RESEARCH ARTICLE

Genetic Variations in TERT-CLPTM1L Genes and Risk of Lung Cancer in a Chinese Population

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Abstract

Background: This study was conducted to investigate the association between single nucleotide polymorphisms (SNPs) in telomerase reverse transcriptase (TERT) and cleft lip and palate transmembrane1-like (CLPTM1L) and lung cancer risk in a Chinese population. Methods: We performed a hospital-based case-control study, including 980 lung cancer cases and 1000 cancer-free controls matched for age and sex. Each case and control was interviewed to collect information by well-trained interviewers. A total of 5 ml of venous blood was collected for genotype testing of TERT rs2736098 and CLPTM1L rs401681 using TaqMan methodology. Results: The results revealed that the variant homozygote TERT rs2736098TT was associated with an increased risk of lung cancer (OR=2.017, 95%CI=1.518-2.681), especially lung adenocarcinoma (OR=2.117, 95%CI=1.557-3.043) and small cell carcinoma (OR=1.979, 95%CI: 1.174-3.334), compared with the TERT rs2736098CC genotype. Similar results were observed in non-smokers. Conclusion: The TERT rs2736098 polymorphism might affect the susceptibility to lung cancer in Chinese populations. The associations need to be verified in larger and different populations.

Keywords: Lung cancer - TERT - CLPTM1L - single nucleotide polymorphism - China

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Introduction

In the worldwide, cigarette smoking has been established as a primarily environmental risk factor of lung cancer, but only a fraction of smokers develop lung cancer during their lifetime. In contrast, there are a significant proportion of lung cancer cases with no history of smoking (Spitz et al., 2003; Lam et al., 2004). Therefore, it is completely credible that lung cancer is a complex disease, which is influenced by genetic and environmental factors and their interactions.

Recently, genetic variants in telomerase reverse transcriptase (TERT) and cleft lip and palate transmembrane1-like (CLPTM1L) locus at chromosome 5p15.33 have been identified to be related to lung cancer by several genome-wide association studies (GWAS) (McKay et al., 2008; Wang et al., 2008; Rafnar et al., 2009; Miki et al., 2010; Yoon et al., 2010). TERT is the reverse transcriptase component of telomerase, which serves to maintain telomere length (Lantuejoul et al. 2007). Telomeres are highly conserved nucleic acid-protein complexes at the distal parts of chromosomes that protect chromosomes from degradation, fusion and rearrangement and allows continued cell dividing indefinitely (Weinrich et al., 1997; Feldser et al., 2003; Rodier et al., 2005). Hence, the structural proteins like TERT that regulate activity of telomerase and synthesis of telomere, play a vital role in cellular immortality and carcinogenesis of various cancers (Hanahan et al., 2000). Normally, TERT is only expressed in embryonic stem cells and germ cells; however, abnormal expression of TERT mRNA and protein has been observed in multiple types of tumors, including lung cancer (Hiyama et al., 1995; Falchetti et al., 2000; Wang et al., 2002). CLPTM1L gene encodes a predicted transmembrane protein, but its function is largely unknown. Several studies have reported that the common polymorphisms of CLPTM1L are associated with the risk for development of various cancers, such as bladder cancer, skin cancer, lung cancer, breast cancer (Savage et al. 2007; Wang et al., 2008; Gago-Dominguez et al., 2011; Nan et al., 2011).

Recently, some genome-wide association studies have reported that the variations in the TERT-CLPTM1L (5p15.33) locus contribute to susceptibility to cancers, and found that TERT rs2736098 and CLPTM1L rs401681 were both associated with risk of lung cancer (Wang et al., 2008; Choi et al., 2009; Chen et al., 2012; Myneni et al., 2013). Our previous study which focused on the association between the two genes and lung cancer, found that TERT and CLPTM1L affected the susceptibility to lung cancer in Chinese women non-smokers (Li et al., 2013). In this study, we collected a relatively larger number of lung cancer sample and tested the association between genetic variants of rs2736098 and rs401681 and the risk of lung cancer.

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Materials and Methods

Study subjects

This case-control study consisted of 980 lung cancer patients and 1000 cancer-free controls. Patients were consecutively recruited between January 2002 and December 2010 at Liaoning Cancer Hospital and Institute, Shenyang, China. We included all patients with histopathologically confirmed lung cancer, without previous chemotherapy or radiotherapy. There were no gender or age restrictions. Controls were randomly selected from the same hospital during the same period as the cases were enrolled. The selection criteria for controls included cancer-free individuals and frequency matched to the cases based on gender and age (±5 years). All cases and controls included were ethnically Chinese and resided in the northeast of China. At the time of enrollment, a written informed consent was provided by each subject, and 5 ml blood sample and a standardized questionnaire including demographic information was collected.

