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ORIGINAL ARTICLE

Risk stratification by combining common genetic mutations and *TERT* promoter methylation in papillary thyroid cancer

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Abstract

Purpose Risk stratification based on somatic mutations in *TERT* promoter and *BRAF*/*RAS* has been well established for papillary thyroid cancer (PTC), and there is emerging evidence showed that *TERT* promoter methylation was frequently observed in thyroid cancer patients with adverse features. This study was aimed to comprehensive explore the prognostic value of *BRAF*/*RAS* mutations, *TERT* promoter mutations, and *TERT* promoter methylation in PTC.

Methods The relationships of *BRAF*/*RAS* mutations, *TERT* promoter mutations, and *TERT* promoter methylation with clinical characteristics and outcomes of PTC were analyzed in 382 patients with PTC.

Results *TERT* promoter mutation and hypermethylation were collectively observed in 52 (13.6%) samples and associated with *BRAF*/*RAS* mutation, aggressive clinical characteristics, and poor clinical outcomes of PTC. Coexistence of *BRAF*/*RAS* and *TERT* alterations was found in 45 of 382 (11.8%) PTC patients and strongly associated with old patient age, extrathyroidal extension, advanced pathologic T stage and metastasis. Importantly, patients with both *BRAF*/*RAS* and *TERT* alterations had higher rates of tumor recurrence (13.6% vs 1.5%, $P = 0.042$) and disease progression (24.4% vs 3.3%, $P < 0.001$) than patients without any alterations, and cox regression analysis revealed that the coexistence of *BRAF*/*RAS* and *TERT* alterations, but not *BRAF*/*RAS* or *TERT* alterations alone, increased the risk of progression-free interval with an adjusted HR of 10.35 (95% CI: 1.79–59.81, $P = 0.009$).

Conclusions This study suggested that comprehensively analysis of *BRAF*/*RAS* mutations, *TERT* promoter mutation and methylation is an effective strategy to identify high-risk patients with PTC.

Keywords Papillary thyroid cancer · *BRAF* mutation · *TERT* promoter mutation · DNA methylation · Prognosis

Introduction

Thyroid cancer is the most common malignancy of the endocrine system, diagnosed in 586,202 people over the world in 2020 [1]. Papillary thyroid cancer (PTC) accounts for 85–90% of all thyroid cancer cases and its incidence rises rapidly in the past several decades [2]. Although over 93% of PTC patients have favorable prognosis within 5 years after initial treatment [3], studies with long-term follow-up time showed that tumor recurrence and disease-specific death rates increased up to 29% and 9%, respectively [4, 5]. Precision identification of the high-risk patients remains a major challenge in the clinic, since the risk stratification system based on conventional clinicopathological risk factors is not accurate enough for identifying the patients with poor prognosis [6].

Molecular-based risk stratification of PTC using genetic markers showed great advantages and has been well accepted in recent years [7]. Among the various genetic alterations in PTC, *BRAF* V600E, *RAS* mutations, and hotspot mutations in the promoter of telomerase reverse transcriptase (TERT) had been well investigated [8]. *BRAF* V600E, the most common driver mutation in PTC, is capable of constitutively activating the mitogen-activated protein kinase (MAPK) pathway. The *RAS* mutation occurs in about 10% of PTC cases and mutually exclusive with *BRAF* mutation. The two mutually exclusive point mutations (C228T and C250T) in *TERT* promoter, collectively occurs

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in about 11% of PTC [9], generates de novo binding sites for the GABP complex and leads to *TERT* activation and telomere length maintain [10–12]. Numerous studies have demonstrated that either *BRAF* or *TERT* mutations has been associated with high risk of tumor recurrence and PTC-specific mortality [13–16]. Interestingly, *TERT* promoter mutations tend to coexist with *BRAF* and *RAS* mutations, and the patients harboring both *BRAF*/*RAS* and *TERT* mutations showed more aggressive characteristics and worse clinical outcomes than the ones harboring *TERT* or *BRAF*/*RAS* mutation alone [9, 17–20]. It should be noticed that the frequency of cases harboring both *BRAF*/*RAS* and *TERT* promoter mutations is 6–8%, which is lower than the proportion of patients that showed aggressive characteristics in the clinic. Therefore, other biomarkers are needed to be identified and enrolled in the molecular -based risk stratification system.

