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ORIGINAL ARTICLE

Study on association between ERCC5 single nucleotide polymorphism and susceptibility to esophageal cancer

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Summary

Purpose: *DNA repair genes play important roles in the genesis of esophageal cancer, and their functional single nucleotide polymorphism (SNP) loci may affect the susceptibility to esophageal cancer through changing the capability of DNA damage repair.*

Methods: *A total of 557 patients with esophageal squamous cell carcinoma and 1503 age- and gender-matched healthy people were selected in this study. The hospital-based case-control method and the candidate gene and functional locus-based SNP selection strategy were used to screen three functional SNPs, loci on excision repair cross complement 5 (ERCC5): rs2296147, rs873601 and rs2094258. Genotyping was performed using Taqman method. A logistic regression model was used to analyze the relationship between the selected loci and the risk of esophageal cancer.*

Results: *rs2296147 reduced the risk of esophageal cancer (CC vs TT: OR=0.79, 95% CI=0.64-0.97, p=0.027; additive model: OR=0.80, 95% CI=0.68-0.94, p=0.007). The results of stratified analysis showed that rs2296147 could reduce the susceptibility to esophageal cancer in women, non-smokers, drinkers and non-drinkers. No correlation between rs873601 and rs2094258 and susceptibility to esophageal cancer was found. However, the combined effect analysis showed that rs2296147, rs873601 and rs2094258 could increase the risk of esophageal cancer (p_{trend}=0.006).*

Conclusion: *The results of this case-control study showed that the polymorphic locus on ERCC5, rs2296147, could reduce the risk of esophageal cancer, which will help further understand the pathogenesis of esophageal cancer.*

Key words: *ERCC5, esophageal cancer, single nucleotide polymorphism, susceptibility*

Introduction

Esophageal cancer is one of the common epithelial tissue-derived malignant tumors in humans. Its incidence ranks eighth among the malignant tumors, and its mortality ranks sixth worldwide. Esophageal cancer has a poor prognosis, with mean survival of 7-8 months in the middle and advanced stages, poorer survival after treatment and 5-year survival less than 20% [1,2]. Most epidemiological studies argue that the external environmental risk factors affecting the occurrence of esophageal cancer include excessive smoking and drinking, obesity and malnutrition [3,4]. However, only a small number of people suffer from this disease even in the high-prevalence areas of esophageal cancer, so the reasons of esophageal cancer development cannot be fully explained only by environmental-related factors. This suggests that inherent genetic factors may determine the susceptibility of individuals to esophageal cancer.

Carcinogenic factors can cause damage to cell DNA, resulting in DNA structure and sequence

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changes, and increasing the genomic instability of proliferating cells. In humans, DNA damage is repaired by the DNA repair system, and the inadequate repair capacity can increase the susceptibility of individuals to esophageal cancer. At present, it is believed that the difference in DNA repair capacity of different individuals is determined by the heredity and caused by the combined effect of a number of functional SNPs of some core genes in the DNA repair pathway [5]. ERCC5 is a key gene in the DNA repair pathway. A number of studies have suggested that the SNPs of this gene are associated with the susceptibility to a variety of tumors [5,6]. However, esophageal cancer is rare in foreign countries, so there is little research on it. Besides, the reliability of statistical analyses is not enough due to small research sample size in China, resulting in poor repeatability.

Therefore, in the comparison of 557 patients with esophageal squamous cell carcinoma of the Han nationality in China and 1503 control subjects without a history of tumor, the relationship between the 3 functional SNPs loci on ERCC5 and the occurrence of esophageal cancer was analyzed. This study provided a theoretical basis for revealing the functional SNP involved in the occurrence of esophageal cancer and its possible biological mechanism.

Methods

Ethics Committee approval

All participants in the study were informed of the purpose and significance of this study, signed the informed consent and provided the necessary biological samples for testing and analysis. All personal information, disease history, family history and gene information of the objects were strictly kept secret. This study was approved by the Ethics Committee of Affiliated Tumor Hospital to Shanghai Fudan University.

Patients and epidemiological data

All patients were Chinese Han population without blood relationship with Eastern China, including Shanghai, Jiangsu and the surrounding areas. Data from 557 patients histopathologically diagnosed with esophageal squamous cell carcinoma, who were admitted and treated in Fudan University Shanghai Cancer Center from March 2014 to September 2016, were collected. Patients with esophageal adenocarcinoma, small cell carcinoma and metastatic cancer were excluded. Patients did not receive radiotherapy and/or chemotherapy before blood collection. The control group included 1503 randomly selected healthy people in the same period. They had no tumor history or biological relevance, and were matched according to gender and age (±5 years). A unified epidemiological questionnaire was designed to investigate the demographic data and environmental exposure data of each factor, including the gender, age, race, weight, height, smoking, drinking, individual history of disease and family history of tumor. Five mL venous blood was collected from each subject using the vacuum anticoagulant tube (containing ethylenediamine tetraacetic acid [EDTA]) and cryopreserved at -70°C.

