Relationship between genetic polymorphisms of metabolizing enzymes CYP2E1, GSTM1 and Kazakh’s esophageal squamous cell cancer in Xinjiang, China

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AIM: To analyze the relationship between genetic polymorphisms of metabolizing enzymes CYP2E1, GSTM1 and Kazakh’s esophageal squamous cell cancer in China.

METHODS: The genotypes of cytochromes P450 (CYP) 2E1 and glutathione S-transferase (GST) M1 were investigated by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) following PCR in 104 Kazakh’s patients with esophageal cancer (EC) and 104 non-cancer controls.

RESULTS: The frequency of CYP2E1 c1/c1 genotype was significantly higher in patients with cancer (77.9%) than in control subjects (24.0%) (P<0.05; OR, 11.13; 95%CI, 5.84-21.22). The difference of GSTM1 null was significantly more frequent in the cancer (34.6%) vs the control group (3.8%) (P<0.05; OR, 13.24; 95%CI, 4.50-38.89). On the other hand, the combination of GSTM1 presence and CYP2E1 c1/c1 genotypes increased the risk for cancer (P<0.05; OR, 13.42; 95%CI, 6.29-28.3).

CONCLUSION: The CYP2E1 c1/c1, GSTM1 deletion genotypes are genetically susceptible biomarkers for ESCC in Kazakh population. Individuals with allele c1 of Rsal polymorphic locus for CYP2E1 may increase the risk of ESCC. Moreover, CYP2E1 wild type (c1/c1) increased the susceptibility to ESCC risk in Kazakh individuals with GSTM1 presence genotype.

Key words: Polymorphisms; CYP2E1; GSTM1; Kazakh’s esophageal squamous cell cancer

INTRODUCTION

It has been revealed that carcinogenesis may result from mutations or deletions in cancer-related genes. In recent years, a relatively new field of cancer research has focused on the interaction between genes and environment to understand the etiology of cancer[1]. Primary candidates for gene-environment interaction studies are those which encode enzymes related to the metabolism of established cancer risk factors. It has been revealed that most carcinogens require metabolic activation in the human body for the carcinogenic effects. Two major enzyme systems can metabolize potential carcinogens, either synthetic or naturally occurring in the body, which have been classified as phases I and II. Generally, phase I enzymes can activate the carcinogen directly and produce more active metabolites. Phase II enzymes can detoxify and process the activated metabolites for final breakdown or excretion. Therefore, the genotypes with high phase I enzyme activity and low phase II enzyme level are considered to pose a high risk of cancer development[2]. Cytochrome P450 (CYP) isoenzymes are one major kind of phase I enzymes and play an important role in the oxidation of chemical compounds, such as polycyclic aromatic hydrocarbons (PAH), often resulting in the formation of highly reactive compounds that are the ultimate carcinogens[3]. Glutathione S-transferases (GSTs) are phase II enzymes and responsible for catalyzing the biotransformation of a variety of electrophiles, and have a central role in the detoxification of activated metabolites of procarcinogens produced by phase I reactions. GSTM1 conjugates xenobiotics with glutathione[4] which promotes the removal of activated carcinogens from the human body. Esophageal cancer (EC) is one of the most common malignant diseases worldwide with a sharp variation in its geographic distribution[5]. The ratio in incidence between high- and low-risk areas could be as great as 13.4:1 in Xinjiang[6]. The high incidence in special areas indicates the importance of environmental factors in esophageal carcinogenesis. However, only a small part of individuals in the high-risk area for EC develop into EC, although all the residents in that area share very...
similar environment-related risk factors and life style, suggesting that host susceptibility factors, such as the polymorphisms of phases I and II enzymes, may play an important role in increased risk for EC. Thus, the present study was undertaken to assess the genetic polymorphisms of CYP2E1 and GSTM1 in Xinjiang to correlate these genetic polymorphisms and susceptibility to EC between Kazakh's esophageal squamous cell cancer and control group.

