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Original Article

Genetic association between PIK3CA gene and oral squamous cell carcinoma: a case control study conducted in Chongqing, China

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Abstract: PIK3CA has been shown to be involved in many malignant tumors. This study was designed to determine the expression level of PIK3CA in oral squamous cell carcinoma (OSCC) and the association of gene polymorphisms of PIK3CA with OSCC in Chinese population. The expression of PIK3CA was detected by real-time PCR in tumor and pericarcinomatous tissues of 10 OSCC patients. Nine single-nucleotide polymorphisms (SNPs) of PIK3CA (rs1607237, rs17849079, rs2677764, rs2699887, rs4855094, rs4975596, rs6443624, rs7651265 and rs7736074) in blood of 113 OSCC patients and 184 normal controls were genotyped using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) assay. The gene expression of PIK3CA was significantly higher in tumor tissues of OSCC patients than that in pericarcinomatous tissues (P = 0.012). An increased frequency of the C allele of PIK3CA rs1607237 was observed in OSCC patients as compared with controls; However, the significance was lost after Bonferroni correction (P = 0.048, p_c = 0.576). In further stratification analysis, although the frequencies of PIK3CA rs4975596 A allele in male patients and rs1607237 C allele in female patients were increased (P = 0.032, P = 0.020, respectively), the significance was also missing when Bonferroni correction was performed (P_c = 0.384, P_c = 0.24, respectively). The prevalence of other SNPs of PIK3CA did not differ between OSCC patients and controls. The expression of PIK3CA was increased in OSCC tumors; however, none of the nine tested SNPs of PIK3CA was associated with susceptibility to OSCC in the studied population.

Keywords: Oral squamous cell carcinoma, PIK3CA, single nucleotide polymorphisms, gene expression, MALDI-TOF MS, RT-PCR

Introduction

Oral cancer is a prevalent worldwide malignant tumor, the epidemiological statistics displayed that annual incidence is around 263,000 and the death number is about 128,000 in the world [1-3]. Squamous cell carcinoma is one of the most common pathological types of oral cancer, and it is especially common in China [2, 4]. In recent years, studies of tumor mechanism are gradually deepened, but according to existing results, effective measures for accurate prevention and early diagnosis of OSCC are rare. Due to high recurrence rate and propensity of lymph node metastasis in OSCC, the 5-year survival rate is less than 50%, and the poor prognosis is also hard to accept for patients and their families [5].

Smoking, alcohol drinking and chewing betel nuts are high risk factors to oral squamous cell carcinoma, and they could play a synergistic role in tumor genesis and tumor progression. Every year, the OSCC deaths with former smoking accounted for 42% of worldwide OSCC death tolls, and with former drinking accounted for 16% [6]. Malignant tumor is the result of combined action of many factors. In addition to the above mentioned risk factors, gene factors are also proven to be involved in the pathogenesis of cancers [7].

PIK3CA is located in 3q26.3, encodes 1068 amino acids and produces a 124 kD protein namely PI3K p110 catalytic subunit alpha (PI3K p110α) [8]. PIK3CA was reported to participate in tumorigenesis and development process of

Table 1. Primer sequences and experimental conditions for the gene expression of PIK3CA analysis

Gene	Primer sequence (5'-3')	Product length (bp)	Tm (°C)
PIK3CA	F: CGTTTCTGCTTTGGGACAAC	100	60.67
	R: CCTGATGATGGTCGTGGAG	100	60.05

Tm, temperature.

cancers as an oncogene [8, 9]. Mutations make gene duplication overactive, result in PIK3CA appearing amplification and the enhancive function of PI3K p110α. Through catalytic function of PI3K p110α, PIP3 increase, which is an important medium of activating PI3K/AKT signaling pathway. PIP3 is combined with the C-terminal pleckstrin homology (PH) domain of phosphoinositide dependence protein kinase 1 (PDK1), activate PDK1. Activated PDK1 phosphorylates the 308th threonine of AKT. On this basis, phosphoinositide dependent protein kinase 2 (PDK2) phosphorylate the 473th serine of AKT. Thus, PI3K/AKT signaling pathway is activated [10].

