Frequent Somatic *TERT* Promoter Mutations in Thyroid Cancer: Higher Prevalence in Advanced Forms of the Disease

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Background: *TERT* encodes the reverse transcriptase component of telomerase, which adds telomere repeats to chromosome ends, thus enabling cell replication. Telomerase activity is required for cell immortalization. Somatic *TERT* promoter mutations modifying key transcriptional response elements were recently reported in several cancers, such as melanomas and gliomas.

Objectives: The objectives of the study were: 1) to determine the prevalence of *TERT* promoter mutations C228T and C250T in different thyroid cancer histological types and cell lines; and 2) to establish the possible association of *TERT* mutations with mutations of *BRAF*, *RAS*, or *RET/PTC*.

Methods: *TERT* promoter was PCR-amplified and sequenced in 42 thyroid cancer cell lines and 183 tumors: 80 papillary thyroid cancers (PTCs), 58 poorly differentiated thyroid cancers (PDTCs), 20 anaplastic thyroid cancers (ATCs), and 25 Hurthle cell cancers (HCCs).

Results: *TERT* promoter mutations were found in 98 of 225 (44%) specimens. *TERT* promoters *C228T* and *C250T* were mutually exclusive. Mutations were present in 18 of 80 PTCs (22.5%), in 40 of 78 (51%) advanced thyroid cancers (ATC + PDTC) ($P = 3 \times 10^{-4}$ vs PTC), and in widely invasive HCCs (4 of 17), but not in minimally invasive HCCs (0 of 8). *TERT* promoter mutations were seen more frequently in advanced cancers with *BRAF/RAS* mutations compared to those that were *BRAF/RAS* wild-type (ATC + PDTC, 67.3 vs 24.1%; $P < 10^{-4}$), whereas *BRAF*-mutant PTCs were less likely to have *TERT* promoter mutations than *BRAF* wild-type tumors (11.8 vs 50.0%; $P = .04$).

Conclusions: *TERT* promoter mutations are highly prevalent in advanced thyroid cancers, particularly those harboring *BRAF* or *RAS* mutations, whereas PTCs with *BRAF* or *RAS* mutations are most often *TERT* promoter wild type. Acquisition of a *TERT* promoter mutation could extend survival of *BRAF*- or *RAS*-driven clones and enable accumulation of additional genetic defects leading to disease progression. **(*J Clin Endocrinol Metab* 98: E1562–E1566, 2013)**

Telomerase, the specialized DNA polymerase that adds telomere repeat segments to the ends of telomeric DNA, is usually absent in nonimmortalized cells but is expressed at functionally significant levels in the vast majority of human cancer cells, enabling their replicative immortality (1). The *TERT* gene encodes the reverse transcriptase component of the telomerase complex, and its overexpression in mouse models, such as in K5-Tert transgenic mice, leads to an increased incidence of cancer (2, 3). High telomerase activity and TERT expression have been reported in thyroid

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Abbreviations: ATC, anaplastic thyroid cancer; ETS, E twenty-six; HCC, Hurthle cell cancer; PDTC, poorly differentiated thyroid cancer; PTC, papillary thyroid cancer.

tumors, particularly in the advanced forms of the disease, but are absent in normal thyroid tissues (4–6).

Mutations in the proximal promoter of *TERT* have been identified recently as a highly frequent event in melanoma, particularly in the metastatic forms of the disease. The two recurrent, nonoverlapping somatic mutations identified (chr5:1,295,228C>T and chr5:1,295,250C>T, hereafter named C228T and C250T, respectively) conferred a 2- to 4-fold increase in TERT transcriptional activity, presumably through the generation of novel consensus binding sites in the *TERT* promoter for E twenty-six (ETS) transcription factors (7, 8).

Although *TERT* C>T transitions could be enhanced by exposure to UV light, these mutations were not restricted to melanoma and were present in 24 of 150 (16%) cell lines from the Cancer Cell Line Encyclopedia. Killela et al (9) extended the original study by surveying 1230 tumors of 60 different types and concluded that *TERT* promoter mutations occurred frequently (>15%) in a subset of 9 tumor types derived from cells with low rates of self-renewal, including gliomas, liposarcomas, and hepatocellular carcinomas. These reports prompted us to evaluate the extent and characteristics of *TERT* promoter mutations in follicular cell-derived thyroid cancer specimens.