In addition to the two hotspot mutations, DNA hypermethylation in the upstream of the transcription start site (UTSS) of *TERT* gene was frequently observed in multiple types of human cancer [21, 22]. And there is accumulating evidence that *TERT* promoter hypermethylation was associated with disease progression and poor prognosis of several cancers, including pediatric brain tumor [23], breast cancer [24], bladder cancer [25], pancreatic cancer [26], and pituitary adenoma [27]. For thyroid cancer, few preliminary studies had shown a correlation between *TERT* methylation and aggressive features and/or poor outcome [28–30], suggesting that *TERT* promoter methylation might be a prognostic marker for PTC. Herein, in this study, we comprehensively analyzed the association of *BRAF*/*RAS* mutations, *TERT* promoter mutations and methylation with clinicopathologic outcomes of PTC and assessed the possibility of enrolling *TERT* promoter methylation into the well-established risk stratification system based on the genetic duet of *BRAF*/*RAS* and *TERT* mutations.

Materials and methods

Patients

The patients with PTC used for this study were originated from The Cancer Genome Atlas (TCGA) thyroid cancer (THCA) dataset. Only the samples containing all the following information were included in the present study: *BRAF* mutation, *RAS* mutation, *TERT* promoter mutation, *TERT* methylation, and *TERT* mRNA expression.

Data acquisition

The mutation status of *BRAF*, *RAS,* and *TERT* promoter were obtained from the 2014 TCGA thyroid cancer paper [31], the methylation level (β-value) of a specific CpG probe (cg11625005) in *TERT* promoter and relative mRNA expression of *TERT* (from Illumina HiSeq) in PTC patients were downloaded from TCGA database by the UCSC Xena platform [32]. The methylation levels of cg11625005 in TCGA normal thyroid tissues were downloaded by the Shiny Methylation Analysis Resource Tool (SMART) [33]. The following clinical characteristics and outcomes as well as related follow-up times for each PTC patients were downloaded and collected from the TCGA Clinical Data Resource [34]: age at diagnosis, sex, extrathyroidal extension, pathologic T/N/M, overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI) and progression-free interval (PFI).

Definition of *TERT* hypermethylation

The methylation level of cg11625005 was selected for representing the overall methylation level of *TERT* promoter region [24, 25]. The cut-off β-value for *TERT* promoter hypermethylation in thyroid cancer was defined as the mean β-value + 2*SD of the normal samples [22]. According to this formula, the cut-off β-value was set at 0.494 for *TERT* hypermethylation in the current study.

Statistical analysis

The categorical data were summarized with frequencies and percentages, and compared by using chi-square test or Fisher's exact test. The continuous data were summarized with means ± standard errors or medians and interquartile ranges (IQR), and compared by independent *t* test or Wilcoxon–Mann–Whitney test, respectively. Kaplan–Meier survival curves with log-rank test were used to compare the progression-free interval (PFI) between different genotypes. Univariate and multivariate survival analyses were performed using Cox regression to assess the association between genomic alterations and clinical outcomes of PTC. All *P* values were two sided, and statistical significance was set at $P < 0.05$. Statistical analyses were performed using Stata (version 10.1; Stata Corp., College Station, TX, USA) and GraphPad Prism (version 7; GraphPad Software, San Diego, CA, USA).