Selection strategy of SNP

Based on the progress in molecular epidemiological studies, some genes that might be closely related to tumorigenesis or genes that were related to the onset risk of esophageal cancer according to the literature were studied. Finally, the representative DNA repair gene, ERCC5, was selected. The software Haploview and 3 databases, HapMap ('http://www.hapmap.org'), NCBI dbSNPr (http://www.ncbi.nlm.nih.gov) and OfDSNPinfo (http://snpinfo.niehs.nih.govA), were used to select the common SNP loci with potential functions. Inclusion criteria: 1) Loci located in the gene control region, such as 5' near gene, 5' untranslated region (UTR), 3' UTR, 3' near gene, splice site and protein coding region; 2) Loci with the minor allele frequency (MAF) S% in Chinese Han population in Beijing (CHB) reported by HapMap or NCBI dbSNP database; 3) Loci that may affect the activity of miRNA binding site or may affect the activity of transcription factor binding site (TFBS) in the promoter region or change the amino acid sequence of exon or affect the splicing signal; 4) Loci with linkage disequilibrium (LD), R2/D<0.80. Finally, three SNP loci were selected.

Genotyping

DNA was extracted from all samples using the traditional phenol-chloroform method, and the genotype was identified by TaqMan genotyping method using the ABI7900 real-time fluorescent quantitative PCR instrument. Two blank controls and two duplicate samples were randomly selected in each 384-well detection unit as quality control.

Statistics

Data collection and statistical analysis were performed using the statistical software Statistics Analysis System (SAS) (version 9.1; SAS Institute, Gary, NC). A p value <0.05 suggested that the difference was statistically significant, and all statistical tests were two-sided probability tests. Differences in the distribution frequencies of demographic characteristics, environmental exposure parameters (smoking and alcohol drinking) and genotype between case and control group were compared using x^2 test. The odds ratio (OR) and 95% confidence interval (CI) were calculated using the univariate and multivariate logistic multiple regression models to present the relative risk, and all OR values were corrected for gender, age, smoking status and alcohol drinking status. Stratified analysis was performed using logistic regression according to the gender, age, smoking and alcohol drinking and the combined effect analysis of the three loci selected was performed.

Results

Characteristics in esophageal cancer cases and controls

As shown in Table 1, the study included the case group (n=557, esophageal squamous cell carcinoma) and the control group (n=1503); for the average age there was not significant difference (p=0.601). The case group included 208 females (36.3%) and 349 males (62.7%) versus 545 females (36.3%) and 958 males (63.7%) in the control group; (p=0.651). The number of smokers was 266 (47.8%) in the case group, which was higher than that in the control group (n=633, 42.1%; p=0.222). The number of drinkers was 245 (44.0%) in patients with esophageal cancer, which was significantly higher compared with the control group (n=502; 33.4%; p<0.001).

Table 1. Selected characteristics in esophageal cancer cases and controls

Variables	*Cases n (%)*	*Controls n (%)*	*p value**
All subjects	557(100)	1503(100)	
Age, years			0.601
<60	250(44.9)	694(46.2)	
≥60	307(55.1)	809(53.8)	
Gender			0.651
Female	208 (737.3)	545(36.3)	
Male	349 (62.7)	958(63.7)	
Smoking status			0.022
Never	291(52.2)	870(57.9)	
Ever	266 (47.8)	633(42.1)	
Alcohol drinking status			<0.001
Never	312(56.0)	1001(66.6)	
Ever	245(44.0)	502(33.4)	

*Two-sided chi-square test

Primary information and minor allele frequencies (MAFs) of selected SNPs

The basic information of the loci selected is shown in Table 2. P values for Hardy-Weinberg equilibirum (HWE) were >0.05. MAF of each locus in the control and case group was >0.05.

Summary of the association between selected SNPs and esophageal cancer risk

As shown in Table 3, logistic regression analysis showed that there was a significantly negative correlation between rs2296147 and the occurrence of esophageal squamous cell carcinoma. The distribution frequencies of three genotypes, TT, CT and CC, in the case group were 56.6, 38.1 and 5.4%, and those in the control group were 50.2, 41.9 and 7.9%, respectively. Compared with carriers of TT genotype, the prevalence risk of esophageal squamous cell carcinoma in individuals with CT was decreased (corrected OR=0.79, 95%CI=0.64-0.97, p=0.027), and that in individuals with CC it was also decreased (corrected OR=0.79, 95%CI=0.64-0.97, p=0.027). The results of the additive model also showed that there was a significantly negative correlation between rs2296147 and the occurrence of esophageal squamous cell carcinoma (corrected OR=0.80, 95%CI=0.68-0.94, p=0.007). No other SNP loci were found to be associated with risk of esophageal cancer.

