TERT promoter mutations in thyroid cancer: a report from a Middle Eastern population

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Abstract

Telomerase reverse transcriptase (*TERT*) promoter mutations C228T and C250T have recently been described in follicular cell-derived thyroid cancer (TC) in patients from North America and Europe. In this study, we explored whether these findings could be replicated in patients from a different ethnic group. We screened 17 benign thyroid adenomas and 265 TC samples from patients in the Middle East for these mutations by PCR and direct sequencing using DNA isolated from paraffin-embedded tumor tissues. None of the 17 benign adenomas harbored *TERT* promoter mutations. Of 265 TC, 34 (12.8%) harbored *TERT* promoter mutations, including 10/153 (6.5%) conventional papillary TC (CPTC), 8/57 (14.0%) follicular variant PTC, 9/30 (30%) tall cell variant PTC, 1/3 (30%) Hurthle cell thyroid cancer (HTC), 1/5 (20%) follicular TC, and 5/13 (38.5%) poorly differentiated TC. C250T mutation was present in only 6/265 (2.3%) cases, while C228T mutation was present in a total of 28/265 (10.6%) cases. These two mutations were mutually exclusive. *TERT* promoter mutations were significantly more common in older (≥45 years) than younger patients and were associated with larger tumour size, vascular invasion, higher TNM stage (stage III and IV), $BRAF^{V600E}$ mutation and persistent/recurrent disease at 6–12 months after initial treatment and at the last follow up. These associations were stronger in non-CPTC. Thus, this study on a large cohort of TC patients from Middle East demonstrates that *TERT* promoter mutations are relatively common, especially in the non-CPTC, and are associated with more aggressive histopathological features, $BRAF^{V600E}$ mutation, and disease persistence/recurrence than the WT *TERT*.

Key Words
- thyroid
- carcinoma

Endocrine-Related Cancer (2015) **22**, 901–908

Introduction

Differentiated thyroid cancer (DTC) is the most common endocrine malignancy (Jemal *et al.* 2011). Its incidence has been rapidly increasing over the last four decades (Jemal *et al.* 2011, 2013, Jung *et al.* 2014). Although the widespread use of neck ultrasonography (US) has contributed to the discovery of an increasing number of cases of small DTC that would otherwise remain undiagnosed, some data have suggested a true increase in the incidence of this cancer (Enewold *et al.* 2009).

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-15-0396

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Major progress in our understanding of the molecular pathogenesis of DTC has taken place over the last decade (Xing 2013). The contribution of genetic mutations in the MAPK and phosphatidylinositide 3-kinases (PI3KCa)-AKT pathways in the pathogenesis of DTC are well established, and new pathways and genes are being discovered as additional drivers for the initiation and progression of DTC (Agrawal *et al*. 2014). These new genetic aberrations include mutations in the telomerase reverse transcriptase (*TERT)* promoter (Landa *et al*. 2013, Liu *et al*. 2013, 2014*a*, Vinagre *et al*. 2013, Liu & Xing 2014, Melo *et al*. 2014, Ngeow & Eng 2014, Wang *et al*. 2014*a*, Xing *et al*. 2014, Gandolfi *et al*. 2015). TERT is the catalytic subunit of telomerase which is composed of a ribonucleoprotein complex that is responsible for adding telomeric fragments to chromosomes, maintaining the telomere length that usually shortens over time in the dividing somatic cells (Smekalova *et al*. 2012). This mechanism plays a major role in cellular immortality (Smekalova *et al*. 2012, Mocellin *et al*. 2013). The activity of TERT varies between different cells, but it is frequently up-regulated in many types of cancer cells (Smekalova *et al*. 2012, Mocellin *et al*. 2013). This seems to provide cancer cells with increasing survival advantage and contributes to their extended division and survival (Blackburn 2005). Although mutations in the coding regions of the *TERT* gene are rare, *TERT* promoter mutations (C288T and C250T) have recently been described in many types of human cancers, including the follicular cell-derived TC (Landa *et al*. 2013, Liu *et al*. 2013, Vinagre *et al*. 2013). They were also found to be associated with more aggressive tumors and with high rate of $BRAF^{V600E}$ mutation (Landa *et al*. 2013, Liu *et al*. 2013, 2014*a*,*b*, Liu & Xing 2014, Melo *et al*. 2014, Wang *et al*. 2014*a*, Xing *et al*. 2014, Gandolfi *et al*. 2015, Shi *et al*. 2015). These studies have been mostly conducted in North American and European populations. The frequency and significance of *TERT* promoter mutations in follicular cell-derived TC have not been well studied in other ethnic groups. In this study, we report the prevalence and prognostic value of *TERT* mutations in a large series of patients with TC from the Middle East.