Results

BRAF/*RAS* mutation and *TERT* alteration in PTC

According to the patients' enrollment criteria, a total of 382 PTC patients were included in the current study. The *BRAF* and *RAS* mutations were detected in 236 (61.8%) and 49 (12.8%) of the samples, respectively; *TERT* promoter

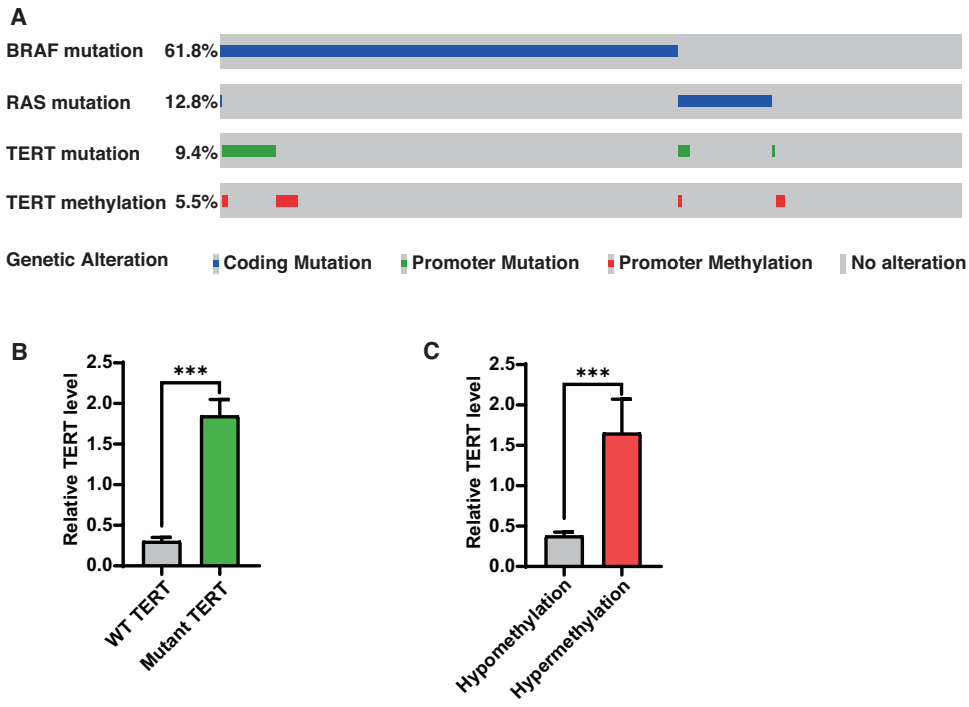

Fig. 1 The association of *TERT* alterations with *BRAF/RAS* mutation and TERT expression. **A** Distribution of *BRAF* mutation, *RAS* mutation, *TERT* promoter mutations and hypermethylation in 382 papillary thyroid cancer (PTC) patients from the TCGA dataset. **B** Relative *TERT* expression in *TERT* promoter wild-type (wt) and mutated PTC samples. **C** Relative *TERT* expression in *TERT* promoter hypomethylated and hypermethylated PTC samples. ***$P < 0.001$

mutation and hypermethylation occurred in 36 (9.4%) and 21 (5.5%) samples, respectively (Fig. 1A). Compared with the patients harboring wild-type *TERT* promoter, patients harboring *TERT* promoter mutations had a significant increased level of *TERT* expression ($P < 0.001$, Fig. 1B); Similarly, *TERT* expression was significantly higher in patients with *TERT* promoter hypermethylation than that in patients with hypomethylated *TERT* promoter ($P < 0.001$, Fig. 1C). Therefore, we combined *TERT* promoter mutation and hypermethylation into one group (named as the *TERT* alteration group) in the following analysis. Collectively, *BRAF* or *RAS* mutation occurred in 284 (74.3%) samples and *TERT* alteration occurred in 52 (13.6%) samples.

