TERT promoter mutations in thyroid cancer

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Abstract

The 2013 discovery of Telomerase reverse transcriptase (*TERT*) promoter mutations chr5, 1,295,228 C>T (C228T) and 1,295,250 C>T (C250T) in thyroid cancer represents an important event in the thyroid cancer field and much progress has occurred since then. This article provides a comprehensive review of this exciting new thyroid cancer field. The oncogenic role of *TERT* promoter mutations involves their creation of consensus binding sites for E-twenty-six transcriptional factors. *TERT* C228T is far more common than *TERT* C250T and their collective prevalence is, on average, 0, 11.3, 17.1, 43.2 and 40.1% in benign thyroid tumors, papillary thyroid cancer (PTC), follicular thyroid cancer, poorly differentiated thyroid cancer and anaplastic thyroid cancer, respectively, displaying an association with aggressive types of thyroid cancer. *TERT* promoter mutations are associated with aggressive thyroid tumor characteristics, tumor recurrence and patient mortality as well as *BRAF* V600E mutation. Coexisting *BRAF* V600E and *TERT* promoter mutations have a robust synergistic impact on the aggressiveness of PTC, including a sharply increased tumor recurrence and patient mortality, while either mutation alone has a modest impact. Thus, *TERT* with promoter mutations represents a prominent new oncogene in thyroid cancer and the mutations are promising new diagnostic and prognostic genetic markers for thyroid cancer, which, in combination with *BRAF* V600E mutation or other genetic markers (e.g. *RAS* mutations), are proving to be clinically useful for the management of thyroid cancer. Future studies will specifically define such clinical utilities, elucidate the biological mechanisms and explore the potential as therapeutic targets of *TERT* promoter mutations in thyroid cancer.

Key Words
- *TERT* promoter mutation
- thyroid cancer
- telomerase reverse transcriptase
- *BRAF* V600E mutation
- genetic molecular markers
- prognosis
- diagnosis

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Introduction

Telomerase reverse transcriptase in human cancer

Telomerase reverse transcriptase (TERT) is the catalytic protein subunit of telomerase, which, together with an integral RNA subunit and several species-specific assessor proteins, functions to add telomeres – tandem repeats of TTAGGG sequence – at the end of chromosomes (Moyzis *et al.* 1988), maintaining chromosomal integrity and genome stability (Greider & Blackburn 2004, Blasco 2005). Telomerase is well expressed in germ line and stem cells but less expressed or repressed in most somatic human cells; in the latter, telomere loss occurs during each round of cell division, causing cells to enter senescence when a critical length of telomeres is reached (Harley & Villeponteau 1995, Blasco 2005). In contrast, telomerase is reactivated in many human cancers (Kim *et al.* 1994,

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Shay & Bacchetti 1997), which prevents the happening of critical telomere shortening, thus enabling cancer cells acquire replicative immortality (Hanahan & Weinberg 2011). Compared with the RNA subunit, TERT plays a dominant role in the activation of telomerase during malignant transformation of cells (Feng *et al*. 1995, Meyerson *et al*. 1997, Bodnar *et al*. 1998, Hahn *et al*. 1999, Janknecht 2004). TERT can also modulate the expression of growth-controlling genes (Smith *et al*. 2003), directly regulate NFκB-dependent gene expression (Ghosh *et al*. 2012), and stabilize MYC levels on chromatin (Koh *et al*. 2015).

Given these and other functions of TERT, it has been proposed that TERT may act as an oncogene in a telomere-independent manner (Wyatt *et al*. 2010). In fact, overexpression of TERT, or naturally occurring alternatively spliced TERT variants that lack telomerase activities, stimulated rapid cell proliferation without changes of telomere length in human and murine cells (Smith *et al*. 2003, Sarin *et al*. 2005, Choi *et al*. 2008, Hrdlickova *et al*. 2012). Induced expression of TERT was also shown to promote the development of mammary carcinomas and epidermal tumors *in vivo* (Gonzalez-Suarez *et al*. 2001, Stewart *et al*. 2002). Inversely, TERT knockdown decreased cell proliferation and suppressed tumor growth in a xenograft model (Ghosh *et al*. 2012). Reactivation of TERT has also been linked to several other cancer hallmark behaviors, such as resistance to antigrowth signals, angiogenesis, resistance to apoptosis, invasion and metastasis, inflammation and immune surveillance, reprogramming of energy metabolism and genome instability (Low & Tergaonkar 2013).

Genome-wide association studies have identified multiple variants at the *TERT* locus that are associated with telomere length and risk of several cancers (Rafnar *et al*. 2009, Bojesen *et al*. 2013, Codd *et al*. 2013, Wang *et al*. 2014*a*), strongly suggesting that this locus is a common susceptibility locus for human cancer. The most significant advance in understanding the genetic role of TERT in human cancer was the landmark finding of mutations in the promoter of the *TERT* gene in melanoma early in 2013 through whole-genome sequencing (Horn *et al*. 2013, Huang *et al*. 2013), which were quickly found also in other human cancers, such as bladder cancer and glioblastoma (Killela *et al*. 2013, Liu *et al*. 2013*a*) as well as thyroid cancer (Liu *et al*. 2013*b*). There are two common recurrent *TERT* promoter mutations in human cancer that are located at two hotspots: chr5, 1,295,228 C>T (C228T) and 1,295,250 C>T (C250T), corresponding to the positions 124 and 146 bp respectively upstream of the *TERT* translation start site. These two *TERT* promoter mutations occurred in a mutually exclusive manner, with C228T being far more dominant than C250T in most cancers. Both mutations generate a consensus binding site in the *TERT* promoter for E-twenty-six (ETS) transcription factors, which has been shown to confer the *TERT* promoter increased transcriptional activities (Horn *et al*. 2013, Huang *et al*. 2013, Bell *et al*. 2015). This provides a mechanistic explanation for the oncogenic role of *TERT* promoter mutations in human cancer.

