Epoxide hydrolase Tyr113His polymorphism is not associated with susceptibility to esophageal squamous cell carcinoma in population of North China

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INTRODUCTION

China is one of the prevalent areas of esophageal squamous cell cancer (ESCC). Exposure to environmental carcinogens is considered as the main risk factors of ESCC[1,2]. Among them, chemical carcinogens such as polycyclic aromatic hydrocarbons (PAHs) in consumed tobacco or ingested food may contribute to the high incidence of ESCC in China[3,4]. Metabolization of PAHs involves a complex enzymatic mechanism. Microsomal epoxide hydrolase (mEH) is an enzyme that hydrolyzes epoxides such as PHA, yielding corresponding trans-dihydriodols. Usually, this hydrolysis acts as a detoxifying step, although in some instances, trans-dihydriodols generated from PAHs are highly toxic and mutagenic. Therefore, mEH plays a dual role in the detoxification and activation of procarcinogens, and its role in carcinogenesis may depend on exposures to different environmental substrates[5].

There are two polymorphic sites that affect the enzyme activity in human mEH gene. One variant is characterized by substitution of histidine for tyrosine at the amino acid position 113, the other is characterized by substitution of arginine for histidine at the position 139. The proteins encoded by polymorphic alleles demonstrated different enzyme activities in vitro[6]. mEH polymorphism has been associated with chemical carcinogen-induced cancers occurring in lung[7,8], ovary[9], colorectum[10] and liver[11]. The correlation of mEH polymorphism with susceptibility to ESCC has not been reported so far. Therefore, the current study investigated the Tyr113His polymorphism in mEH exon 3 in ESCC patients and healthy individuals from North China.

MATERIALS AND METHODS

Subjects

This study included 257 patients with histologically confirmed esophageal squamous cell carcinomas and 252 healthy individuals without overt cancer. The cancer patients were hospitalized for surgery in the Fourth Affiliated hospital, Hebei Medical University between 2001 and 2003. The healthy subjects were from the same hospital for physical examination in the same period. All of the patients and control subjects were from Shijiazhuang city or its surrounding regions. Information of sex, age, smoking habits and family history was obtained from cancer patients and healthy controls by interview following sampling. The smokers were defined as former or current smoking 5 cigarettes per day for at least two years. The individuals with at least one first-degree relative or at least two second-degree relatives having esophageal/cardiac/gastric cancer were defined as having family history of upper gastrointestinal cancers (UGIC). The smoking status and family history were available from some of the cases and controls. Informed consent was obtained from all the recruited subjects. The study was approved by the Ethics Committee of Hebei Cancer Institute.

AIM: To investigate the possible association of microsomal epoxide hydrolase (mEH) Tyr113His polymorphism with susceptibility to esophageal squamous cell carcinoma (ESCC) in a population of North China.

METHODS: The mEH Tyr113His genotypes were determined by polymerase-chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis in 257 patients with esophageal squamous cell carcinoma (ESCC) and 252 healthy subjects as a control group.

RESULTS: The frequencies for Tyr and His alleles were 44.2 %, 55.8 % in ESCC patients, and 44.0 % and 56.0 % in healthy subjects, respectively. No statistic difference in allele distribution was observed between ESCC patients and controls (χ²=0.008, P=0.929). The overall genotype distribution difference was not observed between cancer cases and controls (χ²=2.116, P=0.347). Compared with Tyr/Tyr genotype, neither His/His genotype nor in combination with Tyr/His genotype significantly modified the risk of the development of ESCC, the adjusted odds ratio was 1.076 (95 % CI=0.850-1.361) and 0.756 (95 % CI=0.493-1.157), respectively. When stratified for sex, age, smoking status and family history of upper gastrointestinal cancer, His/His genotype alone or in combination with Tyr/His genotype also did not show any significant influence on the risk of developing ESCC.

CONCLUSION: MEH Tyr113His polymorphism may not be used as a stratification marker in screening individuals at a high risk of ESCC.

Zhang JH, Jin X, Li Y, Wang R, Guo W, Wang N, Wen DG, Chen ZF, Kuang G, Wei LZ, Wang SJ. Epoxide hydrolase Tyr113His polymorphism is not associated with susceptibility to esophageal squamous cell carcinoma in population of North China. World J Gastroenterol 2003; 9(12): 2654-2657

http://www.wjgnet.com/vol1007-9327/9/2654.asp
DNA extraction

Five ml of venous blood from each subject was drawn in Vacutainer tubes containing EDTA and stored at 4 °C. Genomic DNA was extracted within one week after sampling by using proteinase K digestion followed by a salting out procedure.

