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Original Article

Role of single nucleotide polymorphisms of KRAS and BRAF genes in susceptibility for papillary thyroid carcinoma and patients' prognosis

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Abstract: Genetic variations within oncogene are frequently detected in many kinds of malignancies, including papillary thyroid carcinoma (PTC). Rs712 within 3'-untranslated region (UTR) of *KRAS* and *BRAF* rs3748093 have been reported to influence expression and function of the two genes. So we speculated that the two SNPs would implicate in activation of the two genes and increase disposition to PTC. For this, a hospital-based case-control study, including 330 PTC cases and 364 healthy check-up controls, was carried out to investigate the association between them. Our results showed that no significant association was found between *BRAF* rs3748093 and *KRAS* rs712 and risk of PTC in co-dominant, dominant, recessive, over-dominant and allele models in overall and subgroups. However, genotype TA and allele A of rs3748093 were significantly associated with TNM II stage, node and distant metastasis, respectively. And there were positive associations between genotype GT and allele T of rs712 and poor differentiation in cases. These findings suggested that rs3748093 and rs712 were involved in progression of PTC rather than risk, genotype TA and allele A of rs3748093 and genotype GT and allele T of rs712 could be emerged as poor prognostic factors for PTC in Chinese population. With limitation of our study, well designed, multiple centers and larger sample size case-controls studies are of great value to validate our findings.

Keywords: *BRAF*, *KRAS*, single nucleotide polymorphism, papillary thyroid carcinoma

Introduction

Thyroid carcinoma is originated from follicular thyrocytes or parafollicular thyroid cells and has become to be one of the most common cancers in endocrine system [1]. According to the report of cancer statistics from American cancer association, approximately 62,450 individuals will be diagnosed as new cases and 1,950 cancer patients will be dead due to thyroid cancer in 2015 [2]. In China, it was most likely occurred in females and 67,788 new individuals were confirmed as thyroid cancer patients in 2011 [3]. There are many kinds of thyroid cancer, such as papillary thyroid carcinoma (PTC), medullary thyroid cancer and anaplastic carcinoma. Among them, incidence of PTC has significantly increased in past decade [4]. Although the etiology of PTC carcinogenesis and metastasis remains elusive, accumulating evidences indicate that activation of oncogenes or inhibition of tumor suppressed genes which is triggered by mutation or abnormal methylation can lead to carcinogenesis of PTC [4-6]. So we speculated that genetic variation of oncogene would modulate susceptibility to PTC.

KRAS, a member of RAS gene family, is one of the most important oncogenes turmorigenesis. It encodes p21 protein, and the product can bind both guanosine triphosphate (GTP) and guanosine diphosphate (GDT) and plays an important role in regulation of normal signal transduction [7-9]. It can recruit RAF protein to activate RAS-RAF-MEK-ERK-MAP kinase pathway [7], and the pathway is frequently activated in cancer cell and plays a vital role in cell proliferation and cycling [10]. RAF gene family consists of three members, including *BRAF*. The BRAF encoded protein, which is a serine/threonine kinase, is a downstream protein of

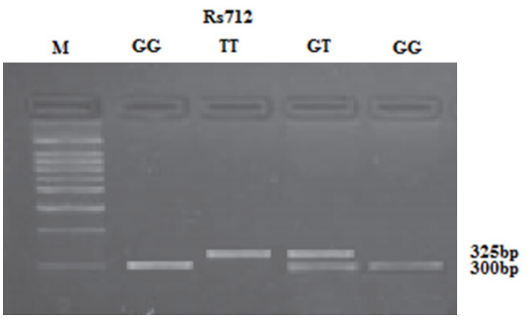

Figure 1. The results of genotype analysis for rs72 by electrophoresis (genotype TT: 325 bp band; genotype GG: 300 bp, 25 bp band; genotype GT: 325 bp, 300 bp, 25 bp band; the 25 bp band wasn't showed in the figure; M: DNA size marker (100 bp ladder).

KRAS in RAS-RAF-MEK-ERK-MAP signal pathway [11]. Both the two genes are prone to mutation in a series of cancers, such as colorectal cancer, lung malignancy and PTC [12-14]. Several studies have reported mutations in *KRAS* and *BRAF* can result in activation of the signal pathway [11, 15], leading to turmorigenesis and metastasis.

Recently, single nucleotide polymorphisms (SNPs) within *KRAS* and *BRAF* has been reported to be functional in regulation of the two oncogenes and are associated with risk of cancer [9, 16, 17]. Rs61764370 in the sixth let-7 complementary binding site (LCS6) of *KRAS* 3'-untranslated region(UTR) was reported to be significantly associated with risk of primary breast and ovarian cancer [18]. Rs712, a SNP located in the LCS1 of *KRAS*, was significantly associated with risk of cancer in Chinese population [9, 19]. Allele G of the locus could alter *KRAS* expression and its secondary spatial structure to affect the binding affinity between miR-181, Let-7 and its mRNA [20]. Rs3748093, an allele A>T alternation in intron of *BRAF*, was reported as a susceptible locus for PTC in Chinese population [16]. However, a recent study conducted by Zhang showed that the locus wasn't associated with PTC in 368 cases and 564 controls [21].