Patients and methods

Patients

We studied 282 surgically removed thyroid tumors obtained from 265 non-selected patients with TC and 17 patients with benign multinodular goitre seen during the period January 2008 to November 2011 at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. The TC patients comprised 201 females (75.8%) and 64 males (24.2%) with a median age of 34 years (range, 11–78 years) at the initial treatment. The tumors included 153 (57.7%) conventional papillary TCs (CPTCs), 57 (21.1%) follicular variant PTCs (FVPTC), 30 (11.3%) tall-cell PTCs (TC-PTC), 4 (1.5%) diffuse sclerosing type TCs, 3 (1.1%) Hurthle cell cancer, 5 (1.9%) follicular TCs (FTCs), and 13 (4.9%) poorly DTCs (PDTCs). Data were extracted from the medical and pathological reports of the patients, and the study protocol was approved by the Institutional Review Board. All patients with DTC underwent near-total or total thyroidectomy, and 227 of them (85.7%) underwent unilateral or bilateral neck dissection. Two hundred and 43 patients (91.7%) underwent radioactive iodine (RAI) ablation 6–12 weeks after thyroidectomy with a median dose of 122 mCi (range, 29–210). The patients were treated with L-thyroxine suppression and followed regularly every 6–12 months. TNM staging revealed that 179 patients (67.5%) were in stage I, 26 (9.8%) in stage II, 18 (6.8%) in stage III, and 34 (12.8%) in stage IV. Eight (3%) cases were non-stageable due to incomplete pathological information. Remission was defined by stimulated thyroglobulin (Tg) level of ≤ 1 ng/dl in the absence of anti-Tg autoantibodies and negative neck US and other imaging studies (when done). When Tg was undetectable but anti-Tg autoantibody levels were elevated, we relied on the findings of US, diagnostic radioiodine whole body scan (DxWBS) and other imaging studies. Persistence was defined by any or a combination of the following findings: a stimulated Tg level of >1 ng/dl, positive US-guided fine-needle aspiration (FNA), or positive radioiodine body scan when performed. Recurrence was considered similar to persistence after at least a 6-month period of remission. Patients in whom the status could not be determined due to inadequate data or elevated Tg levels in the presence of elevated Tg autoantibodies were excluded from the analysis of outcome.

DNA isolation, PCR and direct sequencing

The pathological specimen from each patient was examined by an experienced endocrine pathologist (H A), and the tumor tissue was carefully dissected from formalin-fixed paraffin-embedded tissue blocks. We did not exclude patients with concomitant Hashimoto's thyroiditis or colloid goiter but the dissection was carefully performed to ensure that the specimens contain tumor tissue only with minimal non-tumor tissue. DNA was extracted using a commercial DNA extraction kit (QIAamp DNA FFPE Tissue Kit, Qiagen, Catalog No. 56404)

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-15-0396

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according to the manufacturer's instructions. DNA was quantified using a nanodrop2000 spectrophotometer (Thermo Scientific, Willmington, DE, USA) and DNA purity was assured by the A260/280 ratio, with a ratio of ≥1.8 indicating good purity. We used PCR and direct sequencing using Big Dye terminator v3.1 cycle-sequencing reaction kit and an ABI PRISM 3730Xl genetic analyzer (Applied Biosystems) to detect *TERT* promoter mutations using the same primers and PCR conditions described previously (Liu *et al*. 2013). $BRAF^{V600E}$ mutation was examined by amplifying and sequencing exon 15 of the *BRAF* gene using previously described primers and PCR conditions (Liu *et al*. 2013).

Statistical analysis

The Statistical Package for Social Sciences version 20 (IBM SPSS Statistics, Armonk, NY, USA, IBM Corp.) was used to analyze the data. Continuous data were summarized as median and range or mean±S.D., and the *t* test was used for significance analysis. Categorical data were expressed as numbers and percentages or ratios and Fisher exact and χ^2 tests were used for the significance analysis. A two-tailed *P* value <0.05 was considered significant.

