

Clinical significance of *BRAF*^{V600E} and *TERT* promoter mutation in papillary thyroid microcarcinoma*

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Abstract

Objective The objective of this study was to analyze the correlation between *BRAF*^{V600E} and *TERT* promoter mutations and papillary thyroid microcarcinoma (PTMC) risk factors, and their importance in the risk assessment of papillary thyroid microcarcinoma.

Methods This study retrospectively analyzed 107 cases of PTMC, which were diagnosed after the surgery in the department of head and neck surgery in Gansu Province Tumor Hospital from October 2014 to June 2016. The mutations of *BRAF*^{V600E} and *TERT* promoter were detected by PCR direct sequencing. We analyzed the data using χ^2 test and binary Logistic regression analysis.

Results Among 107 patients with PTMC, the *BRAF*^{V600E} and *TERT* promoter mutation rates were 68.2% and 11.2%, respectively. Single factor analysis showed that there was a significant difference between the presence of membrane invasion, lymph node metastasis, and *BRAF*^{V600E} mutations ($P < 0.01$). The age, gender, thyroid capsular invasion, poor pathologic subtype, and lymph node metastasis of patients, was significantly associated with the *TERT* promoter mutation ($P < 0.05$) and the coexistence of the *BRAF*^{V600E} and *TERT* promoter mutations; although, there was a difference between the association of these factors with the *TERT* promoter mutation and the association of these factors with the coexistence of the *BRAF*^{V600E} and *TERT* promoter mutations. The multifactorial analysis showed that the factors closely related to the *BRAF*^{V600E} mutation included capsular invasion ($P = 0.012$) and lymph node metastasis ($P = 0.000$). The following factors were closely associated with the *TERT* promoter mutant: male ($P = 0.004$), aged < 45 years ($P = 0.026$), capsular invasion ($P = 0.004$), pathological subtype ($P = 0.030$), and lymph node metastasis ($P = 0.043$). The following factors were closely related to the simultaneous mutation of *BRAF*^{V600E} and *TERT*: male ($P = 0.022$), capsular invasion ($P = 0.023$), poor pathological subtype ($P = 0.041$), and lymph node metastasis ($P = 0.030$).

Conclusion The risk of recurrence increases significantly when mutations in *BRAF*^{V600E} and *TERT* promoters occur simultaneously in PTMC and may have adverse outcomes. Combined detection of *BRAF*^{V600E} and *TERT* promoter mutations is of great value in risk assessment of PTMC.

Key words: papillary thyroid microcarcinoma (PTMC); *BRAF*^{V600E}; *TERT*; mutation

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Papillary thyroid microcarcinoma (PTMC) accounts for a large proportion of thyroid cancers with a high annual incidence [1]. PTMC is defined as small papillary thyroid carcinoma that is less than 1.0 cm [2]. Most prognosis of PTMC are considered to be excellent, but some PTMCs have poor prognosis. If the molecular markers of PTMC can be discovered, they can be used to accurately predict prognosis and guide the improvement of clinical diagnosis and treatment. This study will examine *BRAF*^{V600E} and telomerase reverse transcriptase (*TERT*) promoter mutation and their relationship with PTMC risk factors; This will hopefully help balance the treatment-associated

benefit and risk of PTMC.

Material and methods

Research object and methods

One hundred and seven patients with PTMC without metastasis were included (24 men and 83 women), with a median age of 44.0 ± 11.8 years (ranging from 19 to 76 years). Of these patients, 48 were less than 45 years old and 59 were over 45 years old after the initial diagnosis and treatment in the department of head and neck surgery in Gansu Provincial Tumor Hospital from October 2014

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to June 2016. There were 38 cases where the size of tumor was less than 5 mm and 69 cases with sizes more than 5 mm, 28 cases with multifocal carcinoma and 79 cases with solitary carcinoma, 41 cases with thyroid capsule invasion and 66 cases with tumor encapsulation. There were 19 cases with poor pathologic types (high cell subtype, columnar cell subtype, eosinophil subtype, etc.) and 88 cases without it, 64 cases had no lymphoid involvement (stage N0) and 43 cases had lymph node involvement (stage N1 N1a + N1b) (Table 1). All subjects had no previous history of tumors, and standardized surgical treatment and pathological diagnosis were performed. This study was approved by the Ethics Committee of Gansu Provincial Tumor Hospital, and the patients were prior informed and consented.