Materials and Methods

Patient tissue samples

Our series comprised 183 thyroid tumors obtained from surgical pathological specimens and included 80 papillary thyroid cancers (PTCs; 29 from Memorial Sloan-Kettering Cancer Center [MSKCC], New York, and 51 from Nagasaki University, Japan), 58 poorly differentiated thyroid cancers (PDTCs), 20 anaplastic thyroid cancers (ATCs), and 25 Hurthle cell cancers (HCCs). In addition, we screened 42 human thyroid cancer cell lines. The MSKCC cases of PTC, PDTC, HCC, and ATC were randomly selected from the pathology department files of the institution. The thyroid carcinomas were classified according to the last World Health Organization classification of endocrine tumors, except for PDTC (10). The latter tumor was defined as a carcinoma displaying high mitotic activity (≥5 mitosis/10 high-power fields, ×400) and/or tumor necrosis, and showing follicular cell differentiation at the morphological or immunohistochemical level (11). The study was approved by the Institutional Review Board of MSKCC. Informed consent was also obtained for all Japanese samples.

TERT mutation testing

The *TERT* proximal promoter was amplified from sample DNA by a nested PCR approach, using primers and conditions previously described (8), and was subsequently sequenced on an ABI3730 capillary sequencer (Applied Biosystems). Genotyping for *BRAF* (all known point mutations) or *RAS* mutations (codons 12, 13, and 61 for all 3 *RAS* genes) was performed by either mass spectrometry or Sanger sequencing as previously described (12). RET/PTC rearrangements were detected on tumor cDNA as previously reported (12).

Statistical differences in mutation distributions were assessed by a two-sided Fisher's exact test using GraphPad Prism software version 5.04 (GraphPad Software, Inc).

Results

TERT promoter mutations were present in 44% (98 of 225) of the thyroid cancer specimens (Table 1). *TERT* C228T was more common (67 of 225) than C250T (31 of 225), and the two did not overlap (Figure 1).

TERT mutations showed a significantly uneven distribution between tumors of different histological grades (Table 1). They were comparatively infrequent in well-differentiated PTCs (18 of 80, or 22.5%). In contrast, advanced thyroid tumors (ie, ATC and PDTC) were al-

Table 1. *TERT* Promoter Mutations in Thyroid Cancer Tumors and Cell Lines

		TERT Promoter Mutations, n (%)				
Group	n	Wild-Type	C228T	C250T	C228T or C250T	*P* Value[a]
PTC (MSKCC)	29	21 (72.4)	5 (17.2)	3 (10.3)	8 (27.6)	
PTC (Japan)	51	41 (80.4)	5 (9.8)	5 (9.8)	10 (19.6)	
PTC (all)	80	62 (77.5)	10 (12.5)	8 (10.0)	18 (22.5)	
PDTC	58	28 (48.3)	18 (31.0)	12 (20.7)	30 (51.7)	.0005
ATC	20	10 (50.0)	10 (50.0)	0 (0.0)	10 (50.0)	.0241
Advanced thyroid cancers (PDTC + ATC)	78	38 (48.7)	28 (35.9)	12 (15.4)	40 (51.3)	.0003
HCC, minimally invasive (HCC-MIN)	8	8 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
HCC, widely invasive (HCC-WIDE)	17	13 (76.5)	3 (17.6)	1 (5.9)	4 (23.5)	.2689
Thyroid cancer cell lines	42	6 (14.3)	26 (61.9)	10 (23.8)	36 (85.7)	<.0001

[a] *P* values were derived from Fisher's exact test, using "PTC (all)" as the reference group, with the exception of HCC, where "HCC-MIN" was used.