MATERIALS AND METHODS
Patients and controls
The primary Kazakh's ESCC tissues were obtained from 63 patients who underwent surgery in the Department of Surgery, 1st Teaching Hospital of Xinjiang Medical University, from 1999 to 2003, and from 41 patients who underwent surgery in the Department of Surgery, the People's Hospital of Uygur Autonomous Region, Xinjiang, China, between 1998 and 2000. None of the patients received prior treatment. Other simultaneous malignancies were excluded in the 104 cases of ESCC, including 54 males with a mean age of 57 years (57±8.9) and 50 females with a mean age of 54 years (54±9.0). Meanwhile, 104 non-cancer subjects with matched age and sex frequencies were randomly selected as control group from the same region during the field surveys between 1998 and 2000. None of the patients received prior treatment. Other simultaneous malignancies were excluded in the 104 cases of ESCC, including 54 males with a mean age of 56 years (56±8.5) and 50 females with a mean age of 55 years (55±8.8). Cancer tissues obtained from surgically resected esophageal SCC patients, which confirmed pathologically, were fixed in 40 g/L formaldehyde and embedded by paraffin, genomic DNA samples subjected to PCR and enzymatic digestion with RsaI revealed the expected fragment lengths and resulted in three genotypes of CYP2E1 5' area (Figure 2). The frequency of wild homozygous, heterozygous and mutated homozygous variant genotype detected in the controls and ESCC was 24.0% (25/104) and 76.0% (79/104); 77.9% (81/104) and 22.1% (23/104), respectively (Table 2), the difference being significant (P<0.05; OR, 13.24; 95% CI, 5.84-21.22). The c1 allele frequency was significantly higher than c2 allele frequency in Kazakh's population (P<0.05; OR, 4.74; 95% CI, 2.89-7.78).

RESULTS

GSTM1 genetic polymorphism (Table 1)
Figure 1 shows the PCR-amplified fragment of GSTM1. Table 1 shows the deletion of GSTM1. There was a significant difference in GSTM1 between the controls (3.8%) and ESCC (34.6%) (P<0.05; OR, 13.24; 95% CI, 4.50-38.89).

CYP2E1 genetic polymorphism (Table 2) and allele frequency (Table 3)
DNA samples subjected to PCR and enzymatic digestion with RsaI revealed the expected fragment lengths and resulted in three genotypes of CYP2E1 5' area (Figure 2). The frequency of wild homozygous, heterozygous and mutated homozygous variant genotype detected in the controls and ESCC was 24.0% (25/104) and 76.0% (79/104); 77.9% (81/104) and 22.1% (23/104), respectively (Table 2), the difference being significant (P<0.05; OR, 11.13; 95% CI, 5.84-21.22). The c1 allele frequency was significantly higher than c2 allele frequency in Kazakh's population (P<0.05; OR, 4.74; 95% CI, 2.89-7.78).

PCR-RFLP analysis of CYP2E1 gene polymorphism
Genes of the metabolizing enzymes CYP2E1 were amplified by polymerase chain reaction (PCR). The CYP2E1 RsaI polymorphism in the 5' flanking region of the gene featured a distinct base substitution that creates RsaI restriction sites. The primers used are as follows: the forward 5'-CCA GTCGAGTCTACATTGTCA-3' and reverse 5'-TTCA-TTCTGTCTTCTAACTGG-3'. The genotypes of the CYP2E1 gene were identified by restriction fragment length polymorphism (RFLP). The PCR-amplified DNA fragments, including the polymorphic site, were digested with RsaI, and subjected to electrophoresis on 3.0% agarose gel.

Statistical analysis
The χ² test was used to examine the differences in genotype distribution between patients and controls by SPSS 12.0 software. Odds ratios (ORs) with 95% confidence intervals (95%CI) were also calculated. The difference was considered significant in case of a two-tailed P value less than 0.05.

Table 1  Genotypes of GSTM1 in controls and subjects with cancer n (%)

|           | GSTM1 (+) | GSTM1 (-) | OR (95%CI) |
|-----------|-----------|-----------|------------|
| Control   | 100 (96.2) | 4 (3.8)   | 1.0        |
| ESCC      | 68 (65.4)  | 36 (34.6) | 13.24 (4.50-38.89) |

*P<0.05 vs ESCC group.

Table 2  Distribution of CYP2E1 genetic polymorphism in controls and subjects with cancer n (%)

|           | cl/c1 | cl/c2+c2/c2 | OR (95%CI) |
|-----------|-------|-------------|------------|
| Control   | 25 (24.0) | 79 (76.0)   | 1.0        |
| ESCC      | 81 (77.9)  | 23 (22.1)   | 11.13 (5.84-21.22) |

*P<0.05 vs control group.

