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Phospholipase C ε-1 gene polymorphisms and prognosis of esophageal cancer patients from a high-incidence region in northern China

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Abstract. Recent genome-wide association studies identified susceptibility loci for esophageal squamous cell carcinoma (ESCC), the most common histological type of esophageal cancer, in the phospholipase C ε-1 gene (*PLCE1*). The aim of the present study was to investigate whether polymorphisms of *PLCE1* were associated with the prognosis of ESCC patients in a high-incidence region of northern China. The *PLCE1* rs2274223 A/G and rs11599672T/G single-nucleotide polymorphisms (SNPs) were genotyped by polymerase chain reaction-ligase detection reaction method in 207 ESCC patients with survival information. The mean age ± standard deviation of the 207 ESCC patients was 60.3±7.9 years. Sex, age, smoking status and family history of upper gastrointestinal cancer were not found to be associated with the survival time of ESCC patients. The mean survival time of rs2274223 SNP A/A, A/G and G/G genotype carriers were 42.9, 43.4 and 46.3 months, respectively; for rs11599672 SNP T/T, T/G and G/G genotype carriers the survival time were 42.8, 43.8 and 42.7 months, respectively. There was no significant difference in survival time among the ESCC patients with different genotypes of rs2274223 and rs11599672 SNPs. In conclusion, *PLCE1* rs227423 and rs11599672 SNPs cannot be used as predictive markers for the survival of ESCC patients from a high-incidence region of northern China.

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Abbreviations: GWAS, genome-wide association study; ESCC, esophageal squamous cell carcinoma; SNP, single-nucleotide polymorphism; IP3, inositol 1,4,5-triphosphate; DAG, diacylglycerol; UGIC, upper gastrointestinal cancer

Key words: phospholipase C ε-1 gene, polymorphism, prognosis, esophageal squamous cell carcinoma

Introduction

The Ci county of Hebei Province is one of the high-incidence regions of esophageal cancer in China. Over the past 40 years, the incidence and mortality rate of esophageal cancer have significantly decreased with the application of primary and secondary prevention measures. However, in 2011, the age-adjusted esophageal cancer incidence and mortality rates were $106.74/10^5$ and $103.07/10^5$, respectively, in men, and $75.41/10^5$ and $53.52/10^5$, respectively, in women (1). Therefore, esophageal cancer remains a major public health concern in this region. A number of environmental and genetic factors chronically interact, leading to esophageal cancer development. Genome-wide association studies (GWAS) is a powerful tool for identifying genetic markers for various diseases with high-throughput genotyping technology (2). Recent GWAS identified susceptibility loci for esophageal squamous cell carcinoma (ESCC), the most common histological type of esophageal cancer, in the phospholipase C ε-1 gene (*PLCE1*) (3-5). Subsequently, several independent studies were conducted to validate the association between *PLCE1* single-nucleotide polymorphisms (SNPs) and ESCC, with controversial results (6-12). PLCE1 is a member of the phospholipase C family, a group of proteins able to convert phosphoinositol 4,5-bisphosphate to two second messengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 and DAG participate in Ca^{2+} immobilization and protein kinase C activation, respectively (13). It is noteworthy that PLCE1 may be regulated by multiple signaling inputs from both Ras family GTPases and G proteins (14). Hence, PLCE1 plays a crucial role in regulating cell growth, differentiation, apoptosis and angiogenesis (15). It was previously demonstrated that PLCE1 was involved in the development and progression of various cancers, including esophageal cancer (3,10,16-22). In addition, the expression of PLCE1 was associated with the survival of patients with esophageal cancer (23,24). In fact, identifying applicable biomarkers for ESCC prognosis may be helpful in improving the outcome of ESCC patients. Our previous studies indicated that the *PLCE1* rs2274223 and rs11599672 SNPs were associated with susceptibility to ESCC in a high-incidence population from the Ci county of Hebei province in northern China (25). To the best of our knowledge, whether these two SNPs may be used as predictive biomarkers for the prognosis of ESCC patients in this high-incidence region has not been determined to date.

Materials and methods

Study subjects. The survival information of 207 ESCC patients was collected. All the study subjects were ethnically homogeneous (of Han descent) and permanent residents of the Ci county, and they were recruited during an endoscopic screening campaign between 2008 and 2012. The patients had histologically confirmed ESCC. Information on the sex, age, smoking habits and family history of upper gastrointestinal cancer (UGIC) was obtained from the cancer patients by two professional interviewers directly after blood sampling. Smokers were defined as those who formerly or currently smoked no less than five cigarettes per day for at least 2 years. Individuals who had at least one first-degree relative or at least two second-degree relatives who had esophageal/cardiac/gastric cancer were defined as having a family history of UGIC. The present study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University. Informed consent forms were obtained from all recruited subjects.

DNA extraction. Venous blood (5 ml) from each subject was collected in Vacutainer tubes containing EDTA and stored at 4˚C. After sampling, genomic DNA was extracted within 1 week by proteinase K (Merck, Darmstadt, Germany) digestion, followed by a salting out procedure according to the method published by Miller *et al* (26).