Thyroid cancer

Thyroid cancer is the most common endocrine malignancy, which has seen a rapidly rising incidence in recent decades (Siegel *et al*. 2014, Howlader *et al*. 2015). Thyroid cancer is histologically classified into follicular thyroid cell-derived papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC), and parafollicular C cell-derived medullary thyroid cancer (MTC), which classically account for ~80, 10, 5, 2 and 3% of all thyroid malignancies, respectively (Hundahl *et al*. 1998), with PTC emerging to be even more dominant in recent years (Howlader *et al*. 2015). PTC can be further classified into several histological variants, the most common of which are conventional PTC (CPTC), follicular variant PTC (FVPTC) and tall-cell PTC (TCPTC). Among follicular cell-derived thyroid cancers, PTC and FTC are differentiated thyroid cancer with a generally indolent disease course while ATC, albeit uncommon, is an undifferentiated thyroid cancer with a rapid aggressive disease course; PDTC has an intermediate aggressive course between DTC and ATC. There is also thyroid Hürthle cell cancer (HCC), which is uncommon and conventionally treated as a type of FTC. Since our first report of *TERT* promoter mutations in various types of thyroid cancer in 2013 (Liu *et al*. 2013*b*), there have been a large number of more studies on this topic published, reporting and characterizing *TERT* promoter mutations in thyroid cancer (Landa *et al*. 2013, Vinagre *et al*. 2013, Cancer Genome Atlas Research Network 2014, Liu & Xing 2014, Liu *et al*. 2014*a*,*b*, Melo *et al*. 2014, Wang *et al*. 2014*b*,c, Xing *et al*. 2014*a*, de Biase *et al*. 2015, Chindris *et al*. 2015, Crescenzi *et al*. 2015, Dettmer *et al*. 2015, Gandolfi *et al*. 2015, Lee *et al*. 2015, Muzza *et al*. 2015, Qasem *et al*. 2015, Shi *et al*. 2015). This is an exciting new thyroid cancer research field, which has progressed rapidly just in this past 2 years; much has now already been known about the biological role and clinical significance of *TERT* promoter

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mutations in thyroid cancer. This review focuses on *TERT* promoter mutations in thyroid cancer and summarizes the progresses in recent research and clinical development of this young but rapidly mushrooming field. We included all the studies on *TERT* promoter mutations in thyroid cancer published in English in PubMed as of November 2015 with identifiable information on thyroid tumor types. The pooled analyses included nonselective cases from different studies available for the analyzed parameters as presented in the corresponding tables and figures and did not include four studies which were each focused on highly selective special cases, including pediatric thyroid cancer (Ballester *et al*. 2015), papillary thyroid microcarcinomas (PTMC) (de Biase *et al*. 2015), tall-cell PTC (Dettmer *et al*. 2015) and distant-metastasis PTC (Gandolfi *et al*. 2015).

Common occurrence of *TERT* promoter mutations in thyroid cancer

The most common *TERT* promoter mutations are C228T and C250T in human cancers. As initially demonstrated (Liu *et al*. 2013*b*), studies have generally shown no overlap between *TERT* C228T and C250T mutations and the former is far more prevalent than the latter in thyroid cancer. This pattern is uniformly seen regardless of the ethnic and geographical backgrounds of the studies (Table 1). Specifically, on the overall analysis, the prevalences of C228T and C250T are 9.7 and 2.1% in PTC, 15.7 and 2.5% in FTC, 33.8 and 15.0% in PDTC, 37.7 and 4.1% in ATC respectively,. The relative distribution of *TERT* C228T between the two mutations is dominantly 82.5, 86.2, 69.2 and 90.2% in PTC, FTC, PDTC and ATC respectively. The mutual exclusivity of the two *TERT* promoter mutations suggests that either may function sufficiently to play an important role in thyroid tumorigenesis although which one is more powerful oncogenically has not been established at this time. Unless otherwise specified, the analyses and discussions in this review will be on the two *TERT* promoter mutations collectively.

Our initial study reported a prevalence of *TERT* C228T and C250T mutations to be collectively 0% in benign thyroid tumors, 38–46% in PDTC and ATC, and 12–14% in PTC and FTC (Liu *et al*. 2013*b*). This pattern of prevalence was confirmed in many other studies as summarized in Table 2. Specifically, on the analysis of the pooled data, the two *TERT* promoter mutations were found collectively in 11.3, 17.1, 14.6, 43.2 and 40.1% of PTC, FTC, HCC, PDTC and ATC respectively. No *TERT* promoter mutation was

Table 1 Distribution of the two common *TERT* promoter mutations – C228T and C250T – in thyroid cancer

Study No.	PTC		FTC		PDTC		ATC		Reference
	C228T	C250T	C228T	C250T	C228T	C250T	C228T	C250T	
1	30/257 (11.7)	0/257 (0)	9/79 (11.4)	2/79 (2.5)	3/8 (37.5)	0/8 (0)			Liu *et al*. (2013*b*)
2	10/80 (12.5)	8/80 (10.0)			18/58 (31.0)	12/58 (20.7)	10/20 (50.0)	0/20 (0.0)	Landa *et al*. (2013)
3			8/52 (15.4)	1/52 (1.9)					Wang *et al*. (2014*b*)
4	12/51 (23.5)	1/51 (2.0)					8/20 (40.0)	2/20 (10.0)	Liu *et al*. (2014*a*)
5	39/408 (9.6)	7/408 (1.7)	7/22 (31.8)	1/22 (4.6)					Liu *et al*. (2014*b*)
6	27/384 (7.0)	8/384 (2.1)							TCGA (2014)
7	20/243 (8.2)	6/243 (2.5)	1/5 (20.0)	0/5 (0)	6/14 (42.9)	0/14 (0)			Qasem *et al*. (2015)
8	3/30 (10.0)	0/30 (0)	0/1 (0)	0/1 (0)					Crescenzi *et al*. (2015)
9							37/106 (34.9)	4/106 (3.8)	Shi *et al*. (2015)
Overall (1)	141/1453 (9.7)	30/1453 (2.1)	25/159 (15.7)	4/159 (2.5)	27/80 (33.8)	12/80 (15.0)	55/146 (37.7)	6/146 (4.1)	
Overall (2)	141/171 (82.5)	30/171 (17.5)	25/29 (86.2)	4/29 (13.8)	27/39 (69.2)	12/39 (30.8)	55/61 (90.2)	6/61 (9.8)	

Overall (1): *n*/total cases; Overall (2): *n*/TERT mutant cases. The 80 cases of PTC in Study No. 2 consisted of 29 cases from Memorial Sloan-Kettering Cancer Center (MSKCC) and 51 cases from Nagasaki University in Japan. The 384 cases of PTC from the Cancer Genome Atlas (TCGA) Research Network (Study No. 6) contained 47 cases from Johns Hopkins University and 75 cases from MSKCC, which could partially overlap with Study Nos 1 and 2 respectively. Therefore, 47+29=76 out of 1453 cases of PTC or 5.2%, at most, could potentially overlap in the overall analysis for PTC at the bottom of the table. Data used here are from those studies that provided available information specifically on the occurrence of both C228T and C250T. PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer.

