopical and histological diagnosis. Controls (n=94) were recruited from healthy women without cervical cancer, history of conization, hysteretomy or diseases associated with known risk factors for cervical cancer. The subjects were pooled; cases and controls were matched by 5-year age classes then divided into groups according to their smoking status. Prior to this study, the subjects were examined for p53 codon 72 polymorphism (Settheetham-Ishida et al., 2004) and HPV infection (Settheetham-Ishida et al., 2005). The patients were informed of the purpose and experimental procedures of the study and written informed consent obtained. This study was approved by the Ethics Committee of Khon Kaen University.

GSTM1 and GSTT1genotyping

DNA was extracted from peripheral blood cells. The *GSTM1* and *GSTT1* genotypes were determined using PCR methods. The primers for the *GSTM1* genotype were 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and 5'-GTT GGG CTC AAA TAT ACG GTG G-3', and for the *GSTT1* genotype 5'-TTC CTT ACT GTC CTC ACA TCT C-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3'. Co-amplification of the human β-globin using primers 5'-AAC TTC ATC CAC GTT CAC C-3' and 5'-GAA GAG CCA AGG ACA GGT AC -3' was used to confirm the true *GSTM1*- and *GSTT1*-null genotype as opposed to a failure in the PCR assays. Only samples that gave a β-globin PCR positive result were recruited. The PCR products were electrophoresed on 2.5% agarose gel and visualized with ethidium bromide staining. The PCR product of *GSTM1*, *GSTT1* and β-globin was 215, 408 and 268 base pairs in length, respectively.

Statistical analyses

The $\chi 2$-test was used to compare the genotype frequency of *GSTM1* or *GSTT1* polymorphism between the cervical carcinoma patients and the controls. Associations between the GST genotypes and the risk of cervical cancer were tested using odds ratios and 95% confidence intervals (OR and 95% CI), calculated by multivariate logistic regression analysis with 800-STATA-PC. A p-value <0.05 was considered significant.

Results

Table 1 shows the genotype distribution of *GSTM1* and *GSTT1* for the cases and controls. The prevalence of the *GSTM1*-null genotype in the control and SCCA patients was 59.6% and 60.0%, respectively, whereas that of the *GSTT1*-null genotype in the control and SCCA patients was 40.4% and 46.7%, respectively. Neither the *GSTM1*- nor *GSTT1*-null genotypes increased the risk for SCCA (p>0.05). The combination of the *GSTM1*- and *GSTT1*-null genotypes showed a trend to increasing the risk of developing cervical cancer with an adjusted OR of 2.72 (95%CI=0.82-9.03, p= 0.10). This trend was also observed when we tested smokers (Table 2); namely, the *GSTM1*- or *GSTT1*-null genotype did not increase the risk

Table 1. Genetic Polymorphisms of *GSTM1* and *GSTT1* in Cervical Cancer Cases

Genotypes	Subjects, Cases	Controls	OR [95%CI,p-value]	Adjusted OR* [95%CI,p-value]
GSTM1				
+	36(40.0)	38(40.4)	1	
-	54(60.0)	56(59.6)	1.02 [0.54-1.91, 0.95]	0.66 [0.30-1.48, 0.32]
GSTT1				
+	48(53.3)	56(59.6)	1	
-	42(46.7)	38(40.4)	1.29 [0.69-2.41,0.39]	0.72 [0.29-1.80, 0.48]
GSTM1/T1				
+/+	20(22.2)	18(19.1)	1	
+/-	28(31.1)	38(40.4)	0.67 [0.35-1.78, 0.19]	-**
-/+	16(17.8)	20(21.3)	0.80 [0.38-1.77, 0.55]	-**
-/-	26(28.9)	18(19.1)	1.72 [0.82-3.64, 0.12]	2.72 [0.82-9.03, 0.10]

OR [95% CI] = odds ratios [95% confidence interval];*Adjusted for age, p53 genotypes, smoking and HPV status; **drop because of co-linearity

Table 2. Genetic Polymorphisms of *GSTM1* and *GSTT1* and Risk for SCCA among Smokers

Genotypes	Subjects, Cases	Controls	OR [95%CI,p-value]	Adjusted OR* [95%CI,p-value]
GSTM1				
+	30(41.1)	24(42.1)	1	1
-	43(58.9)	33(57.9)	1.00 [0.46-2.15, 1.00]	0.78 [0.30-2.03, 0.62]
GSTT1				
+	39(53.4)	34(59.6)	1	1
-	34(46.6)	23(40.4)	1.35 [0.63-2.91, 0.43]	1.74 [0.68-4.45, 0.25]
GSTM1/T1				
+/+	16(21.9)	12(21.4)	1	1
+/-	23(31.5)	22(39.3)	0.71 [0.32-1.57, 0.36]	-**
-/+	14(19.2)	11(19.6)	0.97 [0.37-2.61, 0.95]	-**
-/-	20(27.4)	11(19.6)	1.54 [0.62-3.96, 0.31]	1.82 [0.43-7.67, 0.41]

OR [95% CI] = odds ratios [95% confidence interval];*Adjusted for age, p53 genotypes, smoking and HPV status; **drop because of co-linearity

for SCCA in smokers (p>0.05), whereas an increased risk for SCCA with an adjusted OR=1.82 (95%CI=43-7.67, p=0.41) was observed in the GST double negative carriers.