Polymorphism genotyping. The genotypes of *PLCE1* polymorphisms were determined by the Shanghai Generay Biotech Co., Ltd. (Shanghai, China) using the polymerase chain reaction-ligase detection reaction method, which has been described in detail in our previous study (25).

Statistical analysis. Statistical analysis was performed using the SPSS version 22.0 software package (IBM Corp., Armonk, NY, USA). $P<0.05$ was considered significant for all statistical analyses. Survival time was calculated from the date of ESCC diagnosis to the date of death or the last follow-up. The associations of survival time with demographic characteristics and *PLCE1* SNPs were estimated using the Kaplan-Meier method and the log-rank test. Univariate or multivariate Cox regression analysis was fitted to estimate the crude hazard ratios (HRs), adjusted HRs and 95% confidence intervals (CIs).

Results

Patient characteristics. The mean age of the 207 ESCC patients was 60.3±7.9 years. Sex, age, smoking status and UGIC family history were not found to be associated with the survival time of the ESCC patients (Table I).

Effect of different SNP genotypes on survival. The mean survival time of rs2274223 SNP A/A, A/G and G/G genotype carriers was 42.9, 43.4 and 46.3 months, respectively. Compared with the A/A genotype, the A/G and G/G genotypes did not modify the mortality risk of ESCC patients (HR=0.924 and 0.986, 95% CI: 0.585-1.459 and 0.439-2.216, respectively). For rs11599672 SNP T/T, T/G and G/G genotype carriers, the mean survival time was 42.8, 43.8 and 42.7 months, respectively. Compared with the T/T genotype, the T/G and G/G genotypes did not significantly affect the mortality risk of ESCC patients (HR=0.902 and 0.993, 95% CI: 0.575-1.414 and 0.436-1.994, respectively) (Table II, Fig. 1).

Discussion

The TNM staging system, which is based on tumor depth (T), presence and number of regional nodes with metastatic disease (N) and presence or absence of distant metastasis (M) is used to predict the prognosis of cancer patients. However, the overall survival of the patients with similar TNM stage varies, which may be attributed to overlooking the biological or molecular characteristics of each individual tumor. Therefore, it is necessary to detect the alterations in the patients' genomic, epigenetic and/or proteomic profile, and even in single markers, and to identify useful biomarkers for the prognosis of cancer patients (27). In the present study, it was investigated whether the *PLCE1* rs2274223 and rs11599672 SNPs may be used to predict the outcome of ESCC patients; however, no correlation was observed between these two SNPs and the survival of ESCC patients.

Two studies demonstrated that the *PLCE1* rs2274223 SNP was associated with the risk of ESCC (3,10). Subsequently, Wang *et al* investigated the PLCE1 protein expression of ESCC tissues with different genotypes of rs2274223 SNP and found a higher expression level in ESCC tissues with the A/G genotype compared with those with the A/A genotype (28). Similar results were observed in the study by Hu *et al* (10). However, it remains controversial whether PLCE1 acts as an oncogene or a tumor suppressor in ESCC development and progression. Hu *et al* (10) and Li *et al* (24) reported decreased mRNA in ESCC tumors compared with that in the adjacent normal esophagus, but no difference in protein expression. By contrast, ESCC tissues exhibited a higher PLCE1 protein expression compared with normal tissues in other studies (3,23,28-30). This disparity may be attributed to differences in sample size, genetic background or experimental methods. It is worth mentioning that downregulation of PLCE1 inhibited ESCC cell proliferation and promoted cell apoptosis *in vitro* (23,29).

As regards the association of PLCE1 expression with the survival of ESCC patients, the results were inconsistent. Specifically, one study reported that high PLCE1 protein expression in ESCC was associated with poor survival (23), while increased tumor/normal-fold change of mRNA and protein expression in ESCC was associated with improved survival in another study (24). To the best of our knowledge, the present study is the first to evaluate the association between the rs2274223 SNP and survival of ESCC patients of Han nationality, and found no correlation, which is similar to the results in the northern Indian population (7).The *PLCE1* rs2274223 SNP, a non-synonymous SNP causing an amino acid change from His to Arg in the 26th exon, is located at the calcium-binding domain, indicating its crucial functional significance. However, data analysis by bioinformatics software suggests rs2274223 SNP to be benign and tolerated and the variation between wild-type and mutated structures negligible, which may partly explain the null results (7). Interestingly, the same SNP played a different role in ESCC susceptibility and prognosis of ESCC patients.

Table I. ESCC patient characteristics and survival.