The association of *BRAF/RAS* mutation and *TERT* alteration with clinical characteristics and outcomes of PTC

Compared with the patients without *BRAF* or *RAS* mutation, the *BRAF/RAS* mutation positive patients had higher rates of extrathyroidal extension (33.1% vs. 14.9%, $P = 0.001$) and advanced pathologic T stage (39.6% vs 26.8%, $P = 0.024$). Disease progression was 32 of 284 (11.3%) in *BRAF/RAS* mutation positive patients versus 4 of 98 (4.1%) in *BRAF/RAS* mutation negative patients ($P = 0.043$, Table 1). In addition to extrathyroidal extension and pathologic T stage, the *TERT* alterations were found to be significantly correlated with old patient age and pathologic M stage (Table 2). And patients with *TERT* alterations had higher rates of

Table 1 Association of BRAF/RAS mutation with clinical characteristics and outcomes of PTC

Characteristics and outcomes	Overall	BRAF/RAS mutation		*P*
		Yes	No	
No.	382	284	98	
Age	46 (35–58)	47 (35–58)	46 (34–61)	0.990
Gender, male	100/382 (26.2)	77/284 (27.1)	23/98 (23.5)	0.479
Extrathyroidal extension	99/347 (28.5)	86/260 (33.1)	13/87 (14.9)	0.001
Pathologic T				
T1	114 (30.0)	86 (30.4)	28 (28.9)	
T2	128 (33.7)	85 (30.0)	43 (44.3)	
T3	123 (32.4)	98 (34.6)	25 (25.8)	
T4	15 (3.9)	14 (5.0)	1 (1.0)	0.032
T3 + T4	138 (36.3)	112 (39.6)	26 (26.8)	0.024
Pathologic N	164/343 (47.8)	129/260 (49.6)	35/83 (42.2)	0.237
Pathologic M	7/221 (3.2)	7/175 (4.0)	0/46 (0)	0.350
Overall mortality	14/382 (3.7)	10/284 (3.5)	4/98 (4.1)	0.761
Disease specific mortality	5/376 (1.3)	3/279 (1.1)	2/97 (2.1)	0.607
Tumor recurrence	14/273 (5.1)	13/199 (6.5)	1/74 (1.4)	0.122
Disease progression	36/382 (9.4)	32/284 (11.3)	4/98 (4.1)	0.043

Table 2 Association of TERT alteration with clinical characteristics and outcomes of PTC

Characteristics and outcomes	TERT alteration (mutation/hypermethylation)		*P*
	Yes	No	
No.	52	330	
Age	60 (46–70)	46 (34–56)	<0.001
Gender, male	15/52 (28.9)	85/330 (25.8)	0.638
Extrathyroidal extension	20/47 (42.6)	79/300 (26.3)	0.022
Pathologic T			
T1	10 (19.6)	104 (31.6)	
T2	13 (25.5)	115 (35.0)	
T3	18 (35.3)	105 (31.9)	
T4	10 (19.6)	5 (1.5)	<0.001
T3 + T4	28 (54.9)	110 (33.4)	0.003
Pathologic N	27/51 (52.9)	137/292 (46.9)	0.427
Pathologic M	4/33 (12.1)	3/188 (1.6)	0.011
Overall mortality	7/52 (13.5)	7/330 (2.1)	<0.001
Disease specific mortality	4/50 (8.0)	1/326 (0.3)	0.001
Tumor recurrence	3/27 (11.1)	11/246 (4.5)	0.150
Disease progression	12/52 (23.1)	24/330 (7.3)	<0.001

overall mortality (13.5% vs 2.1%, $P < 0.001$), disease-specific mortality (8.0% vs 0.3%, $P = 0.001$) and progression (23.1% vs 7.3%, $P < 0.001$) than patients without any *TERT* alteration (Table 2).

We next performed Kaplan-Meier and Cox-regression analyses of progression-free interval (PFI) by genotype. Although the PFI curve had a modest decline in patients harboring *BRAF* or *RAS* mutation, the *BRAF/RAS* mutation was not significantly associated with PFI (log-rank $P = 0.071$; HR = 2.52, 95% CI: 0.89–7.12, $P = 0.082$; Fig. 2A and Table 3). While the Kaplan–Meier curve showed that the presence of *TERT* alteration was significantly associated with PFI in PTC (log-rank $P < 0.001$, Fig. 2B), and the hazard ratio (HR) of *TERT* alteration for PFI was 3.48 (95% CI: 1.74–6.96, $P < 0.001$), which lost significance after adjustment for aggressive tumor behaviors (Table 3).

