Retraction Notice

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Expression of Concern:
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Correction:
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Comment:

The authors have more requirements on indexing.

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The Relationship between BRAF V600E Mutation and the Clinicopathological Features of the Papillary Thyroid Carcinoma

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Abstract

There has been a dramatic increase in the morbidity of papillary thyroid carcinoma (PTC), which accounts for more than 85% to 90% of all thyroid malignancies. A somatic BRAF V600E oncogenic mutation has been deemed as the most frequent genetic alteration that occurs in approximately 32% - 83% of papillary thyroid cancer cases, associated with more aggressive clinical behaviors and worse prognosis. Nevertheless, the prognostic value of the BRAF V600E mutation remains to be confirmed. Herein, we examined BRAF V600E mutation by performing qPCR as well as immunohistochemistry (IHC), and analyzed the relationship between BRAF V600E mutation status and multiple clinicopathological features of a total of 188 patients with PTC. There was no significant difference in the prevalence of BRAF V600E mutation, with regard to patient gender (χ^2 = 1.252, P = 0.263), ethnicity (χ^2 = 0.756, P = 0.384) and age (younger than 40 years versus 40 years or older, χ^2 = 0.002, P = 0.957). Also, our study found no association between the frequency of V600E mutation and various pathological characteristics of PTC, including calcification (χ^2 = 0.2186, P = 0.640), hashimoto (χ^2 = 0.072, P = 0.789), tumor size (χ^2 = 1.453, P = 0.228), tumor multifocality (χ^2 = 0.183, P = 0.668), thyroid capsular invasion (χ^2 = 0.138, P = 0.710), vascular invasion (χ^2 = 1.132, P = 0.860) and lymph node metastasis (χ^2 = 0.080, P = 0.777). Considering that the mutation is widespread in the PTC, we speculate that this mutation is related to tumorigenesis rather than tumor progression. Moreover, we evaluated the clinical value of immunohistochemical strategy for detecting V600E mutation in comparison to qPCR method. Of the 188 patients with PTCs in the present study, the mutation was identified in 153 (81.38%) cases by immunohistochemistry (IHC), while it was detected in 160 (85.11%) patients by using PCR assay. we can conclude that PCR method presents a superior sensitivity compared to IHC.

Keywords

Papillary Thyroid Carcinoma, BRAF V600E, Tumorigenesis, qPCR, Immunohistochemical Methods

1. Introduction

According to papers published during the last decades, there has been a rapid and continuous increase worldwide in the incidence rate of thyroid cancer (TC), the most common endocrine malignancy [1] [2]. Papillary thyroid cancer (PTC) accounts for the vast majority (more than 85% to 90%) of all thyroid malignancies [3]. Despite the steadily raising morbidity, mortality related to PTC appears to be stationary [1] [4]. Indeed, due to the intrinsic indolent behavior of the disease and the effective initial management, the prognosis of patients affected with PTC is generally promising with a 10-year overall survival rate of approximately 85% [5] [6]. However, a relapse from complete remission has been reported in about 5% to 25% of the patients [7] [8]. In case a tumor metastasis occurs, the 10-year survival rate of PTC can be reduced to 40%.

Interestingly, the incidence rates of different TC histotypes vary considerably: the rates of PTC have exclusively increased for last decades, particularly the follicular variant of PTC [9], whereas an extremely modest increase in the morbidity of follicular thyroid cancers (FTC) has been observed [10] and the rates of anaplastic thyroid cancer are stable or even have shrunk [11]. This suggests that some carcinogenetic factors are involved in PTC onset by dysregulating specific molecular signaling.

BRAF is a Ser/Thr specific protein kinase gene, which is a member of the Raf kinase family of growth signal transduction protein kinases. This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion. The BRAF V600E mutation results in substitution of valine to glutamate in codon 600 of a serine- or threonine-specific protein kinase named BRAF, consequently leading to constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway [12]. It occurs in approximately 36% - 83% of PTC cases [13] and plays an essential role in maintaining and progression of thyroid cancer [14]. Actually, recent studies reported that there has been an increasing trend of patients with PTC carrying the BRAF V600E oncogenic mutation [15] [16]. BRAF V600E mutation is the most frequent genetic aberration correlated with more aggressive tumors based upon conventional staging, and patients harboring BRAF V600E mutation are more susceptible to an increased risk for recurrence and lymph node metastasis in comparison to those patients with PTC devoid of the mutation [17]. However, the value of the BRAF V600E mutation as a prognostic hallmark in PTC was controversial and unclear. To this end, the present study aimed to define the correlation between the BRAF V600E mutation and PTC-related clinicopatho-

logical characteristics. In addition, the study evaluated the clinical values of qPCR and immunohistochemical strategies for the identification of V600E mutation.

