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Original Article

Clinicopathological significance of BRAF^{V600E} mutation in Uyghur Chinese patients with papillary thyroid carcinoma

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Abstract: Background: The BRAF^{V600E} mutation is the most common genetic alteration found in papillary thyroid carcinoma (PTC). Although several studies have demonstrated that this mutation occurs more frequently in patients with PTC showing aggressive clinicopathologic features, its significance in Uyghur Chinese population remains unclear. The aim of this study was to evaluate the prevalence of the BRAF^{V600E} mutation in tumor samples and its association with clinicopathologic features in Uyghur Chinese patients with PTC. Methods: Formalin fixed paraffin embedded tissue specimens taken from 66 patients undergoing thyroidectomy from June 2007 to August 2011, at the First Affiliated Hospital of Xinjiang Medical University, Urumqi, China were investigated. PCR was used to amplify exon 15 of the BRAF gene from the specimens, followed by direct sequencing to detect the BRAF^{V600E} mutation. Results: The BRAF^{V600E} mutation was found in 19/26 (73.1%) patients with PTC, but not detected in other types of malignant and benign thyroid lesions. The rate of BRAF^{V600E} mutation was different in classic PTC (15/19 (78.9%)) and other types of PTC (4/4 (100%) in tall-cell variant and 0/3 (0%) in follicular variant). The BRAF^{V600E} mutation was associated with extrathyroidal extension (15/17 (88.2%) with extension and 4/9 (44.4%) without, $P = 0.028$). Age, sex, lymph node metastases and advanced disease stage were not significantly different between the patients with and without the BRAF^{V600E}. Conclusion: The BRAF^{V600E} mutation is common in Uyghur PTC patients. The BRAF^{V600E} mutation may be a potential prognostic factor for PTC in these patients.

Keywords: Thyroid cancer, papillary, BRAF protein, human, carcinoma/genetics, mutation

Introduction

Thyroid cancer ranks first in the head and neck neoplasms, and is the most common malignant tumor of the endocrine system [1]. Papillary thyroid carcinoma (PTC) accounts for 80-90% of all thyroid cancer [2]. The incidence of thyroid cancer is currently around 2%, and this is continuing to rise [3], so vigorous efforts have been given to improve its diagnosis and treatment. PTC generally shows low malignancy and invasiveness. Up to 80% of patients can live 10 more years. However, in some cases the disease can recur, transform and cause death [4]. The increasing incidence of thyroid cancer is mostly due to increased PTC [5]. Consequently, current thyroid cancer medicine has mostly focused on diagnosis and treatment of PTC.

In many countries, the standard treatments for PTC include surgical thyroidectomy and subsequent radioiodine ablation [3]. Although mortality of patients with PTC has reduced with these treatments, the recurrence rate has not been changed, and it often develops into advanced disease that cannot be surgically treated and lacks iodine affinity [4]. Appropriate design of the initial treatment is the key to prevent recurrence of PTC [5]. PTC can be classified into different variants according to prognostic implications these include classic PTC, follicular, tall cell, columnar cell, diffuse sclerosing, oxyphilic (Hurtle cell), and de-differentiated papillary [6]. Consequently, accurate preoperative assessment of the risk, predicting prognosis, is very important to optimize the management of patients with PTC. This is usually accomplished

by using the traditional risk stratification system, based on clinicopathological characteristics, including age and gender of patients, tumor size, extrathyroidal extension status and lymph node metastasis, and tumor stages [7]. However, it is often neither reliable nor complete to use this system to assess the PTC prognosis, especially in apparently low-risk patients [8]. This unreliability results in controversies over the treatment of PTC, so more reliable methods for predicting thyroid cancer prognosis are constantly being investigated [9].

