Spectrum of TERT promoter mutations and mechanisms of activation in thyroid cancer

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Abstract

Background: Reactivation of telomerase reverse transcriptase (TERT) is an important event in cancer. Two hotspot mutations in the TERT promoter region, c.-124C > T (C228T) and c.-146C > T (C250T), occur in various cancer types including thyroid cancer. They generate de novo binding sites for E-twenty-six (ETS) transcription factors causing increased TERT transcription. The aim of this study was to search for novel TERT promoter mutations and additional mechanisms of TERT activation in thyroid cancer.

Methods: We studied 198 papillary thyroid carcinomas (PTCs), 34 follicular thyroid carcinomas (FTCs), 40 Hürthle cell carcinomas (HCCs), 14 poorly differentiated/anaplastic thyroid carcinomas (PDTC/ATC), and 15 medullary thyroid carcinomas (MTCs) for mutations in an −424 bp to +64 bp region of TERT. The luciferase reporter assay was used to functionally characterize the identified alterations. Copy number variations (CNVs) in the TERT region were analyzed using TaqMan copy number assay and validated with fluorescence in situ hybridization (FISH).

Results: We detected the hotspot c.-124C > T and c.-146C > T mutations in 7% PTC, 18% FTC, 25% HCC, and 86% PDTC/ATC. One PTC carried a c.-124C > A mutation. Furthermore, we identified two novel mutations resulting in the formation of de novo ETS-binding motifs: c.-332C > T in one MTC and c.-104_-83dup in one PTC. These genetic alterations, as well as other detected mutations, led to a significant increase in TERT promoter activity when assayed using luciferase reporter system. In addition, 5% of thyroid tumors were found to have ≥3 copies of TERT.

Conclusions: This study confirms the increased prevalence of TERT promoter mutations and CNV in advanced thyroid cancers and describes novel functional alterations in the TERT gene promoter, including a point mutation and small duplication. These mutations, as well as TERT copy number alterations, may represent an additional mechanism of TERT activation in thyroid cancer.

Keywords
copy number change, promoter activity, promoter mutation, TERT activation, thyroid cancer
1 | INTRODUCTION

Human telomerase reverse transcriptase (TERT) gene encodes the catalytic subunit of telomerase that, together with an RNA component, maintains chromosomal integrity by telomere elongation.1,2 Telomerase is expressed in germline and stem cells, but it is normally repressed in postnatal somatic cells.3 Reactivation of TERT in cancer cells prevents telomere shortening, thus allowing unlimited cellular proliferation essential for transformation.4,5 Telomerase activity is upregulated in ~85%-90% of aggressive tumors, making this event a hallmark of cancer.6,7

Somatic mutations in the promoter region of TERT are known to occur in cancer, providing a mechanism for TERT reactivation.8-10 Specifically, two recurrent mutations, located 124 bp and 146 bp upstream of the ATG translation start site and referred to as C228T (chr5, 1 295 228 C > T) and C250T (chr5, 1 295 250 C > T), have been reported in various cancers including thyroid cancer.11-14 Both mutations stimulate TERT promoter transcription activity through the generation of de novo consensus binding sites “(T/A)TCC” for E-twenty-six (ETS) family of transcription factors, which in turn can be upregulated by MAPK signaling.15-19

Indeed, as reported in thyroid and other cancer types, TERT promoter mutations lead to an increase in TERT mRNA expression levels as compared to wild-type (WT) TERT.20-25

Other mechanisms of TERT reactivation include amplification of the TERT gene, as reported in some cancers, including follicular thyroid cancers.26-28

Thyroid cancer is the most common type of endocrine tumors.29 The majority of thyroid tumors arise from thyroid follicular cells, comprising well-differentiated thyroid papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), poorly differentiated thyroid carcinoma (PDTC) and anaplastic (undifferentiated) thyroid carcinoma (ATC).30 Initiation and progression of thyroid cancer occur through gradual accumulation of early and late genetic and epigenetic events, which lead to the activation of the MAPK and PI3K-AKT signaling pathways.31,32 Early genetic events in thyroid cancer progression (ie, BRAF mutations) are frequently found in both well-differentiated thyroid cancer and in PDTC or ATC, therefore they are involved in tumor initiation and in the predisposition of the tumor to dedifferentiation. On the contrary, the late genetic events (ie, TP53 mutations) occur with increasing frequency in tumors that progressively lose thyroid differentiation and therefore are associated with tumor progression and unfavorable outcome.31,33 In thyroid cancer, TERT mutations represent a late event and are found in more aggressive thyroid cancers, being more common in PDTC and ATC (up to ~70% of cases) than in well-differentiated PTC and FTC (~25%).34,35 Moreover, TERT mutations have been established as an independent predictor of recurrence, distant metastases, poor prognosis, and cancer-related mortality in well-differentiated PTC and FTC.36-39 Such mutations, C228T and C250T, have also been identified in Hürthle cell carcinoma (HCC) but not in medullary thyroid carcinoma (MTC).34 However, other regions of TERT promoter have not been fully characterized in thyroid tumors.

