Original article

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The BRAF^{V600E} mutation in papillary thyroid microcarcinoma with intermediate-risk to high-risk features: does the mutation have an effect on clinical response to radioiodine therapy?

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Objectives **Preclinical studies showed that BRAF^{V600E} mutation significantly reduced radioiodine uptake and decreased the sensitivity to radioactive iodine (RAI) therapy. However, clinical data regarding its role in therapeutic decision making with respect to RAI therapy are currently insufficient. Thus, this study aimed to evaluate the effect of BRAF mutation on the clinical response to RAI therapy for papillary thyroid microcarcinoma (PTMC) with intermediate-risk to high-risk features.**

Patients and methods **From January 2012 and October 2015, consecutive patients with PTMC with intermediate-risk to high-risk features who underwent RAI therapy were retrospectively included. The data about BRAF mutation status were also obtained. The association between clinicopathological characteristics and mutation was investigated. After a median follow-up of 40 months, the clinical response to RAI therapy was also compared between positive and negative mutation groups.**

Results **A total of 236 patients were included, of whom 147 (62.3%) had positive mutation. The clinicopathological features did not show significant correlation with BRAF mutation status except the sex, extrathytoidal extension and T stage. Patients with PTMC with BRAF mutation showed an increased likelihood of having advanced T stage and extrathyroidal extension. In addition, this mutation did not affect the clinical outcome of RAI therapy.**

Conclusion **The status of BRAF^{V600E} mutation may not affect the clinical response to RAI therapy for patients with PTMC with intermediate-risk to high-risk features. More trials examining the role of BRAF mutation in guiding postoperative RAI therapy are needed.** ***Nucl Med Commun*** **40:8–13**

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Keywords: BRAF^{V600E} mutation, intermediate to high risk, ongoing risk stratification, papillary thyroid microcarcinoma, radioiodine therapy

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Introduction

In recent years, the prevalence of papillary thyroid carcinoma (PTC) has significantly increased [1]. In particular, papillary thyroid microcarcinoma (PTMC), which is defined by the WHO as PTCs with a maximum diameter up to 10 mm, has increased faster than other types of PTCs [1,2].

The vast majority of PTMC were inert carcinomas, and for this entity, the clinical outcomes are excellent, with disease-specific mortality rates less than 1%, loco-regional recurrence rates of 2–6%, and distant recurrence rates of 1–2% [3,4]. The newest guidelines in 2015 suggested that radioactive iodine (RAI) therapy was not routinely recommended after thyroidectomy for patients with PTMC in the absence of other adverse features [5]. Conversely, a relatively small percentage of patients with PTMC have reported to present with high-risk features (e.g. extrathyroidal extension and macroscopic lymph node metastases), even clinically significant distant metastases [6,7]. These patients definitely fall within the intermediate-risk or high-risk group of PTC, and RAI therapy may be beneficial for decreasing the risk of recurrence [8,9].

The BRAF^{V600E} mutation is the most common genetic alteration in PTC, occurring in ~45–60% of PTC cases [10]. Many studies demonstrated that this mutation was significantly associated with aggressive clinicopathological features such as extrathyroidal extension, larger tumor size, lymph node metastases or advanced stage [11–13]. As a subgroup of PTC, PTMC also showed a significant correlation between BRAF mutation and aggressive behaviors [14,15]. A meta-analysis of 2247 patients with PTMC showed that patients with positive mutation had a higher likelihood for recurrence with odds ratio of 2.09 [16]. The BRAF^{V600E} mutation might help to specifically identify patients with PTMC who will show progression and have regional or distant metastases.

Several molecular mechanisms have been reported to clarify the role of BRAF^{V600E} mutation in the aggressive behavior, including promoting upregulation of many tumor-promoting genes and downregulation of tumor-

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suppressor genes, silencing the expression of iodine-handling genes, and impairing the sensitivity to RAI therapy [17,18]. A recent study showed that $BRAF^{V600E}$ mutation did not affect the clinical response to RAI therapy in patients with PTC without distant metastases [19]; however, for patients with PTMC with high-risk features, clinical data are currently insufficient. Thus, this study aimed to evaluate the role of $BRAF^{V600E}$ mutation in guiding postoperative RAI therapy for patients with PTMC with intermediate-risk to high-risk features.