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found in MTC. Of the total of 363 benign thyroid neoplasms from various studies, only one sample was reported to harbor *TERT* promoter mutation (C228T). It should be noted, though, that this case of patient with *TERT* promoter mutation-positive benign thyroid neoplasm later developed tumor recurrence and died of FTC, raising question on the histological nature of the benign tumor (Wang *et al*. 2014*b*). Thus, the prevalence of *TERT* promoter mutations in the large number of true benign thyroid neoplasms is 0%.

TERT promoter mutation is a rare genetic event in PTMC as demonstrated in the study of de Biase *et al*. (2015) which examined the *TERT* promoter mutation status in 431 PTMC and found a prevalence of 4.7%; this prevalence is much lower than that seen on the analyses of thyroid cancers of all tumor sizes (Table 2). *TERT* promoter mutation appears to also be uncommon in pediatric thyroid cancers as suggested by the study of Ballester *et al*. (2015), which found no *TERT* promoter mutation in 27 pediatric thyroid cancers, including 25 PTC, 1 FTC and 1 MTC. Larger studies on pediatric thyroid cancer, however, are needed to confirm this finding. Our initial study (Liu *et al*. 2013*b*) demonstrated a prevalence distribution of *TERT* promoter mutations among the three main variants of PTC in the order of TCPTC≫CPTC>FVPTC. Several subsequent studies have analyzed the distribution pattern of *TERT* promoter mutations in PTC variants and generally found the prevalence to be similarly highest in TCPTC and lowest in FVPTC. Specifically, as summarized in Table 3, the prevalence of *TERT* promoter mutations is 25.0% in TCPTC, 9.6% in CPTC and 8.0% in FVPTC. Lee *et al*. (2015) did not found *TERT* promoter mutation in ten cases of hobnail-variant PTC analyzed.

The presence of *TERT* promoter mutations provides a genetic mechanisms for the upregulation of *TERT* in thyroid cancer since these mutations create binding consensus sites in the *TERT* promoter for ETS transcriptional factors (Horn *et al*. 2013, Huang *et al*. 2013, Bell *et al*. 2015), which upregulate genes, including the *TERT* gene. In fact, two studies directly demonstrated an association of *TERT* promoter mutations with increased TERT expression in thyroid cancers (Vinagre *et al*. 2013, Muzza *et al*. 2015). This seems to be consistent with the previous findings that telomerase activity and *TERT* expression were undetectable in normal thyroid tissues

Table 2 Frequency of *TERT* promoter mutations in various thyroid tumors

Study No.	Frequency (mutation/total (%))							Reference
	PTC	FTC	HCC	PDTC	ATC	MTC	Benign neoplasm	
1	30/257 (11.7)	11/79 (13.9)		3/8 (37.5)	25/54 (46.3)	0/16 (0)	0/85 (0)	Liu *et al*. (2013*b*)
2	18/80 (22.5)		4/25 (16.0)	30/58 (51.7)	10/20 (50.0)			Landa *et al*. (2013)
3	13/169 (7.7)	9/64 (14.1)		3/14 (21.4)	2/16 (12.5)	0/28 (0)	0/72 (0)	Vinagre *et al*. (2013)
4	5/111 (4.5)	4/18 (22.2)					0/166 (0)	Liu & Xing (2014)
5		9/52 (17.3)					1/58 (1.7)	Wang *et al*. (2014*b*)
6						0/39 (0)		Wang *et al*. (2014*c*)
7	13/51 (25.5)	8/36 (22.2)			10/20 (50%)	0/37 (0)		Liu *et al*. (2014*a*)
8	46/408 (11.3)	8/22 (36.4)					0/44 (0)	Liu *et al*. (2014*b*)
9	25/332 (7.5)	12/70 (17.1)		9/31 (29.0%)	12/36 (33.3%)	0/28 (0)	0/72 (0)	Melo *et al*. (2014)
10	61/507 (12.0)							Xing *et al*. (2014*a*)
11	36/384 (9.4)							TCGA (2014)
12	26/243 (10.7)	1/5 (20.0)	1/3 (33.3)	6/14 (42.9)				Qasem *et al*. (2015)
13	3/30 (10.0)	0/1 (0)					0/17 (0)	Crescenzi *et al*. (2015)
14			8/61 (13.1)					Chindris *et al*. (2015)
15					41/106 (38.7)			Shi *et al*. (2015)
16	22/182 (12.1)	8/58 (13.8)				0/14 (0)	0/6 (0)	Muzza *et al*. (2015)
Overall	250/2217 (11.3)	49/287 (17.1)	13/ 89 (14.6)	48/111 (43.2)	73/182 (40.1)	0/97 (0)	1/363 (0.3)	

Study Nos 1, 4 and 10 were from one group of the same investigators; Study Nos 3 and 9 were from another group of the same investigators; and Study Nos 5, 6 and 7 were from still another group of the same investigators. To avoid overlap, only the cases from the largest study from each of the three groups of investigators are included for the overall analyses at the bottom of the table. For the reason explained in the footnotes of Table 1, 76/2,217 or 3.4%, at most, could potentially overlap on the overall analysis for PTC at the bottom of the table. The one case of patient with *TERT* promoter mutation-positive benign thyroid neoplasm later developed tumor recurrence and died of FTC, raising question on the histological nature of the benign tumor (Wang *et al*. 2014*b*). Thus, the prevalence of *TERT* promoter mutations in the large number of remaining true benign thyroid neoplasms is 0%. PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; HCC, thyroid Hürthle cell cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer; MTC, medullary thyroid cancer.

