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Polymorphisms in lncRNA PTENP1 and the risk of oral squamous cell carcinoma in a Chinese population

C. XIN, J.-L. LI, Y.-X. ZHANG, Z.-H. YU

Department of Stomatology, Central Hospital Affiliated to Qingdao University, Qingdao, China

Abstract. – OBJECTIVE: PTENP1, a long non-coding RNA, has previously been reported to be involved in tumorigenesis and cancer progression. The relationship between PTENP1 and susceptibility tumors is reported, while, an association of PTENP1 with the risk of oral squamous cell carcinoma (OSCC) in Chinese population is lacked. This research is designed to investigate the association of PTENP1 with susceptibility of OSCC.

PATIENTS AND METHODS: In this research, TaqMan technology was used to test genotype in 342 OSCC patients and 711 healthy controls, so as to analyze the association between PTENP1 polymorphisms (rs7853346 rs865005 and rs10971638) and susceptibility of oral squamous cell carcinoma.

RESULTS: The results of this research showed that rs7853346 [Additive model: Adjusted odds ratio (OR) = 0.81, 95% confidence interval (CI) = 0.66-0.99] was related to the OSCC risk. It was not found that the other two sites were associated with the susceptibility of OSCC.

CONCLUSIONS: This research indicated that rs7853346 is statistically correlated with the OSCC risk.

Key Words
LncRNA, PTENP1, Polymorphism, OSCC.

Introduction

Oral squamous cell carcinoma (OSCC) is one of the six most frequent cancers in the world, with high incidence and poor prognosis, seriously threatening human life and health. As reported, more than five hundred thousand new OSCC cases occur worldwide each year[1]. In the United States alone, there are forty thousand new cases and eight thousand deaths of OSCC each year[2]. Despite advances in cancer diagnosis and treatment, the 5-year survival rate of patients with OSCC is still only about 50%[3,4]. Continuous exposure to tobacco, alcohol use, and human papillomavirus infection are the major risk factors for OSCC[5,6]. At present, the pathogenesis of oral cavity cancer remains unclear, but it is generally believed that oral cavity cancer is attributed to a combined effect of various risk factors and genetic and epigenetic changes.

Long non-coding RNAs are defined as transcripts longer than 200 nucleotides, which can be involved in a series of biological processes by interacting with DNA, RNA or protein[7]. PTEN has been reported as a tumor suppressor in many cancers[8,9]. Long non-coding RNA PTENP1, as a pseudogene of PTEN, which is highly homologous with PTEN and located in 9p13.3, is involved in the occurrence and development of many kinds of human diseases. It can negatively regulate PI3K/Akt/mTOR signaling pathway to inhibit the development of tumor by interacting with small molecule RNA and affecting the expression of PTEN. At the same time, studies have proved that PTENP1, as a competing endogenous small-molecule RNA for the targeted regulation of PTEN, can regulate the occurrence and development of tumor[8,10,11]. Many studies have demonstrated that the expression of PTENP1 is downregulated or deleted in a variety of tumors, including head and neck squamous cell carcinoma (HNSCC)[12], OSCC[13], breast cancer[14], and gastric cancer[15]. Liu et al[12] reported that the expression level of PTENP1 in HNSCC is decreased, and the decreased expression of PTENP1 is associated with the poor survival of patients with HNSCC. Gao et al[13] found that PTENP1 is aberrantly expressed in OSCC and there is a significant correlation between the expression levels of PTENP1 and PTEN.

Based on the aberrant expression of PTENP1 in OSCC, three tag single nucleotide polymorphisms (tagSNPs) (rs7853346, rs865005, and rs10971638) in lncRNA PTENP1 were selected from 1000 Genomes data. The effect of gene polymorphism of PTENP1 on susceptibility to OSCC in Chinese population was investigated by genotyping.

Corresponding Author: Zaihu Yu, BM; e-mail: debbielee806@163.com

Patients and Methods

Patients

A case-control study was carried out, involving 342 newly diagnosed OSCC cases confirmed by pathological diagnosis and 711 healthy controls without cancer history from the community. All the subjects were unrelated Chinese Han population. All the OSCC cases were collected from Central Hospital Affiliated to Qingdao University without restriction of gender and age and history of other malignancies. The controls were randomly selected from the healthy population who took part in chronic disease screening at the same period in Shandong Province, and the frequency of age (±5 years) and gender were matched with OSCC cases. Information on subjects was collected *via* face-to-face interviews. This study was approved by the Ethics Committee of Central Hospital Affiliated to Qingdao University, China. All the subjects were required to sign informed consent. 5 mL peripheral blood was taken.

A total of 20 paired cancer tissues and para-carcinoma normal tissues of OSCC patients were collected and stored in the liquid nitrogen. OSCC was diagnosed by two pathologists independently.