Stratified analysis for associations between rs2296147 variant genotype and esophageal cancer risk

As shown in Table 4, the rs2296147 locus was examined in the stratified analysis according to the gender, age, smoking status and alcohol drinking status. Logistic regression analysis showed that females and non-smokers were less susceptible to esophageal cancer (corrected OR=0.78, 95% CI=0.64-0.96, p=0.019 and corrected OR=0.72, 95% CI=0.58-0.91, p=0.004, respectively).

Cumulative effect of rs2296147 rs873601 and rs2094258 variants on the esophageal cancer risk

As shown in Table 5, the three SNP mutant sites (OR>1.0) of ERCC5 were combined to study their combined effects on susceptibility to esophageal squamous cell carcinoma. The risks of ERCC5 were rs2094258 T+ rs873601G+ rs2296147T. With the increase of risk genotypes, the prevalence risk of esophageal squamous cell carcinoma showed good effects (additive model: corrected OR=1.15, 95%CI=1.04-1.27, p_{trend}=0.006).

Table 2. Primary information and minor allele frequencies (MAFs) of selected SNPs

SNP	*Chr*	*Position*	*Alleles*	*HWE**	*MAF*	
					Cases	*Controls*
rs2296147	13q33	5'UTR	T>C	0.427	0.244	0.289
rs873601	13q33	5'near gene	C>T	0.151	0.410	0.329
rs2094258	13q33	3'UTR	G>A	0.744	0.434	0.420

*P values for Hardy-Weiberg equilibrium (HWE) tests.

MAF: minor allele frequency, SNP: single nucleotide polymorphism

Table 3. Summary of association between selected SNPs and esophageal cancer risk

SNP	*Controls n (%)*	*All cases n (%)*	*Adjusted OR* (95%CI)*	*p value**
rs2296147			1	
TT	754(50.2)	315(56.6)	0.82(0.67- 1.00)	0.053
CT	630(41.9)	212(38.1)	0.79(0.64-0.97)	0.027
CC	119(7.9)	30(5.4)	0.80(0.68- 0.94)	0.007
Additive model				
rs873601			1	
CC	689(45.8)	213 (49.7)	0.89(0.72- 1.10)	0.293
CT	639(42.5)	275 (39.3)	0.97(0.83-1.14)	0.703
TT	175(11.6)	91(11.0)	0.94(0.82-1.09)	0.422
Additive model				
rs2094258			1	
GG	509(33.9)	178(32.0)	1.08(0.86-1.35)	0.508
AG	726(48.3)	275(49.4)	1.08(0.93-1.25)	0.303
AA	268(17.8)	104(18.7)	1.07(0.93-1.23)	0.353
Additive model				

*Logistic regression with adjustment for age, sex, smoking status and alcohol drinking status in additive model

Table 4. Stratified analysis for associations between rs2296147 variant genotype and esophageal cancer risk

Variables	*rs2296147*		*Adjusted OR*(95%CI)*	*p value**
	Cases CC/CT/TT	*Controls CC/CT/TT*		
Age, years				
<60	13/96/141	55/296/343	0.80(0.62-1.01)	0.064
≥60	17/116/174	64/334/411	0.81(0.65-1.01)	0.055
Sex				
Females	15/140/194	78/404/476	0.78(0.64-0.96)	0.019
Males	15/72/121	41/226/278	0.79(0.60-1.03)	0.080
Smoking				
Never	16/106/169	79/362/429	0.72(0.58-0.91)	0.004
Ever	14/106/146	40/268/325	0.83(0.64-1.07)	0.141
Drinking				
Never	19/118/175	78/426/497	0.80 (0.65-0.99)	0.041
Ever	11/94/140	41/204/257	0.76 (0.59-1.00)	0.041

*Adjusted by age, sex, smoking status and alcohol drinking status

Table 5. Cumulative effect of rs2296147 rs873601 and rs2094258 variants on the esophageal cancer risk

*Number of variants**	*Cases n (%)*	*Controls n (%)*	*Adjusted OR†(95%CI)*	*p value†*
0-2	193 (42.0)	708 (45.9)	1.00	
3	130 (28.3)	481 (31.2)	1.52 (1.09-2.12)	0.013
4	137 (29.8)	355 (23.0)	1.59 (1.15-2.18)	0.005
5-6			1.69 (1.20-2.37)	0.003
Trend	--	--	1.15 (1.04-1.27)	0.006
Binary classification				
0-3	323 (70.2)	1189 (77.0)	1.00	
4-6	137 (29.8)	355 (23.0)	1.24 (1.01-1.51)	0.039

*The rs2296147 T and rs873601 C and rs2094258 A allele were assumed as risk alleles based on main effect of individual locus.
†Adjusted by age, sex, smoking status and alcohol drinking status.