Impacts of *BRAF/RAS* mutation, *TERT* alteration and their coexistence on clinicopathologic outcomes of PTC

Since it has been well established that *TERT* promoter mutation tends to be coexist with *BRAF/RAS* mutation, we next analyzed the association of *TERT* alterations (*TERT* promoter mutation + hypermethylation) with *BRAF/RAS* mutation. As a result, *TERT* alteration was found in 7 of 98 (7.1%) *BRAF/RAS* mutation -negative patients versus 45 of 284 (15.8%) *BRAF/RAS* mutation -positive patients, while *BRAF/RAS* mutation was found in 239 of 330 (72.4%)

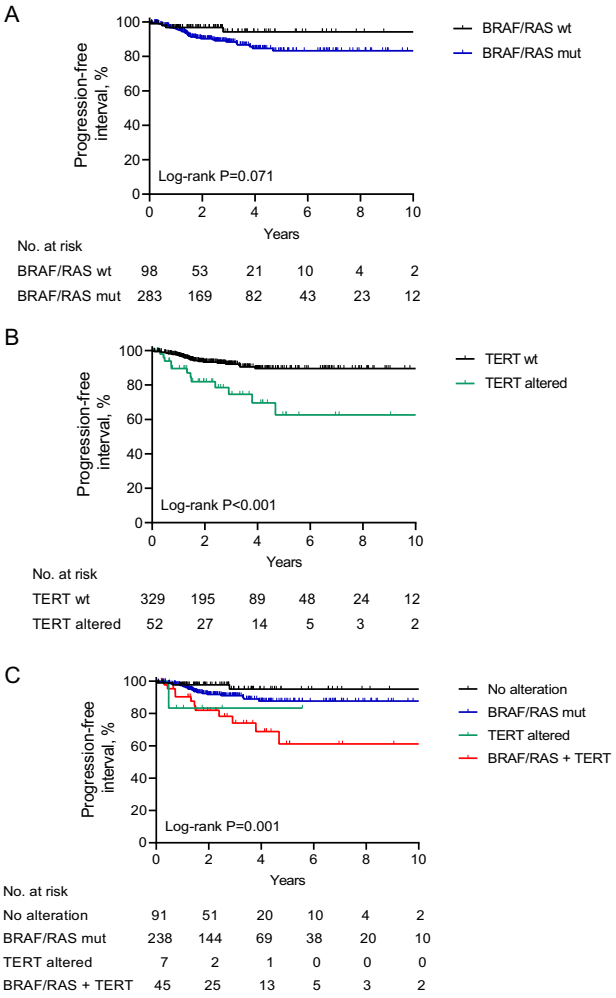

Fig. 2 Kaplan–Meier analyses of the impacts of *BRAF/RAS* mutation and *TERT* alterations on progression-free interval (PFI) of patients with papillary thyroid cancer. **A** Effect of *BRAF/RAS* mutation on PFI. **B** Effect of *TERT* alteration on PFI. **C** Effects of *BRAF/RAS* mutation or *TERT* alteration alone or their coexistence on PFI

TERT alteration -negative patients versus 45 of 52 (86.5%) *TERT* alteration -positive patients ($P = 0.030$), suggesting a significant positive association between the presence of *TERT* alteration and *BRAF/RAS* mutation in PTC. Coexistence of *BRAF/RAS* and *TERT* alteration was found in 45 of 382 (11.8%) PTCs. Next, we divided the patients into 4 groups according to *BRAF/RAS* and *TERT* alteration status, and analyzed the association of *BRAF/RAS* mutation alone, *TERT* alteration alone, and the coexisting of *BRAF/RAS* and *TERT* alterations with clinicopathologic characteristics and outcomes of PTC.