A more accurate preoperative prediction of PTC prognosis, has been suggested by $BRAF^{V600E}$ mutation detection [10-12]. $BRAF^{V600E}$ mutation as a novel and highly specific prognostic molecular markers has important clinical practical value in helping optimize the diagnosis and treatment of the PTC. Many well-designed studies have confirmed a correlation between BRAF mutation and the occurrence, development and prognosis of PTC [13-19]. The BRAF gene (also known as murine sarcoma viral oncogenic homolog B1) belongs to the RAF gene family, located on human chromosome 7q34, T1799A single point mutation is the most common type of BRAF mutation, that is a single conversion of T→A on the 1799 nucleotide in exon 15, leading Glutamic acid to substitute to Valine at codon 600 in protein products (V600E). $BRAF^{V600E}$ kinase has a strong carcinogenic effect, because of aberrantly and constitutively activating the Ras→Raf→MAP kinase/ERK pathway (MAP kinase pathway) [20]. The role of preoperative assessment of $BRAF^{V600E}$ mutation status is still controversial, but this method can assist with the treatment decision making process when considered alongside other conventional risk factors [21].

Meanwhile, studies have shown that molecular markers are race-specific. Many recent studies confirm that the BRAF gene in different countries and nationalities has different mutation rates, mainly between 29% and 83% [22]. For example Korean, $BRAF^{V600E}$ mutation rates are relatively high, ranging from 58% to 83% [23-26]; Western countries have lower rates from 31% to 49% [27-31], Saudi Arabia has 51.7% [32], Taiwan 47% [33], and Japan 23.8% to 38.4% [34, 35].

China is very multiethnic country while the majority of the population belongs to the Han ethnic group, in Xinjiang on the northwest border of China where silk road trade occurred historically, the Uyghur ethnic group is more common at around half the population [36]. The Uyghur are considered to have eastern and western Eurasian lineages [36].

The aim of this investigation was to evaluate the prevalence of the $BRAF^{V600E}$ mutation in Uyghur Chinese patients with PTC and to analyze the association between clinicopathologic characteristics and the $BRAF^{V600E}$ mutation in Uyghur PTC patients.

Materials and methods

Patients and sample collection

In total, 92 formalin fixed paraffin embedded (FFEP) tissue specimens were obtained from 66 Uyghur patients who underwent thyroidectomy surgery from 2007 to 2011 at the First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, including 26 specimens of papillary thyroid carcinoma (PTC), 26 specimens of normal thyroid tissues surrounding the PTC tissues, 12 specimens of nodular goiter, 9 specimens of thyroid adenoma, 7 specimens of Hashimoto's thyroiditis, 5 specimens of follicular carcinoma, 4 specimens of medullary carcinoma and undifferentiated carcinoma in 3 specimens. All tissue samples were FFEP tissue and were confirmed by histopathological examination. All of the patients from whom the samples were taken had not received chemotherapy, radiation therapy or other cancer therapy prior to surgery. The study was approved by the Ethics and Research Committees in the hospital and was conducted in accordance with the Declaration of Helsinki Principles. All participants provided written informed consent.

DNA extraction

DNA from different thyroid paraffin-embedded tissue specimens was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen, USA), DNA extraction was performed according to the manufacturer's instruction. Quality and quantity of successfully extracted DNA samples were determined spectrophotometrically by Nucleic acid protein analyzer.

PCR amplification

224 basepair (bp) fragments of the BRAF gene (Genbank accession number: NG_007873.3) exon 15 were amplified by PCR. The primers were as follows: forward: 5'-TCATAATGCTTGCTCTGATAGGA-3'; reverse: 5'-GGCCAAAAATTTAAT-