Discussion

The underlying background for the present and related studies is that the enzyme activity of GSTs in detoxification protects from cancer development, which results in the higher cancer incidence with *GST*-null genotypes. However, not every *GSTM1*- or *GSTT1*-null genotype increased the risk for SCCA (p>0.05). These observations are in agreement with studies among Caucasians (Warwick et al., 1994a; 1994b; Chen et al., 1999; Goodman et al., 2001), Indians (Sharma et al., 2004; Sobti et al., 2006), and Japanese (Niwa et al., 2005); which reported no difference in the frequency of the *GSTM1*- and *GSTT1*-null genotypes between the controls and cervical carcinoma cases.

There are controversial reports on women carrying both the *GSTT1* and *GSTM1* null genotypes having an increased risk for cervical carcinoma among Koreans (Kim et al., 2000) and a risk for high-grade cervical neoplasia and invasive cervical cancers among Caucasians (Sierra-Torres et al.,2003). These controversial results are frequently observed in studies on *GST* and cancer susceptibility. It is interesting that persons who carry both the *GSTM1* and *GSTT1* null genotype showed a trend for an increased risk of SCCA. As phase II detoxifying enzymes, GSTM1 and GSTT1 have no stringent substrate spectra and different contributions of *GST* null-genotype to the risk of cancer; perhaps, then, it is attributable to the different properties between GSTM1 and GSTT1.

Our previous research showed that passive tobacco smoking contributes to an increased risk of SCCA development among Northeast Thai women (Ishida et al., 2004). Tobacco-related carcinogens in a smoking sex partner's seminal fluid are applied directly to the cervix mucus membrane during sexual intercourse so they may play some role in the pathogenesis of cervical cancer (Kulikauskas et al., 1985; Lahdetie et al., 1986).

The tobacco smoke constituents are modified by metabolizing enzymes and may promote malignant cellular growth (Prokopczyk et al., 1997). The mode of action is through the activation and detoxification of tobacco carcinogens; thus, one might expect the polymorphism of GSTs may alter the risk of cancer among smokers. The lack of GST activities caused by an inherited deletion of the *GST* have been reported to increase the risk of several tobacco-related cancers (Heagerty et al., 1994; Gawronska-Szklarz et al., 1999; Autrup et al., 1999; Nazar-Stewart et al., 1999; Setiawan et al., 2000; Deng et al., 2001;Spurdle et al., 2001; Kietthubthew et al., 2001;van der Hel et al., 2003;Sweeney et al., 2003; Lee et al., 2002). It was therefore hypothesized that smoking status and the *GST* genotype may synergistically influence cancer development. In India, the absence of the *GSTM1* and *GSTT1* gene increased the risk of cervical cancer among passive smokers 7.0- and 10.2-fold, respectively (Sobti et al., 2006). No significant interaction was found between tobacco smoking and the genetic background of *GSTM1* on the risk of cervical squamous intraepithelial lesion in Hawaii (Goodman et al., 2001). The effect of the *GST* null-genotype on the increased risk for cervical cancer among smokers was not observed in our study; even though the combination of genotypes failed to increase the risk among subjects with exposure to tobacco smoke. Strong contributions of phase I detoxifying enzymes may mask the effects of *GST* null genotypes.

The prevalence of the null genotypes for the *GSTM1* (0.60) and *GSTT1* (0.40) among Northeast Thais was comparable to the results reported for other Thais; in the Central region (0.60 and 0.38) (Pakakasama et al., 2005; Tiwawech et al., 2005) and the South (0.66 and 0.36) (Kietthubthew et al., 2001) and other Asian populations. The high prevalence of the null genotypes may, therefore, give a clue to explaining the controversial results.

If the enzyme activity protects cancer development, the null alleles are deleterious and should be eliminated from the population by negative selective pressures. The high frequencies suggest that the lack of GST activity has unknown advantage(s) and maintains the persistence of these alleles in the population. Since (i) the substrate specificity of GSTs is relatively low, (ii) compensation of enzyme activity between GSTs may exist, and (iii) little exposure to tobacco smoke is expected in cervical cancer. These conditions may conceal the true influence of the null allele: their low specificity and bifunctional property also limits consistent interpretations throughout related cervical cancer studies, because the environmental conditions of each subjected population is different and the effects of/from this difference cannot be ruled out.

The relationships between the null-genotype for *GSTM1* and *GSTT1* and cancer susceptibility suggests a large-scale study with simultaneous analyses of phase I detoxifying enzyme genes. Currently, we just test for genotype or null-genotype presence; exact genotyping of wild-homozygous, heterozygous or null-homozygous, should be done in order to identify the cryptic effects of *GST* genotypes on the development of cervical cancer, with special reference to smoking status.

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