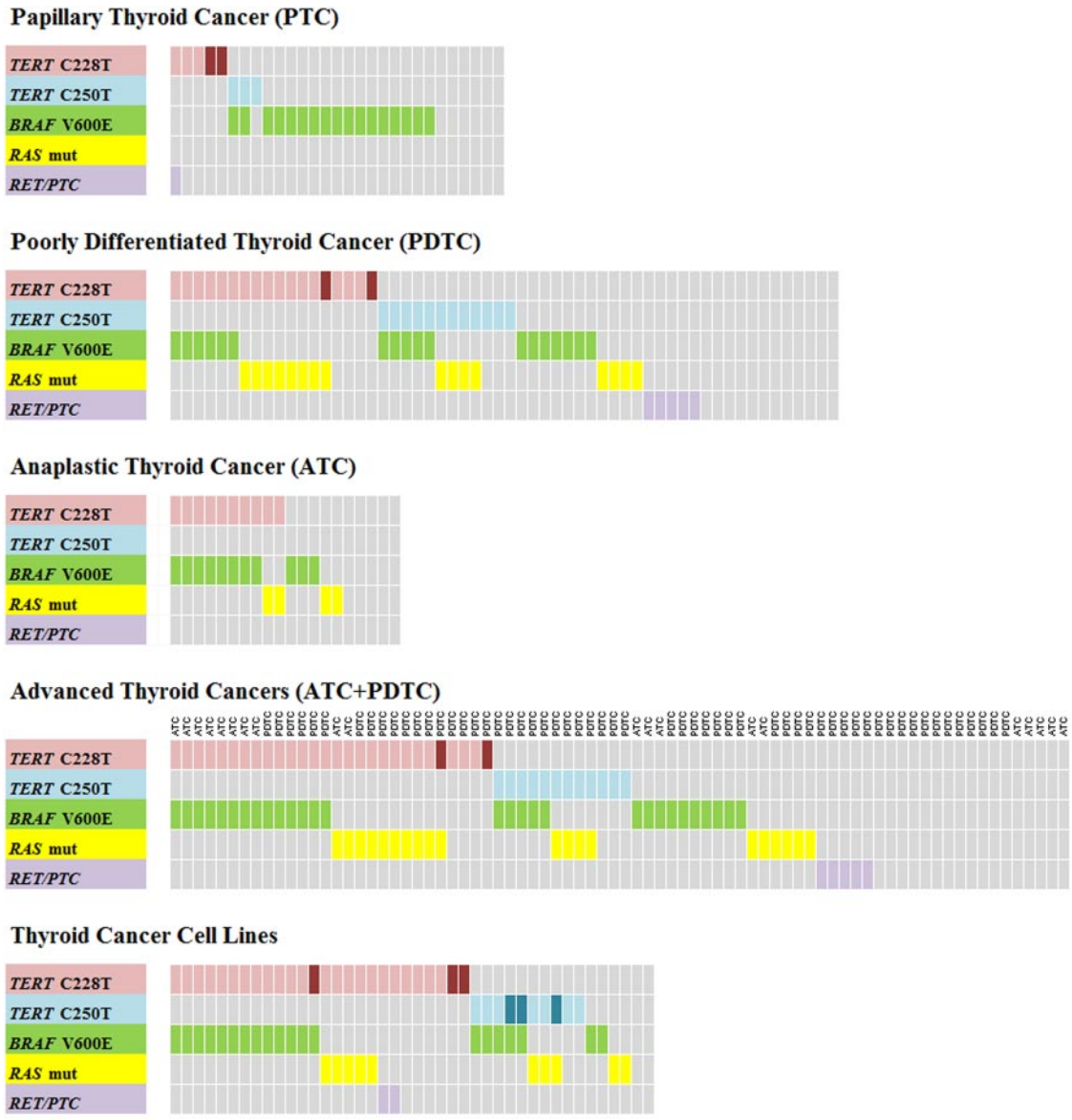

Figure 1. Concordance of *TERT* promoter and known thyroid driver genes (*BRAF*, *RAS*, *RET/PTC*) in thyroid cancer tumors and cell lines. Each cell represents one sample. Colored and gray shadings denote mutant and wild-type status, respectively. For *TERT* mutations, darker shading represents homozygous mutations. Detailed numbers can be found on Supplemental Table 2.

most twice as likely to harbor *TERT* mutations (40 of 78, or 51.3%; $P = .0003$). Regarding the less frequent HCC group, only widely invasive tumors harbored *TERT* mutations (4 of 17, or 23.5%), whereas none of their minimally invasive counterparts had these defects (0 of 8). Cell lines showed the highest rate of *TERT* mutations (37 of 42, or 88.1%), suggesting that this may be a common requirement for immortalization in cell culture (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