Genotype analysis

Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and phenol-chloroform extraction. Genotyping of TERT rs2736098 and CLPTM1L rs401681 was performed using the Taqman real-time polymerase chain reaction (pCR) assays from Applied Biosystems (Foster City, CA). The PCR program was heating to 95°C for 10 min followed by 47 cycles of 92°C for 30s and 60°C for 1 min. An ABI Prism 7500 Sequence Detection System was applied to read the reacted plates and analyze the endpoint fluorescence. Primers and probes were supplied by Applied Biosystems. For quality control, genotyping analysis was performed by investigators blinded to case-control status; and 10% randomly selected samples were duplicated, with the 100% concurrence rate of the duplicate sets.

Statistical analysis

Cases and controls were compared using Student's t-test for continuous variables and Pearson's x^2 test for categorical variables. Hardy-Weinberg equilibrium (HWE) for genotypes of each SNP was tested with a goodness-of-fit x^2 test among controls. Associations of the SNPs with lung cancer were estimated by calculating the odds ratios (ORs) and 95% confidence intervals (CIs) from logistic regression model after adjustment for gender and age. All tests were two sided, and P <0.05 was used as the criterion of statistical significance. All statistical analyses were conducted by SPSS v13.0 software.

Results

The demographic characteristics for 980 lung cancer cases and 1000 cancer-free controls are shown in Table 1. There were no significant differences in the distributions of age (p= 0.117) and gender (p= 0.629) between the cases and controls enrolled in this study with a similar mean age (58.68±11.43 years for cases and 57.81±13.13 years for controls). 26.9% of the cases were smokers compared to 18.8% of controls. Cases included 504 adenocarcinomas, 220 squamous cell carcinomas, 146 small-cell carcinoma and 110 other tumors with a variety of different pathologies (including large-cell, mixed cell carcinomas and undifferentiated carcinomas).

The distribution of TERT rs2736098 and CLPTM1L rs401681 genotypes in cases and controls are presented in Table 2. The genotype frequencies among controls were both in agreement with Hardy-Weinberg equilibrium (HWE), P value is 0.365 for TERT rs2736098 and 0.502 for CLPTM1L rs401681, respectively. In the multivariate logistic regression model adjusted for age and sex, the individuals carrying the TERT rs2736098 TT genotype had a 2.017-fold risk of lung cancer compared to those with the homozygous CC genotype (95%CI: 1.518-2.681,

Table 1. Distribution of General Characteristics among Lung Cancer Cases and Cancer-free Controls

Variables	Cases (n=980)	Controls (n=1000)	*p* value
Gender			
Male	366	363	0.629[b]
Female	614	637	
Age			
Mean±SD	58.68±11.43	57.81±13.13	0.117[a]
Smoking status			
Smoker	264 (27.0%)	188 (18.8%)	0.000[b]
Never-smoker	650 (66.3%)	807 (80.7%)	
Unknown	66 (6.7%)	5 (0.5%)	
Histology			
Adenocarcinoma	504 (51.4%)		
Squamous cell carcinoma	220 (22.5%)		
Small-cell carcinoma	146 (14.9%)		
Others	110 (11.2%)		

[a]*p* value was calculated by the t-test; [b]*p* value was calculated by the chi-square test; Abbreviations: SD, standard deviation

Table 2. Association of TERT and CLPTM1L Gene Polymorphisms with Lung Cancer

Genotype		Cases(%)	Controls(%)	*P* of HWE	Adjusted OR[a]	95%CI	*p* value
TERT rs2736098 C>T	CC	337(35.4)	406(42.5)	0.365	Ref		
	CT	438(46.0)	443(46.4)		1.174	0.964, 1.431	0.11
	TT	177(18.6)	106(11.1)		2.017	1.518, 2.681	0.000b
Dominant model	CC	337(35.4)	406(42.5)		Ref		
	CT+TT	615(64.6)	549(57.5)		1.335	1.108, 1.608	0.002
CLPTM1L rs401681 C>T	CC	436(45.8)	437(45.8)	0.502	Ref		
	CT	424(44.6)	424(44.4)		0.993	0.820,1.201	0.94
	TT	91(9.6)	93(9.8)		0.981	0.711,1.352	0.905
Dominant model	CC	436(45.8)	437(45.8)		Ref		
	CT+TT	515(54.2)	517(54.2)		0.991	0.826, 1.188	0.918