Results

Prevalence of *TERT* promoter mutations

The initial characteristics of the TC patients included in this study are summarized in Table 1. None of the 17 benign multinodular goiters harbored *TERT* promoter mutations. Overall, C250T and C228T mutations were collectively found in 34 out of 265 cases of TC (12.8%). This included 10/153 (6.5%) CPTCs, 8/57 (14.0%) FVPTCs, 9/30 (30%) TC-PTCs, 1/3 (30%) Hurthle cell thyroid cancers (HTCs) 1/5 (20%) FTCs and 5/13 (38.5%) PDTCs (Table 1). The C250T mutation was present in only six cases (2.3%), including 3/153 CPTC (2%), 2/57 FVPTC (3.5%) and 1/30 (3.3%) TC-PTC (Table 2). The C228T mutation was present in a total of 28/265 (10.6%) cases, including 7/153 (4.6%) CPTC, 6/57 (10.5%) FVPTC, 8/30 (26.7%) TC-PTC, 1/3 (33.3%) HTC, 1/5 (20%) FTC and 5/13 (38.5%) PDTC (Table 2). These two mutations were mutually exclusive.

Association of *TERT* promoter mutations with poor clinicopathological outcomes

TERT mutations were more common in older (≥45 years) than younger patients (19/77 (24.7%) vs 15/188 (8%), *P*<0.0001) (Table 3). The mean±S.D. age was 50.4±16.7 years for patients with *TERT* mutations vs 33.7±15.7 years for patients with WT *TERT* (*P*<0.0001). Compared to patients with WT *TERT*, those with *TERT* promoter mutations showed larger tumor size (4.75±2.6 cm vs 3.2±2.2 cm, *P*<0.0001), more frequent vascular invasion (56.2% vs 30.8%, *P*=0.009), higher TNM stage (stage III and IV) (53.3% vs 15.9%, *P*<0.0001) and more common persistent/recurrent disease at 6–12 months after RAI remnant ablation (71.4% vs 50.7%, *P*=0.045) and at the last follow-up visits (58.1% vs 34.1%, *P*=0.017) (Table 3). However, *TERT* promoter mutations were not associated with gender, tumor multifocality, tumor extrathyroidal extension, lymph node metastasis, distant metastasis and the need for additional therapeutic interventions after the initial management (Table 3). The number of foci in the multifocal TC was not different between those with *TERT* promoter mutations (median 2, range 2–5 foci) and those with WT *TERT* (median 3, range 2–4 foci) (*P*=0.31).

When we restricted the analysis to CPTC only, none of the preceding factors were associated with *TERT* promoter mutations (Table 4). However, the total number of *TERT* promoter mutations in CPTC is small (10/153, 6.5%), and this lack of association might be due to the small number of cases with positive *TERT* promoter mutations (Table 2). Similarly, when the analysis was

Table 1 Characteristics of 265 patients with thyroid cancer

Characteristic	Number (%)
Median age (range), years	34 (11–78)
Gender (female:male)	201:64
Tumour size, median (range) cm	3.0 (0.9–13)
Tumour multifocality	119 (46.5)
Extrathyroidal invasion	120 (47.4)
Vascular Invasion	80 (34.3)
Lymph node metastasis	136 (61)
Distant metastasis	30 (11.4)
TNM stage[a]	
I	179 (67.5)
II	26 (9.8)
III	18 (6.8)
IV	34 (12.8)
Radioactive iodine remnant ablation	224 (84.5)
Radioactive iodine administered activity	109.8±52.3
Additional therapeutic intervention	79 (29.8)
Persistent disease at 6–12 months after RAI	130 (53.3)
Persistent disease at the last follow up	93 (37.1)
$BRAF^{V600E}$ mutation	110 (41.5)
C228T *TERT* promotor mutation	28 (10.6)
C250T *TERT* promotor mutation	6 (2.3)
Both *TERT* promotor mutations	34 (12.8)

[a]Data of eight cases were inadequate to allow staging.