We also evaluated the co-occurrence of *TERT* promoter mutations with alterations in known thyroid cancer driver genes, such as *BRAF*, *RAS*, and *RET/PTC* (Figure 1 and Supplemental Table 2). We observed a significant co-occurrence of *TERT* mutations with advanced thyroid tumors harboring *BRAF* or *RAS* alterations compared to those that were wild-type for these genes (n = 78; ATC + PDTC, 67.3 vs 24.1%; $P = .0004$; Figure 1). Accordingly, most of the advanced tumors without mutations in known drivers clustered in the *TERT* wild-type group (17 of 24). By contrast, we found a reciprocal association in the subset of PTCs that were genotyped for driver gene alterations. Thus, tumors were less likely to have *TERT* promoter mutations if they harbored a mutation in *BRAF* or *RAS* (n = 29; 11.8 vs 50.0%; $P = .04$; see Figure 1). *TERT* promoter mutations generate de novo consensus binding sites for the ETS family of transcription factors. Because these are components of the MAPK transcriptional output, these data suggest that a MAPK-independent altera-

tion might be driving the transformation of *TERT* promoter wild-type PDTCs and ATCs.

Finally, mutation at *TERT* c.-57A>C, described in the germline of a family with cutaneous melanoma (8), was not found in any of the thyroid tumors assessed for that specific locus (0 of 82).

Discussion

This is the first study reporting a high frequency of *TERT* promoter mutations in follicular cell-derived thyroid carcinomas. We found an overrepresentation of *TERT* promoter mutations in advanced thyroid cancers, as well as a significant co-occurrence with mutations in *BRAF* and *RAS* in this subset of tumors. *TERT* C228T and C250T mutations appeared in a strict nonoverlapping fashion, suggesting that either is sufficient to drive the phenotype.

Mutations in the promoter of *TERT* were recently identified as common events in melanomas, glioblastomas, bladder carcinomas, and other tumors (7–9, 13). *TERT* mutations are enriched in advanced cancers, such as metastatic melanomas (8) and adult primary glioblastomas (9) with respect to their less aggressive counterparts. Our results show that this is also the case in thyroid cancers, where *TERT* mutations were more prevalent in advanced forms of the disease (51%) compared to well-differentiated tumors (22%). Hence, *TERT* promoter mutations may be biomarkers of tumor progression. Deep-sequencing methods would be more rigorous screening approaches to identify *TERT* mutations in advanced disease, particularly for ATC tumors with heavy macrophage infiltration (14, 15) because it is likely that Sanger sequencing underrepresented the *TERT* mutation prevalence in these cancers.

Killela et al (9) proposed that *TERT* promoter mutations may be more common in cancers derived from terminally differentiated cells, which have a low self-renewing capacity, whereas tissues that are rapidly renewing have alternative mechanisms to maintain telomerase lengthening, and thus would be less likely to benefit from activating mutations in *TERT*. Thyroid cells have a very low mitotic rate postnatally (16). Hence, the high rate of mutations observed in thyroid cancer is consistent with this hypothesis.

We found a significant overrepresentation of *TERT* promoter mutations in thyroid tumors harboring alterations in *BRAF* or *RAS* genes. A likely functional consequence of both C228T and C250T is to create de novo consensus binding sites for ETS factors in the *TERT* promoter (7, 8). MAPK activation, through either *BRAF* or *RAS* mutations, induces expression of members of the ETS transcription factor family (17). Conceivably, acquisition of a *TERT* promoter mutation could extend the lifespan of *BRAF*- or *RAS*-driven clones and enable accumulation of additional genetic defects leading to the development of more advanced forms of the disease. This may help explain the paradoxical finding that well-differentiated PTCs harboring *BRAF* or *RAS* mutations are less likely to harbor *TERT* promoter mutations than PTCs that are wild-type for these oncogenes, whereas *BRAF*- or *RAS*-mutant PDTCs and ATCs are markedly enriched for these *TERT* defects. These data raise the possibility that *TERT* promoter mutations may be relevant prognostic markers in thyroid cancer and should help refine the molecular taxonomy of the disease. It should be noted that mutations in the *TERT* promoter, although remarkably frequent, may be only 1 of the potential mechanisms of illegitimate activation of TERT, which may include aberrant methylation of the *TERT* promoter (18) or inactivating mutations in the *ATRX* gene, a Rad54-like ATP-driven DNA translocase, the loss of function of which leads to telomere lengthening (19).

Acknowledgments

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