The aim of this study was to detect novel mechanisms of TERT activation in thyroid cancer by mutational screening of the extended promoter region of TERT and evaluating copy number variations (CNVs) involving the TERT chromosomal locus in different thyroid cancers.

2 | MATERIALS AND METHODS

2.1 | Tumor samples and cell lines

Snap-frozen and formalin-fixed paraffin-embedded (FFPE) tissues from surgically removed thyroid samples and fine-needle aspiration samples were collected at the Department of Pathology, University of Pittsburgh Medical Center and also obtained from the University of Pittsburgh Health Sciences Tissue Bank (HSTB). Study was done in accordance with the US Federal Policy for the Protection of Human Subjects and approved by the University of Pittsburgh Institutional Review Board. Thyroid cancer tissues were fixed with 4% phosphate-buffered paraformaldehyde for 24 hours. Histologic sections were reviewed to confirm the diagnosis. Overall, 301 thyroid tumors were collected with following diagnosis: PTC (n = 198), FTC (n = 34), HCC (n = 40), PDTC/ATC (n = 14), and MTC (n = 15).

The following cell lines were used: normal thyroid HTori-3 cells were purchased from European Tissue Culture Collection (ECACC) (cat# 90011609); follicular thyroid cancer FTC-133 cells (Sigma/ECACC, cat# 94060901), papillary thyroid cancer K1cells (Sigma/ECACC, cat# 92030501), TPC-1cells (Sigma cat# SCC147), and anaplastic thyroid cancer TTA1 cells were obtained from Dr Rebecca Schwepp (University of Colorado Cancer Center); anaplastic thyroid cancer SW1739 (Cell Lines Service, cat# 300453), C643 (Cell Lines Service, cat# 300298), and T241 cells were obtained from Dr Jeffrey Knauf and Dr James Fagin (Memorial Sloan Kettering Cancer Center).

2.2 | DNA extraction

Genomic DNA was isolated from snap-frozen and FFPE tissues using QIAmp DNA Mini Kit (Cat#51304, Qiagen) and QIamp DNA FFPE Tissues Kit (Cat #56404, Qiagen), respectively.

2.3 | Mutational analysis for TERT promoter

TERT promoter mutation status was determined using targeted polymerase chain reaction (PCR) amplification followed by Sanger sequencing of the −424 to +64 (relative
to ATG) TERT promoter region (chr5: 1 295 528 to chr5: 1 295 040 (hg19). Briefly, PCR reactions were performed in a total reaction volume of 50 μL, containing 1X AmpliTaq Gold 360 Master Mix (Cat #4398881, Life Technologies), 10 μL of 300 GC enhancer, 0.2 μM of each primer, and ~60 ng of DNA template, using primers reported in Table S1. The PCR products were purified using MinElute PCR Purification kit (Cat #28006, Qiagen), and then sequenced by Sanger sequencing. Sanger sequencing was performed in both directions using the BigDye Terminator Kit v3.1 (Cat #4337456, Thermo) and an ABI 3130xl DNA Sequencer (Applied Biosystems).

2.4 | Plasmids

TERT promoter regions encompassing −424 to −1 bp (relative to ATG) with WT sequence and with c.-104_-83dup mutation were synthesized and cloned into the multiple cloning site of pEZX-GA01 Gaussia Luciferase (GLuc) and secreted alkaline phosphatase (SEAP) dual reporter vector (Cat #ZX103, GeneCopoeia) as GeneScript according to company’s protocols using primers listed in Table S1. The GLuc reporter was driven by a cytomegalovirus (CMV) promoter that was used as an internal control. To generate mutations corresponding to c.-124C > T, c.-146C > T, c.-124C > A, c.-332C > T, and c.-104_-83dup_Mut (c.-104_-83dup with eliminated ETS-binding motifs: “TTCCTTTCC” changed to “TGCTTCTCA”) and the variant allele for rs2853669 polymorphism, site-directed mutagenesis was then performed by GeneScript according to company's protocols using primers listed in Table S1.