[a]Adjusted for age and sex; [b]p<0.05

Table 3. Results from Stratified Analysis of TERT-CLPTM1L and Lung Cancer Risk

Genotypes		Cases (%)	Controls (%)	Adjusted OR[a]	95% CI	*p* value
TERT rs2736098						
Adenocarcinoma	CC	169 (34.6)	406 (42.5)	Ref		
	CT	219 (44.9)	443 (46.4)	1.199	(0.937, 1.533)	0.149
	TT	100 (20.5)	106 (11.1)	2.177	(1.557, 3.043)	0.000[b]
Squamous cell carcinoma	CC	82 (38.7)	406 (42.5)	Ref		
	CT	101 (47.6)	443 (46.4)	1.085	(0.782, 1.506)	0.624
	TT	29 (13.7)	106 (11.1)	1.436	(0.889, 2.320)	0.139
Small cell carcinoma	CC	52 (36.4)	406 (42.5)	Ref		
	CT	66 (46.1)	443 (46.4)	1.175	(0.796, 1.736)	0.417
	TT	25 (17.5)	106 (11.1)	1.979	(1.174, 3.334)	0.01
CLPTM1L rs401681						
Adenocarcinoma	CC	230 (47.1)	406 (42.5)	Ref		
	CT	210 (43.0)	443 (46.4)	0.922	(0.729, 1.167)	0.5
	TT	48 (9.9)	106 (11.1)	0.931	(0.629, 1.377)	0.72
Squamous cell carcinoma	CC	104 (48.4)	406 (42.5)	Ref		
	CT	91 (42.3)	443 (46.4)	0.916	(0.667, 1.258)	0.589
	TT	20 (9.3)	106 (11.1)	0.941	(0.549, 1.613)	0.824
Small cell carcinoma	CC	59 (42.5)	406 (42.5)	Ref		
	CT	68 (48.9)	443 (46.4)	1.197	(0.823, 1.740)	0.347
	TT	12 (8.6)	106 (11.1)	0.981	(0.505, 1.905)	0.955

[a]Adjusted for age and sex; [b]$p<0.05$

Table 4. Relationship between the TERT-rs2736098 and CLPTM1L-rs401681 Genotypes and Lung Cancer in Non-smokers

Genotypes		CT *vs* CC		TT *vs* CC	
		ORa (95% CI)	*p* value	ORa (95%CI)	*p* value
TERT rs2736098	Overall	1.129 (0.899,1.418)	0.297	1.999 (1.455,2.744)	0.000[b]
	Adenocarcinoma	1.203 (0.917,1.578)	0.183	2.179 (1.515,3.134)	0.000[b]
	Squamous cell carcinoma	0.858 (0.566,1.302)	0.471	1.204 (0.669,2.167)	0.535
	Small cell carcinoma	1.135 (0.700,1.843)	0.607	1.558 (0.795,3.056)	0.196
CLPTM1L rs401681	Overall	0.992 (0.797,1.236)	0.946	0.921 (0.637,1.332)	0.662
	Adenocarcinoma	0.952 (0.735,1.232)	0.708	0.902 (0.588,1.386)	0.639
	Squamous cell carcinoma	0.805 (0.538,1.205)	0.292	0.814 (0.407,1.628)	0.561
	Small cell carcinoma	1.294 (0.806,2.078)	0.285	0.781 (0.315,1.938)	0.594

[a]Adjusted for age and sex; [b]$p<0.05$

$p<0.001$). The dominant model (CT+TT genotype) was performed to increase statistical power, and significant association was found under the model (adjusted OR=1.335, 95%CI: 1.108-1.608). When stratified by histological type, we found a significant relationship between rs2736098 TT and adenocarcinoma (adjusted OR=2.177, 95%CI: 1.557-3.043) and small cell carcinoma (adjusted OR=1.979, 95%CI: 1.174-3.334) (Table 3). Likewise, similar results were observed among non-smokers (for TT *vs* CC of overall: OR=1.999, 95%CI: 1.455-2.744; for TT *vs* CC of adenocarcinoma: OR=2.179, 95%CI: 1.515-3.134) (Table 4). For CLPTM1L rs401681 SNP, the frequencies of genotype were not significantly different between the cases and controls, or between none of the histological subgroups and controls.