Patients and methods

Patient enrollment

This study was approved by the ethical committee of West China Hospital of Sichuan University, and written informed consents were obtained. We retrospectively screened a total of 3187 consecutive patients with differentiated thyroid carcinoma who received thyroid surgery and RAI therapy between January 2012 and October 2015. The inclusion criteria were as follows: (a) patients with PTC with a maximum diameter up to 10 mm, (b) patients aged 18 years or older, (c) patients who underwent total thyroidectomy with bilateral central lymph node dissection with or without lateral lymph node dissection, (d) patients who had available data of $BRAF^{V600E}$ mutation analysis, and (e) patients divided into intermediate-risk to high-risk category based on the newest ATA guidelines [5]. Patients with distant metastases or incomplete tumor resection at the time of PTMC diagnosis were excluded. Patients with positive serum thyroglobulin (Tg) antibody were also excluded from this study. Eventually, 236 patients with PTMC were included in this study, with positive BRAF mutation in 147 patients.

Mutational testing

Thin-section paraffin-embedded tissues were used to extract genomic DNA with a QIAamp DNA FFPE Tissue Kit (cat. 56404; Qiagen, Hilden, Germany). Based on the BRAF sequence, the PCR primers were designed, including forward primer (5′-TGCTTGCTCTGATAGGAAAATG-3′) and reverse primer (5′-AGCCTCAATTCTTTACCATCCA-3′). The thermal cycling protocol was set as follows: 94°C 3 min, 35 cycles of 94°C 30 s, 60°C 30 s, and 72°C 30 s, and 72°C for 5 min. The sequencing of PCR products was performed using a ABI PRISM 3500 machine (Applied Biosystems, Foster City, California, USA). Positive signals were detected by intercalation of fluorescent dye, and the threshold cycle value was obtained to evaluate the BRAF mutation status.

Treatment and follow-up

All included patients underwent total thyroidectomy (TT) with central lymph node dissection (CLND). The lateral lymph node dissection was performed as clinically indicated, such as biopsy-proven lymph node metastases, suspicious findings on preoperative neck ultrasound, or macroscopic extension during surgery. With intermediate-risk to high-risk features, all of our patients received RAI therapy for remnant ablation or adjuvant therapy or therapy based on postoperative Tg level and imaging findings [neck ultrasound and chest computed tomography (CT)], followed by levothyroxine replacement. All cases were followed up with clinical examinations including thyroid-stimulating hormone, serum Tg, Tg antibodies, diagnostic radioiodine whole-body scintigraphy and neck ultrasound every 6 months for the initial 2 years, and annually thereafter. When recurrence or metastasis was suspected, additional examinations such as neck and chest CT, ^{18}F-FDG PET/CT, and fine needle aspiration were performed. During follow-up, ongoing risk stratification was used to assess the clinical response to therapy [5]. Patients were divided into four categories including excellent response (ER), biochemical incomplete response, structural incomplete response, and indeterminate response [5].

Statistical analysis

Tumor size was expressed as mean ± SD, and the difference between groups was compared using independent-sample Student's *t*-test. The association between $BRAF^{V600E}$ mutation and other clinicopathological characteristics was evaluated using χ^2test or for small cell values, Fisher's exact test. The clinical response to RAI therapy between positive and negative mutation groups was compared using Mann–Whitney *U*-test. *P* value less than 0.05 was considered statistically significant. The statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Baseline characteristics of patients with papillary thyroid microcarcinoma

We evaluated 236 patients with PTMC with intermediate-risk to high-risk features at the time of patient enrollment. The baseline characteristics are shown in Table 1. Among the included patients, 178 (75.4%) were women and 58 were men. The mean age was 42.33 year, and most patients (89.8%) were younger than 55 years at the time of diagnosis. A total of 174 patients underwent TT + CLND, whereas 62 received TT + CLND + lateral lymph node dissection. Multifocality was observed in 36.4% of patients, and 25% of patients had extrathyroidal extension, with 17.4% divided into T3b and 7.6% into T4 (T4a or T4b). Lymph node metastasis was noted in 217 (92.0%) patients, including 168 (71.2%) patients in N1a and 49 (20.8%) in N1b. Approximately 99% of patients were classified as stage I or II (89.9 and 9.3%, respectively) whereas only two (0.9%) patients as stage III. A total of 217 (91.9%) patients were identified as having an intermediate-risk of recurrence, and 19 (8.1%) patients having a high-risk of recurrence. All these patients underwent radioiodine therapy, of whom 31 patients received less than 100 mCi for remnant ablation,