2. Materials and Methods

2.1. Patients

A total of 188 patients with primary papillary thyroid carcinoma diagnosed at the First Affiliated Hospital of Xinjiang Medical University (Urumqi, Xinjiang, China) from June 2015 to December 2015 were recruited in this study. Tumor tissues were obtained by surgical resection during the surgery. And we record and statistical analysis the gender, ethnicity, age, calcification, tumor diameter, hashimoto, thyroid focality, thyroid capsular invasion, vascular invasion, lymph node metastasis, BRAF immunity and BRAF V600E PCR results in these cases.

Written informed consents were given by all included patients and the sample collection as well as investigation were approved by the local ethics committee of the First Affiliated Hospital of Xinjiang Medical University.

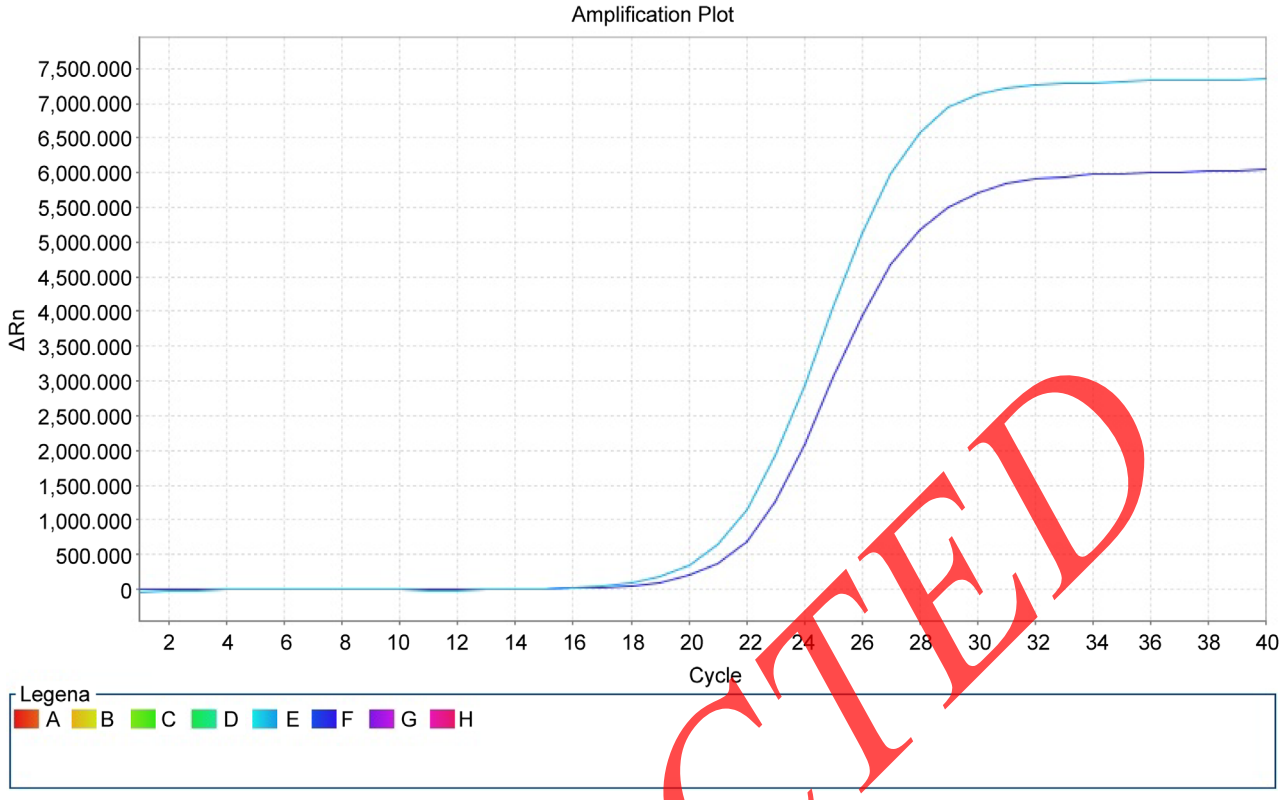
2.2. Quantitative PCR Assay

Total DNA was extracted from paraffin-embedded tissues. The resuspended DNA samples were subjected for PCR analysis with the human BRAF V600E detection kit (ACCB Biotech Ltd., Beijing, China) according to the manufacturer's protocol. The amplification was performed in an ABI 7300 plus machine (Applied Biosystems, Foster City, CA, USA) (**Figure 1**). The amplification profile was started with an initial denaturation at 95˚C for 10 min, followed by 40 cycles of denaturation 95˚C for 15 s, annealing at 60˚C for 30 s, and extension at 72˚C for 1 min.