Discussion

In this study, it was found that rs2296147 was significantly associated with the occurrence of esophageal squamous cell carcinoma, and the risk of esophageal squamous cell carcinoma was decreased with the increase of mutant effect.

ERCC5, also known as human xeroderma pigmentosum G (XPG) gene, is mainly involved in the nucleotide excision repair mechanism. So far, there are three reports on the relationship between rs2296147 and the risk of tumor, with 1059 patients with squamous cell carcinoma of head and neck (SCCHN) and 1066 normal controls in white population [7], 722 patients with endometrial cancer and 726 normal controls in mixed population [8], and 1125 patients with gastric cancer and 1196 normal controls in Chinese Han population [9] , respectively. The results of these studies showed no correlation between rs2296147 and the tumor development, but positive correlation was found between rs873601 and gastric cancer development

[9]. There was only one report on the relationship between rs2094258 and the susceptibility to SCCHN [7], which was consistent with the results in esophageal squamous cell carcinoma which showed no statistically significant difference. Differences in the results of the above studies might be due to different genetic backgrounds and etiologies. Another reason is the different pathogenesis of various tumors.

ERCC5 plays an important role in the DNA repair pathway, so we believe that, from the biological perspective, its functional SNPs may affect the susceptibility to esophageal cancer. As the structurally specific endonuclease and 5'-3'exonuclease, ERCC5 protein is involved in the transcription-coupled nucleotide excision repair and whole-genome nucleotide excision repair, the former of which first cuts the transcription-coupled DNA strand damage, and the latter of which cuts the whole-genome DNA strand damage [10,11]. Rare mutations in ERCC5 can induce abnormal cell apoptosis in DNA repair-deficiency diseases and lead to a significant increase in cancer risk [12,13]. In addition, ERCC5 also plays a key role in the transcriptional regulation [14,15]. Studies have confirmed that changes in ERCC5 transcripts will regulate the transcriptional domain-related repair capability [16,17] and other important phenotypes, including individual differences in tumorigenesis [18,19]. The locus rs2296147 is located in the 5'UTR, possibly the p53 recognition site [20] and the transcription factor binding site (TFBS). Although the biological function of the locus is not very clear at present, many studies have shown that SNP located in TFBS may cause carcinogenesis by altering the binding of transcription factors and DNA, and affecting the genetic expression [21,22]. In fact, previous functional studies have revealed that the genetic variation of TP53 recognition site (rs2296147 T allele) and E2F1/YY1 response site (rs751402 allele) have a cis-acting in normal bronchial epithelial cells of lung cancer patients, and increase the allele-specific ERCC5 transcription level [23]. This is consistent with our findings that rs2296147 C allele has a protective effect on esophageal squamous cell carcinoma and can reduce the risk for cancer development. Interestingly, data from HapMap did not show that rs2296147 allele was associated with low expression of B lymphoblastoid cell line ERCC5 mRNA, maybe because the influence of rs2296147 was small, and the existing sample size was not enough to find a weak effect, with other unknown functional SNP, jointly changing the functions of ERCC5. In addition, rs2296147 may regulate the post-transcriptional processing, because the length of 5'UTR of ERCC5 affects the number of polyribosome-containing cells and increases the cell translation level at the time of DNA damage response [24]. In general, there is no direct biological evidence, so we cannot confirm that the rs2296147 locus is the pathogenic SNP.

In this study, there were the inherent deficiencies of case-control studies, which might lead to biased results. Firstly, the case group came from the hospital, while the control group came from the community, which might lead to biased selection. However, gender and age between case and control group were matched, thus minimizing the possible biased selection. Secondly, only the functional SNP loci rather than TagSNP were assessed in this study, so loci were not completely covered and important SNP loci might be missed, failing to fully analyze the overall effect or joint effect of genetic variation. Besides, as a retrospective study, the detailed information of other exposure factors, such as occupational exposure and eating habits, could not be obtained, except smoking and drinking status, which might have interactions with genes or be potential confounding factors.

In conclusion, this study is one of the few large-scale case-control studies on the correlation between DNA repair gene functional SNPs and susceptibility to esophageal cancer. The research results further proved the important role of genetic variation of DNA repair genes in the occurrence of esophageal cancer, which still needs to be verified in larger-sample and population-based case-control studies. At the same time, further functional studies will contribute to deep understanding the mechanism of genetic variation in the occurrence of esophageal cancer.

Conflict of interests

The authors declare no confict of interests.

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