Previous studies have shown PI3K/AKT signaling pathway was frequently activated by gene mutations to promote tumor growth [11]. Furthermore, genetic polymorphisms of PIK3CA were also reported to be associated with head neck squamous cell carcinoma [9], esophageal squamous cell carcinoma [12], non-small cell lung cancer [13], breast cancer [8], endometrial cancer [14], gastric cancer [15] and rectal cancer [16]. Elevated PIK3CA levels have been reported in tumor tissues and exacerbated the clinical symptoms of patients with colorectal cancer, esophageal squamous cell carcinoma [17, 18]. Whether PIK3CA is involved in the development of OSCC patients in Chinese Han population is not yet known and was therefore the subject of the current study.

Materials and methods

Study population

113 OSCC patients and 184 geographically and ethnically matched normal controls were included in this study for SNP analysis of PIK3CA. Ten pairs of tumor and pericarcinomatous tissues of OSCC patients (6 man and 4 women, with an average age of 53.6 years) were randomly selected for measurement of PIK3CA mRNA expression. Pericarcinomatous tissues were adjacent to carcinoma tissue at least 2 mm. All samples were obtained from the Stomatological Hospital of Chongqing Medical University between August 2013 and November 2014. The diagnosis of OSCC cases were strictly based on the histopathological confirmation. Allele and genotype frequencies of patients and controls were in accord with the Hardy-Weinberg equilibrium. The study was approved by the Local Ethics Research Committee and all the investigated subjects provided informed consent before collection of blood and tissues. All procedures were in compliance with the principles of Declaration of Helsinki.

Genotyping

Genomic DNA was extracted by Tiangen DNA blood genome extraction kit (Tiangen, Beijing, China). The gene polymorphisms were genotyped by the Sangon Biotechnology Company (Shanghai, China) using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) assay.

Real-time PCR

All total RNA were extracted from soft tissues using Takara RNAios Plus (Takara, Dalian, China), followed by reverse transcription using Takara PrimeScrtipit TM 1st Strand cDNA Synthesis Kit (Takara, Dalian, China) with one micrograms of total RNA. Primers synthesized by Sangon Biotechnology Company (Shanghai, China) were designed for the amplification of a 100 bp fragment of PIK3CA cDNA (**Table 1**). Real-time PCR assay was performed on the Biorad CFX Connect™. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Hardy-Weinberg equilibrium was evaluated using Chi-square test. The Chi-square test was also used to compare clinical characteristics, genotype and allele frequencies between groups. For genotype analysis, a single genotype vs. the others was used to compare the genotype distribution in controls and patients. In SNP analysis, the *p* value was corrected by Bonferroni correction and $p_c<0.05$ was considered statistically significant. Gene expression level was analyzed by paired-samples T test. All data were analyzed by SPSS 17.0 (SPSS, Inc Chicago, IL, U S).

Table 2. Clinical features between OSCC patients and controls (n, %)

Characters	OSCC patients (N = 113)	Controls (N = 184)	*P value*
Age, years			
<60	49 (43.4)	100 (54.3)	0.074
≥60	64 (56.6)	84 (45.7)	
Gender			
Male	78 (69.0)	96 (53.2)	0.005
Female	35 (31.0)	88 (46.8)	
Smoking status			
Never	52 (46.0)	98 (53.3)	0.234
Ever	61 (54.0)	86 (46.7)	
Drinking status			
Never	75 (66.4)	148 (80.4)	0.009
Ever	38 (33.6)	36 (19.6)	