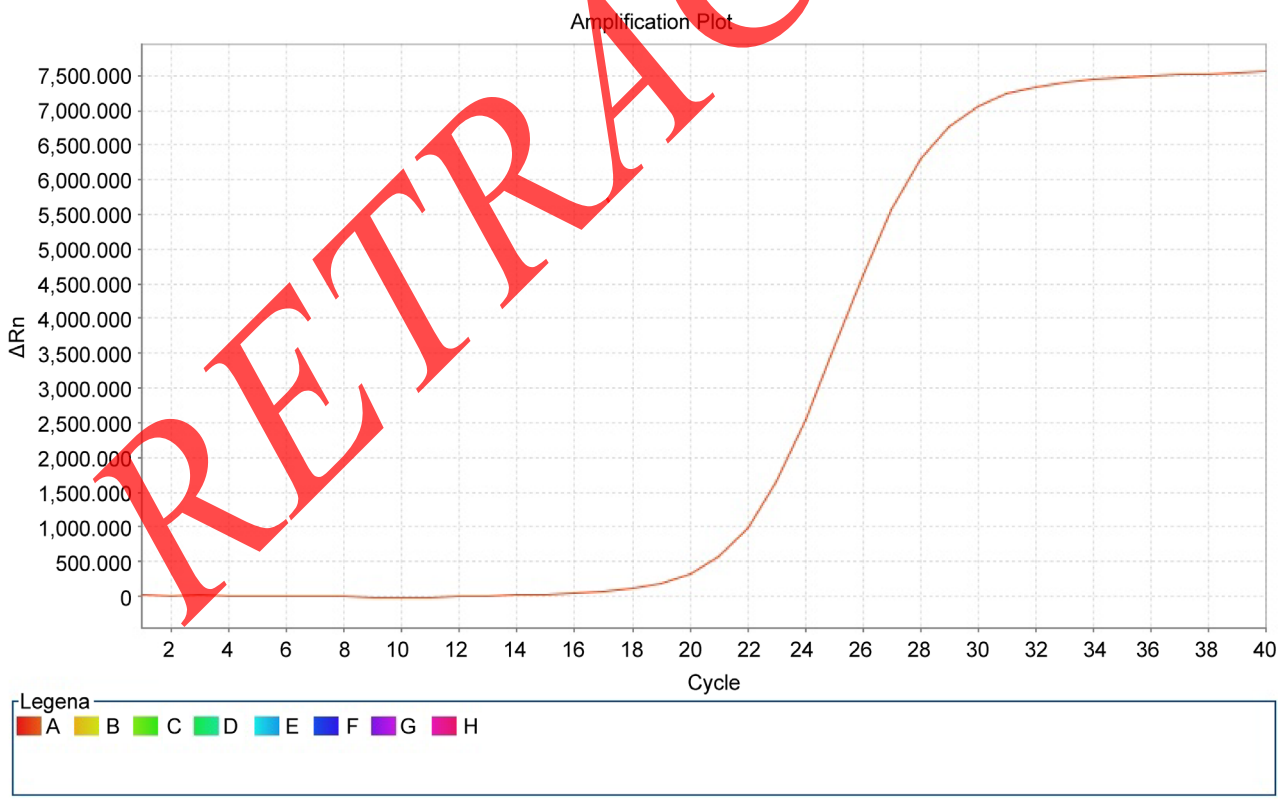
2.3. Immunohistochemistry

Immunohistochemistry was performed as previously described [18]. Briefly, formalin-fixed, paraffin-embedded tissue sections (4 μm-thick) were deparaffinized and rehydrated, and were incubated in retrieval solution at 60˚C and overnight to retrieve antigens. After inhibition of the preprimary peroxidase and blocking in 1% BSA in TBS for 2 hrs at room temperature, slides were incubated with the anti-BRAF V600E (VE1) (1:50, Roche, Ventana Medical Systems, Tucson, AZ, USA) mouse monoclonal primary antibody at 37˚C for 30 minutes. Afterwards, the primary antibodies were detected using an OptiView DAB IHC Detection kit (Ventana Medical Systems) following incubation with hematoxylin and a bluing reagent (for 4 minutes each).

Statistical analysis was performed using SPSS software (version 11.0; SPSS Inc., Chicago, IL). Chi square test was used to compare frequencies of BRAF V600E mutation between groups. Categorical variables were expressed as numbers and percentages. Statistical significance was assumed when the P value was < 0.05.



(a)



(b)

Figure 1. (a) BRAF gene mutant-type in FAM channel; (b) BRAF gene Wild-type in FAM channel.

3. Results

3.1. Clinicopathological Features of Patients with PTC

With the extensive application of ultrasound-guided fine needle aspiration and imaging studies, there have been an increasing number of PTC cases over the past several decades in our hospital. During the study period, a total of 188 patients were diagnosed with PTC, of which 160 patients carried BRAF V600E mutation (85.1%) and 28 were mutation-negative (14.9%) patients. The basic demographics of PTC patients was retrospectively analyzed and summarized in Table 1. The majority of the patients were women (69.7%), Han people (74.5%), and older than 40 years (63.8%). Calcification was detected in 60 cases (31.9%) and hashimoto was present in 63 cases (33.5%).Multifocal tumors were observed in 114 patients (60.6%). The frequencies of thyroid capsular invasion, vascular invasion and the lymph node metastasis were 36.2%, 4.8% and 30.9% respectively. 62 patients (33.0%) had thyroid tumors with a diameter more than 1 cm.