Table 3  Distribution of CYP2E1 allele frequency in controls and subjects with cancer n (%)

|           | Allele c1 | Allele c2 | OR (95%CI) |
|-----------|-----------|-----------|------------|
| Control   | 124 (59.6) | 84 (40.4)  | 1.0        |
| ESCC      | 182 (87.5) | 26 (12.5)  | 4.74 (2.89-7.78) |

*P<0.05 vs control group.
The frequency of the individuals carrying both GSTM1 presence and CYP2E1 c1/c1 genotypes was higher in patients with cancer (52/68; 76.5%) than in the controls (19/97; 19.6%) (P<0.05; OR, 13.42; 95%CI, 6.29-28.3).

**DISCUSSION**

Under similar environmental carcinogens exposure, different individuals responded differently to environmental exposures. The different liability to cancer was called genetic susceptibility to cancer. Genetic susceptibility can affect in every step of carcinogenesis, including modifying the effect of environmental carcinogens[8-13]. Cancer susceptible genes include types I and II metabolism enzyme genes, DNA repair gene and those affecting cell proliferation rate.

In recent years, the evidence has been accumulated to support the hypothesis that cancer susceptible genes may be of importance in determining individual susceptibility to cancer[14-19]. EC is a disease determined by multi-factors, including environmental risk factors and genetic factors. However, little is known about the impact of GSTM1 and CYP2E1 genetic polymorphisms on the susceptibility to EC in the Kazakh population. This is the first study that simultaneously evaluated the GSTM1 and CYP2E1 polymorphisms in Kazakh patients with ESCC.

The gene of GSTM1, one of the most important phase 2 enzymes, has attracted much attention with reference to EC. GSTM1 can detoxify a number of reactive electrophilic compound substances, including the carcinogens PAHs. In individuals with GSTM1 deletion genotype, the ability of detoxifying the carcinogens decreased. The null genotype leads to loss of enzyme activity and individuals with GSTM1 deletion could have increased risk of cancers[20,21,22]. In China there were similar researches on GSTM1 deletion genotype and the risks of lung cancer (OR = 2.56)[23], and stomach cancer (OR 1.90, 95%CI 1.01-3.56)[25]. However, it was reported that in Henan Province, a high incidence area of EC in China, GSTM1 deletion genotype did not show significant relation with EC susceptibility[26]. In the present study, dramatical difference was found in GSTM1 null genotype between control (3.8%) and ESCC (34.6%) (P<0.05). It indicated that GSTM1 deletion genotype was a genetic susceptibility risk factor for EC (OR, 13.24; 95%CI, 4.50-38.89), which interacted synergistically with CYP2E1 genetic polymorphism. The proportions of GSTM1 genotypes in the control group were similar to those in previous case-control studies[8].

CYP2E1 plays an important role in the metabolic activation of various TSNA, benzene, styrene, butadiene and urethane, including several potent precarcinogens, such as 4-methylisotrisaminio-1,3-pyridyl-1-butane and N'-nitrosonornicotine[26,27]. In addition, it effectively reduces dioxygen to give rise to radical species, thus contributing to lipid peroxidation and oxidative inhibition[28]. Individuals with the variant Rsal allele (c1/c2 or c2/c2) have a lower basal CYP2E1 activity. Over-representation of the variant CYP2E1 Rsal alleles was reported in ESCC[29] and a lower frequency of the Rsal variant allele was also found in ESCC patients than in controls[30]. In agreement with the result, studies in Japan and Brazil also found a significant increase in ESCC risks associated with the wild type Rsal[31,32]. Similarly, two studies in Chinese found an association between the CYP2E1 Rsal variant allele and decreased risk of ESCC[30,33]. However, two studies conducted in Brazil and Canada failed to reproduce this observation[34,35]. Results in our study indicated that CYP2E1 c1/c1 or c1 allele increased the susceptibility to ESCC risk in Kazakh population (P<0.05; OR, 13.24; 95%CI, 4.50-38.89), and that individuals with combined GSTM1 presence genotype and CYP2E1 wild type showed a dramatically increased (OR 13.4) risk of ESCC, which is higher than that due to the respective genotypes. These findings indicate that GSTM1 presence genotype had synergistic interactions with CYP2E1 c1/c1 of ESCC. Our study was in accordance with the findings of Shi Yun et al[36], who observed an association between the risk of ESCC and CYP2E1 wild allele.