Hence, we used a retrospective study of 330 clinical confirmed PTC patients and 364 healthy check-up individuals to comprehensively investigate *KRAS* rs712 and *BRAF* rs3748093 as potential susceptible factors for PTC in Chinese population.

Materials and methods

A total of 330 clinicopathologically confirmed PTC cases and 364 healthy check-up individual with free of clinical symptom and any other disease which recruited from Pingxiang People's Hospital and The Third Affiliated Hospital of Guizhou Medical University in the interval of 2013 March to May 2015 were included in present study. All included individuals were Han nationality, which consisted of more than 95% of population in China. 1 ml EDTA-anticoagulated peripheral blood sample was collected from all eligible individuals and stored in -80°C. The detail demographic characteristics such as gender and year, status of smoking and drinking and clinical pathological features were obtained from medical record of each included patients. All written informed consents were signed by all included individuals and the study was approved by the ethical committee of Pingxiang People's Hospital and The Third Affiliated Hospital of Guizhou Medical University, respectively. According to the manufacturer's protocol, human genomic DNA of each participant was extracted from 200 μl blood sample using Tiangen human genomic DNA isolation kit (Tiangen, Beijing, China). Concentration and purity of all DNA samples were detected using ultraviolet spectrophotometer (Eppendorf, Hambrug, German) and concentration of all eligible sample should be higher than 200 ng/μl and DNA purify should be within the interval of 1.8-2.1. *KRAS* rs712 was genotyped using polymerase-chain reaction and followed by digestion with Taq I (**Figure 1**). *BRAF* rs3748093 was genotyped by ABI 7500 TaqMan-PCR genotyping assay (ABI, Foster city, CA). Primer and probe sequences, reaction conditions of the loci were used according to the descriptions of Zhang et al and Pan et al [21, 22], and 5% PCR products were randomly selected to DNA sequencing.

Genotype and allele frequencies in two groups were obtained by direct counting. Hardy-Weinberg equilibrium (HWE) and distribution difference of genotype and allele of the loci in two groups were examined using personal χ^2 test. Possible strength between two SNPs and PTC risk was assessed by odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were performed using the SPSS 17. 0 statistic software (SPSS Inc, Chicago, IL, USA) and $P<0.05$ was considered as significance.

Table 1. Clinical features of case and control groups

Variables	Cases (330)	Percentage (%)	Controls (364)	Percentage (%)	*P*-value
Age (years, M+SD)	35.8±5.78		36.5±6.00		0.106
Male/female	30/300	9.1%/90.9%	39/325	10.7%/89.3%	0.475
Smoking (Yes/No)	37/293	11.2%/88.8%	44/320	12.1%/87.9%	0.720
Drinking (Yes/No)	45/285	13.6%/86.4%	42/322	11.5%/88.5%	0.405
Differentiation (Well/Poor)	173/157	52.4%/47.6%			
TNM (I/II)	189/141	57.3%/42.7%			
Invasion (T1+T2/T3+T4)	46/284	13.9%/86.1%			
Node metastasis (No/N1)	195/135	59.1%/40.9%			
Distant Metastasis (M0/M1)	189/141	57.3%/42.7%			

Table 2. Genotype and allele distributions of *BRAF* rs3748093 and *KRAS* rs712 in two groups