3.2. The Relationship of Clinicopathological Features of Patients with PTC and BRAF V600E Mutation

As shown in Table 1, of the 57 male patients, 46 (80.70%) were BRAF V600E-mutation-carriers, and 114 (87.02%) of the 131 female patients were BRAF V600E-mutation-positive. Therefore, no significant correlation was found between gender and BRAF V600E mutation ($\chi^2 = 1.252$, P = 0.263).

To assess the correlation between BRAF V600E mutation and age, 188 patients were divided into two groups: the younger segment aged less than 40, the older segment at least 40 years of age. BRAF V600E mutation was detected in 102 (85%) of the 120 patients at least 40 years of age, and 58 (85.29%) of the 68 patients younger than 40. There was neither a direct association between BRAF V600E and patient age ($\chi^2 = 0.002$, P = 0.957).

Also, PTC patients were divided into two groups according to whether they were Han people or not. However, there was no significant difference existed between the groups when considering the incidence of BRAF V600E (86.43% *vs* 81.25%, $\chi^2 = 0.756$, P = 0.384).

We next analyzed the association between BRAF V600E mutation and the presence of thyroidcalcification and hashimoto. For the 128 patients without thyroidcalcification, 110 (85.94%) of them were BRAF V600E-positive, whereas 50 (83.33%) of the 60 patients with calcification carried BRAF V600E. Likewise, 53 (84.13%) of 63 PTC patients with hashimoto were BRAF V600E-positive carriers, while 107 (85.60%) of the 125 patients devoid of hashimoto harbored BRAF V600E mutation. There seems no significant associations between the BRAF V600E mutation and thyroid calcification or hashimoto ($\chi^2 = 0.2186$, P = 0.640; $\chi^2 = 0.072$, P = 0.789, respectively).

The associations between BRAF V600E mutation and focal number as well as tumor size were also evaluated. Multifocality was seen in 60.64% of all the involved patients with PTC. For the 74 patients with monofocal PTC, 64 (86.49%)

Table 1. The association between clinicopathological characteristics of PTC and BRAF V600E mutation status in patients.

Items	Cases	Positive rate (%)	χ^2	P value	Power
Gender			1.252	0.263	0.999
Male	57	87.02			
Female	131	80.70			
Ethnicity			0.756	0.384	0.677
Han	140	86.43			
non-Han	48	81.25			
Age			0.002	0.957	0.999
<40	68	85.29			
≥40	120	85			
Calcification			0.2186	0.640	0.952
None	128	85.94			
Yes	60	83.33			
Tumor diameter			1.453	0.228	0.999
≤1 cm	126	87.30			
>1 cm	62	80.64			
Hashimoto			0.072	0.789	0.973
None	125	84.12			
Yes	63	85.60			
Thyroid focality			0.183	0.668	0.999
Monofocal	74	86.49			
Multifocal	114	84.21			
Thyroid capsular invasion			0.138	0.710	0.993
None	120	85.83			
Yes	68	83.82			
Vascular invasion					
None	179	85.47	1.132	0.860	0.047
Yes	9	77.78			
Lymph node metastasis			0.080	0.777	0.903
None	130	84.62			
Yes	58	86.21			
BRAF immunity			78.054	1.00269E−18	0.378
Positive	153	96.08			
Negative	35	37.14			

patients carried the mutation, whereas 96 (84.21%) in the 114 patients with multifocal PTC harbored V600E mutation. There was no significant difference for the positive rate between these two groups (χ^2 = 0.183, P = 0.668). The 188 patients were also divided into two groups according to the tumor size. The size of the thyroid tumor was greater than 1 cm in 62 (32.98%) patients, whereas 67.02% of the tumor were smaller than 1 cm. In the greater tumor group, 50 (80.65%) were BRAF V600E mutation-positive patients, while 110 (87.30%) of the 126 tumor which smaller than 1 cm were BRAF V600E mutation-positive. No significant association was defined between the BRAF V600E mutation and tumor size (χ^2 = 1.453, P = 0.228).