As shown in Table 4, in comparison with the 91 patients with neither *BRAF/RAS* mutation nor *TERT* alteration, *BRAF/RAS* mutation alone was associated with extrathyroidal extension (30.6% vs 14.8%, $P = 0.006$), *TERT* alteration alone was not associated with any of the aggressive characteristics of PTC, while coexistence of

Table 3 Hazard ratios of BRAF/RAS or TERT alteration for progression-free interval of PTC

Genomic alteration	1000-person years	Crude HR (95% CI)	Adjusted HR (95% CI)
BRAF/RAS mutation			
Negative	14.28 (5.36–38.05)	1.00	1.00
Positive	33.41 (23.63–47.25)	2.52 (0.89–7.12)	2.42 (0.73–8.03)
TERT alteration			
Negative	22.12 (14.83–33.00)	1.00	1.00
Positive	78.54 (44.60–138.29)	3.48 (1.74–6.96)	2.16 (0.93–5.03)

Table 4 Association of BRAF/RAS or TERT alteration or their coexistence on clinicopathologic outcomes of PTC

Characteristics and outcomes	No alteration	BRAF/RAS mutation	*P*	TERT alteration	*P*	BRAF/RAS + TERT	*P*
	No. (%)	No. (%)		No. (%)		No. (%)	
No.	91	239		7		45	
Age	47 (34–61)	44 (34–54)	0.140	37 (28–46)	0.174	62 (51–70)	<0.001
Gender, male	21/91 (23.1)	64/239 (26.8)	0.492	2/7 (28.6)	0.665	13/45 (29.9)	0.529
Extrathyroidal extension	12/81 (14.8)	67/219 (30.6)	0.006	1/6 (16.7)	1.000	19/41 (46.3)	<0.001
Pathologic T							
T1	25 (27.5)	79 (33.2)		3 (50.0)		7 (15.6)	
T2	42 (46.1)	73 (30.7)		1 (16.7)		12 (26.7)	
T3	24 (26.4)	81 (34.0)		1 (16.7)		17 (37.8)	
T4	0 (0)	5 (2.1)	0.047	1 (16.7)	0.030	9 (20.0)	<0.001
T3 + T4	24 (26.4)	86 (36.1)	0.093	2 (33.3)	0.657	26 (57.8)	<0.001
Pathologic N	32/76 (42.1)	105/216 (48.6)	0.328	3/7 (42.9)	1.000	24/44 (54.5)	0.188
Pathologic M	0/42 (0)	3/146 (2.1)	1.000	0/4 (0)		4/29 (13.8)	0.024
Overall mortality	3/91 (3.3)	4/239 (1.7)	0.400	1/7 (14.3)	0.260	6/45 (13.3)	0.059
Disease specific mortality	1/90 (1.1)	0/236 (0)	0.276	1/7 (14.3)	0.140	3/43 (7.0)	0.099
Tumor recurrence	1/69 (1.5)	10/177 (5.7)	0.300	0/5 (0)	1.000	3/22 (13.6)	0.042
Disease progression	3/91 (3.3)	21/239 (8.8)	0.100	1/7 (14.3)	0.260	11/45 (24.4)	<0.001

Table 5 Hazard ratios of BRAF/RAS or TERT alteration or their coexistence for progression-free interval of PTC

Genomic alteration	1000-person years	Crude HR (95% CI)	Adjusted HR (95% CI)
No alteration	11.21 (3.62–34.76)	1.00	1.00
BRAF/RAS mutation	25.69 (16.75–39.40)	2.48 (0.74–8.31)	3.30 (0.74–14.81)
TERT alteration	80.24 (11.30–569.61)	5.87 (0.59–58.06)	15.17 (0.77–297.57)
BRAF/RAS + TERT	78.39 (43.41–141.54)	7.25 (2.02–26.01)	10.35 (1.79–59.81)