Locus	Model	Genotype and allele	Cases	Controls	*P*-value	Crude OR (95% CI)	Adjusted OR (95% CI)*
RS3748093	Co-dominant	TT	240 (72.7%)	271 (74.5%)			
		TA	75 (22.7%)	82 (22.5%)	0.824	0.960 (0.670-1.375)	0.973 (0.677-1.398)
		AA	15 (4.6%)	11 (3.0%)	0.502	0.770 (0.359-1.652)	0.779 (0.363-1.672)
	Dominant	TT	240 (72.7%)	271 (74.5%)			
		TA/AA	90 (27.3%)	93 (25.5%)	0.666	0.928 (0.663-1.301)	0.939 (0.668-1.319)
	Recessive	TT/TA	315 (95.4%)	353 (97.0%)			
		AA	15 (4.6%)	11 (3.0%)	0.515	0.778 (0.364-1.660)	0.785 (0.366-1.680)
	Over-dominant	TA	75 (22.7%)	82 (22.5%)			
		TT/AA	255 (77.3%)	282 (77.5%)	0.881	1.028 (0.719-1.468)	1.017 (0.709-1.458)
Rs712	Co-dominant	GG	212 (64.2%)	252 (69.2%)			
		GT	104 (31.5%)	101 (27.8%)	0.228	0.817 (0.588-1.135)	0.816 (0.585-1.137)
		TT	14 (4.3%))	11 (3.0%)	0.314	0.661 (0.294-1.487)	0.642 (0.284-1.454)
	Dominant	GG	212 (64.2%)	252 (69.2%)			
		GT/TT	118 (35.8%)	112 (30.8%)	0.163	0.798 (0.582-1.096)	0.800 (0.581-1.102)
	Recessive	GG/GT	316 (95.7%)	353 (97.0%)			
		TT	14 (4.3%)	11 (3.0%)	0.389	1.422 (0.636-3.177)	1.394 (0.621-3.130)
	Over-dominant	GT	104 (31.5%)	101 (27.8%)			
		GG/TT	226 (68.5%)	263 (72.2%)	0.277	1.198 (0.865-1.661)	1.198 (0.862-1.665)

Results

The clinical baseline features in each group were described in **Table 1**. As shown in **Table 1**, there was no significant difference in age, gender and status of smoking and drinking in two groups. All of the included cases were TNM-I-II stage patients, and proportion of female cancer individual was high up to 90.9%, ages of the cases were less than 45, and average age was only 35.8±5.78. Proportions of well and poor differentiation in cases were 52.4% and 47.6%, respectively. Percentages of PTC patients with deep invasion, node and distant metastasis were 86.1%, 40.9% and 42.7%, respectively.

Genotype and allele frequencies of two loci were summarized in **Table 2**. *P*-value of HWE of rs712 and rs3748093 in controls were 0.820 and 0.126, respectively, indicating that genotype distributions were fit for HWE in both two SNPs. Genotype GG, GT and TT frequencies of rs712 in case and control groups were and 64.2%, 31.5%, 4.3% and 69.2%, 27.8%, 3.0%, respectively. No significant distribution difference was found in comparison of GT vs. GG (31.5% vs. 27.8%, P=0.228, adjusted OR= 0.816, 95% CI=0.585-1.137), TT vs. GG (4.3% vs. 3.0%, P=0.314, adjusted OR=0.642, 95% CI=0.284-1.454) in two groups, respectively. Moreover, rs712 was not associated with risk of PTC in dominant (35.8% vs. 30.8%, P=0.163, adjusted OR=0.800, 95% CI=0.581-1.102 for GT+TT vs. GG), recessive (4.3% vs. 3.0%, P=0.389, 95% CI=0.621-3.130 for TT vs. GT+GG) and over-dominant (68.5% vs. 72.2%, P=0.277, adjusted OR=1.198, 95% CI=0.862-1.665 for GT vs. TT+GG) models. Genotype TT, TA, and AA of rs3748093 in cases are 72.7%,

Table 3. Genotype distributions of *BRAF* rs3748093 and *KRAS* rs712 in subgroups stratified by smoking and drinking

Locus	Genotype	Smoking			Non-smoking			Drinking			Non-drinking		
		Cases	Controls	*P*-value	Cases	Controls	*P*-value	Cases	Controls	*P*-value	Cases	Controls	*P*-value
Rs3748093	TT	28	31		212	240		29	31		212	240	
	TA	8	11	0.405	67	71	0.734	14	10	0.407	61	72	0.833
	AA	1	2	0.156	14	9	0.191	2	1	0.535	13	10	0.367
Rs712	GG	25	30		187	222		31	28		181	224	
	GT	10	11	0.866	94	90	0.226	12	13	0.703	92	88	0.151
	TT	2	1	0.593	12	10	0.419	2	1	1.000	12	10	0.366

Table 4. *BRAF* rs3748093 and *KRAS* rs712 and clinical pathological baseline in cases

Variables	Rs3748093						Rs712					
	TA vs. TT	*P*-value	AA vs. TT	*P*-value	A vs. T	*P*-value	GT vs. GG	*P*-value	TT vs. GG	*P*-value	T vs. G	*P*-value
TNM-1	32/150		7/150		52/326		58/122		9/122		76/302	
TNM-II	43/90	0.002	8/90	0.221	59/223	0.015	46/90	0.764	5/90	0.621	56/226	0.937
Invasion (T1+T2)	12/31		9/137		18/74		18/27		1/27		20/72	
Invasion (T3+T4)	63/209	0.497	6/103	0.825	87/481	0.301	86/185	0.274	13/185	0.538	112/456	0.653
Node metastasis (NO)	34/155		6/155		46/344		57/130		8/130		73/317	
Node metastasis (N1)	41/85	0.003	9/85	0.056	59/221	0.001	47/82	0.268	6/82	0.756	59/211	0.322
Distant metastasis (MO)	37/145		7/145		51/327		58/122		9/122		76/302	
Distant metastasis (M1)	38/95	0.090	8/95	0.292	54/228	0.049	46/90	0.764	5/90	0.621	56/226	0.937
Differentiation (Well)	43/121		9/121		61/285		65/99		9/99		83/263	
Differentiation (Poor)	32/119	0.295	6/119	0.471	44/270	0.204	39/113	0.008	5/113	0.202	49/265	0.007