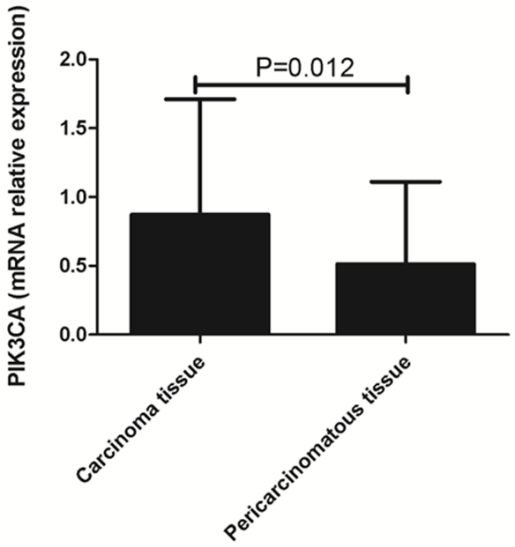

Figure 1. PIK3CA mRNA expression in paired tumor and pericarcinomatous tissues of OSCC patients. Relative gene expression of the PIK3CA in paired tumor and pericarcinomatous tissues of OSCC patients (n = 10) was measured by real time PCR. The results of these experiments are presented as expression relative to β-actin. Paired-samples T test was used for statistical analysis. The data are presented as mean ± standard deviation.

Results

Clinical features between OSCC patients and controls

The clinical characteristics of OSCC patients and controls are presented in **Table 2**. We found no significant differences in terms of distributions on age and smoking status between OSCC patients and controls. However, in contrast with the controls, the OSCC patients had significantly more drinking (P = 0.009). There was a significant difference of gender between OSCC cases and controls (P = 0.005), cases were more likely to be male.

Expression of PIK3CA in the tissues of OSCC patients

Gene expression level of PIK3CA was assayed in 10 paired tumor and pericarcinomatous tissues of OSCC patients using Real-time PCR. The gene expression was significantly higher in tumor tissues (0.87±0.84) compared with pericarcinomatous tissues (0.51±0.598, P = 0.012, **Figure 1**). The expression levels of PIK3CA in tumor tissues were 1.7-fold higher than that in pericarcinomatous tissues.

Allele and genotype frequencies of SNPs in patients and controls

A total of 9 SNPs of PIK3CA (rs1607237, rs17849079, rs2677764, rs2699887, rs4855094, rs4975596, rs6443624, rs7651265, rs7736074) were genotyped by assaying blood samples of 113 OSCC patients and 184 controls. Allele and genotype frequencies of PIK3CA did not deviate from Hardy-Weinberg equilibrium. All dates of allele and genotype frequencies were presented in the **Table 3**. The data indicated the frequency of the C allele of rs1607237 was increased in OSCC patients compared with controls (P = 0.048, OR = 1.465, 95% CI = 1.003 to 2.140). However, there was no significant difference when Bonferroni correction was performed (P_c = 0.576, n = 12). Compared with controls, OSCC patients had no statistically significant difference to relate to allele and genotype of the other eight SNPs. In the further stratification analysis, we found that the frequency of the rs4975596 A allele in male patients was significantly increased (P = 0.032, OR = 1.610, 95% CI = 1.041 to 2.491), and a significantly higher frequency of the rs1607237 C allele was observed in female patients (P = 0.020, OR = 2.256, 95% CI = 1.123 to 4.532). But when Bonferroni correction was performed, there were also no significant difference between cases and controls (P_c = 0.384, P_c = 0.24, respectively, **Tables 4** and **5**). No differences were found of the else tested SNPs based on other clinical characteristics of OSCC patients and controls in stratification analysis (results were not shown).

Table 3. Frequencies of genotypes and alleles of PIK3CA polymorphisms in patients with OSCC and controls