Sequencing method

The *BRAF*^{V600E} and *TERT* promoter mutations were detected by polymerase chain reaction (PCR) and direct sequencing. The *TERT* promoters included the C228T and C250T sites. Tumor tissues were fixed with a 4% formaldehyde solution, embedded in paraffin, and sliced at a thickness of 5 to 10 μ m. One slice was taken for routine hematoxylin and eosin (HE) staining, and then identified by the pathologist and evaluated for tumor cell content. Two to four sections were taken to extract genomic DNA, which were then subjected to PCR amplification. After electrophoresis was used to detect the quality of the PCR amplification product, the higher quality PCR amplification products were subjected to

DNA sequencing. These results were compared with the *BRAF*^{V600E} and *TERT* gene sequences, to confirm whether or not mutations occurred.

Statistical analyses

Statistical analysis of relevant data was performed using SPSS v19.0 software. Univariate analysis of each variable was performed using the χ^2 test and multivariate analysis was performed using binary logistic regression analysis; $P < 0.05$ was considered statistically significant.

Results

BRAF^{V600E} mutation

The *BRAF*^{V600E} mutation rate in patients with PTMC was 68.2% (73/107). There were no significant correlations between gender, age, tumor size, number of primary lesions, adverse pathological subtypes and *BRAF*^{V600E} mutations ($P > 0.05$). However, there was a significant correlation between thyroid capsule invasion, lymph node metastasis, and *BRAF*^{V600E} mutations ($P < 0.01$). Multivariate logistic analysis revealed significant factors associated with *BRAF*^{V600E} mutations, including thyroid capsule invasion ($P = 0.012$) and lymph node metastasis ($P = 0.000$) (Table 1 and 2).

TERT promoter mutation

The mutation rate of *TERT* promoter in PTMC patients was 11.2% (12/107), among which, the *TERT* C228T site mutation rate was 66.7% (8/12) and the *TERT* C250T site

Table 1 Relationship of *BRAF*^{V600E} and *TERT* promoter mutations with Clinicopathological features of PTMC (n, %)

Features	n (%)	BRAF (n = 73)	χ^2	P	TERT (n = 12)	χ^2	P	BRAF + TERT (n = 9)	χ^2	P
Gender										
Male	24 (22.4)	18 (75.0)	0.655	0.418	7 (29.2)	10.014	0.002	5 (20.8)	6.197	0.013
Female	83 (77.6)	55 (66.3)			5 (6.0)			4 (4.8)		
Age (years)										
< 45	48 (44.9)	29 (60.4)	2.448	0.118	1 (2.1)	7.290	0.007	1 (2.1)	4.525	0.033
\geq 45	59 (55.1)	44 (74.6)			11 (18.6)			8 (13.6)		
Tumor size (d/mm)										
\leq 5	38 (35.5)	23 (60.5)	1.611	0.204	3 (7.9)	0.652	0.419	2 (5.3)	0.758	0.384
> 5	69 (64.5)	50 (72.5)			9 (13.0)			7 (10.1)		
Multifocal										
Yes	28 (26.2)	19 (67.9)	0.002	0.961	5 (17.9)	1.680	0.195	3 (10.7)	0.261	0.609
No	79 (73.8)	54 (68.4)			7 (8.9)			6 (7.6)		
Capsular invasion										
Yes	41 (38.3)	34 (82.9)	6.628	0.010	10 (24.4)	11.588	0.001	7 (17.1)	6.474	0.011
No	66 (61.7)	39 (59.1)			2 (3.0)			2 (3.0)		
Pathological subtype										
Low risk	88 (82.2)	58 (65.9)	1.225	0.268	7 (7.9)	5.291	0.021	5 (5.7)	4.792	0.029
High risk	19 (17.8)	15 (78.9)			5 (26.3)			4 (21.1)		
Lymph node Metastasis										
N0	64 (59.8)	34 (54.7)	16.748	0.000	4 (6.3)	3.943	0.047	2 (3.1)	5.777	0.016
N1(N1a + N1b)	43 (40.2)	39 (88.4)			8 (18.6)			7 (16.3)		

Table 2 Multivariate Logistic regression analysis of $BRAF^{V600E}$ and $TERT$ promoter mutations of PTMC