Table 1 Baseline clinicopathological characteristics of patients with papillary thyroid microcarcinoma with high-risk features

	n (%)
All of included patients	236 (100)
Sex	
Male	58 (24.6)
Female	178 (75.4)
Age (years)	
Mean ± SD (range)	42.33 ± 10.67 (19–77)
≥ 55	24 (10.2%)
< 55	212 (89.8%)
Tumor size (cm)	0.75 ± 0.23 (0.1–1.0)
Multifocality	86 (36.4)
Bilaterality	58 (24.6)
Extrathyroidal extension	59 (25.0)
Surgery	
TT + CLND	174 (73.7)
TT + CLND + LLND	62 (26.3)
T stage[a]	
1	177 (75)
3b	41 (17.4)
4	18 (7.6)
N stage[a]	
0	19 (8)
1a	168 (71.2)
1b	49 (20.8)
TNM stage[a]	
T3b-4N0M0	19 (8.1)
T1aN1M0	177 (75)
T3b-4N1M0	40 (16.9)
Risk of recurrence	
Intermediate	217 (91.9)
High	19 (8.1)
AJCC stage[a]	
I	212 (89.8)
II	22 (9.3)
III	2 (0.9)
Radioiodine dose (mCi)	
< 100	31 (13.1)
100	199 (84.3)
150	6 (2.6)
Follow-up (median) (months)	40 (15–60)

CLND, central lymph node dissection; LLND, lateral lymph node dissection; TT, total thyroidectomy.
[a]AJCC Cancer Staging Manual, 8th edition.

199 received 100 mCi for remnant ablation or potential adjuvant therapy, and six received 150 mCi for adjuvant therapy.

The association of clinicopathological features with BRAF mutation

There were 147 (62.3%) patients in BRAF-positive mutation group, and 89 (37.7%) in BRAF-negative group. The comparison of clinicopathological features according to mutation status is presented in Table 2. Sex was significantly associated with BRAF mutation, with larger percentage of male in BRAF-positive group. A significant correlation was observed between BRAF mutation status and extrathyroidal extension and T stage ($P = 0.05$), that is, patients with PTMC with BRAF mutation showed an increased likelihood of having advanced T stage and extrathyroidal extension. Other characteristics such as tumor size, age, lymph node involvement, multifocality, or risk stratification showed no significant correlation with the BRAF mutation status.

Table 2 The association of clinicopathological characteristics of patients with papillary thyroid microcarcinoma with BRAF mutation

	Positive BRAF ($n = 147$) [*n* (%)]	Negative BRAF ($n = 89$) [*n* (%)]	χ^2/t	*P*
Sex				
Male	43 (29.3)	15 (16.9)	4.597	0.032
Female	104 (70.7)	74 (83.1)		
Age				
≥ 55	18 (12.2)	6 (6.7)	1.838	0.175
< 55	129 (87.8)	83 (93.3)		
Tumor size (cm)	0.77 ± 0.23	0.72 ± 0.23	−1.722	0.086
Multifocality				
Yes	55 (37.4)	31 (34.8)	0.160	0.689
No	92 (62.6)	58 (65.2)		
Extrathyroidal extension				
Yes	43 (29.3)	16 (18.0)	3.758	0.050
No	104 (70.7)	73 (82.0)		
T stage[a]				
1	104 (70.7)	73 (82.0)	3.758	0.050
3 + 4	43 (29.3)	16 (18.0)		
N stage[a]				
0	12 (8.2)	7 (7.9)	0.254	0.881
1a	106 (72.1)	62 (69.7)		
1b	29 (19.7)	20 (22.5)		
Risk of recurrence				
Intermediate	134 (91.2)	83 (93.3)	0.331	0.630
High	13 (8.8)	6 (6.7)		
AJCC stage[a]				
I	129 (87.8)	83 (93.3)	2.418	0.298
II	17 (11.6)	5 (5.6)		
III	1 (0.7)	1 (1.1)		
Radioiodine dose (mCi)				
≤ 100	144 (98.0)	86 (96.6)	0.396	0.529
> 100	3 (2.0)	3 (3.4)		

[a]AJCC Cancer Staging Manual, 8th edition.