Aggressive characteristics of PTC were analyzed for define the factors related to BRAF V600E mutation. Thyroid capsular invasion, vascular invasion and lymph node metastasis occurredin 68 (36.17%), 9 (4.79%) and 58 (30.85%) patients respectively. Among the 120 patients without thyroid capsular invasion and 68 patients who hadthyroid capsular invasion, the number of BRAF V600E mutation positive PTCs in the former was 103, accounting for 85.83% of them, whereas that in the latter was 57 (83.82%). There was no direct correlation of the mutant frequency and the incidence of thyroid capsular invasion (χ^2 = 0.138, P = 0.710). In 9 patients with vascular invasion, the BRAF mutation rate was 77.78%, while the non-invasive patient, the mutation rate was 85.47%. There was no statistical difference between the two groups (χ^2 = 1.132, P = 0.860). Of the 58 patients with lymph node metastasis, the frequency of BRAF V600E mutation was 86.21% (50/58) and in patients who had no lymph node metastasis, the mutation frequency was 84.62% (110/130). No significant differences in the mutation frequency were noticed between the metastatic and non-metastatic PTCs (χ^2 = 0.080, P = 0.777).

In aggregate, we analyzed the relationship between the incidence of BRAF V600E mutation and diverse clinicopathological factors including age, gender, ethnicity, tumor size, and the presence of calcification, hashimoto, multifocality, thyroid capsular invasion, vascular invasion and lymph node metastasis. However, Univariate analysis showed that the presence of mutation was not apparently influenced by these factors.

3.3. Comparison of the BRAF V600E Mutation by Using IHC and PCR

We also performed the mutation detection by using IHC, todetermine if the PCR method can be used as a reliable substitute for BRAF V600E detection. Using IHC, the mutation was found in 153 cases (**Figure 2**). The positive rate was 81.38%, relatively lower compared with that using PCR molecular test (85.11%). In the 153 V600E mutation-positive cases which were detected by IHC, 147 (96.08%) could also betested by PCR, whereas in the 35 mutation-immuno-negative cases, 13 was detected by PCR. Giving the superior performance of PCR strategy in detecting barely detectable cells carrying a V600E mutation, probably due to

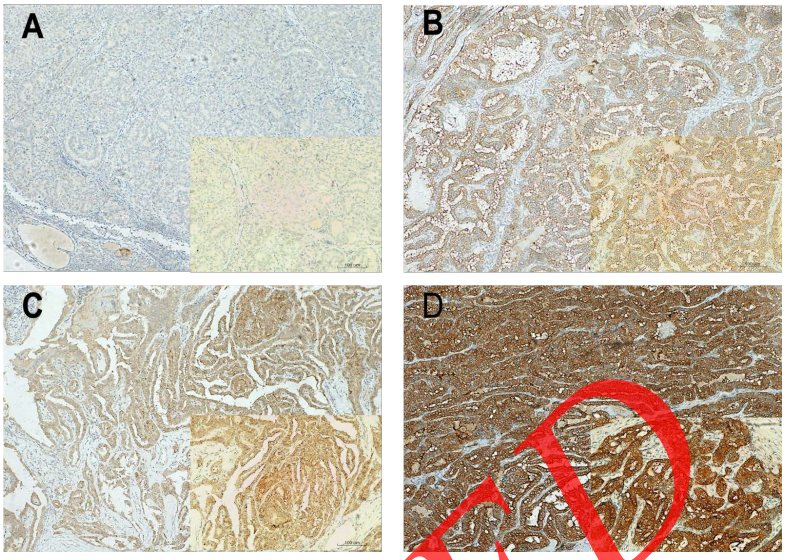


Figure 2. (IHC): (A)-(D) was the expression of BRAF V600E in thyroid papillary carcinoma. (A) Negative; (B) Weakly positive; (C) Medium positive; (D) Strongly positive statistical analysis.

its amplification effect on the mutant sequences froma fraction of BRAF mutated tumor cells in tissues, we can conclude that the PCR presents a superior sensitivity compared to IHC method.

4. Discussion

A worldwide increase in the PTC incidence results in a relevant public health concern. Nowadays, clinical decision-making remains controversial in absence of specific assessment of the malignancy [19]. Despite the importance of prognostic implications of other tumor-specific genetic alterations is increasingly apparent [20], their clinical predictive power is limited.

The RAS-ERK-MAPK signaling pathway is involved in cell responses to environmental stimuli and plays a crucial part in human carcinomas [19]. This pathway comprises RAS, MEK, ERK and the protein kinases RAF, which includes three protein kinases with nonredundant functions. On incitement of cytokines or hormones, active RAS recruits RAF to themembrane and the latter phosphorylates and activates the scaffold protein MEK, which afterwards activates ERK. BRAF is one of the RAF kinase family members and acts as an important transduction factor in the RAS-ERK-MAPK signaling pathway that can regulate a variety of biological events such as cell survival, division, differentiation, senescence, apoptosis, and secretionin normal cells [20].