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DOI: 10.1530/ERC-15-0396

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Table 2 Prevalence of *TERT* promotor and $BRAF^{V600E}$ mutations in different subtypes of TC

Tumor subtype	Total no.	C228T	C250T	C228T+C250T	$BRAF^{V600E}$
CPTC	153	7 (4.6)	3 (2)	10 (6.5)	63 (41.2)
FVPTC	56	5 (8.9)	2 (3.6)	7 (12.5)	13 (23.2)
TC-PTC	30	8 (26.7)	1 (3.3)	9 (30)	28 (93.3)
DS-PTC	4	0	0	0	1 (25)
PDTC	14	6 (42.9)	0	6 (42.9)	5 (35.7)
HTC	3	1 (33.3)	0	1 (33.3)	0
FTC	5	1 (20)	0	1(20)	0
Total	265	28 (10.6)	6 (2.3)	34 (12.8)	110 (41.5)

CPTC, conventional papillary thyroid cancer; FVPTC, follicular variant PTC; TC-PTC, tall cell PTC; DS-PTC, diffuse sclerosing type PTC; PDTC, poorly differentiated thyroid cancer; HTC, Hurthle cell thyroid cancer; FTC, follicular thyroid cancer.

limited to FVPTC, no association with any of the preceding factors was noted (Table 4). On the other hand, when the analysis was limited to all other types of TC (excluding CPTC), *TERT* promoter mutations were associated with age, vascular invasion, high TNM stage, and persistent/recurrent disease at 6–12 months after the initial treatment and at the last follow-up visit (Table 3).

Association of *TERT* promoter mutation with $BRAF^{V600E}$ mutations

$BRAF^{V600E}$ mutation was more common in patients with *TERT* mutations (20/34, 58.8%) than in those without *TERT* promoter mutations (90/231, 39%; $P=0.045$). Similarly, *TERT* promoter mutations were more common in patients with $BRAF^{V600E}$ mutations (20/110, 18.2%) than in those with WT $BRAF^{V600E}$ (14/155, 9%; $P=0.045$). As reported previously, $BRAF^{V600E}$ mutation itself was associated with extrathyroidal invasion, distant metastasis and higher TNM stage (data not shown).

Discussion

TERT promoter mutations have recently been reported to be common in DTC in patients from North America and Europe (Landa *et al*. 2013, Liu *et al*. 2013, Vinagre *et al*. 2013, Wang *et al*. 2014*a*). Studies from these regions have shown that *TERT* promoter mutations are associated with more aggressive histopathological features and worse outcomes (Landa *et al*. 2013, Liu *et al*. 2013, 2014*a*,*b*, Vinagre *et al*. 2013, Liu & Xing 2014, Melo *et al*. 2014, Ngeow & Eng 2014, Wang *et al*. 2014*a*, Xing *et al*. 2014, Gandolfi *et al*. 2015, Shi *et al*. 2015). Similar and consistent findings from other ethnic groups would support these conclusions and firmly establish the association between *TERT* promoter mutations and the risk of aggressiveness of DTC. This was the rationale for undertaking this study. Indeed, we found that *TERT* promoter mutations were similarly common in our patients and that they were also associated with more aggressive histopathological features and worse outcomes of TC (Tables 2 and 3).

Table 3 The association between *TERT* promotor mutations and different demographic, histopathological features and TC outcome

	All types of TC			All types of TC except CPTC		
Characteristic	*TERT*+	*TERT*−	*P* value	TERT+	TERT−	*P* value
Age	50.4±16.8	33.7±15.7	<0.0001	55.0±13.6	37.4±14.9	<0.0001
Sex (M:F)	11:23	53:178	0.33	8:17	24:78	0.53
Tumour size (cm)	4.75±2.6	3.2±2.2	<0.0001	5.2±2.2	3.6±2.5	0.005
Tumour multifocality	11/34 (32.4)	108/222 (48.6)	0.11	8/25 (32.0)	42/100 (42.0)	0.49
Extrathyroidal invasion	17/33 (51.5)	103/220 (46.8)	0.75	13/24 (54.2)	41/98 (41.8)	0.39
Vascular invasion	18/32 (56.2)	62/201 (30.8)	0.009	15/23 (65.2)	33/94 (35.1)	0.017
Lymph node metastasis	13/23 (56.5)	123/200 (61.5)	0.81	8/16 (50.0)	38/85 (44.7)	0.91
Distant metastasis	6/33 (18.2)	24/230 (10.4)	0.31	6/24 (25.0)	13/101 (12.9)	0.24
High TNM stage (III and IV)	16/30 (53.3)	36/227 (15.9)	<0.0001	14/21 (66.7)	20/101 (19.8)	<0.0001
Persistent/recurrent disease at 6–12 months	21/29 (72.4)	109/215 (50.7)	0.045	17/20 (85.0)	43/92 (46.7)	0.004
Additional therapeutic interventions	12/31 (38.7)	67/223 (30.0)	0.44	10/22 (45.5)	23/96 (24.0)	0.08
Persistent/recurrent disease at last follow up	18/31 (58.1)	75/220 (34.1)	0.017	15/22 (68.2)	29/96 (30.2)	0.002