Table 3 Response to radioiodine therapy between positive and negative BRAF mutation groups at the end of follow-up

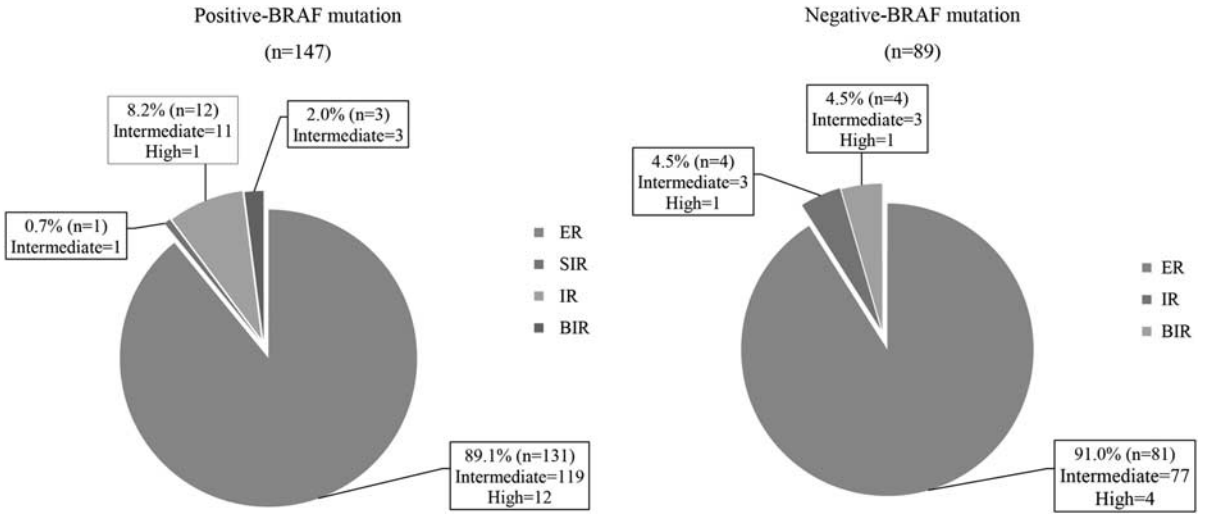
Clinical response	BRAF-positive mutation [*n* (%)]	BRAF-negative mutation [*n* (%)]	*Z*	*P*
ER	131 (89.1)	81 (91)	−0.413	0.680
IR	12 (8.2)	4 (4.5)		
BIR	3 (2.0)	4 (4.5)		
SIR	1 (0.7)	0 (0)		

BIR, biochemical incomplete response; ER, excellent response; IR, indeterminate response; SIR, structural incomplete response.

Effect of BRAF mutation on clinical outcome of radioiodine therapy

As shown in Table 3, during median follow-up of 40 months, disease-related mortality was not noted, and only one patient with positive-BRAF mutation had disease recurrence. The recurrent lesion was localized to the right lateral lymph node. Most patients in both groups achieved ER, with 131 (89.1%) patients in positive-BRAF group and 81 (91%) patients in negative-BRAF group. In fact, 97.3% (143/147) of positive mutation patients initially classified as intermediate-risk/high-risk shifted to ER or indeterminate response, whereas 95.5% (85/89) of negative mutation patients had the same trend (Fig. 1). In addition, the univariate analysis demonstrated no significant association between BRAF mutation status and clinical response to RAI therapy ($P = 0.680$).

Fig. 1



Comparison of response to RAI therapy based on ongoing risk stratification between positive and negative mutation groups, showing risk estimates evolvement over time (from initial risk stratification at receiving RAI therapy to ongoing risk stratification at the end of follow-up). BIR, biochemical incomplete response; ER, excellent response; IR, indeterminate response; RAI, radioactive iodine; SIR, structural incomplete response.