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Table 3 *TERT* promoter mutations in three main variants of papillary thyroid cancer

Study No.	Frequency (mutation/total (%))			Reference
	CPTC	FVPTC	TCPTC	
1	23/187 (12.3)	2/56 (3.6)	4/13 (30.8)	Liu *et al.* (2013*b*)
2	12/110 (10.9)	0/39 (0)		Vinagre *et al.* (2013)
3	19/230 (8.3)	5/74 (6.8)	0/2 (0)	Melo *et al.* (2014)
4	47/383 (12.3)	8/103 (7.8)	5/19 (26.3)	Xing *et al.* (2014*a*)
5	25/270 (9.3)	5/81 (6.2)	6/29 (20.7)	TCGA (2014)
6	10/153 (6.5)	7/56 (12.5)	9/30 (30)	Qasem *et al.* (2015)
7	12/143 (8.4)			Muzza *et al.* (2015)
Overall	113/1179 (9.6)	25/314 (8.0)	20/80 (25.0)	

Study Nos 1 and 4 were from a group of the same investigators. Study Nos 2 and 3 were from another group of the same investigators. To avoid overlap, only the cases from the largest study of each group are included in the overall analysis at the bottom of the table. For the reason explained in Tables 1 and 2, overall, 47/1,573, or 3.0%, at most, has a potential overlap with the TCGA data. CPTC, conventional papillary thyroid cancer; TCPTC, tall-cell papillary thyroid cancer; FVPTC, follicular-variant papillary thyroid cancer.

and infrequently detectable in benign thyroid tumors, but TERT reactivation was frequently observed in thyroid cancers (Umbricht *et al.* 1997, Saji *et al.* 1999, Asaad *et al.* 2006). Compared with PTC and FTC, high *TERT* expression was more commonly observed in ATC (Ito *et al.* 2005), which is consistent with the distribution patterns of *TERT* promoter mutations among these thyroid cancers (Tables 1 and 2). Interestingly, knockdown of TERT using the antisense approach in human thyroid cancer cells inhibited telomerase activity *in vitro* and diminished tumor growth *in vivo* (Teng *et al.* 2003), providing the functional evidence for an oncogenic role of TERT in thyroid tumorigenesis. These data are all consistent with and support an important role of the *TERT* promoter mutations in thyroid tumorigenesis.

Association of *TERT* promoter mutations with clinicopathological outcomes of thyroid cancer

A striking feature of *TERT* promoter mutations in thyroid cancer is their association with poor clinicopathological outcomes of the tumor, which has been consistently observed in different studies. The relationship between *TERT* promoter mutations and various clinicopathological parameters of thyroid cancer is summarized in Table 4. All the studies, except one on ATC (Liu *et al.* 2014*a*), consistently demonstrated a significant association between *TERT* promoter mutations and older patient age. On the pooled analysis, the patient age is 59.2 ± 15.5 vs 44.9 ± 15.6 years in *TERT* mutation-positive vs mutation-negative patients ($P < 0.001$). Interestingly,

Table 4 Summary of association between *TERT* promoter mutations and clinicopathological outcomes of thyroid cancer

Clinicopathological outcomes	All types of thyroid cancer			Papillary thyroid cancer		
	TERT WT	TERT mutant	*P*	TERT WT	TERT mutant	*P*
Age (*n*, mean ± S.D.)[a]	2110, 44.9 ± 15.6	327, 59.2 ± 15.5	<0.001	1781, 43.4 ± 14.8	212, 55.7 ± 15.7	<0.001
Gender (male/total, %)[b]	580/2335 (24.8)	132/353 (37.4)	<0.001	408/1786 (22.8)	80/212 (37.7)	<0.001
Tumor size (cm) (*n*, mean ± S.D.)[a]	1666, 2.69 ± 1.93	263, 3.93 ± 2.68	<0.001	1389, 2.30 ± 1.52	161, 2.92 ± 1.97	<0.001
Multifocality (pos/total, %)[b]	364/872 (41.7)	43/123 (35.0)	0.152	332/770 (43.1)	36/97 (37.1)	0.260
Extrathyroidal invasion (pos/total, %)[b]	516/1430 (36.1)	119/214 (55.6)	<0.001	405/1226 (33.0)	66/146 (45.2)	0.003
Vascular invasion (pos/total, %)[b]	265/933 (28.4)	49/116 (42.2)	0.002	200/831 (24.1)	26/86 (30.2)	0.207
Lymph node metastasis (pos/total, %)[b]	531/1210 (43.9)	91/178 (51.1)	0.070	469/1079 (43.5)	63/120 (52.5)	0.059
Distant metastasis (pos/total, %)[b]	109/1037 (10.5)	64/193 (33.2)	<0.001	61/872 (7.0)	25/108 (23.1)	<0.001
Stage (III + IV) (pos/total, %)[b]	434/1687 (25.7)	128/218 (58.7)	<0.001	358/1523 (23.5)	88/172 (51.2)	<0.001
Tumor recurrence (pos/total, %)[b]	179/956 (18.7)	75/144 (52.1)	<0.001	142/817 (17.4)	50/111 (45.0)	<0.001
Death (pos/total, %)[b]	23/413 (5.6)	26/62 (41.9)	<0.001	6/303 (2.0)	9/32 (28.1)	<0.001

Case overlap among the data from different studies is avoided in the analyses in this table except for the potential overlap of a small number of cases of PTC from the TCGA database as explained in Tables 1, 2 and 3

[a]Comparisons were performed using independent *t*-test.

[b]Comparisons were performed using χ^2 test. Information on certain parameters was not available in all studies and this table summarizes the data from studies that provided the available information as detailed in the Supplemental Table S1.

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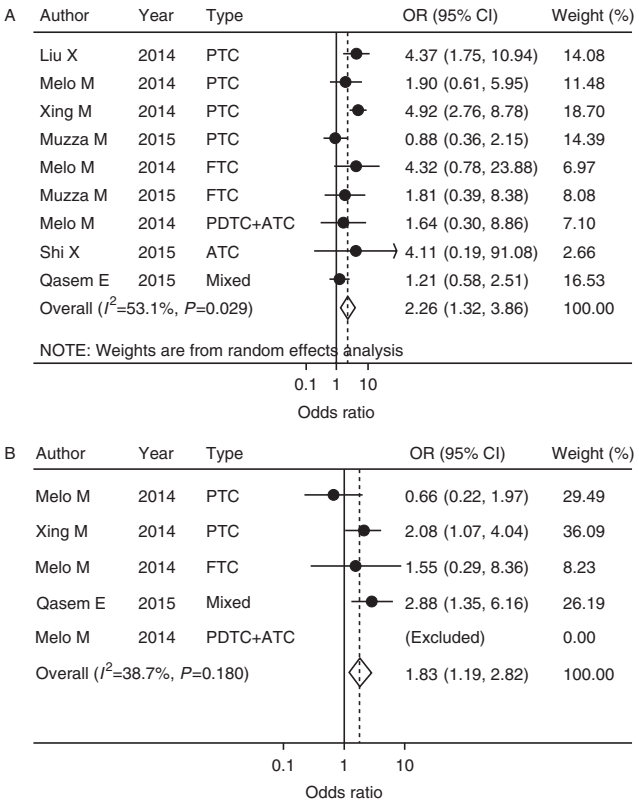