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DOI: 10.1530/ERC-15-0396

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Table 4 The association between *TERT* promotor mutations and different demographic, histopathological features and outcome of CPTC and FVPTC

Characteristic	CPTC only			FVPTC only		
	TERT+	*TERT*−	*P* value	TERT+	TERT−	*P* value
Age	37.4±18.7	30.8±15.7	0.23	49.0±14.6	37.7±14.5	0.059
Sex (M:F)	3:6	29:100	0.74	1:6	9:40	1.0
Tumour size (cm)	3.7±3.5	2.8±1.8	0.20	4.9±2.9	3.5±2.5	0.21
Tumour multifocality	3/9 (33.3)	66/122 (54.1)	0.39	2/7 (28.6)	19/48 (39.6)	0.70
Extrathyroidal invasion	4/9 (44.4)	62/122 (50.8)	0.98	1/7 (14.3)	13/48 (27.1)	0.66
Vascular invasion	3/9 (33.3)	29/107 (27.1)	0.99	4/7 (57.1)	10/46 (21.7)	0.07
Lymph node metastasis	5/7 (71.4)	85/115 (73.9)	1.0	2/5 (40.0)	10/38 (26.3)	0.61
Distant metastasis	0/9 (0)	11/129 (8.5)	1.0	1/7 (14.3)	6/48 (12.5)	1.0
High TNM stage (III and IV)	2/9 (22.2)	16/126 (12.7)	0.34	3/7 (42.9)	8/48 (16.7)	0.13
Persistent/recurrent disease at 6–12 months	4/9 (44.4)	66/123 (53.7)	0.73	2/5 (40.0)	19/45 (42.2)	1.0
Additional therapeutic interventions	12/31 (38.7)	67/223 (30.0)	0.44	0/6 (0)	8/47 (17.0)	0.57
Persistent/recurrent disease at last follow up	2/9 (22.2)	44/127 (34.6)	0.72	2/6 (33.3)	13/46 (28.3)	1.0

TERT promoter mutations were initially found to occur in melanoma, CNS and bladder tumors (Horn *et al.* 2013, Huang *et al.* 2013, Vinagre *et al.* 2013). Previous studies screening different types of tumors for *TERT* promoter mutations have shown that the rates of these mutations were 59, 43, 29 and 10% in bladder, CNS, melanoma and thyroid tumors respectively (Vinagre *et al.* 2013). Liu *et al.* (2013) studied *TERT* promoter mutations in different subtypes of TCs and thyroid cell lines and found that the two mutations occurred in 13.9, 46.3 and 91.7% of the samples of FTC, ATC and thyroid cell lines respectively. C250T mutation was much less common, but both mutations were mutually exclusive. The C228T mutation was found in 12.3% of CPTCs, 3.6% of FVPTCs, 30.8% of TC-PTC samples and in none of 16 medullary TC samples. $BRAF^{V600E}$ mutation was more frequent in cases with C228T mutations than in those without mutation. Several subsequent studies from North America and Europe showed that *TERT* promoter mutations occurred in about 10–15% TCs (Landa *et al.* 2013, Vinagre *et al.* 2013, 2014, Fredriksson *et al.* 2014, Liu *et al.* 2014*a*, Melo *et al.* 2014, Wang *et al.* 2014*a*,*b*, Gandolfi *et al.* 2015, Muzza *et al.* 2015, Shi *et al.* 2015). These mutations were more common in the poorly differentiated and anaplastic TC and were associated with more aggressive features of DTC (Landa *et al.* 2013, Liu *et al.* 2013). Further studies showed a synergistic prognostic effect of these mutations with the $BRAF^{V600E}$ mutation – a well-known mutation in DTC with worse prognostic features (Xing *et al.* 2014).