BRAF/*RAS* mutation and *TERT* alteration was strongly associated with several high-risk clinicopathologic characteristics, including older age (mean value of 62 vs 47, $P < 0.001$), extrathyroidal extension (46.3% vs 14.8%, $P < 0.001$), advanced pathologic T stages (T3 + T4, 57.8% vs 26.4%, $P < 0.001$) and metastasis (13.8% VS 0%, $P = 0.024$). Importantly, patients with both *BRAF*/*RAS* mutation and *TERT* alteration had higher rates of tumor recurrence (13.6% vs 1.5%, $P = 0.042$) and disease progression (24.4% vs 3.3%, $P < 0.001$) than patients without any alterations in *BRAF*, *RAS* or *TERT* (Table 4).

On Kaplan–Meier analysis of the impacts of the 4 groups on PFI of patients with PTC, the PFI curve of patients harboring neither alteration was almost flat, the curves of patients harboring *BRAF*/*RAS* mutation alone or *TERT* alteration alone showed modest decline, while the curve of patients with coexisting of *BRAF*/*RAS* and *TERT* alterations declined more sharply and dramatically than the other 3 groups (log-rank $P = 0.001$, Fig. 2C). PFI per 1000-person years in patients with neither alteration, *BRAF*/*RAS* mutation alone, *TERT* alteration alone, or both *BRAF*/*RAS* mutation and *TERT* alteration were 11.21 (95% CI:

3.62–34.76), 25.69 (95% CI: 16.75–39.40), 80.24 (95% CI: 11.30–569.61), and 78.39 (95% CI: 43.41–141.54), respectively (Table 5). In the Cox regression analysis, the HRs of *BRAF*/*RAS* mutation alone and *TERT* alteration alone for PFI were not significant; the HR of coexisting *BRAF*/*RAS* and *TERT* alterations for PFI was 7.25 (95% CI: 2.02–26.01, $P = 0.002$), which remained significant at 10.35 (95% CI: 1.79–59.81, $P = 0.009$) after adjustment for multiple aggressive tumor characteristics (Table 5). Moreover, compared with patients harboring *BRAF*/*RAS* mutation, patients with both *BRAF*/*RAS* mutation and *TERT* alteration had a higher risk of PFI (HR = 2.98, 95% CI: 1.44–6.19, $P = 0.003$).

Discussion

The discovery of *TERT* promoter mutation is a milestone in the genetic field of thyroid cancer. The diagnostic and prognostic value of *TERT* promoter mutation have been well established in the past 10 years. A large number of studies have shown that *TERT* promoter mutation associated with almost all the adverse features of PTC and patients harboring *TERT* mutation were at high risk of radioiodine refractory, tumor recurrence and death from thyroid cancer [15, 16, 35–37]. Mechanismly, the GABPA/BABPB transcription factor complex and ETV5 selectively binds to and activates the mutant *TERT* promoter, leading to increased *TERT* expression and thus confers oncogenic functions in thyroid tumorigenesis and development [38, 39].

In addition to promoter mutation, pan-cancer analysis revealed that *TERT* overexpression was seen in patients harboring several other types of genetic and epigenetic alterations, including *TERT* amplification, structural variation, and DNA methylation [21]. Although whether *TERT* promoter methylation activates its expression is still controversial [22, 40–44], emerging studies have shown that *TERT* methylation was associated with increased *TERT* expression and aggressive clinical behaviors in thyroid cancer [28–30]. Consistently, in this study, we found that *TERT* expression is significantly higher in PTC patients with *TERT* hypermethylation than that in patients with *TERT* hypomethylation.

In this study, the *TERT* promoter mutation was observed in 9.4% of PTC patients, *TERT* hypermethylation was observed in 5.5% of PTCs, and they were collectively present in 13.6% of patients with PTC. These two types of *TERT* alterations were associated with old patient age, extrathyroidal extension, advanced T and M stage, and poor outcomes. These results are in line with a recent study indicating that both *TERT* promoter mutation and methylation were frequently observed in clinically aggressive thyroid cancers [30].