In conclusion, our study suggests that CYP2E1 Rsal wild allele or c1 allele, GSTM1 null genotype were genetically susceptible risk factors for ESCC risk in the Kazakh population. Moreover, we found that CYP2E1 wild type increased the susceptibility to ESCC risk in Kazakh people with GSTM1 presence genotype.

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REFERENCES

1. Mucci LA, Wedren S, Tamimi RM, Trichopoulou D, Adami HO. The role of gene-environment interaction in the aetiology of human cancer: examples from cancers of the large bowel, lung and breast. J Intern Med 2001; 249: 477–493
2. Kihara M, Kihara M, Noda K. Risk of smoking for squamous and small cell carcinomas of the lung modulated by combinations of CYP1A1 and GSTM1 gene polymorphisms in a Japanese population. Carcinogenesis 1995; 16: 2331–2336
3. Guengerich FP. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. Cancer Res 1988; 48: 2946–2954
4. Sram RJ. Effect of glutathione S-transferase M1 polymorphisms on biomarkers of exposure and effects. Environ Health Perspect 1998; 106 Suppl 1: 231–239
5. Wang LD, Zhou Q, Feng CW, Liu B, Qi YJ, Zhang YR, Gao SS, Fan ZM, Zhou Y, Yang CS, Wei JP, Zheng S. Intervention and follow-up on human esophageal precancerous lesions in Xinjiang, Xinjiang Uyghur Autonomous Region, northern China, a high-incidence area for esophageal cancer. Xinjiang Yi Xue Yuan Xue Bao 1998; 11: 139–144
6. Diffenbach CW, Dveksler GS. PCR Primer: A Laboratory Manual. Cold Spring Harbor Laboratory Press 1995: 64–69
7. Hayashi S, Watanabe J, Kawajiri K. Genetic polymorphisms in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. J Biochem 1991; 109: 559–565
8. Casson AG, Zheng Z, Chiasson D, MacDonald K, Riddell DC, Guernsey JR, Guernsey DL, McLaughlin J. Associations between genetic polymorphisms of Phase I and II metabolizing enzymes, p53 and susceptibility to esophageal adenocarcinoma. Cancer Detect Prev 2003; 27: 139–146
9. Tan W, Song N, Wang GQ, Liu Q, Tang HJ, Kadlubar FF, Lin DX. Impact of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferases M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China. Cancer Epidemiol Biomarkers Prev 2000; 9: 551–556
10. van Lieshout EM, Roelofs IM, Dekker S, Mulder CJ, Wobbes T, Jansen JB, Peters WH. Polymorphic expression of the glutathione-S-transferase P1 gene and its susceptibility to Barrett’s esophagus and esophageal cancer. Cancer Res 1999; 59: 586–589
11. Rothen M, Dawsey SM, Wang G, Tangrea JA, Zhou B, Ratnasinghe DA, Woodson KG, Olivera OA, Poitier MC, Frie BL, Taylor PR, Weston A. Association between GSTM1*0 and squamous dysplasia of the esophagus in the high risk region of Linxian, China. Cancer Lett 2000; 156: 73–81
12. Butler WJ, Ryan P, Roberts-Thomson IC. Metabolic genotypes and risk for colorectal cancer. J Gastroenterol Hepatol 2001; 16: 631–635
13. Xing D, Tan W, Song N, Lin D. Genetic polymorphism in hOGG1and susceptibility to esophageal cancer in Chinese. Zhongguo Yixueyixue Xiehuan Zazhi 2000; 17: 377–380
14. Song C, Xing D, Tan W, Wei Q, Lin D. Methylene tetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res 2001; 61: 3272–3275
15. Lee JM, Lee YC, Yang SY, Yang PW, Luh SP, Lee CJ, Chen CJ, Wu MT. Genetic polymorphisms of XRCC1 and risk of the esophageal cancer. Int J Cancer 2001; 95: 240–246
16. Tan W, Chen GF, Xing FY, Song CY, Kadlubar FF, Lin DX. Frequency of CYP2A6 gene deletion and its relation to risk of lung and esophageal cancer in the Chinese population. Int J Cancer 2001; 95: 96–101