Discussion

During the past 20 years, the incidence of thyroid cancer has showed a dramatic increase [20], and up to 50% of the increase is mainly owing to the identification of PTMC [21,22]. Current theories demonstrate that increased identification of PTMC is attributable to more sensitive imaging methods and increased access to healthcare as well as the increased exposures from environment [23]. The appropriate management of PTMC cases has become a crucial issue. PTMC has been reported to consist of two biologically different subtypes, that is, most of PTMC are indolent tumors that barely have disease progression whereas a relatively small number of PTMCs have aggressive behaviors with clinically significant regional or distant metastases [24]. For asymptomatic PTMC or low-risk PTMC without clinically evident metastases or local invasion, an active surveillance management can be considered as an alternative to immediate surgery based on the newest guidelines, although there is still controversy about this issue [5]. However, both strategies are not applicable to patients with PTMC with high-risk features such as extrathyroidal extension or lymph node metastases that are always associated with a poor prognosis. They may require more aggressive treatment including total thyroidectomy with lymph node dissection as well as postoperative RAI therapy [5]. Unfortunately, no clinical features can reliably differentiate the relatively small percentage of patients with PTMC who will show disease progression from the larger population of PTMC with indolent tumor [25–27].

In recent years, $BRAF^{V600E}$ mutation as a well-known thyroid cancer oncogene has been evaluated in the tumorigenesis, progression and aggressiveness of PTMC, especially for PTMC with high-risk characteristics. The prevalence of BRAF mutation in aggressive PTMC with lymph node metastases or tumor recurrence was higher than that in nonaggressive PTMC (77 vs. 32%, $P=0.001$) [26]. Then, several studies further found that PTMCs with BRAF-positive mutation were more likely to manifest aggressive behaviors (extrathyroid extension and lymph node metastases) [14,28]. Two recent meta-analyses with large samples showed that BRAF mutation status was significantly associated with the aggressiveness and recurrence of PTMC [16,29]. This study suggested the similar results that patients with PTMC in the positive mutation group have more likelihood to present extrathyroidal extension and advanced T stage.

In fact, preclinical studies revealed that $BRAF^{V600E}$ mutation is associated with loss of radioiodine uptake by silencing of thyroid iodine-handing genes and impairing the sensitivity to RAI therapy [30]. Then, some clinical studies also found that this mutation was highly prevalent in radioiodine-refractory PTC [31,32]. In the study of Xing *et al.* [32], 54% of patients with recurrent PTC with positive mutation (7/13) lacked radioiodine avidity in their recurrent lesion, whereas none of negative mutation group (0/7) lacked radioiodine avidity, indicating that BRAF mutation was more frequently associated with absence of radioiodine avidity in loco-regional lesions. Similarly, Yang *et al.* [33] reported that in the mutation group, 84.2% (16/19) of patients had non-iodine-avid

distant lesions, whereas in the wild-type group, only 5.6% had the same situation, suggesting the value of $BRAF^{V600E}$ mutation in predicting the status of radioiodine uptake in distant metastases. So, whether the BRAF mutation status in PTC may affect the clinical response or outcome of radioiodine therapy? In a recent subgroup analysis of 282 patients with T1–T2N0M0 treated with RAI therapy, after 5 years of follow-up, the rate of biochemical recurrence in positive mutation groups was 9.3% (9/97), whereas the rate was 2.2% (4/185) in negative group [13]. The rate of macroscopic structural recurrence was also higher in positive mutation group (8.2%, 8/97) than in negative group (1.6%, 3/185) [13]. The findings indicated that BRAF mutation predicted a poor therapeutic efficacy of RAI therapy in patients with PTC with low risk. In contrast, another recent study showed no significant difference regarding clinical response to timely postsurgical RAI therapy between positive and negative mutation groups for patients with PTC without distant metastases [19]. Consistent with their findings, for patients with PTMC with high risk, the BRAF mutation status did not affect the clinical response of RAI therapy in our study. One possible reason is that the therapeutic efficacy of radioiodine might be stronger than the role of BRAF mutation in aggressiveness. Several studies and the newest guidelines have showed the benefit of RAI therapy for patients with PTMC with high-risk features [5,8,34].

Our study has several notable limitations. The sample was small, especially the number of patients with high-risk features. In fact, only a very small percentage of patients with PTMC showed high-risk features. In addition, the median follow-up time of 40 months might be too short to capture sufficient recurrence events. Finally, owing to insufficient data, we did not further investigate the effect of the combination of BRAF mutation with other genetic mutations on the RAI therapy.

Conclusion

The status of $BRAF^{V600E}$ mutation may not affect the clinical response to RAI therapy for patients with PTMC with high-risk features. More research examining the role of BRAF mutation in guiding postoperative RAI therapy is needed.

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Conflicts of interest

There are no conflicts of interest.

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