In our study, *TERT* promoter mutations were not found in any of the 17 benign thyroid tumors. This is consistent with previous studies. In six previous studies that included 476 benign thyroid tumors of different types, none was found to harbor *TERT* promoter mutations (Liu *et al.* 2013, 2014*a*, Vinagre *et al.* 2013, Liu & Xing 2014, Melo *et al.* 2014, Muzza *et al.* 2015). However, in one study, one out of 58 benign follicular adenomas and three of 18 atypical follicular adenomas harbored C228T *TERT* promoter mutation, suggesting that this mutation is an early molecular event in thyroid tumorigenesis (Wang *et al.* 2014*a*). This finding has not been substantiated in any other study, including our study. CPTC is the most common type of TC. In our study, the prevalence of *TERT* promoter mutations in CPTC was relatively low (6.5%). Other studies showed rates that ranged between 4.5 and 22.5%, with most studies showing a frequency of *TERT* promoter mutations of around 12% in CPTC (Landa *et al.* 2013, Liu *et al.* 2013, 2014*a*, Vinagre *et al.* 2013, Liu & Xing 2014, Melo *et al.* 2014, Xing *et al.* 2014, Gandolfi *et al.* 2015, Muzza *et al.* 2015). Similar to previous studies, ours showed *TERT* promoter mutations in 8/57 (14%) cases of FVPTC. This is similar to the rate reported in a large series of TC in which 18 out of 103 cases (14.6%) of FVPTC harbored *TERT* promoter mutations (Xing *et al.* 2014) and another smaller study from Europe showing a similar rate (Gandolfi *et al.* 2015).

In our study, the rate of *TERT* promoter mutations was significantly higher in other subtypes of DTC compared to those in CPTC and FVPTC. *TERT* promoter mutations were found in 30% of TC-PTC, 30% HTC, 20% FTC and 38.5% PDTC (Table 2). Previous studies showed rates of *TERT* promoter mutations varying between 13.9 and 36.4% in FTC with most studies showing rates of around 14–17% (Landa *et al.* 2013, Liu *et al.* 2013, 2014*b*, Vinagre *et al.* 2013, Melo *et al.* 2014, Shi *et al.* 2015). In one study that included only HTC, *TERT* promoter mutations were

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DOI: 10.1530/ERC-15-0396

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screened in a subset of 61 cases, of which 8 (13.1%) harbored C228T mutation (Chindris *et al*. 2015). In another study that included 25 cases of HTC, 4 (16%) of them harbored *TERT* promoter mutations (Landa *et al*. 2013). The rates of *TERT* promoter mutations varied between 21 and 51.7% in PDTC (Landa *et al*. 2013, Liu *et al*. 2013, 2014*b*, Vinagre *et al*. 2013, Melo *et al*. 2014) and between 13 and 50% in ATC (Landa *et al*. 2013, Liu *et al*. 2013, 2014*b*, Vinagre *et al*. 2013, Melo *et al*. 2014, Shi *et al*. 2015).

The association between *TERT* promoter mutations and aggressive features of TC has been demonstrated in most previous studies (Landa *et al*. 2013, Liu *et al*. 2013, 2014*a*, Melo *et al*. 2014, Liu & Xing 2014, Xing *et al*. 2014, Gandolfi *et al*. 2015, Shi *et al*. 2015). However, the relative significance of each of those features varies among these studies. A consistent finding is that *TERT* promoter mutations occur more frequently at an older age and are associated with distant metastasis, higher tumor stage and poorly differentiated types of TC (Vinagre *et al*. 2013, Liu *et al*. 2014*a*, Melo *et al*. 2014, Wang *et al*. 2014*a*, Gandolfi *et al*. 2015, Muzza *et al*. 2015, Shi *et al*. 2015). Interestingly, tumor size, extrathyroidal invasion, tumor multifocality, lymph node metastasis and vascular invasion are not consistently associated with *TERT* promoter mutations. These conflicting findings between different studies might be due to the differences in characterizing the patients studied, sample size and proportions of different subtypes of TC included. In our study, overall, there was a strong association with patient's age and tumor size, vascular invasion and short- and long-term persistent/recurrence rates. However, there was no association with extrathyroidal extension, tumor multifocality, lymph node and distant metastasis. Interestingly, when we limited the analysis to CPTC or FVPTC, no association with any of these factors was noted (Table 4). In contrast, a stronger association was seen between these factors and *TERT* promoter mutations in non-CPTC types (Table 3). This suggests that *TERT* promoter mutations are more characteristic of high-grade TC and that they may have a particularly useful prognostic value in these subtypes of TC. This idea is consistent with the fact that previous studies showed a much higher rate of *TERT* promoter mutations in high-grade non-CPTC subtypes of TC (Landa *et al*. 2013, Liu *et al*. 2013, Xing *et al*. 2014).