Although the hotspot mutations in *BRAF* and *RAS* genes had been identified 10 years earlier than *TERT* mutation, the association between *BRAF*/*RAS* mutation and prognosis of PTC is still controversial. However, there is no doubt that *BRAF* mutation is not an effective biomarker for aggressive PTCs since the frequency of *BRAF* mutation is much higher than the frequency of aggressive cases among all the patients with PTC. Unlike other well-known somatic mutations, the *RAS* mutations could be frequently identified not only in malignant thyroid nodules but also in benign nodules, two recent cohort studies with large patient number showed that *RAS* mutation alone was likely to be a favorable marker of thyroid cancer [45], while coexisting of *RAS* mutation with additional oncogenic alteration associated with more aggressive phenotype and increased risk of recurrence and mortality in differentiated thyroid cancer [46]. Consistently, in this study, we found that patients harboring *BRAF* or *RAS* mutation had higher rates of extrathyroidal extension and advanced pathologic T stage, but the *BRAF*/*RAS* mutation was not an independent risk factor for the prognosis of PTC.

Importantly, it has been well established that *TERT* promoter mutations were associated with *BRAF*/*RAS* mutations in PTC, and the patients harboring both *BRAF*/*RAS* and *TERT* mutations were associated with the most aggressive behaviors and worst clinical outcomes of PTC. In this study, we showed that the presence of both *BRAF*/*RAS* mutation and *TERT* alteration significantly associated with multiple aggressive characteristics of PTC, including old age, extrathyroidal extension, advanced pathologic T stage and metastasis. Moreover, we observed that patients harboring both *BRAF*/*RAS* and *TERT* alterations had remarkable increased rate of DFI and PFI. Importantly, Cox regression analysis revealed that the coexistence of *BRAF*/*RAS* and *TERT* alterations, but not *BRAF*/*RAS* alone or *TERT* alterations alone, increased the risk of PFI of PTC, suggesting a cooperative role of *BRAF*/*RAS* and *TERT* alterations in PTC progression. These data suggested an updated risk stratification model for PTC prognosis with a risk order of the coexisting of *BRAF*/*RAS* mutation and *TERT* alteration »» *BRAF*/*RAS* mutation alone = *TERT* alteration alone > wild type for the 3 genes.

Here we enrolled *TERT* methylation in the risk stratification system for PTC, compared with previous established *BRAF*/*RAS* and *TERT* mutations -based risk stratification system, enrollment of *TERT* methylation as a high-risk marker will identify more patients which potentially at high risk of poor outcomes. This is particularly the case for PTC patients in Asian, since *TERT* promoter mutation was reported in 2–3% of PTC patients in Asian countries and the frequency of coexisting of *BRAF*/*RAS* and *TERT* mutations was 1–2% in Asian patients with PTC [47–49], which is much lower than the frequency of clinically high-risk patients.

Conclusions

In conclusion, *TERT* promoter mutation and hypermethylation are common events in PTC and they associate with adverse features and poor clinical outcome of PTC. Patients harboring both *TERT* alterations and *BRAF*/*RAS* mutations showed more aggressive characteristics and worse prognosis than patients harboring *TERT* alterations alone, *BRAF*/*RAS* mutations alone, or no alteration. Comprehensively analysis of hotspot somatic mutations in *BRAF*, *RAS,* and *TERT* genes, in combination with *TERT* promoter methylation could identify high-risk patients and lead to a better management for the patients with PTC.

Data availability

Publicly available TCGA thyroid cancer datasets analyzed in this study can be found at https://portal.gdc.cancer.gov/ or https://xena.ucsc.edu/. Further specific inquiries can be directed to the corresponding author.

Author contributions R.L. and Y.S. designed the research; Y.S., G.H., J.X., M.C., and S.H. collected the data; Y.S., G.H., J.X., and R.L. analyzed the data; R.L. and Y.S. drafted the manuscript, with inputs from all authors. All authors have read and agreed to the published version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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