SNP	Genotype/Allele	OSCC (N = 113)	Controls (N = 184)	χ^2	*P*	p_c	OR (95% CI)
rs1607237	CC	65 (0.575)	88 (0.478)	2.635	0.105	NS	1.477 (0.921-2.368)
	CT	43 (0.381)	78 (0.424)	0.546	0.460	NS	0.835 (0.517-1.348)
	TT	5 (0.044)	18 (0.098)	2.813	0.094	NS	0.427 (0.154-1.184)
	C	173 (0.765)	254 (0.690)	3.925	0.048	NS	1.465 (1.003-2.140)
	T	53 (0.235)	114 (0.310)	3.925	0.048	NS	0.683 (0.467-0.997)
rs17849079	CC	112 (0.991)	183 (0.995)	0.122	0.727	NS	0.612 (0.038-9.883)
	CT	1 (0.009)	1 (0.005)	0.122	0.727	NS	1.634 (0.101-26.384)
	C	225 (0.996)	367 (0.997)	0.122	0.727	NS	0.613 (0.038-9.850)
	T	1 (0.004)	1 (0.003)	0.122	0.727	NS	1.631 (0.102-26.207)
rs2677764	AG	12 (0.106)	23 (0.125)	0.238	0.626	NS	0.832 (0.396-1.745)
	GG	101 (0.894)	161 (0.875)	0.238	0.626	NS	1.202 (0.573-2.523)
	A	12 (0.053)	23 (0.062)	0.223	0.637	NS	0.841 (0.410-1.725)
	G	214 (0.947)	345 (0.938)	0.223	0.637	NS	1.189 (0.580-2.439)
rs2699887	AG	12 (0.106)	23 (0.125)	0.238	0.626	NS	0.832 (0.396-1.745)
	GG	101 (0.894)	161 (0.875)	0.238	0.626	NS	1.202 (0.573-2.523)
	A	12 (0.053)	23 (0.062)	0.223	0.637	NS	0.841 (0.410-1.725)
	G	214 (0.947)	345 (0.938)	0.223	0.637	NS	1.189 (0.580-2.439)
rs4855094	GG	113 (1.000)	184 (1.000)	NS	NS	NS	NS
	G	226 (1.000)	368 (1.000)	NS	NS	NS	NS
rs4975596	AA	45 (0.398)	56 (0.304)	2.749	0.097	NS	1.513 (0.926-2.470)
	AG	57 (0.504))	102 (0.554	0.701	0.402	NS	0.818 (0.512-1.309)
	GG	11 (0.097)	26 (0.141)	1.240	0.265	NS	0.655 (0.310-1.384)
	A	147 (0.650)	214 (0.582)	2.790	0.095	NS	1.339 (0.950-1.887)
	G	79 (0.350)	154 (0.418)	2.790	0.095	NS	0.747 (0.530-1.052)
rs6443624	AA	1 (0.009)	1 (0.005)	0.122	0.727	NS	1.634 (0.101-26.384)
	AC	16 (0.142)	29 (0.158)	0.140	0.709	NS	0.882 (0.455-1.707)
	CC	96 (0.850)	154 (0.837)	0.083	0.773	NS	1.100 (0.576-2.102)
	A	18 (0.080)	31 (0.084)	0.039	0.843	NS	0.941 (0.513-1.724)
	C	208 (0.920)	337 (0.916)	0.039	0.843	NS	1.063 (0.580-1.948)
rs7651265	AA	98 (0.867)	153 (0.833)	0.683	0.409	NS	1.324 (0.680-2.578)
	AG	13 (0.115)	29 (0.158)	1.045	0.307	NS	0.695 (0.345-1.400)
	GG	2 (0.018)	2 (0.011)	0.246	0.629	NS	1.640 (0.228-11.806)
	A	209 (0.925)	335 (0.910)	0.379	0.538	NS	1.211 (0.658-2.229)
	G	17 (0.075)	33 (0.090)	0.379	0.538	NS	0.826 (0.449-1.520)
rs7736074	CC	43 (0.381)	56 (0.304)	1.828	0.176	NS	1.404 (0.858-2.298)
	CG	52 (0.460)	100 (0.543)	1.944	0.163	NS	0.716 (0.447-1.146)
	GG	18 (0.159)	28 (0.152)	0.027	0.869	NS	1.056 (0.554-2.011)
	C	138 (0.611)	212 (0.576)	0.690	0.406	NS	1.154 (0.823-1.618)
	G	88 (0.389)	156 (0.424)	0.690	0.406	NS	0.867 (0.618-1.215)

CI, confidence intervals; OR, odds ratios; NS, not significant; P_c, the Bonferroni correction *P* values.

Discussion

In present study, we investigated the expression of PIK3CA in OSCC patients. The result indicated a significantly higher gene expression of PIK3CA in tumor tissues compared with the paired pericacinomatous tissues. Moreover, we respectively examined nine single nucleotide polymorphisms of PIK3CA. Our findings showed that there was no association of these tested SNPs with OSCC.