BRAF V600E mutation is the most frequent genetic event in PTC, and it has been found in approximately 32% - 83% of PTC cases, whereas this alterations carcely appears in other thyroid tumors [13]. The V600E mutation has been considered to simulate constitutive phosphorylation on T599 and S602 residues, thereby abnormal activation of BRAF protein kinase [21]. Indeed, it was reported that BRAF V600E was 500-fold activated, leading to constitutively acti-

vated ERK-MAPK signaling and inducting proliferation and survival of cancer cells. This activating mutation BRAF V600E has been extensively observed in PTC, colorectal cancer, melanoma, and non-small-cell lung cancer [22] [23] [24]. The clinical researchers using the BRAF inhibitors vemurafenib, dabrafenib improved the survival rate among patients with advanced melanoma [25] and initiated an objective response in refractory hairy-cell leukemia (HCL) [26]. And the BRAF-selective inhibitor vemurafenib restrained growth of BRAF mutated anaplastic thyroid cancerin mice [27]. These data validated BRAF V600E mutation is a therapeutic target in many cancers and, furthermore, BRAF-selective drugs have been applied in the clinic and display excellent effects in patients with BRAF mutant melanomas [28].

Although results from some individual studies suggested that BRAF V600E mutation was associated with multiple aggressive clinicopathological characteristics of PTCs, including extrathyroid extension, multifocality, advanced tumor stage, and lymph node metastasis [29] [30], it is still controversial and indeterminate. We herein carried out an additional study involving a larger number of patients with PTC, to determine whether the frequency of BRAF V600E mutation is correlated with the aforementioned aggressive clinicopathological features as well as other characteristics such as patients age, gender, ethnicity, tumor size, the presence of hashimoto and calcification. The features of a total of 188 patients with PTC were retrospectively studied, and qPCR analysis was used to identify if the BRAF V600E mutation were existed. By chi-square analysis, we found that all variables showed no correlation to BRAF V600E mutation, indicating that each of these factors cannot function alone. Thus we speculated that two or more factors might be collectively involved in the presence of V600E mutation. However, the amount of BRAF V600E negative specimens was not enough for us to accurately evaluate the phenotypic differences between mutation-positive and negative tumors. On the other hand, we assumed that the mutation was preferably contributed to the tumorigenesis rather than progression of PTC. And this study has potential limitations that we did not analysis subtypes (the classical type, the follicular variant, the oncocytic variant and the tall cell variant) of PTC.

Recent studies have demonstrated that the BRAF V600E mutation has emerged as a useful diagnostic hallmark and a specific factor for poor clinical outcomes of PTCs [13] [31] [32]. An efficient and sensitive method with higher specificity therefore needs to be developed to detect the presence of BRAF V600E mutation. Of the 188 patients with PTCs in the present study, the mutation was identified in 153 cases by immunohistochemistry (IHC), while it was detected in 160 patients by using PCR assay. The detection rate for the mutation by IHC was 81.38%, slightly lower compared with PCR method (85.11%). In the 153 V600E mutation-positive cases which were detected by IHC, 147 (96.08%) could be also tested by PCR, whereas in the 35 mutation-immunonegative cases, 13 was detected by PCR. Giving the preferable performance of PCR strategy in detecting

barely detectable cells carrying a V600E mutation, we can conclude that it presents a superior sensitivity compared to IHC method. Therefore, it is recommendable to combine the results obtained from PCR molecularstrategy together with conventional IHC method for clinical evaluation of BRAF V600E mutation.

The differential diagnostic value of the BRAF V600E mutation in thyroid tumors has been widely recognized and applied. The results of this study indicate that the sensitivity of the ARMS PCR assay to the detection of the BRAF V600E mutation to diagnose PTC is as high as 85%comparingwith an average rate of 44% (36% - 83%), [33]. This is similar to Li *et al*. [34] (83.1%), Wang *et al*. [35] (86.8%). This shows that the ARMS PCR method has a better clinical application prospect than the direct sequencing method [36].

Disclosure

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

[1] Davies, L. and Welch, H. (2014) Current Thyroid Cancer Trends in the United States. *JAMA Otolaryngology-Head & Neck Surgery*, **140**, 317-322. https://doi.org/10.1001/jamaoto.2014.1

[2] Benhamiche, A.-M., Faivre, C., Minello, A., *et al*. (1998) Time Trends and Age-Period-Cohort Effects on the Incidence of Primary Liver Cancer in a Well-Defined French Population: 1976-1995. *Journal of Hepatology*, **29**, 802-806. https://doi.org/10.1016/S0168-8278(98)80262-6

[3] Davies, L. and Welch, H. (2006) Increasing Incidence of Thyroid Cancer in the United States, 1973-2002. *The Journal of the American Medical Association*, **295**, 2164-2167. https://doi.org/10.1001/jama.295.18.2164

[4] Oh, C.-M., Jung, K.-W., Won, Y.-J., *et al*. (2015) Age-Period-Cohort Analysis of Thyroid Cancer Incidence in Korea. *Cancer Research and Treatment: Official Journal of Korean Cancer Association*, **47**, 362-369. https://doi.org/10.4143/crt.2014.110