22.7%, 4.6%, and 74.5%, 22.5%, 3.0% in controls, respectively. However, we didn't observed significant association of *BRAF* rs3748093 with risk of PTC in comparison of co-dominant (22.7% vs. 22.5%, P=0.824, adjusted OR=0.973, 95% CI=0.677-1.398 for TA vs. TT; 4.6% vs. 3.0%, P=0.502, adjusted OR=0.779, 95% CI=0.363-1.672 for AA vs. TT), dominant (27.3% vs. 25.5%, P=0.666, 95% CI=0.939, 95% CI=0.668-1.319 for TA+AA vs. TT), recessive (4.6% vs. 3.0%, P=0.515, adjusted OR=0.785, 95% CI=0.366-1.680 for AA vs. TA+TT), over-dominant (77.3% vs. 77.5%, P=0.881, adjusted OR=1.017, 95% CI=0.709-1.458 for TA vs. AA+TT) models. After stratifying overall group into smoking, non-smoking, drinking and non-smoking subgroups, we still didn't find the significant association between KRAS rs712, BRAF rs3748093 and susceptibility to PTC in these subgroups (all P>0.05) (**Table 3**).

Additionally, results of the possible association between two loci and clinical baseline characteristics in case group showed that allele T (P=0.007) and genotype GT of rs712 (P=0.008) were significantly associated with poor differentiation of PTC, allele A of rs3748093 were significantly associated with TNM II stage (P=0.015), node (P=0.001) and distant (P=0.049) metastasis, respectively, genotype TA of the locus were positively associated with TNM II stage (P=0.002) and node metastasis (P=0.003) (**Table 4**).

Discussion

KRAS and *BRAF,* encoding two important of signal effective proteins in RAS-RAF-MEK-ERK-MAP kinase pathway, are two important oncogenes in onset of malignancies including PTC [23, 24]. Genetic variants in *KRAS* and *BRAF* have been reported to be significantly associated with cancer susceptibility, anti-EGFR therapeutic efficacy and poor prognosis in many kinds of malignancies [12, 13, 25-27]. Recently, few studies reported the association between *KRAS* and *BRAF* polymorphisms and susceptibility to PTC [16, 21, 28]. A meta-analysis reported that rs712, rather than rs61764370 was significantly associated with cancer risk in Chinese population [9]. However, inconsistent results were reported between *BRAF* rs3748093 and susceptibility to PTC [16, 21].

In our study, a hospital based case-control study was performed to investigate the possible association between *KRAS, BRAF* polymorphisms and risk of PTC. The results showed that *KRAS* rs712 and *BRAF* rs3748093 genotype and allele distributions weren't associated with susceptibility to PTC in co-dominant, dominant, recessive, over-dominant and allele models, respectively, suggesting that rs712 and rs3748093 might be not involved in thyroid turmorigenesis, allele and genotypes of two loci couldn't be considered as susceptible factors for PTC in Chinese population. Additionally, genotype TA and allele A of rs3748093 were significantly associated with TNM II stage, node and distant metastasis, respectively, and there were positive associations between genotype GT and allele T of rs712 and poor differentiation in cases, indicating that the SNPs were implicated in PTC progression, and genotype TA and allele A of rs3748093 and genotype GT and allele T of rs712 could predict poor prognosis of the disease in Chinese population. Our results were inconsistent with case-control studies conducted by Jin et al and Jiang et al [16, 28]. The following reasons may be account for the contradictory results. Firstly, thyroid cancer is a kind of heterogeneous diseases, our study's sample size is still small, and it can't obtain a significant result [29]. Moreover, although the loci's genotype distributions are fit for HWE, it is a hospital-based case-control study and it may have a risk to represent the general population. Additionally, PTC is a heterogeneous complex disease with interaction of environmental factors and personal genetic background, however, environmental exposure is not available in our study, which prevent our further analysis of the effect of gene-environment interaction on PTC risk.

In conclusion, our data show that genotype TA and allele A of rs3748093 and genotype GT and allele T of rs712 could be emerged as poor prognostic factors for PTC in Chinese population. Further well designed and large sample size epidemiological studies are warrant to verify our findings in Chinese population.

Disclosure of conflict of interest

None.

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