Factors	Partial regression coefficient	Standard deviation	Wald	df ^a	P	Exp ^b (B)	95.0% CI for Exp (B)	
							Lower	Upper
BRAF								
Capsular invasion	-1.213	0.485	6.260	1	0.012	0.297	0.115	0.769
Lymph node Metastasis	2.152	0.582	13.687	1	0.000	8.603	2.751	26.903
TERT								
Gender	-1.860	0.644	8.346	1	0.004	0.156	0.044	0.550
Age	2.377	1.064	4.986	1	0.026	10.771	1.337	86.755
Capsular invasion	-2.334	0.805	8.411	1	0.004	0.097	0.020	0.469
Pathological subtype	1.419	0.653	4.719	1	0.030	4.133	1.149	14.866
Lymph node Metastasis	1.232	0.648	3.816	1	0.043	3.429	0.962	12.215
BRAF + TERT								
Gender	-1.648	0.718	5.272	1	0.022	0.192	0.047	0.786
Age	1.998	1.080	3.423	1	0.064	7.373	0.888	61.194
Capsular invasion	-1.885	0.829	5.167	1	0.023	0.152	0.030	0.771
Pathological subtype	1.488	0.727	4.186	1	0.041	4.427	1.064	18.408
Lymph node Metastasis	1.796	0.829	4.699	1	0.030	6.028	1.188	30.589

^adf: Degree of freedom, ^bExp (B): The OR value of the corresponding variable(Advantage ratio), CI: Confidence interval

mutation was 33.3% (4/12). Age, gender, capsule invasion, adverse pathological subtypes, and lymph node metastasis were significantly associated with $TERT$ promoter mutations ($P < 0.05$). There was no significant correlation between tumor size and the number of primary lesions with $TERT$ promoter mutations ($P > 0.05$). Multivariate logistic analysis showed that male patients ($P = 0.004$), aged < 45 years ($P = 0.026$), who had thyroid capsule invasion ($P = 0.004$), an adverse pathological subtypes ($P = 0.030$), and lymph node metastasis ($P = 0.043$) were significant associated with the $TERT$ promoter mutation (Table 1 and 2).

Coexistence of $BRAF^{V600E}$ and $TERT$ promoter mutations

Twelve PTMC patients had $TERT$ promoter mutations, and nine of them (75%) had $BRAF^{V600E}$ mutations as well. Age, gender, capsule invasion, adverse pathological subtypes, and lymph node metastasis were significantly associated with $BRAF^{V600E}$ and $TERT$ mutations ($p < 0.05$). There was no significant correlation between tumor size and the number of primary lesions with the $BRAF^{V600E}$ and $TERT$ mutations ($P > 0.05$). Multivariate logistic analysis showed significant correlation between male patients ($P = 0.022$), who had thyroid capsule invasion ($P = 0.023$), an adverse pathological subtype ($P = 0.041$), and lymph node metastasis ($P = 0.030$) with $BRAF^{V600E}$ and $TERT$ mutations (Table 1 and 2).

Discussion

PTMC is a common type of papillary thyroid carcinoma, and most prognoses of PTMC are considered to be

excellent with a 15-year survival rate of approximately 90.7% [3]. However, some PTMC have highly invasive clinicopathological features, which may result in cervical lymph node involvement or even multiple metastases with small primary lesions. The treatment of PTMC has been controversial for many years [4-6], including how to screen high-risk patients in PTMC and achieve accurate treatment, which depends on further development of molecular etiology and molecular imaging as well as other related disciplines [7-10].

At present, the key indicators for PTMC risk assessment, such as tumor diameter, multifocal, capsule invasion, adverse pathological subtypes, and lymph node involvement, are mostly based on retrospective studies of postoperative pathological results and have limited value in preoperative evaluations and intraoperative guidance [11]. There is an urgent need to discover more molecular markers for diagnosis, prognostic evaluation, and therapeutic targets. It is extremely important for formulating rational and standardized treatment protocols to screen out valuable molecular biomarkers of PTMC.

The $BRAF^{V600E}$ gene is a DNA sequence that can be transfected into NIH3T3 cells in human Ewing's sarcoma. The mutation of T1799A in the $BRAF$ gene can activate the MAPK pathway, which promotes cell proliferation and carcinogenesis, and it is closely related to the occurrence and development of thyroid cancer [12-13]. $BRAF^{V600E}$ is one of the most common mutant genes in thyroid cancer, with high diagnostic specificity and a sensitivity of 85.1% [14]. However, with the increasing sensitivity of molecular biology techniques, the detection rate of the $BRAF^{V600E}$ gene mutation in thyroid cancer is increasing. Furthermore, the role of the $BRAF$ gene

mutation, as an independent prognostic indicator, is controversial [15].