Figure 1
Meta-analysis of the association of *TERT* promoter mutations with extrathyroidal invasion (A) and vascular invasion (B) of thyroid cancer. The between-study heterogeneity was tested by a χ^2-based *Q*-test and quantified by the I^2 metric, which ranged from 0 to 100% and was considered low for I^2 <25%, modest for 25–50%, and large for >50% (Higgins *et al*. 2003). The combined OR was calculated by the fixed-effects model (the Mantel–Haenszel method) when between-study heterogeneity was absent (Mantel & Haenszel 1959); otherwise the random-effects model (the Dersimonian and Laird method) was used (Dersimonian & Laird 1986). The circles and horizontal lines correspond to the study-specific OR and 95% CI respectively. The combined ORs and their 95% CIs are indicated by the diamonds. PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer. OR, odds ratio.

male sex of patients is also associated with *TERT* promoter mutations, being 37.4% male in mutation-positive patients vs 24.8% in mutation-negative patients, $P<0.001$. Larger tumor size is associated with *TERT* promoter mutations, being 3.93±2.68 vs 2.69±1.93 cm ($P<0.001$) in *TERT* mutation-positive tumors vs *TERT* mutation-negative tumors. *TERT* promoter mutations are also significantly associated with extrathyroidal invasion, vascular invasion, distant metastasis and stage III/IV of thyroid cancer. Specifically, extrathyroidal invasion of thyroid cancer is seen in 55.6% of *TERT* mutation-positive patients vs 36.1% of *TERT* mutation-negative patients ($P<0.001$). Meta-analysis reveals that *TERT* promoter mutations significantly increase the risk of extrathyroidal invasion with an odds ratio (OR) of 2.26 (95% CI, 1.32–3.86; $P=0.003$; Fig. 1A). Vascular invasion is also more common in the *TERT* promoter mutation-positive patients than the mutation-negative patients, being 42.2 vs 28.4% ($P=0.002$), with an OR of 1.83 (95% CI, 1.19–2.82; $P=0.006$; Fig. 1B). There is no significant difference in lymph node metastasis between the *TERT* promoter mutation-positive and mutation-negative patients, although there is a strong trend of higher prevalence in the mutation-positive patients (Table 4). The distant metastasis is far more common in *TERT* promoter mutation-positive patients than mutation-negative patients, being 33.2 vs 10.5% ($P<0.001$) (Table 4), with an OR of 4.87 (95% CI, 2.31–10.26; $P<0.001$; Fig. 2A). Stage III/IV disease is similarly far more common in the *TERT* promoter mutation-positive patients than the mutation-negative patients, being 58.7 vs 25.7% ($P<0.001$) (Table 4), with an OR of 4.08 (95% CI, 3.00–5.54; $P<0.001$; Fig. 2B). Importantly, *TERT* promoter mutations are strongly associated with poor clinical outcomes of thyroid cancer on the analysis of large number of cases, including cancer recurrence and patient mortality (Table 4, Fig. 2C and D). Specifically, recurrence in *TERT* promoter mutation-positive patients vs mutation-negative patients is 52.1 vs 18.7% ($P<0.001$), with an OR of 4.89 (95% CI, 3.36–7.13; $P<0.001$; Fig. 2C). Patient death rate is 41.9% in the *TERT* promoter mutation-positive patients vs 5.6% in the mutation-negative patients ($P<0.001$, Table 4), with an OR of 10.26 (95% CI, 4.11–25.60; $P<0.001$; Fig. 2D). It is worth noting that a significant and strong association of *TERT* promoter mutations with cancer recurrence (Fig. 2C) and patient death (Fig. 2D) was uniformly observed in all studies for PTC and FTC except for one study on ATC. Overall, the association of *TERT* promoter mutations with patient mortality is the strongest and most significant among all the clinicopathological parameters (Table 4 and Fig. 2). In fact, the four most aggressive clinicopathological parameters, including distant metastasis, disease stage III/IV, cancer recurrence and patient mortality, are all far more commonly and significantly associated with *TERT* promoter mutations than other clinicopathological parameters (Table 4 and Fig. 2). An analysis focused on PTC, the most common thyroid cancer, revealed similar results on the genetic–clinicopathological relationship as on the overall analysis of all cancers (Table 4). Analyses on other individual types of thyroid cancer showed mostly also a significant association between *TERT* promoter mutations and aggressive clinicopathological outcomes, particularly

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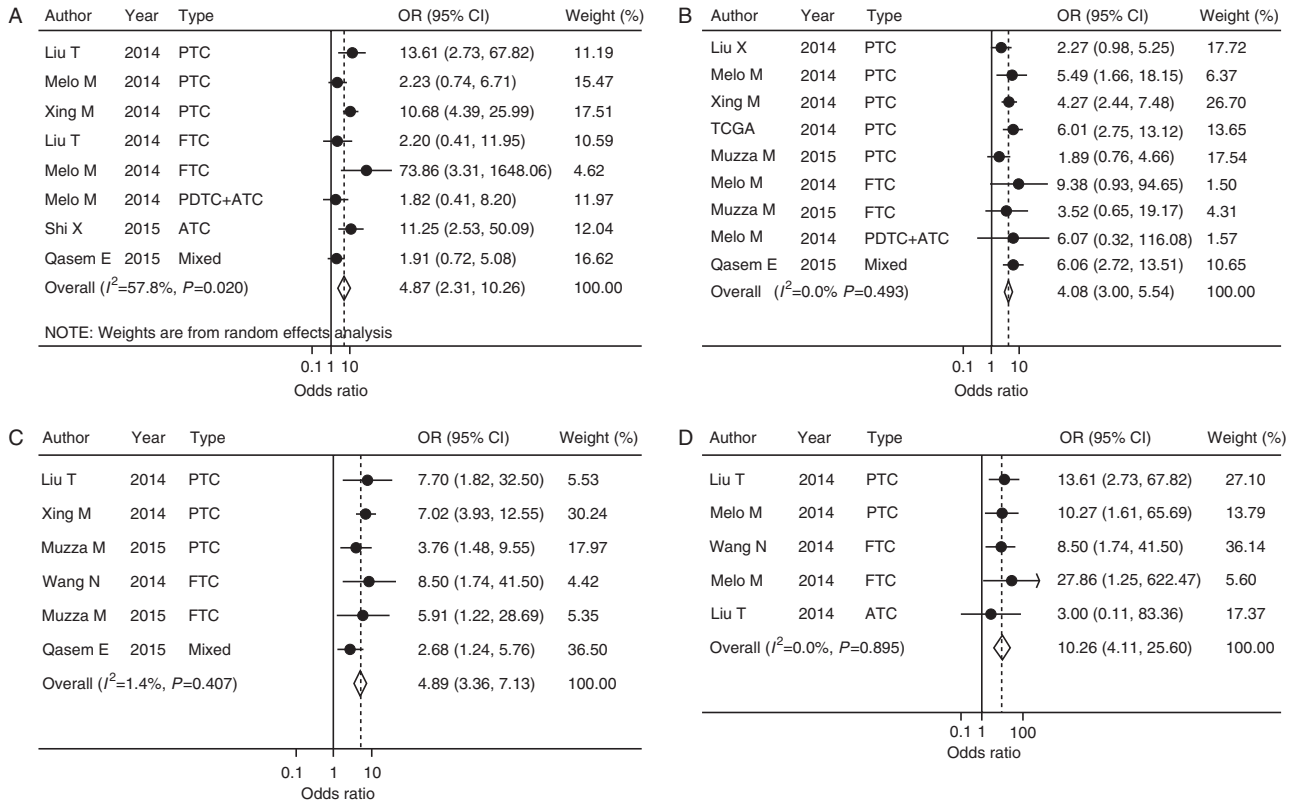