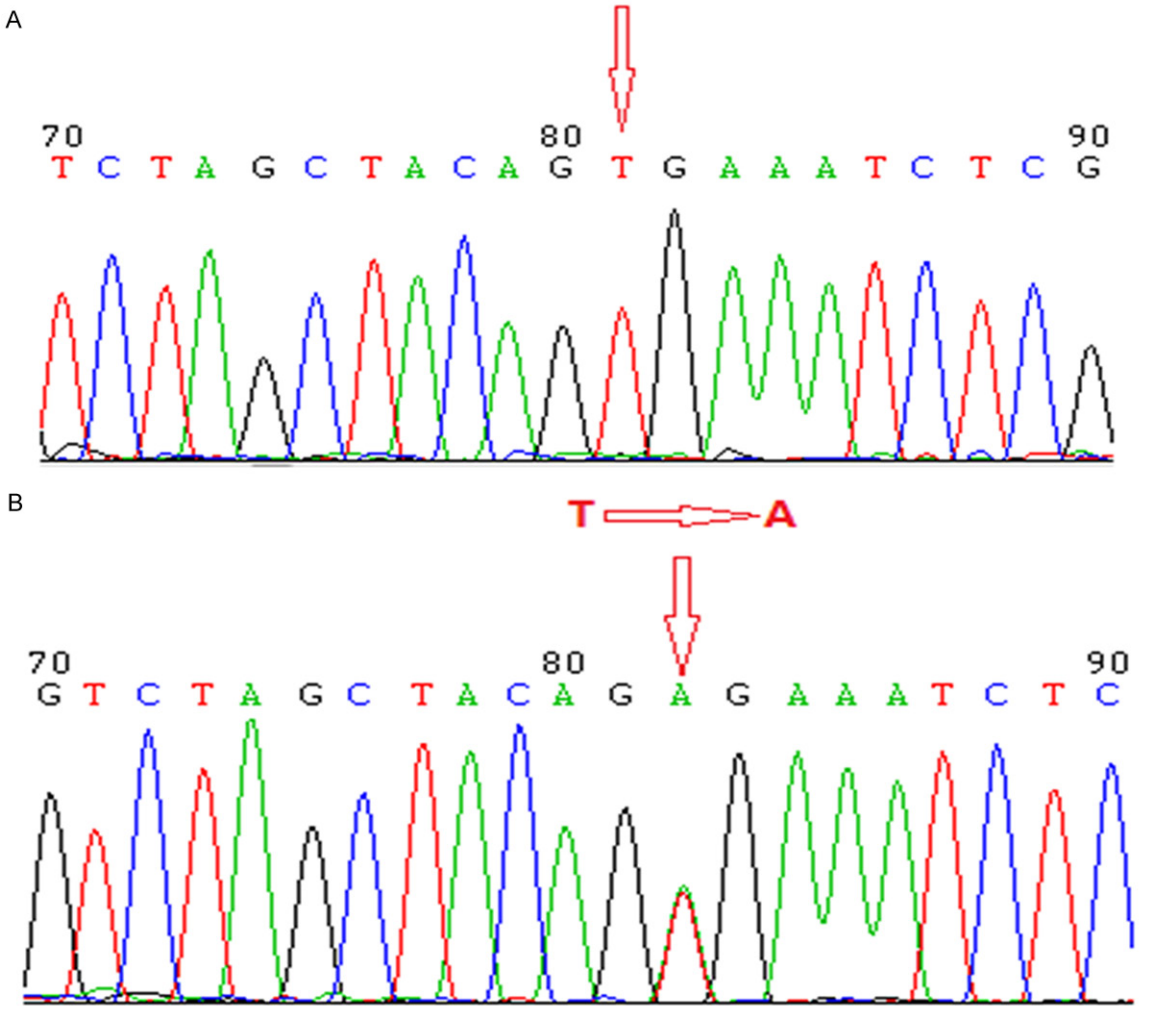

Figure 1. $BRAF^{V600E}$ mutation sequencing. A. Nodular goiter, $BRAF^{V600E}$ mutation negative. B. Papillary thyroid carcinoma (PTC) tissue, $BRAF^{V600E}$ mutation positive. The arrow indicates T-to-A mutation at nucleotide 1799.

CAGTGGA-3'. PCR was performed in 25 μL of reaction mixture containing 2.0 μL of genomic DNA, 10×PCR reaction buffer 2.5 μL, 0.6 μL of each primer, 2.0 μL of each deoxynucleotide, 2.5 mM $MgCL_2$, and 0.2 μL Platinum Taq DNA Polymerase (Fermentas, China), with ddH_2O to 25 μl. The PCR reactions were carried out with following conditions: an initial denaturation step at 95°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 45 seconds, annealing at 58°C for 45 seconds, elongation at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. PCR products were separated by a 2% agarose gel and visualized by ethidium bromide staining. The relative position of the marker bands determined a positive result. Direct DNA sequencing was performed using the dideoxy chain termination method (Sanger sequencing). Sequencing results were compared with the normal BRAF gene sequences in the NCBI genbank to determine whether the mutation was present.