MEH genotyping by PCR and restriction fragment analysis

The exon 3 T to C variant in meH gene, changing tyrosine 113 to histidine, creates an EcoR restriction site (GATATC), which can be exploited for genotyping by PCR and subsequent RFLP analysis. PCR was performed in a 25 µl volume containing 100 ng of DNA template, 2.5 µl of 10×buffer, 1 U of Taq-DNA-polymerase (BioDev-Techn., Beijing, China), 200 µmol of dNTPs and 200 nmol of sense primer (5’-GATCGATAAGTTCCGTTTCACC-3’) and antisense primer (5’-ATCCCTTAGTCTTGAAGTGAGGAT-3’). Initial denaturation at 94 °C for 5 min was followed by 35 cycles at 94 °C for 30 sec, at 56 °C for 30 sec and at 72 °C for 1 min. Subsequently, the PCR products were digested with 10 units of EcoR V (TakaRa Biotechnology Co., Ltd, Dalian, China) overnight at 37 °C and separated on a 3 % agarose gel. RFLP bands were visualized through ethidium bromide staining under UV light. Tyr113 wild-type homozygote was characterized by two bands at the position of 140 bp and 22 bp, while His113 homozygotes were identified by a single band (162 bp) and the heterozygotes by three bands (162 bp, 140 bp and 22 bp). For a negative control, each PCR reaction used distilled water instead of DNA in the reaction system. For 10 % of the samples, the reaction was repeated once.

Table 2 Influence of meH Tyr113His polymorphism on ESCC development

|                         | Tyr/ Tyr | Tyr/ His+His/ His | His/ His | aOR (95%CI) | aOR (95%CI) |
|-------------------------|----------|------------------|---------|-------------|-------------|
| Overall                 |          |                  |         |             |             |
| Normal                  | 76 (30.2)| 176 (69.8)       | 105 (41.7) | 0.756 (0.493–1.157) | 1.076 (0.850–1.361) |
| ESCC                    | 84 (32.7)| 173 (67.3)       | 115 (44.7) |             |             |
| Male                    |          |                  |         |             |             |
| Normal                  | 44 (30.6)| 100 (69.4)       | 60 (41.7) |             |             |
| ESCC                    | 61 (35.7)| 110 (64.3)       | 73 (42.7) | 0.724 (0.427–1.225) | 1.087 (0.811–1.458) |
| Female                  |          |                  |         |             |             |
| Normal                  | 32 (29.6)| 76 (70.4)        | 45 (41.7) |             |             |
| ESCC                    | 23 (26.8)| 63 (73.2)        | 42 (48.8) | 0.825 (0.398–1.710) | 1.047 (0.704–1.558) |
| Age<50                  |          |                  |         |             |             |
| Normal                  | 46 (32.6)| 95 (67.4)        | 60 (42.6) |             |             |
| ESCC                    | 19 (33.9)| 37 (66.1)        | 22 (39.3) | 0.867 (0.450–1.671) | 1.064 (0.738–1.534) |
| Age>50                  |          |                  |         |             |             |
| Normal                  | 30 (27.0)| 81 (73.0)        | 45 (40.6) |             |             |
| ESCC                    | 65 (32.3)| 136 (67.7)       | 93 (46.3) | 0.790 (0.472–1.323) | 1.018 (0.768–1.348) |
| Nonsmoker               |          |                  |         |             |             |
| Normal                  | 31 (27.7)| 81 (72.3)        | 49 (43.7) |             |             |
| ESCC                    | 34 (28.3)| 86 (71.7)        | 60 (50.0) | 0.659 (0.338–1.286) | 1.135 (0.790–1.631) |
| Smoker                  |          |                  |         |             |             |
| Normal                  | 35 (31.8)| 75 (68.2)        | 41 (37.3) |             |             |
| ESCC                    | 46 (35.4)| 84 (64.6)        | 52 (40.0) | 0.901 (0.494–1.644) | 1.022 (0.728–1.433) |
| Negative family history |          |                  |         |             |             |
| Normal                  | 59 (30.9)| 132 (69.1)       | 80 (41.9) |             |             |
| ESCC                    | 49 (37.1)| 83 (62.9)        | 51 (38.7) | 0.660 (0.385–1.134) | 1.237 (0.912–1.678) |
| Positive family history |          |                  |         |             |             |
| Normal                  | 7 (22.6)| 24 (77.4)        | 10 (32.2) |             |             |
| ESCC                    | 29 (27.9)| 75 (72.1)        | 52 (50.0) | 0.638 (0.241–1.689) | 0.946 (0.546–1.639) |

ESCC: esophageal squamous cell carcinoma. a,b. information of smoking status and family history was available from some of subjects. c,d. the age and sex adjusted odds ratio of Tyr/ His+His/ His (c) and His/ His genotype (d) against Tyr/ Tyr genotype.
correlated with gender, age and smoking status both in ESCC patients and in healthy controls (data not shown). The Tyr and His allele frequencies were 44.0 %, 56.0 % in ESCC patients and 44.2 %, 55.8 % in healthy controls, respectively. There was no statistic difference in allele distribution between ESCC patients and controls ($\chi^2=0.008$, $P=0.929$). The frequencies of Tyr/Tyr, Tyr/His and His/His genotype were 30.2 %, 28.2 % and 41.6 % in healthy controls, respectively. The overall mEH genotype distribution in ESCC patients was not significantly different from that in healthy controls ($\chi^2=2.116$, $P=0.347$) (Table 1).