Discussion

In this study, we investigated the association between genetic variants of TERT rs2736098 and CLPTM1L rs401681 and lung cancer, with a total of 980 Chinese cases included. The results suggested that TT genotype of TERT rs2736098 was significantly associated with an increased risk of lung cancer, especially lung adenocarcinoma and small cell carcinoma. In addition, we obtained similar results in further analyses in non-smokers, which was to rule out the interference of smoking.

TERT is a key component of telomerase enzyme, which is responsible for maintaining telomere length (Lantuejoul et al., 2007). The length of telomeres gradually decreases with each cell division, increasing age, or mutations in structural proteins. It has been known that telomere is essential for the preservation of chromosomal integrity and stability, cellular immortality and carcinogenesis of various cancers (Weinrich et al., 1997; Feldser et al., 2003; Rodier et al., 2005). TERT, which regulates activity of telomerase and synthesis of telomere, shows a high-level of expression in many cancers, including lung cancer (Bagheri et al., 2006). Hence, TERT is considered to play a vital role in unlimited cell division and carcinogenesis, especially among Asians (Hanahan et al., 2000; Qi et al., 2012; Zhang et al., 2012). To date, there have been a few studies on TERT rs2736098 polymorphism and susceptibility to lung cancer. Choi et al. (2009) investigated the association of rs2736098 in TERT gene with lung cancer in a Korean population. In

their study, the rs2736098 TT genotype was positively related to increased risk of lung cancer. Li et al. (2013) and Wu et al. (2013) also found that individuals carrying rs2736098 TT genotype had higher risk of lung cancer, particular lung adenocarcinoma in Chinese populations. In concordance with the above studies, in the present study, significant associations between rs2736098 polymorphism and lung cancer were observed. In addition, our research also provided the strong evidence that TT genotype was associated with small cell carcinoma. As far as we know, this discovery is found for the first time in Chinese population.

CLPTM1L gene encodes a predicted transmembrane protein, which has been reported to be involved in cellular response to genotoxic stress and cisplatin resistance (Yamamoto et al., 2001). This gene is widely expressed in different tissues; however, its function is largely unknown. Several studies have revealed that the common polymorphisms of CLPTM1L are associated with multiple types of cancers, including lung cancer (Savage et al., 2007; Wang et al., 2008; Gago-Dominguez et al., 2011; Nan et al., 2011). In the most recently published study, Ke et al. (2013) confirmed the association between the T allele of rs401681 and reduced risk of lung cancer in a Chinese population. To our knowledge, a few genome-wide association studies have focused on the relationship of CLPTM1L rs401681 and lung cancer. It was firstly identified by Wang et al. (2008) in a Caucasion population. Their results revealed that rs401681 was significantly associated with decreased risk of lung cancer. Thereafter, a few GWA studies which performed in different populations replicated the association but with conflicting outcomes. Mckay et al. (2008) and Rafnar et al. (2011) found that rs401681 C allele was a risk allele for the development of lung cancer in Caucasion populations. However, it was recently reported that rs401681 CT genotype was showed protective effect on lung cancer risk in Asian populations (Bae et al., 2012; Chen et al., 2012). In contrast to above studies, Zienolddiny et al. (2009) and Sun et al. (2013) did not find any significant association between rs401681 and lung cancer. Base on our research, the rs401681 polymorphism may not affect individual susceptibility to lung cancer. The possible explanations for the discrepancies of the research results could be that the allele frequencies in different ethnicity were different. The data in HapMap demonstrates that CLPTM1L rs401681 T allele frequency in Caucasian populations was 43%, but 29% in Asian populations (http://snp.cshl.org/cgi-perl/gbrowse/hapmap24_B36/). Moreover, the discrepancies might come from different sources of control group. The exact function of CLPTM1L remains unclear. Further studies are needed to validate the findings.

In conclusion, this case-control study revealed that TT genotype of TERT rs2736098 increased the risk of lung cancer, especially lung adenocarcinoma and small cell carcinoma in a Chinese population. The association of TERT with lung cancer was also observed in non-smokers. Further studies on the association are warranted in larger and well designed studies and in different populations. In addition, the functional mechanisms of the genes of TERT-CLPTM1L need to be clarified in the further studies.

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