Studies have shown that *BRAF* mutations are closely related to the occurrence, development, recurrence, and prognosis of papillary thyroid carcinoma (PTC) and can be used to assess the risk of PTC [16–18]. A meta-analysis found that the *BRAF*^{V600E} mutant had a higher recurrence rate (24.9% vs. 12.6%) than the *BRAF* wild type in 2470 PTC patients, and the *BRAF*^{V600E} mutation correlated with tumor aggressiveness [19]. Some studies have found PTMC patients with the *BRAF* gene mutation have a higher rate of cervical lymph node metastasis; Hence, it is considered as an independent risk factor for this metastasis. The *BRAF* gene mutation in PTMC patients also has a high rate of lateral lymph node metastasis [20–21].

It was also reported that tumor recurrence was mainly associated with lymph node metastasis and thyroid capsule invasion without the *BRAF*^{V600E} mutation. Furthermore, the *BRAF* gene mutation did not increase the invasiveness of PTMC [22–23]. The prognostic value of the *BRAF*^{V600E} mutation, in the recurrence of papillary thyroid carcinoma, was evaluated. The results indicated that the *BRAF*^{V600E} mutation in the high-risk group (aged < 35 years and ≥ 60 years), had local residual and recurrence rates that were 17.28 to 33.49 times higher than those of the low-risk group, *BRAF*^{V600E} [24]. The earliest study on *BRAF* gene mutations in China showed that there was no correlation between the *BRAF* mutation and the prognosis of papillary thyroid carcinoma, except when they were related to the age of the patients [25]. Thus, the prognostic value of *BRAF* gene mutations is a debatable indicator and should be used in combination with other molecules or other clinicopathological factors for tumor prognosis.

This study showed that the *BRAF*^{V600E} mutation rate of PTMC was 68.2% (73/107). There was no significant correlation between gender, age, tumor size, number of primary lesions, and adverse pathological subtypes with the *BRAF*^{V600E} mutations ($P > 0.05$), when associated with capsule invasion and lymph node metastasis ($P < 0.01$). Multivariate logistic analysis showed that the *BRAF*^{V600E} mutation was significantly associated with capsule invasion and lymph node metastasis, which is associated with the prognosis of PTMC [12]. Thus, based on this study, the *BRAF*^{V600E} mutation can be considered as a reference index for evaluating the prognosis of PTMC.

The *TERT* promoter mutation in thyroid cancer was first discovered in 2013, and later studies have found that the mutation rate in patients with PTMC is 4.7% and it was related to the degree of tumor differentiation [26–27]. This study found that the mutation rate of the *TERT* promoter in patients with PTMC was 11.2% (12/107). There was a significant correlation between age, gender, capsule invasion, adverse pathological subtypes, and lymph node

involvement with *TERT* promoter mutations ($P < 0.05$). However, there was no significant correlation between tumor size and number of primary lesions with *TERT* promoter mutations ($P > 0.05$). Multivariate logistic analysis showed that male patients, aged < 45 years old, with capsule invasion, adverse pathological subtypes, and lymph node involvement, were significantly associated with the *TERT* promoter mutations. Therefore, this study suggests that *TERT* mutations are closely related to prognosis in patients with PTMC.

Studies have shown that *BRAF*^{V600E} is correlated and synergistic with *TERT* promoter mutations [28–29]. This study found that *BRAF*^{V600E} mutations in PTMC patients were not significantly associated with gender, age, tumor size, number of primary lesions, and adverse pathological subtypes ($P > 0.05$). However, *BRAF*^{V600E} shows significant correlation ($P < 0.05$) when combined with *TERT* mutations. Multivariate logistic analysis showed that males with thyroid capsule invasion, adverse pathological subtype, and lymph node involvement, which are known risk factors for PTMC recurrence, are associated with *BRAF*^{V600E} and *TERT* mutations. This study showed that *BRAF*^{V600E} and *TERT* promoter mutations have great value for PTMC risk assessment and could be used as primary indicators for predicting prognosis.

While the future of PTMC prognosis is promising, there are controversies surrounding the various treatments and the search for specific molecular markers is ongoing. Many studies about *BRAF*^{V600E} mutations have also been reported. However, studies on *BRAF*^{V600E} mutations and *TERT* promoter mutations have rarely been reported in patients from other countries. Other studies have mainly concentrated on late-stage tumors or tumors with poor prognosis; only a few have studied PTMC. Our study suggested that simultaneous *BRAF*^{V600E} and *TERT* promoter mutations may be associated with poor prognosis of PTMC, but the mechanism is still not clear. *BRAF*^{V600E} and *TERT* promoter mutations are potential molecular markers for PTMC prognosis and their combined detected could be a new prognostic approach for this disease.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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