Figure 2

Meta-analysis of the association of *TERT* promoter mutations with thyroid cancer distant metastasis (A), tumor stage III/IV (B), tumor recurrence (C) and patients mortality (D). The between-study heterogeneity and combined OR were calculated as described in the legend to Fig. 1. The circles and horizontal lines correspond to the study-specific OR and 95% CI. The combined ORs and their 95% CIs are indicated by the diamonds. PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer. OR, odds ratio.

distant metastasis, disease stage III/IV, tumor recurrence and patient mortality, although in some cases just an association trend was seen, likely due to the small numbers of cases (Supplemental Table S1, see section on supplementary data given at the end of this article). Taken together, these data strongly suggest that *TERT* promoter mutations play an important role in the aggressiveness of thyroid cancer and are strong predictors for poor clinical outcomes of thyroid cancer. This conception is also consistent with our initial report that *TERT* promoter mutations were far more common in the aggressive thyroid cancers PDTC and ATC than the generally indolent differentiated PTC and FTC (Liu *et al*. 2013*b*), which have been confirmed in many subsequent reports (Tables 1 and 2). Interestingly, the *TERT* promoter mutation prevalence order of TCPTC≫CPTC>FVPTC (Table 3) is similar to the clinicopathological aggressiveness order of the three PTC variants reported recently (Shi *et al*. 2016), further supporting a role of *TERT* promoter mutations in the development of aggressiveness of thyroid cancer.

Association of *TERT* promoter mutations with *BRAF* V600E mutation in thyroid cancer

BRAF V600E is the most common oncogene in thyroid cancer that plays a fundamental role in the tumorigenesis and progression of thyroid cancer, particularly PTC (Xing 2005, 2007). Our initial study on *TERT* promoter mutations in thyroid cancer also reported an interesting association of *TERT* promoter mutations with the *BRAF* V600E mutation in PTC (Liu *et al*. 2013*b*). This finding was confirmed in many subsequent studies. As summarized in Table 5, on the overall analysis of the large pooled data, *TERT* promoter mutations are found in 146/1 294 (11.3%) *BRAF* V600E-negative cases vs 197/1 104 (17.8%) *BRAF* V600E-positive cases; inversely, *BRAF* V600E is found in 907/2 055 (44.1%) *TERT* mutation-negative cases vs 97/343 (57.4%) *TERT* mutation-positive cases, with an OR of 2.46 (95% CI, 1.89–3.20; $P<0.001$; Fig. 3). Similar results are obtained when analyses are performed only on PTC or advanced types of thyroid cancer, PDTC and ATC

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Table 5 Association of *TERT* promoter mutations with *BRAF* V600E mutation and in thyroid cancer

		TERT mutation		BRAF mutation			
Cancer type	**Study No.**	BRAF −	BRAF +	TERT −	TERT +	***P***	**Reference**
PTC	1	11/153 (7.2)	19/104 (18.3)	85/227 (37.4)	19/30 (63.3)	0.009	Liu *et al.* (2013*b*)
	2	6/12 (50.0)	2/17 (11.8)	15/21 (71.4)	2/8 (25.0)	0.038	Landa *et al.* (2013)
	3	1/93 (1.1)	12/65 (18.5)	53/145 (36.6)	12/13 (92.3)	<0.001	Vinagre *et al.* (2013)
	4	6/158 (3.8)	40/250 (16.0)	210/362 (58.0)	40/46 (87.0)	<0.001	Liu *et al.* (2014*b*)
	5	2/19 (10.5)	11/32 (34.4)	21/38 (55.3)	11/13 (84.6)	0.096	Liu *et al.* (2014*a*)
	6	6/153 (3.9)	18/148 (12.2)	130/277 (46.9)	18/24 (75.0)	0.008	Melo *et al.* (2014)
	7	26/313 (8.3)	35/194 (18.0)	159/446 (35.7)	35/61 (57.4)	0.001	Xing *et al.* (2014*a*)
	8	10/161 (6.2)	26/223 (11.7)	197/348 (56.6)	26/36 (72.2)	0.071	TCGA (2014)
	9	0/10 (0)	3/11 (27.3)	8/27 (29.6)	3/3 (100)	0.041	Crescenzi *et al.* (2015)
	10	12/118 (10.2)	10/64 (15.6)	54/160 (33.8)	10/22 (45.5)	0.281	Muzza *et al.* (2015)
	Subtotal	68/953 (7.1)	145/939 (15.4)	794/1679 (47.3)	145/213 (68.1)	<0.001	
PDTC & ATC	2	21/49 (42.9)	19/29 (65.5)	10/38 (26.3)	19/40 (47.5)	0.053	Landa *et al.* (2013)
	3	1/11 (9.1)	1/5 (20.0)	4/14 (28.6)	1/2 (50.0)	1.000	Vinagre *et al.* (2013)
	6	17/55 (30.9)	4/10 (40.0)	6/44 (13.6)	4/21 (19.0)	0.715	Melo *et al.* (2014)
	11	28/90 (31.1)	9/16 (56.3)	7/69 (10.1)	9/37 (24.3)	0.052	Shi *et al.* (2015)
	Subtotal	66/194 (34.0)	32/55 (58.2)	23/151 (15.2)	32/98 (32.7)	0.001	
Mixed	12	12/147 (8.2)	20/110 (18.2)	90/225 (40.0)	20/32 (62.5)	0.016	Qasem *et al.* (2015)
Overall		146/1294 (11.3)	197/1104 (17.8)	907/2055 (44.1)	197/343 (57.4)	<0.001	

Because of the same investigator origin, there are potential overlaps of cases between Study Nos 1 and 7 and between Study Nos 3 and 6. The data of Study Nos 1 and 3 were excluded from the pooled analyses. *P* value was calculated by χ^2 test or, for small cell sizes, Fisher's exact test. PTC, papillary thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer.