[5] Eustatia-Rutten, C.F., Corssmit, E.P., Biermasz, N.R., *et al*. (2006) Survival and Death Causes in Differentiated Thyroid Carcinoma. *Journal of Clinical Endocrinology & Metabolism*, **91**, 313-319. https://doi.org/10.1210/jc.2005-1322

[6] Cooper, D.S., Doherty, G.M., Haugen, B.R., *et al*. (2006) Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, **16**, 109-142. https://doi.org/10.1089/thy.2006.16.109

[7] Pitoia, F., Bueno, F., Urciuoli, C., *et al*. (2013) Outcomes of Patients with Differentiated Thyroid Cancer Risk-Stratified According to the American Thyroid Association and Latin American Thyroid Society Risk of Recurrence Classification Systems. *Thyroid*, **23**, 1401-1407. https://doi.org/10.1089/thy.2013.0011

[8] Vaisman, F., Momesso, D., Bulzico, D.A., *et al*. (2012) Spontaneous Remission in Thyroid Cancer Patients after Biochemical Incomplete Response to Initial Therapy. *Clinical Endocrinology*, **77**, 132-138. https://doi.org/10.1111/j.1365-2265.2012.04342.x

[9] Jung, C.K., Little, M.P., Lubin, J.H., *et al*. (2014) The Increase in Thyroid Cancer Incidence during the Last Four Decades Is Accompanied by a High Frequency of BRAF Mutations and a Sharp Increase in RAS Mutations. *Journal of Clinical Endocrinology & Metabolism*, **99**, E276-E285. https://doi.org/10.1210/jc.2013-2503

[10] Aschebrook-Kilfoy, B., Kaplan, E.L., Chiu, B.C.-H., *et al*. (2013) The Acceleration in Papillary Thyroid Cancer Incidence Rates is Similar Among Racial and Ethnic Groups in the United States. *Annals of Surgical Oncology*, **20**, 2746-2753. https://doi.org/10.1245/s10434-013-2892-y

[11] Husson, O., Haak, H.R., van Steenbergen, L.N., *et al*. (2013) Rising Incidence, No Change in Survival and Decreasing Mortality from Thyroid Cancer in The Netherlands Since 1989. *Endocrine-Related Cancer*, **20**, 263-271. https://doi.org/10.1530/ERC-12-0336

[12] Davies, H., Bignell, G.R., Cox, C., *et al*. (2002) Mutations of the BRAF Gene in Human Cancer. *Nature*, **417**, 949-954. https://doi.org/10.1038/nature00766

[13] Xing, M. (2005) BRAF Mutation in Thyroid Cancer. *Endocrine-Related Cancer*, **12**, 245-262. https://doi.org/10.1677/erc.1.0978

[14] Nucera, C., Porrello, A., Antonello, Z.A., *et al*. (2010) B-RafV600E and Thrombospondin-1 Promote Thyroid Cancer Progression. *Proceedings of the National Academy of Sciences*, **107**, 10649-10654. https://doi.org/10.1073/pnas.1004934107

[15] Romei, C., Fugazzola, L., Puxeddu, E., *et al*. (2012) Modifications in the Papillary Thyroid Cancer Gene Profile Over the Last 15 Years. *The Journal of Clinical Endocrinology & Metabolism*, **97**, E1758-E1765. https://doi.org/10.1210/jc.2012-1269

[16] Mathur, A., Moses, W., Rahbari, R., *et al*. (2011) Higher Rate of BRAF Mutation in Papillary Thyroid Cancer over Time. *Cancer*, **117**, 4390-4395. https://doi.org/10.1002/cncr.26072

[17] Lee, J.-H., Lee, E.-S. and Kim, Y.-S. (2007) Clinicopathologic Significance of BRAF V600E Mutation in Papillary Carcinomas of the Thyroid. *Cancer*, **110**, 38-46. https://doi.org/10.1002/cncr.22754

[18] Kim, Y.H., Yim, H., Lee, Y.-H., *et al*. (2016) Evaluation of the VE1 Antibody in Thyroid Cytology Using Ex Vivo Papillary Thyroid Carcinoma Specimens. *Journal of Pathology and Translational Medicine*, **50**, 58-66. https://doi.org/10.4132/jptm.2015.10.10

[19] Conzo, G., Avenia, N., Ansaldo, G.L., Calò, P., De Palma, M., *et al*. (2017) Surgical Treatment of Thyroid Follicular Neoplasms: Results of a Retrospective Analysis of a Large Clinical Series. *Endocrine*, **55**, 530-538. https://doi.org/10.1007/s12020-016-0953-2

[20] Marotta, V., Sciammarella, C., Capasso, M., Testori, A., Pivonello, C., *et al*. (2017) Germline Polymorphisms of the VEGF Pathway Predict Recurrence in Nonadvanced Differentiated Thyroid Cancer. *Journal of Clinical Endocrinology & Metabolism*, **102**, 661-671.