SNP Selection

Based on data from the databases (dbSNP, HapMap, and UCSC), tagSNPs of PTENP1 were selected. Haploview software was used to single out tagSNPs, with criteria of minor allele frequency (MAF) ≥0.05, Hardy-Weinberg value ≥0.05, and r^2<0.8 utilized for tagSNP selection. Finally, three tagSNPs (rs7853346 C>G, rs865005 C>T, and rs10971638 G>A) were selected.

DNA Extraction and Genotyping

The traditional phenol-chloroform method was used to extract genomic DNA. The purity and concentration of DNA was detected by UV spectrophotometry, with DNA stored at -20°C before genotyping. DNA was genotyped with TaqMan, using Primer Express 3.0 to synthesize primers and probes. The 10 μL PCR volume included 1 μL DNA template, 5 μL 2× HotTaq PCR Mix, 0.5 μL primer, 0.25 μL probe, and 2.5 μL double distilled water. The success rate of genotyping was more than 99%. 5% samples were selected randomly for repeated assays, obtaining the consistent genotyping results as above.

Statistical Analysis

The goodness-of-fit χ^2-test was conducted to analyze whether every genotype met the Hardy-Weinberg equilibrium in controls. Differences in gender, age, smoking and drinking condition between cases and controls were calculated by the χ^2-test. The odds ratio (OR) and 95% confidence interval (CI) was calculated by the univariate and multivariate Logistic regression models to assess the statistical correlation between the tagSNPs and the risk of OSCC. The stratification analysis was performed with gender, age, smoking and drinking condition. Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for statistical analyses. All statistical tests were two-sided, and p<0.05 suggested that the difference was statistically significant.

Data were expressed as mean ± SD and analyzed using GraphPad Prism 5.0. (La Jolla, CA, USA) Student's *t*-test was performed to compare two different groups.

Results

A total of 342 patients histopathologically diagnosed with OSCC and 711 healthy controls with matched age and gender were included in this study, as shown in Table I. There were no statistically significant differences between the two groups in age and gender. The percentage of smokers had no statistically significant difference between cases and controls (39.8% *vs.* 35.9%, p=0.220), while the percentage of drinkers was

Table I. Selected characteristics in OSCC cases and controls.

Variables	Case N (%)	Control N (%)	p^a
All subjects	342 (100)	711 (100)	
Age			0.179
<60	152 (44.1)	285 (40.1)	
≥60	190 (55.6)	426 (59.9)	
Gender			0.976
Females	161 (47.1)	334 (47.0)	
Males	181 (52.9)	377 (53.0)	
Smoking			0.220
Ever	136 (39.8)	255 (35.9)	
Never	206 (60.2)	456 (64.1)	
Drinking			0.010
Ever	111 (32.5)	177 (24.9)	
Never	231 (67.5)	534 (75.1)	

[a]Two-sided chi-squared test.

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

SNP	Base change	MAF in our controls	HWE
rs7853346	C>G	0.314	0.843
rs865005	C>T	0.235	0.131
rs10971638	G>A	0.252	0.227

HWE=Hardy-Weinberg equilibrium. MAF=minor allele frequency.

higher in cases compared with that in controls, displaying a statistically significant difference (32.5% *vs.* 24.9%, p=0.010).

The information of selected sites is summarized in Table II. All the selected sites in controls were in Hardy-Weinberg equilibrium (p>0.05), with MAF>0.05.

The associations of selected sites with OSCC in Chinese population were analyzed (Table III). The multivariate Logistic regression analysis indicated that rs7853346 significantly decreased OSCC risk (Additive model: adjusted OR=0.81, 95% CI=0.66-0.99) with gender, age, smoking and drinking condition adjusted. The stratification analysis was performed with gender, age, smoking and drinking condition (Table IV). It was found that the association between rs7853346 and the susceptibility to OSCC was more pronounced in non-smokers and drinkers (Adjusted OR=0.74, 95% CI=0.59-0.97, p=0.027; Adjusted OR=0.68, 95% CI=0.48-1.00, p=0.048). However, heterogeneity was not found between stratification in heterogeneity analysis. No statistically significant associations of the other two sites with the risk of OSCC were observed in the multivariate Logistic regression (p>0.05).

Discussion

In the case-control study, the associations between PTENP1 tagSNPs and the risk of OSCC in Chinese population were investigated. Results revealed that rs7853346 was significantly associated with the risk of OSCC. It was observed by RT-PCR that the expression level of PTENP1 was lower in OSCC tissues than that in normal tissues. The study proved for the first time that the genetic variation of PTENP1 may be closely associated with OSCC in Chinese population, which was of highly significance.