The association between $BRAF^{V600E}$ and *TERT* promoter mutations has also been demonstrated in most but not all studies (Liu *et al*. 2014*a*, Melo *et al*. 2014, Xing *et al*. 2014, Gandolfi *et al*. 2015, Muzza *et al*. 2015, Shi *et al*. 2015). Tumors with $BRAF^{V600E}$ mutations are more likely to harbor *TERT* promoter mutations and vice versa (Liu *et al*. 2014*a*, Melo *et al*. 2014, Xing *et al*. 2014, Shi *et al*. 2015). In our study, we have also shown a similar strong association between $BRAF^{V600E}$ and *TERT* promoter mutations. Previous studies have also shown a synergistic prognostic effect of $BRAF^{V600E}$ and *TERT* promoter mutations with significantly more aggressive features and higher risk of recurrence in tumors harboring both mutations compared with those with either one or none of them (Xing *et al*. 2014). Other investigations have shown that *TERT* promoter mutations are stronger prognostically than $BRAF^{V600E}$ mutation, but found no difference in recurrence rates between those tumors that carry both or either one of the mutations (Muzza *et al*. 2015). These studies, however, were relatively small, with a limited number of cases harbouring both mutations. For similar reason, we did not find synergy between *TERT* promoter and $BRAF^{V600E}$ mutations in the present study. These conflicting results might also reflect the role of tumor microenvironment. Recent studies have shown a role for $BRAF^{V600E}$ mutation in thyroid tumors that are mesenchymal in origin (Sadow *et al*. 2014). Sadow *et al*. reported the presence of $BRAF^{V600E}$ mutation in three benign myopericytomas, a rare frequently benign mesenchymal tumor. One of these three myopericytomas was in the thyroid gland. Myopericytomas that harboured $BRAF^{V600E}$ mutation were likely to be multifocal, invasive and recurrent. *In vitro* and *in vivo* studies showed significant effects of $BRAF^{V600E}$ mutation on the tumor microenvironment promoting invasiveness and angiogenesis. These effects were reversible by the specific anti BRAF inhibitor, Vemurafenib, in tumors harbouring $BRAF^{V600E}$ mutation but not in the WT *BRAF* tumors (Sadow *et al*. 2014). This suggests that cells other than the epithelial follicular cells from which TC normally arises might harbour mutations that contribute to the progression of cancer. Similar effects and interactions of *TERT* promoter mutations with tumor microenvironment are possible including their presence in the mesenchymal cells. The study of these mutations in cells from the tumor microenvironment can be facilitated by using orthotopic mouse models of TC (Antonello & Nucera 2014).

In summary, our study shows that *TERT* promoter mutations are consistently detectable in a significant percentage of TC patients from a different ethnic group. The rate of these mutations observed in our study is similar to that in other ethnic populations. *TERT* promoter mutations are more likely to occur in tumors from older patients, in larger and less differentiated tumors and in association with more aggressive tumor features. The

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DOI: 10.1530/ERC-15-0396

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findings of our study confirmed the findings in other ethnic groups and therefore strongly support a general important pathogenetic role of *TERT* promoter mutations in TC. Our findings also highlight the significant prognostic value of *TERT* promoter mutations for the aggressiveness of TC. To our knowledge, this is the first study reporting *TERT* promoter mutations and their association with the aggressiveness of TC in a large cohort of patients with TC from the Middle East, supporting the inter-ethnic consistency of the previous findings on these mutations.

Declaration of interest

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

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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Received in final form 7 September 2015
Accepted 9 September 2015
Made available online as an Accepted Preprint
9 September 2015

Published by Bioscientifica Ltd.