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 onco-genes 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 tumorigenesis. 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...
KRAS and BRAF SNPs and papillary thyroid carcinoma

Materials and methods

A total of 330 clinicopathologically confirmed PTC cases and 364 healthy check-up individuals 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 \( \chi^2 \) 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.

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 tumorigenesis 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.

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).
Table 1. Clinical features of case and control groups

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

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 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 (35.8% vs. 30.8%, P=0.163, adjusted OR=1.198, 95% CI=0.862-1.665) 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=1.198, 95% CI=0.862-1.665) and over-dominant (68.5% vs. 72.2%, P=0.277, adjusted OR=1.198, 95% CI=0.862-1.665) models. Genotype TT, TA, and AA of rs3748093 in cases are 72.7%,
Table 3. Genotype distributions of \textit{BRAF} rs3748093 and \textit{KRAS} rs712 in subgroups stratified by smoking and drinking

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
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<td></td>
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<tr>
<td>\textit{BRAF} rs3748093</td>
<td>TT</td>
<td>28</td>
<td>31</td>
<td>212</td>
<td>240</td>
<td>29</td>
<td>31</td>
<td>212</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>8</td>
<td>11</td>
<td>0.405</td>
<td>67</td>
<td>71</td>
<td>0.734</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>1</td>
<td>2</td>
<td>0.156</td>
<td>14</td>
<td>9</td>
<td>0.191</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>\textit{KRAS} rs712</td>
<td>GG</td>
<td>25</td>
<td>30</td>
<td>187</td>
<td>222</td>
<td>31</td>
<td>28</td>
<td>181</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>10</td>
<td>11</td>
<td>0.866</td>
<td>94</td>
<td>90</td>
<td>0.226</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>2</td>
<td>1</td>
<td>0.593</td>
<td>12</td>
<td>10</td>
<td>0.419</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

22.7%, 4.6%, and 74.5%, 22.5%, 3.0% in controls, respectively. However, we didn’t observe significant association of \textit{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-drinking subgroups, we still didn’t find the significant association between \textit{KRAS} rs712, \textit{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).

Discussino

\textit{KRAS} and \textit{BRAF}, encoding two important of signal effective proteins in RAS-RAF-MEK-ERK-MAP kinase pathway, are two important oncogenes in onsets of malignancies including PTC [23, 24]. Genetic variants in \textit{KRAS} and \textit{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 \textit{KRAS} and \textit{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 \textit{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 tumorigenesis, 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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References

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