(Table 5 and Fig. 3). Specifically, for PTC, *TERT* promoter mutations are found in 68/953 (7.1%) *BRAF* V600E-negative cases vs 145/939 (15.4%) *BRAF* V600E-positive cases; inversely, *BRAF* V600E is found in 794/1 679 (47.3%) *TERT* mutation-negative cases vs 145/213 (68.1%) *TERT* mutation-positive cases, with an OR of 2.37 (95% CI, 1.38–4.05; $P<0.001$; Fig. 3). For PDTC/ATC, *TERT* promoter mutations are found in 66/194 (34.0%) *BRAF* V600E-negative cases vs 32/55 (58.2%) *BRAF* V600E-positive cases; inversely, *BRAF* V600E is found in 23/151 (15.2%) *TERT* mutation-negative cases vs 32/98 (32.7%) *TERT* mutation-positive cases, with an OR of 2.36 (95% CI, 1.26–4.44; $P=0.008$; Fig. 3). One exception to the highly positive association between *TERT* promoter mutations and *BRAF* V600E mutation reported in many studies and confirmed in this meta-analysis is the study by Landa *et al.* (2013) in which the authors reported an inverse relationship between *TERT* promoter mutations and the *BRAF* V600E mutation. It is not clear how to interpret this result, but this is the only study that has reported an inverse relationship between the two mutations. Interestingly, several studies also reported an association between *TERT* promoter mutations and the *BRAF* V600E mutation in melanoma (Horn *et al.* 2013, Griewank *et al.* 2014), suggesting that such a relationship between the two oncogenic genetic events may be a general phenomenon in human cancer.

We have demonstrated in a large study that coexistence of *BRAF* V600E and *TERT* promoter mutations constitutes a unique genetic background that drives particularly aggressive pathogenesis and poor clinical outcomes of PTC (Xing *et al.* 2014*a*). In this study, when the PTC patients were divided into four groups – no mutation, *BRAF* V600E mutation alone, *TERT* promoter mutation alone and coexistence of the two mutations, the group with both mutations was far more robustly associated with virtually all the conventional high-risk factors, such as large-tumor size, lymph-node metastasis, extrathyroidal invasion, vascular invasion, distant metastasis and advanced disease stages III/IV than other groups. *BRAF* V600E and *TERT* promoter mutations each alone in fact had a modest effect and there was a strong incremental effect of the coexisting mutations over either mutation alone. This was even more clearly the case with the structural tumor recurrence patterns of PTC. Specifically, the recurrence of PTC was 8.7, 16.3, 19.2 and 68.6% in the above defined corresponding four groups respectively over a median follow-up time of two years in 507 patients; these corresponded to hazard ratios (95% CI) of 1.0, 2.24 (1.29–3.88), 1.69 (0.65–4.43) and 8.51 (4.84–14.97) respectively (Xing *et al.* 2014*a*). We also demonstrated a similar robust synergistic impact of coexisting *BRAF* and *TERT* promoter mutations on disease-specific mortality of patients with PTC in 607 patients (Xing *et al.* 2014*b*).

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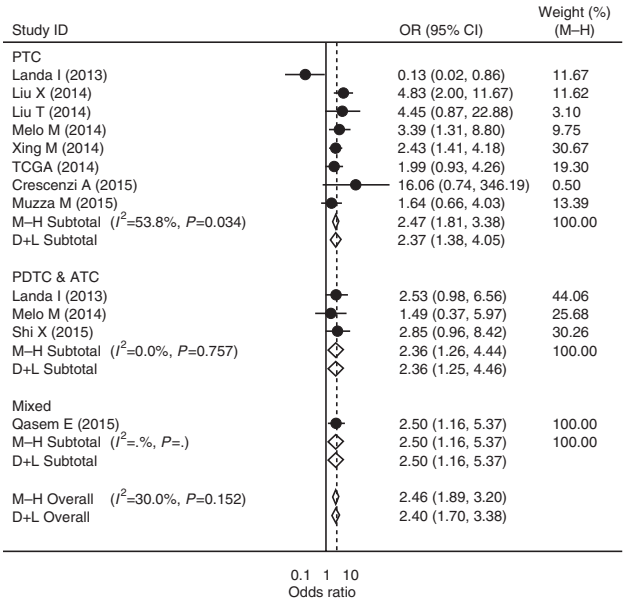

Figure 3
Meta-analysis of the association between *BRAF* V600E and *TERT* promoter mutations in thyroid cancer. The between-study heterogeneity and combined OR were calculated as described in Fig. 1. The circles and horizontal lines correspond to the study-specific OR and 95% CI. The combined ORs and their 95% CIs are indicated by the diamonds. PTC, papillary thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer. OR, odds ratio.

The effect of *BRAF* V600E mutation and *TERT* promoter mutation each alone on the mortality was also modest. In these analyses, the effect of coexisting *BRAF* V600E and *TERT* promoter mutations on PTC recurrence and patient mortality remained strongly significant even after the multivariate adjustment for multiple conventional risk factors and was incremental over either mutation alone. These results suggest that, indeed, *BRAF* and *TERT* team up for trouble in thyroid cancer as commented in a recent commentary by Ngeow & Eng (2014). These results also suggest that the role of *BRAF* V600E mutation previously observed and the role of *TERT* promoter mutations discussed above in the aggressiveness of thyroid cancer are substantially attributable to the synergistic effect of the coexisting two mutations. One potential molecular mechanism to explain this synergistic effect of coexisting *BRAF* V600E and *TERT* promoter mutations is that, as discussed previously (Liu *et al*. 2013*b*, Xing *et al*. 2014*a*), the *BRAF* V600E-acctivated MAP kinase pathway upregulates the ETS transcriptional factors; the latter can then avidly bind to the consensus binding site in the *TERT* promoter created by C228T or C250T mutation to robustly upregulate the expression of *TERT*; highly expressed TERT would play a profound tumor-promoting role in thyroid cancer as in other human cancers as discussed above. As *BRAF* V600E has been shown to play an important role in the oncogenic extracellular micro environmental changes in the pathogenesis of thyroid cancer (Nucera *et al*. 2010, 2011, Nucera 2013), it remains an interesting possibility to be explored that the synergistic effects of *BRAF* V600E and *TERT* promoter mutations on the progression and aggressiveness of thyroid cancer may involve tumor invasion-promoting molecular changes in the micro environments of thyroid cancer.