Statistical analysis

All the statistical analyses were performed by SPSS software (version 18.0, SPSS Inc., Chicago, IL, USA). The fisher's exact test was used for association of BRAF mutational status and clinicopathological features of PTC in Uyghur patients. A P value < 0.05 was considered statistically significant.

Results

$BRAF^{V600E}$ mutation detection in various thyroid diseases

Of 92 samples, 19 were $BRAF^{V600E}$-mutated and all of them were PTC, the remaining 66

Table 1. $BRAF^{V600E}$ mutation status in various thyroid diseases

Histological type	No. of specimens	$BRAF^{V600E}$ mutation, n (%)	
		Positive	Negative
Papillary thyroid carcinoma	26	19 (73.1)	7 (26.9)
Normal tissues surrounding the PTC	26	0 (0.0)	26 (100.0)
Nodular goiter	12	0 (0.0)	12 (100.0)
Thyroid adenoma	9	0 (0.0)	9 (100.0)
Hashimoto's thyroiditis	7	0 (0.0)	7 (100.0)
Thyroid follicular carcinoma	5	0(0.0)	5(100.0)
Anaplastic thyroid carcinoma	3	0 (0.0)	3 (100.0)
Medullary thyroid carcinoma	4	0 (0.0)	4 (100.0)

samples did not have this mutation. The overall mutation rate was 20.7% (19/92) in all types of thyroid diseases and 73.1% in PTC (19/26). Mutations were located at the 1799 nucleotide changing the adenine (A) to thymine (T) mutation (**Figure 1**). All mutations were heterozygous. The prevalence of the $BRAF^{V600E}$ mutation in histologic subtypes of PTC was 78.9 % (15/19), 100% (4/4) and 0% (0/3) in classic variant, tall-cell variant, follicular variant, respectively. No $BRAF^{V600E}$ mutation was found out in other types of thyroid samples including normal thyroid tissues surrounding the PTC tissue (**Table 1**).

$BRAF^{V600E}$ mutation and clinicopathological features of PTC

The fisher's exact test between BRAF mutational status and clinicopathological characteristics of the PTC patients shows that there was no correlation between the BRAF mutation and gender, age, lymph node metastasis and tumor node metastasis (TNM) stage of PTC ($P>0.05$). BRAF mutation was closely associated with extrathyroidal extension, and the difference was statistically significant ($P = 0.028$). Correlation between BRAF mutation and clinicopathological features of PTC are shown in **Table 2**.

Discussion

BRAF mutation status in PTC is now well established as a prognostic marker [10-12]. However, the prevalence of the $BRAF^{V600E}$ mutation in different populations apparently varies according to their genetic background [22]. The aim of this study was to investigate the prevalence of the $BRAF^{V600E}$ mutation in Uyghur patients with PTC and to analyze the association with clinicopathologic characteristics. The results of this study showed that the overall $BRAF^{V600E}$ mutation rate in PTC in Uyghur was 73.1%. There was a close association between the $BRAF^{V600E}$ mutation and extrathyroidal extension, but no significant correlation with gender, age, lymph node metastasis and tumor clinical staging. This study also showed that in a variety of thyroid diseases, $BRAF^{V600E}$ mutation occurs only in PTC, as the other thyroid specimen had no mutations in the BRAF gene. This is in agreement with previous studies [30-35]. Prevalence of $BRAF^{V600E}$ mutation in histologic subtypes of PTC was 78.9%, 100% and 0% in classic variant, tall-cell variant, follicular variant, respectively.