Characteristics	Patients, n (%)	Deaths, n (%)	MST (months)	Log-rank P-value	HR (95% CI)
Sex					
Male	141 (68.1)	60 (42.6)	42.2		1.000
Female	66 (31.9)	2 (39.4)	45.3	0.593	0.883 (0.557-1.399)
Age, years					
≤60	106 (51.2)	44 (41.5)	43.5		1.000
>60	101 (48.8)	42 (41.6)	42.9	0.943	1.015 (0.665-1.550)
Smoking status					
Non-smoker	92 (44.4)	34 (37.0)	45.2		1.000
Smoker	115 (55.6)	52 (45.2)	41.6	0.282	1.265 (0.821-1.949)
Family history of UGIC					
Negative	129 (62.3)	59 (45.7)	41.7		1.000
Positive	78 (37.7)	27 (34.6)	45.8	0.159	0.723 (0.459-1.141)

ESCC, esophageal squamous cell carcinoma; MST, mean survival time; UGIC, upper gastrointestinal cancer; HR, hazard ratio; CI, confidence interval.

Table II. *PLCE1* SNPs and survival of ESCC patients.

SNP	Patients, n (%)	Deaths, n (%)	MST (months)	Log-rank P-value	HR (95% CI)[a]
rs2274223 A/G					
A/A	113 (54.6)	47 (41.6)	42.9		1.000
A/G	81 (39.1)	32 (39.5)	43.4		0.924 (0.585-1.459)
G/G	13 (6.3)	7 (53.8)	46.3	0.904	0.986 (0.439-2.216)
rs11599672 T/G					
T/T	102 (49.3)	43 (42.2)	42.8		1.000
T/G	87 (42.0)	35 (40.2)	43.8		0.902 (0.575-1.414)
G/G	118 (8.7)	8 (44.4)	42.7	0.946	0.993 (0.436-1.994)

[a]Adjusted for sex, age, smoking status and UGIC family history. *PLCE1*, phospholipase C ε-1 gene; SNP, single-nucleotide polymorphism; ESCC, esophageal squamous cell carcinoma; MST, mean survival time; UGIC, upper gastrointestinal cancer; HR, hazard ratio; CI, confidence interval.

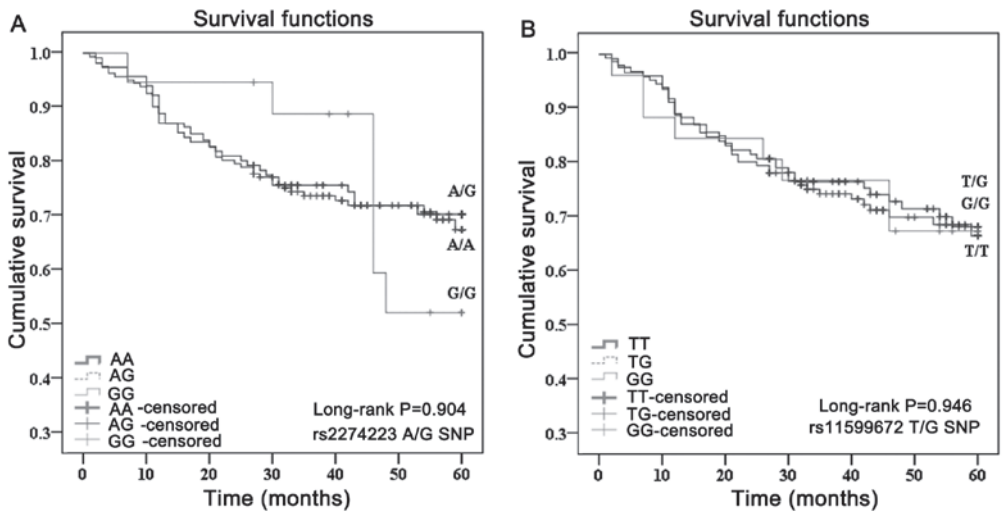

Figure 1. Kaplan-Meier survival curves for esophageal squamous cell carcinoma patients by genotypes of *PLCE1* SNPs. (A) rs2274223 A/G SNP; (B) rs11599672 T/G SNP. *PLCE1*, phospholipase C ε-1 gene; SNP, single-nucleotide polymorphism.

The possible reason for this discrepancy may be the complex structure of PLCE1 and its involvement in various signaling pathways, such as nuclear factor-κB and Ras-mitogen-activated protein kinase (31).

The *PLCE1* rs11599672 SNP is situated within the transcription factor-binding site, and the substitution of T to G may alter the transcriptional activity of *PLCE1*. Our previous study indicated that a family history of UGIC increased the risk of ESCC in subjects with the T/T genotype. In addition, the rs11599672 SNP G/G genotype was found to be associated with a decreased risk of non-oropharyngeal tumors in a non-Hispanic white population (32). However, there was no association between the rs11599672 SNP and the prognosis of ESCC patients in the Ci county, which is a high-incidence region. To date, the functional role of rs11599672 SNP has not been determined. Therefore, defining the function of the rs11599672 SNP, such as the association of PLCE1 expression with different genotypes of rs11599672 SNP, may be useful in providing mechanistic evidence for the findings.

In conclusion, the *PLCE1* rs227423 and rs11599672 SNPs cannot be used as predictive markers for survival of ESCC cases in a high-incidence region of northern China. Further investigation is required to determine the association of other SNPs in *PLCE1* with the prognosis of ESCC patients.

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