17. Shibata J, Eto T, Kataoka A, Inoue H, Ueo H, Suzuki T, Barnard GF, Mori M. Genetic polymorphism of N-acetyltransferase 2 in patients with esophageal cancer. Am J Gastroenterol 2001; 96: 3419–3424
18. Matsuo K, Hamajima N, Shinozaki M, Hataoka S, Inoue M, Takezaki T, Tajima K. Gene-environment interaction between an aldehyde dehydrogenase-2 (ALDH2) polymorphism and alcohol consumption for the risk of esophageal cancer. Carcinogenesis 2001; 22: 913–916
19. Dong CH, Yu SZ, Chen GC, Zhao DM, Hu Y. Association of polymorphisms of glutathione S-transferase M1 and T1 genotypes with elevated aflatoxin and increased risk of primary liver cancer. Shijie Huanen Xiaohua Zazhi 1998; 6: 463–466
20. Cai L, Yu SZ. A molecular epidemiologic study on gastric cancer in Changle, Fujian province. Shijie Huanen Xiaohua Zazhi 1999; 7: 652–655
21. Gao J, Ren C, Zhang Q. CYP2D6 and GSTM1 genetic polymorphism and lung cancer susceptibility. Zhonghua Zhongliu Zazhi 1998; 20: 185–186
22. Cai L, Yu SZ. Preliminary studies on cytochrome P4502E1 and Glutathione S-transferase M1 polymorphism and susceptibility to gastric cancer. Zhongguo Gonggong Weisheng 1999; 15: 895–897
23. Lin D, Tang Y, Lu S. Glutathione S-transferase M1, T1 genotypes and the risk of esophageal cancer: a case-control study. Zhonghua Liuxingbingxue Zazhi 1998; 19: 195–199
24. Gao CM, Takezaki T, Wu JZ, Li ZY, Liu YT, Li SP, Ding JH, Su P, Hu X, Xu TL, Sugimura H, Tajima K. Glutathione-S-transferases M1 (GSTM1) and GSTT1 genotype, smoking, consumption of alcohol and tea and risk of esophageal and stomach cancers: a case-control study of a high-incidence area in Jiangsu Province, China. Cancer Lett 2002; 188: 95–102
25. Belloc G, Dreano Y, Lossach P, Menez JF, Berthou F. Cytochrome P450 metabolic dealkylation of nine N-nitrosodialkylamines by human liver microsomes. Carcinogenesis 1996; 17: 2029–2034
26. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999; 91: 1194–1210
27. Ekstrom G. Ingelman-Sundberg M. Rat liver microsomal cytochrome P450 metabolic dealkylation of nine N-nitrosodialkylamines by ethanol-inducible cytochrome P450 (P450IE1). Biochem Pharmacol 1989; 38: 1313–1319
28. Nishimoto IN, Hanaoka T, Sugimura H, Nagura K, Ihara M, Li XJ, Ariai T, Hamada GS, Kowalski LP, Tsugane S. Cytochrome P450 2E1 polymorphism in gastric cancer in Brazil: case-control studies of Japanese Brazilians and non-Japanese Brazilians. Cancer Epidemiol Biomarkers Prev 2000; 9: 675–680
29. Shi Y, Zhou XW, Zhou YK, Ren X. Analysis of CYP2E1, GSTM1, and GSTT1 genetic polymorphism for its relationship to human lung cancer and esophageal cancer. J Huazhong Univ Sci Tech 2002; 14: 17
30. Itoya S, Nomura F, Makino Y, Tomonaga T, Shimada H, Ochiai T, Iizasa T, Baba M, Fусsawa S, Tardaha S. Tandem repeat polymorphism of the CYP2E1 gene: an association study with esophageal cancer and lung cancer. Alcohol Clin Exp Res 2002; 26: 135–195
31. Godoy W, Albano RM, Moraes EG, Pinho PR, Nunes RA, Saito EH, Higa C, Filho IM, Kruele CD, Schirmer CC, Gurski R, Lang MA, Pinto LF. CYP2A6/2A7 and CYP2E1 expression in tuberculosis. Mammalian Expression System. Lang MA, Pinto LF. CYP2A6/2A7 and CYP2E1 expression in tuberculosis. Mammalian Expression System. Lang MA, Pinto LF. CYP2A6/2A7 and CYP2E1 expression in tuberculosis. Mammalian Expression System. Lang MA, Pinto LF. CYP2A6/2A7 and CYP2E1 expression in tuberculosis. Mammalian Expression System. Lang MA, Pinto LF. CYP2A6/2A7 and CYP2E1 expression in tuberculosis. Mammalian Expression System.