In our study, we found the gene expression of PIK3CA was increased in OSCC tissues, our

Table 4. Frequencies of genotypes and alleles of rs4975596 in male patients with OSCC and controls

SNP	Genotype/Allele	OSCC (N = 78)	Controls (N = 96)	χ^2	*P*	p_c	OR (95% CI)
rs4975596	AA	32 (0.410)	26 (0.271)	3.764	0.052	NS	1.873 (0.990-3.542)
	AG	39 (0.500)	53 (0.552)	0.469	0.494	NS	0.811 (0.446-1.477)
	GG	7 (0.090)	17 (0.177)	2.761	0.097	NS	0.458 (0.180-1.169)
	A	103 (0.660)	105 (0.547)	4.601	0.032	NS	1.610 (1.041-2.491)
	G	53 (0.340)	87 (0.453)	4.601	0.032	NS	0.621 (0.401-0.961)

Table 5. Frequencies of genotypes and alleles of rs1607237 in female patients with OSCC and controls

SNP	Genotype/Allele	OSCC (N = 35)	Controls (N = 88)	χ^2	*P*	p_c	OR (95% CI)
rs1607237	AA	23 (0.657)	42 (0.477)	3.251	0.071	NS	2.099 (0.930-4.736)
	AG	12 (0.343)	36 (0.409)	0.462	0.497	NS	0.754 (0.333-1.706)
	GG	0 (0.000)	10 (0.114)	4.329	0.037	NS	NS
	A	58 (0.829)	120 (0.682)	5.393	0.020	NS	2.256 (1.123-4.532)
	G	12 (0.171)	56 (0.318)	5.393	0.020	NS	0.443 (0.221-0.891)

results are in accord with previous reports indicating the increased PIK3CA expression levels in tumor tissues of patients with colorectal cancer [17], esophageal squamous cell carcinoma [18], head neck squamous cell carcinoma [19] and non-small cell lung cancer [20]. Recent studies have shown that PIK3CA could regulate PIP3 content and promote PIP3 to activate PDK1. AKT activity is regulated by regulating the content of PIP3, which binds AKT to the cell membrane, permitting its activation by activated PDK1. Activated AKT contributes to tumor cells proliferation, survival, metastasis, inhibiting cell apoptosis, even oncogenic transformation [9, 12, 15, 18, 21]. These data collectively indicates that increased PIK3CA expression may result in more activation of AKT, then to promote the development of OSCC.

Although OSCC is thought to be caused by gene aberrant expression, genetic polymorphisms are also considered to play a role in this disease. PIK3CA is emerging as a key factor in tumor cell survival. A study has reported that PIK3CA mutations generally appear late in tumorigenesis, just before or coincident with invasion [15], illustrating that PIK3CA may be closely related with the invasiveness of cancer cells. Moreover, PIK3CA polymorphisms caused enhancement of PI3K signaling pathway in tumor tissues, that also suggested PIK3CA may be involved in tumor formation [12]. In this study, we studied whether polymorphisms of PIK3CA were associated with susceptibility to OSCC. The choice of the SNPs of PIK3CA was based on earlier studies. A total of nine SNPs polymorphisms were reported to be associated with some malignant cancers [14, 16, 22-27] and were selected as candidates in our study. Eight SNPs (rs1607237, rs17849079, rs2677764, rs2699887, rs4975596, rs6443624, rs7651265, rs7736074) have a minor allele frequency of > 1% and another variant (rs4855094) were monozygous. Our results revealed that all selected SNPs were not associated with OSCC. These SNPs based on gender, age, ever smoking or drinking and clinical stage were further analyzed, but did not indicate a detectable association.

In our study, clinical features showed that alcoholic drinking was a high risk factor to OSCC. It suggests that alcoholic drinking may be involved in the pathogenesis of cancers. Moreover, compared with women, men are more likely to drink wine. Which may be the reason that more cases were likely to be male. There were no significant differences in distributions analysis on age and smoking status between OSCC patients and controls.

In conclusion, this study found that the expression of PIK3CA was increased in OSCC tumors. However, none of the nine tested SNPs of PIK3CA was associated with susceptibility to OSCC in the studied population. Reasons for this matter may include 1) the subjects in this study were limited; 2) the chosen SNPs weren't appropriate for this association study. Therefore, more studies are needed to clarify this issue.

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Disclosure of conflict of interest

None.

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