The overall prevalence of the $BRAF^{V600E}$ mutation in the Uyghur PTC patients was probably higher than might have been expected, the reason for this is unclear, but we think that genetic or geographic factors could lead to such differences. While Uyghur eating habits may also account for high $BRAF^{V600E}$ mutation rate of PTC patients. The association between $BRAF^{V600E}$ mutation and eating habits, genetic or geographic factors of the Uyghur population will; however, have to be investigated by further studies.

This study also explored the relationship between $BRAF^{V600E}$ mutation and clinicopathological parameters of patients with PTC. Nikiforova and colleagues studied 104 cases of PTC patients, found a significant association of the $BRAF^{V600E}$ mutation with higher TNM stage (III-IV) and extrathyroidal extension of PTC [37]. Namba and his colleagues reported a close association between the $BRAF^{V600E}$ mutation and advanced tumors and distant metastasis of PTC [34]. Xing demonstrated a strong association of the $BRAF^{V600E}$ mutation with the high-risk clinicopathologic characteristics of PTC, such as lymph node metastasis, advanced tumor, extrathyroidal extension and recurrence [38]. In addition, many recent studies have confirmed the correlation between $BRAF^{V600E}$ mutation and poor prognosis of PTC [30, 32, 39-43]. Even in papillary thyroid microcarcinoma

Table 2. Correlation between $BRAF^{V600E}$ Mutation and Clinicopathological features of PTC

		N	$BRAF^{V600E}$ mutation, n (%)		*P*-value
			Positive	Negative	
Gender	Male	8	6 (75.0)	2 (25.0)	1.000
	Female	18	13 (72.2)	5 (27.8)	
Age, (years)	≤45	10	8 (80.0)	2 (20.0)	0.668
	>45	16	11 (68.8)	5 (31.2)	
Histologic subtypes	Classic variant	19	15 (78.9)	4 (21.1)	-
	Tall-cell variant	4	4 (100.0)	0 (0.0)	
	Follicular variant	3	0 (0.0)	3 (100.0)	
Lymph node metastasis	Present	5	4 (80.0)	1 (20.0)	1.000
	Absent	21	15 (71.4)	6 (28.6)	
Extrathyroidal extension	Present	17	15 (88.2)	2 (11.8)	0.028
	Absent	9	4 (44.4)	5 (55.6)	
TNM stage	I-II	21	16 (76.2)	5 (23.8)	0.281
	III-IV	5	2 (40.0)	3 (60.0)	

Abbreviations: PTC = papillary thyroid carcinoma; TNM = tumor node metastasis.

(PTMC), $BRAF^{V600E}$ mutation is still considered to be associated with invasive pathological features of the tumor, such as lymph node metastasis, extrathyroidal extension, and high TNM stages [28, 44, 45]. In contrast, some research reports no significant correlation between $BRAF^{V600E}$ mutation and clinicopathological features of PTC [46]. The present study also showed a close association of $BRAF^{V600E}$ mutation with extrathyroidal extension, but no significant correlation with gender, age, lymph node metastasis and tumor clinical staging. The number of cases (26) is relatively small, so this may be the reason for the difference with previous studies.

This study has some limitations. The number of patients involved in the study was relatively small in particular there were only 26 PTC patients therefore we could not fully investigate risk factors for PTC by methods such as multivariate analysis. We did not perform any survival analysis to relate the BRAF mutation status with prognosis. Further studies with increased number of cases will be needed to explore the association of $BRAF^{V600E}$ mutation with clinicopathological parameters in patients with PTC in Uyghur patients.

In conclusion, in this study, we analyzed the $BRAF^{V600E}$ mutation status in a variety of thyroid diseases in an Uyghur population in Xinjiang, China. PTC had a high prevalence of $BRAF^{V600E}$ mutation. $BRAF^{V600E}$ mutation only occurred in the PTC tissue. Our study suggests that the $BRAF^{V600E}$ mutation is associated with pathological subtypes and extrathyroidal extension of PTC, but not with the patient's age, gender, or lymph node metastasis. These results also suggest that $BRAF^{V600E}$ mutation is a highly specific molecular marker for PTC in the Uyghur Chinese population, in agreement with many other studies in other population.

Disclosure of conflict of interest

None.

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