Table 1 Demographic characteristics and mEH Tyr113H is polymorphism in ESCC patients and healthy individuals

| Groups                  | Control n (%)       | ESCC n (%)       |
|-------------------------|---------------------|-----------------|
| Sex                     |                     |                 |
| Male                    | 147 (58.3)          | 171 (66.5)      |
| Female                  | 105 (41.7)          | 86 (33.5)       |
| Age (mean±SD)           | 49.4±8.56           | 58.5±9.39       |
| Smoking statusa         |                     |                 |
| Ex-or current smoker    | 110 (49.5)          | 130 (52.0)      |
| Non-smoker              | 112 (50.5)          | 120 (48.0)      |
| Family history of UGICb |                     |                 |
| Positive                | 31 (14.0)           | 104 (44.1)      |
| Negative                | 191 (86.0)          | 132 (55.9)      |
| Genotype                |                     |                 |
| Tyr/ Tyr                | 76 (30.2)           | 49 (37.1)       |
| Tyr/ His                | 71 (28.2)           | 32 (24.2)       |
| His/ His                | 105 (41.6)          | 51 (38.7)       |
| Allele type             |                     |                 |
| T                       | 223 (44.2)          | 226 (44.0)      |
| C                       | 281 (55.8)          | 288 (56.0)      |

ESCC: esophageal squamous cell carcinoma, UGIC: upper gastrointestinal cancer. a. Information of smoking status was available from some of subjects; b. Positive family history of UGIC significantly increased the risk to develop ESCC. A ge and sex adjusted OR=4.06 (95 % CI =2.46-6.69), $\chi^2=98.87$, $P <0.0001$.

By using Tyr/Tyr as the reference genotype, neither His/ His genotype alone nor in combination with Tyr/His genotype significantly modified the risk of ESCC, the adjusted odds ratio was 1.076 (95 % CI=0.850-1.361) and 0.756 (95 % CI=0.493-1.157), respectively. When stratified for sex, age, smoking status and family history of upper gastrointestinal cancer, the frequency of His/His and Tyr/His genotype in ESCC patients was not significantly different from healthy controls. Consistently, His/His alone, or in combination with Tyr/His genotype, did not show any significant influence on the risk of ESCC (Table 2).

DISCUSSION

Chemical carcinogens in consumed alcohol and tobacco, polluted water, ingested food, are in general considered as the main risk factors of ESCC in China. However, not all individuals exposed to the above exogenous risk factors will develop ESCC, indicating that the host susceptibility factors may play an important role in cancer development. In recent years, many polymorphic carcinogen metabolic enzymes, such as aldehyde dehydrogenase-2 (ALDH2)[13], cytochrome P450 (CYP)[14,15], glutathione S-transferase (GST)[15,16], methylenetetrahydrofolate reductase (MTHFR)[17], NAD(P)H, quinone oxidoreductase 1 (NQO1)[18,19] have been found to be able to modify the susceptibility to chemically induced cancers including esophageal and gastric cancer. Therefore, these polymorphic genes, alone or in combination with each other or with other newly developed genetic markers, may be used as predicative parameters for screening individuals at a high risk of ESCC.

MEH is involved in the metabolism of environmental carcinogens. Polymorphisms in mEH gene might affect the enzyme expression and lead to different phenotypes, probably by the alteration of protein stability[6]. Tyr113His substitution in exon 3 could reduce the enzyme expression by about 40 %, producing a slow phenotype with a low epoxide hydrolase activity. In contrast, Arg139 His in exon 4 could increase the expression by about 25 %, producing a fast phenotype with an increased enzyme activity[6]. The relationship between mEH gene polymorphisms and susceptibility to cancers studied had inconsistent conclusions due to different cancer types and populations. The Tyr allele in exon 3 was reported to increase the risk of several cancer types including ovarian cancer[17], oropharyngeal cancer[18] and acute leukemia[19], whereas the His allele was associated with increased susceptibility to cancers occurring in colon[10], liver[11] and cervix[12]. In addition, gene-environment interaction was strongly suggested by some investigations, thus, cumulative cigarette smoking might play a pivotal role in association of His homozygous genotype with lung cancer development, altering the direction of risk from a risk factor in nonsmokers to a relatively protective factor in heavy smokers[9].

Recently, a slight decrease in mEH Tyr113 frequency was observed in esophageal adenocarcinoma (42 %) compared to controls (53 %, $P=0.05$)[21]. In the present study, the frequencies of Tyr/Tyr, Tyr/His and His/His genotype in healthy controls were in consistent with a recent report from a Chinese group[24]. The genotype distribution difference was not found in ESCC patients and healthy controls, as well as in stratification comparison according to the age, sex (>50 or ≤50), smoking status (never smoking or current and ever smoking), and family history of UGIC. The result suggests that although mEH Tyr113His polymorphism is correlated with some cancer types, this genetic alteration may not be associated with susceptibility to ESCC in population of North China.

ACKNOWLEDGEMENTS

We greatly acknowledge Mr. Baoshan Zhao and Mr. Fanshu Meng for their assistance in recruiting study subjects.

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Edited by Su Q and Wang XL