Association of *TERT* promoter mutations with *RAS* mutations in thyroid cancer

RAS mutations are major oncogenic genetic alterations and commonly occur in thyroid cancer, particularly in FTC, PDTC and ATC (Xing 2013). Several studies investigated the relationship between *RAS* mutations and *TERT* promoter mutations in thyroid cancers (Table 6). One individual study on FTC showed a significant association

Table 6 Association of *TERT* promoter mutations with *RAS* mutations in thyroid cancer

		***TERT* mutation**		***RAS* mutation**				
Cancer type	**Study No.**	RAS −	RAS +	TERT −	TERT +	***P***	**OR (95% CI)**	**Reference**
FTC	1	4/50 (8.0)	5/14 (35.7)	9/55 (16.4)	5/9 (55.6)	0.019	6.39 (1.43–28.53)	Vinagre *et al*. (2013)
	2	7/50 (14.0)	4/14 (28.6)	10/53 (18.9)	4/11 (36.4)	0.237	2.46 (0.60–10.05)	Melo *et al*. (2014)
	3	6/44 (13.6)	2/14 (14.3)	12/50 (24.0)	2/8 (25.0)	1.000	1.06 (0.19–5.94)	Muzza *et al*. (2015)
	Subtotal	13/94 (13.8)	6/28 (21.4)	22/103 (21.4)	6/19 (31.6)	0.330	1.71 (0.58–5.02)*	
PDTC & ATC	1	4/29 (13.8)	1/1 (100)	0/25 (0)	1/5 (20.0)	0.167	17.00 (0.59–486.41)	Vinagre *et al*. (2013)
	2	13/44 (29.5)	8/17 (47.1)	9/40 (22.5)	8/21 (38.1)	0.197	2.12 (0.67–6.71)	Melo *et al*. (2014)
	4	26/58 (44.8)	14/20 (70.0)	6/38 (15.8)	14/40 (35.0)	0.052	2.87 (0.97–8.52)	Landa *et al*. (2013)
	Subtotal	39/102 (38.2)	22/37 (59.5)	15/78 (19.2)	22/61 (36.1)	0.026	2.50 (1.14–5.50)†	
Overall		52/196 (26.5)	28/65 (43.1)	37/181 (20.4)	28/80 (35.0)	0.012	2.21 (1.18–4.15)‡	

Because of the same investigator origin, there are potential overlaps of cases between Study Nos 1 and 2. The data of Study No. 1 was excluded from the pooled analyses. The combined odds ratio (OR) and 95% CI were calculated by meta-analysis. *P* value was calculated by χ^2 test or, for small cell sizes, Fisher's exact test. *P=0.327; †P=0.022; ‡P=0.014. FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer.

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between *TERT* promoter mutations and *RAS* mutations (Vinagre *et al*. 2013). The remaining studies individually showed a trend of such association. The pooled data of PDTC and ATC show a significant association between *TERT* promoter mutations and *RAS* mutations, with an OR of 2.50 (95% CI, 1.14–5.50, $P=0.022$). When all the thyroid cancers are pooled together, this association remains significant, with an OR of 2.21 (95% CI, 1.18–4.15) and an even more significant *P* value of 0.014 (Table 6). The biological and clinical significance of this association remains to be investigated. It is possible that, like the coexisting *TERT* promoter mutations and *BRAF* mutations in PTC, coexisting *TERT* promoter and *RAS* mutations may also play a cooperative role in thyroid tumorigenesis and promote tumor aggressiveness and poor clinical outcomes of thyroid cancer. *BRAF* V600E synergizes the role of *TERT* promoter mutations through activating the MAP kinase pathway. *RAS* mutations likely synergize the role of *TERT* promoter mutations through activating the PI3K pathway since *RAS* mutations preferentially activate this pathway over the MAP kinase pathway in thyroid cancer (Xing 2013). As such, coexistence of *TERT* promoter mutations and *RAS* mutations may represent a unique genetic background that is particularly important in FTC, PDTC and ATC in which *RAS* mutations are particularly common and the PI3K pathway plays a particularly important role (Saji & Ringel 2010, Xing 2010, 2013). These remain to be further investigated and established in the future.

Conclusion

It has been <3 years since the initial report on *TERT* promoter mutations in thyroid cancer (Liu *et al*. 2013*b*), while substantial progress has occurred in this exciting new field. Much has been known about the biological and clinical relevance of these mutations in thyroid cancer in this short time. Studies from various populations and regions in the world uniformly found *TERT* promoter mutations to be present in thyroid cancers, but not benign thyroid tumors, and be more common in aggressive types of thyroid cancers. These mutations are also more commonly associated with aggressive tumor behaviors and poor clinical outcomes, including tumor recurrence and patient mortality. A particular interesting and important aspect of *TERT* promoter mutations in PTC is their association with the *BRAF* V600E mutation and the robust synergistic impact of the coexisting two mutations on aggressive clinicopathological outcomes of PTC, particularly tumor recurrence and patient mortality. These results are consistent with the proposed model in which *TERT* promoter mutations create consensus binding sites for ETS transcriptional factors for the latter to activate the expression of TERT, a process that can be upregulated by the *BRAF* V600E/MAP kinase signaling pathway. A similar synergistic effect between *TERT* promoter mutations and *RAS* mutations, likely through activating the PI3K pathway, may also exist in thyroid cancer. These clinicopathological data strongly support a prominent role of *TERT* promoter mutations in the tumorigenesis and progression of thyroid cancer, which is well corroborated by previous results on similar differential expression patterns of TERT in benign and malignant thyroid tumors. As such, *TERT* promoter mutations are promising diagnostic and prognostic genetic markers for thyroid cancer, which is highly applicable clinically as demonstrated recently by applying them to thyroid fine needle aspiration biopsy for such purposes (Liu & Xing 2014, Nikiforov *et al*. 2014, Shrestha *et al*. 2015). Future studies will need to more specifically define such clinical utilities, elucidate the biological mechanisms of the role of *TERT* promoter mutations in thyroid tumorigenesis, and explore and establish the therapeutic utilities of targeting TERT for thyroid cancer.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-15-0533.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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