[21] Wan, P.T.C., Garnett, M.J., Roe, S.M., *et al*. (2004) Mechanism of Activation of the RAF-ERK Signaling Pathway by Oncogenic Mutations of B-RAF. *Cell*, **116**, 855-867. https://doi.org/10.1016/S0092-8674(04)00215-6

[22] Elisei, R., Ugolini, C., Viola, D., *et al*. (2008) BRAF(V600E) Mutation and Outcome of Patients with Papillary Thyroid Carcinoma: A 15-Year Median Follow-Up Study.

Journal of Clinical Endocrinology & Metabolism, **93**, 3943-3949. https://doi.org/10.1210/jc.2008-0607

[23] Gear, H., Williams, H., Kemp, E.G., *et al*. (2004) BRAF Mutations in Conjunctival Melanoma. *Investigative Ophthalmology & Visual Science*, **45**, 2484-2488. https://doi.org/10.1167/iovs.04-0093

[24] Benlloch, S., Payá, A., Alenda, C., *et al*. (2006) Detection of BRAF V600E Mutation in Colorectal Cancer. *The Journal of Molecular Diagnostics*, **8**, 540-543. https://doi.org/10.2353/jmoldx.2006.060070

[25] Chapman, P.B., Hauschild, A., Robert, C., *et al*. (2011) Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. *The New England Journal of Medicine*, **364**, 2507-2516. https://doi.org/10.1056/NEJMoa1103782

[26] Dietrich, S., Glimm Andrulis, H., *et al*. (2012) BRAF Inhibition in Refractory Hairy-Cell Leukemia. *The New England Journal of Medicine*, **366**, 2038-2040. https://doi.org/10.1056/NEJMc1202124

[27] Nehs, M.A., Nucera, C., Nagarkatti, S.S., *et al*. (2012) Late Intervention with Anti-BRAF(V600E) Therapy Induces Tumor Regression in an Orthotopic Mouse Model of Human Anaplastic Thyroid Cancer. *Endocrinology*, **153**, 985-994. https://doi.org/10.1210/en.2011-1519

[28] Schwartz, G.K., Robertson, S., Shen, A., *et al*. (2009) A Phase I Study of XL281, a Selective Oral RAF Kinase Inhibitor, in Patients (Pts) with Advanced Solid Tumors. *Journal of Clinical Oncology*, **27**, 3513-3513.

[29] Moses, W., Weng, J., Sansano, I., *et al*. (2010) Molecular Testing for Somatic Mutations Improves the Accuracy of Thyroid Fine-Needle Aspiration Biopsy. *World Journal of Surgery*, **34**, 2589-2594. https://doi.org/10.1007/s00268-010-0720-0

[30] Mathur, A., Weng, J., Moses, W., *et al*. (2010) A Prospective Study Evaluating the Accuracy of Using Combined Clinical Factors and Candidate Diagnostic Markers to Refine the Accuracy of Thyroid Fine Needle Aspiration Biopsy. *Surgery*, **148**, 1170-1177. https://doi.org/10.1016/j.surg.2010.09.025

[31] Kim, T.H., Park, Y.J., Lim, J.A., *et al*. (2012) The Association of the BRAF(V600E) Mutation with Prognostic Factors and Poor Clinical Outcome in Papillary Thyroid Cancer: A Meta-Analysis. *Cancer*, **118**, 1764-1773. https://doi.org/10.1002/cncr.26500

[32] Tufano, R.P., Teixeira, G.V., Bishop, J., *et al*. (2012) BRAF Mutation in Papillary Thyroid Cancer and Its Value in Tailoring Initial Treatment: A Systematic Review and Meta-Analysis. *Medicine* (*Baltimore*), **91**, 274-286. https://doi.org/10.1097/MD.0b013e31826a9c71

[33] Gray-Schopfer, V., Wellbrock, C. and Marais, R. (2007) Melanoma Biology and New Targeted Therapy. *Nature*, **445**, 851-857. https://doi.org/10.1038/nature05661

[34] Hong, T.L., *et al*. (2018) Relationship between BRAF(V600E) Gene Mutations and Clinicopathological Factors in 192 Thyroid Papillary Carcinomas. *Journal of Xinjiang Medical University*, No. 2.

[35] Xu, H.W., *et al*. (2017) Relationship between BRAFV600E Gene Mutation and Central Lymph Node Metastasis in Cervical Lymph Node-Negative Thyroid Papillary Microcarcinoma. *CJV*, **17**, 1277-1281.

[36] Jin, W.D., *et al*. (2017) Detection of BRAFV600E Mutation in Papillary Thyroid Carcinoma Based on ARMS Method. *Chinese Journal of Otorhinolaryngology Base*, **24**, 71-75.