It has long been recognized that non-coding RNAs are the garbage of transcription without biological functions. However, increasingly more molecular biological events cannot be explained, so non-coding RNAs need introducing. Increasing evidence has demonstrated that non-coding RNAs play an important role in regulating various pathological and physiological processes. Non-coding RNAs can be divided into long non-coding RNAs and short non-coding RNAs according to its length. SiRNAs, a kind of short non-coding RNAs, have been reported with important functions regulating life progress. Long non-coding RNAs refer to RNAs with transcripts more than 200 nt. PTENP1, as a kind of long non-coding RNAs and a pseudogene of PTEN, which is highly homologous with PTEN, play

Table III. Logistic regression analysis for associations between selected SNPs and risk of OSCC.

SNP	Genotype	Case (%)	Control (%)	Adjusted OR [a](95%CI)	p-value[a]
rs7853346	CC	191 (52.9)	351 (47.5)	1.00	
	CG	141 (39.1)	308 (41.7)	0.78 (0.60-1.01)	0.051
	GG	129 (8.0)	80 (10.8)	0.84 (0.64-1.11)	0.223
	Additive model			0.81 (0.66-0.99)	0.042
rs865005	CC	200 (58.5)	409 (57.5)	1.00	
	CT	128 (37.4)	270 (38.0)	0.97 (0.74-1.27)	0.0.802
	TT	14 (4.1)	32 (4.5)	0.93 (0.67-1.29)	0.650
	Additive model			0.95 (0.76-1.19)	0.669
rs10971638	GG	167 (48.8)	392 (55,.1)	1.00	
	GA	148 (43.3)	280 (39.4)	1.20 (0.92-1.57)	0.186
	AA	27 (7.9)	39 (5.5)	1.08 (0.81-1.44)	0.611
	Additive model			1.14 (0.92-1.41)	0.238

[a]Logistic regression with adjustment for age, sex ,smoking and drinking.
Significant values (p<0.05) are in bold.

Table IV. Stratified analysis for associations between variant genotype ofrs7853346and OSCC risk.

Variables	rs7853346		Adjusted[b] OR (95%CI)	*p*[b]
	Cases[a] CC/CG/GG	Controls[a] CC/CG/GG		
Age, yr				
<60	79/66/6	134/129/21	0.805 (0.56-1.79)	0.143
≥60	101/72/17	198/179/48	0.82 (0.63-1.06)	0.130
Gender				
Females	86/64/11	154/148/30	0.79 (0.58-1.06)	0.123
Males	94/74/12	178/160/39	0.80 (0.61-1.07)	0.132
Smoking				
Ever	70/55/11	124/106/25	0.90 (0.64-1.25)	0.524
Never	110/83/12	208/202/44	0.74 (0.59-0.97)	0.027
Drinking				
Ever	66/36/9	84/72/21	0.68 (0.48-1.00)	0.048
Never	114/101/62	248/236/48	0.86 (0.67-1.11)	0.244

[a]Major homozygote/heterozygote/rare homozygote between cases and controls;
[b]Logistic regression with adjustment for age, sex, smoking and drinking.
Significant values ($p<0.05$) are in bold.

important regulating functions in a variety of tumors[16,17]. PTEN is deleted in various tumors, and it can encode a phosphatase and negatively regulate PI3K/Akt/mTOR signaling pathway to inhibit the development of tumor[18,19].

It was reported[20] that the expression level of PTENP1 in hepatocellular carcinoma tissues is significantly lower than that in control tissues. Moreover, PTENP1 can interact with miR-193a-3p to suppress cell migration and invasion in hepatocellular carcinoma. Yu et al[21] found that the expression levels of PTENP1 and PTEN in bladder cancer are significantly reduced, and there is a positive correlation between them. The expression levels of PTENP1 and PTEN are negatively correlated with the expression level of miR-17. Liu et al[12] reported that the expression level of PTENP1 in HNSCC is decreased and associated with the poor survival of patients with HNSCC. Another study[22] manifested that the polymorphic site rs7853346 on PTENP1 significantly reduces the risk of gastric cancer, and the expression level of PTENP1 in the mutant genotype GG/CG is increased. This study suggested that rs7853346 could significantly reduce the risk of OSCC. The mechanism by which the polymorphic site rs7853346 on PTENP1 reduced the risk of OSCC remained unclear. The mutation of rs7853346 may change the folding structure of PTENP1, changing its stability[22]. However, further research is needed to confirm the specific mechanism unknown yet.

There were some deficiencies in this work. Firstly, the cases were selected from hospitals, which may cause selection bias. To reduce the potential bias, strict inclusion criteria for cases were established, and some factors such as age, gender, smoking and drinking were adjusted in the statistical analysis. Secondly, the sample size was relatively small, limiting the statistical power. Therefore, investigations with larger sample size are needed to validate the initial findings, and functional research should be carried out to explore the specific mechanism.

Conclusions

We demomstrated for the first time that rs7853346 significantly reduced the risk of OSCC, but the specific mechanism remains unclear, so further functional research should be carried out to illuminate it.

Conflict of Interests:

The authors declared no conflict of interest.

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