2.5 | Cell culture and luciferase reporter assay

For the luciferase reporter assay, HTori-3, FTC-133, and TTA-1 cells were seeded at a density of 3-4 × 10^5 per well in 6-well plates and transiently transfected the following day with 1 μg of each dual reporter construct using Lipofectamine 2000 (Cat #11668019, Invitrogen). Independent transfection experiments were performed six (for WT and c.-104_-83dup constructs) or three times (for rs2853669, c.-124C > T, c.-146C > T, c.-124C > A, c.-332C > T, and c.-104_-83dup_Mut constructs) on each cell line. After 24 hours, the culture medium was collected and stored at −80°C. The GLuc and SEAP activities were measured in triplicates per each experiment with the secreted-pair dual luminescence kit (Cat #LF032, GeneCopoeia) using Spark™ 10M multimode microplate reader (Tecan) according to the manufacturer's instructions. The GLuc/SEAP luminescence ratios from each replicate measurement were averaged and considered as transcriptional activity of TERT promoter variant per experiment. The transcriptional activity of each TERT promoter variant was normalized to the average (from six transfection experiments) transcriptional activity of WT TERT promoter in each cell line and defined as “normalized-to-WT transcriptional activity.”

2.6 | TaqMan copy number assay

Copy number variations affecting the TERT gene were analyzed using the TaqMan copy number assay targeting intron 6 (ID: Hs06005815_cn; Applied Biosystems) on a 7500 Real-Time PCR System (Applied Biosystems), according to the manufacturer’s recommendations. RNase P TaqMan Copy Number Reference Assay (ID: 4403326; Applied Biosystems) was used for assay normalization. Briefly, all reactions were performed in quadruplicate in 20-μL final volume containing 5-ng DNA substrate, 10 μL of TaqMan Genotyping Master Mix (ID: 4371353, Applied Biosystems), 1 μL of TaqMan Copy Number Assay, which contains two primers and a carboxyfluorescein (FAM) dye-labeled MGB probe to detect the genomic DNA target sequence, and 1 μL of TaqMan Copy Number Reference Assay, which contains two primers and a 2’-chloro-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) and TAMRA dye-labeled probe to detect the genomic DNA reference sequence. The cycling conditions were: 95°C for 10 minutes, followed by 40 cycles each of 95°C for 15 seconds and 60°C for 1 minute. Average CT values were 24.73 to 30.117 for FAM and 25.46 to 33.63 for VIC. Raw data were analyzed for copy number using CopyCaller software (Applied Biosystems), as recommended. Briefly, CopyCaller Software performed relative quantification (RQ), following the comparative delta-delta threshold cycle (ΔΔCT) method. Normal thyroid DNA samples were used as diploid controls.

2.7 | Fluorescence in situ hybridization (FISH)

Copy number variations affecting the TERT gene were further validated using FISH. Tumor touch imprints were prepared from snap-frozen tissues. For FISH probes generation, RP11-990A6 (TERT gene 5p15.33 locus) and RP11-461014 (reference 5q31 locus) BAC clones (BAC/PAC Resources, Children's Hospital Oakland Research Institute) were labeled by Nick translation kit (Cat #07j00-001, Abbott Molecular) with SpectrumOrange-dUTP (Cat # 02N33-050, Abbott Molecular) and SpectrumGreen-dUTP (Cat # 02N32-050, Abbott Molecular), respectively. Hybridization was done as previously described.40 Microscopy was performed using Leica SP5 TCS confocal laser scanning microscope (Leica Microsystems) and images were captured using 63X, 1.4 NA oil PlanApo objective and 5X digital zoom.
2.8 | Statistical analysis

Statistical analysis of the transcriptional activity of the WT and mutated TERT promoter regions was performed in pairs using Mann-Whitney test in GraphPad Prism 6 (GraphPad Software Inc.). *P* were two-sided and considered significant if <.05.

3 | RESULTS

3.1 | Detection of canonical and novel TERT promoter mutations in thyroid cancer

To search for novel TERT mutations, 301 thyroid tumor samples representing common types of thyroid cancers were subjected to Sanger sequencing analysis of the extended TERT promoter region (424 bp upstream and 64 bp downstream of the translation start site). As expected, the majority of the detected genetic alterations in the TERT promoter was presented by known c.‐124C > T (C228T) and c.‐146C > T (C250T) mutations. The most common c.‐124C > T TERT mutation was found in 10 (5%) PTC, 6 (17.6%) FTC, 10 (25%) HCC and 8 (57.1%) PDTC/ATC, but not in any MTC analyzed (Table 1; Figure S1A). In one (0.5%) PTC, the analysis showed a TERT mutation at the same hotspot position of −124 bp but with a different nucleotide substitution, c.‐124C > A. The second common mutation, c.‐146C > T, was detected in four (2%) PTC and four (28.6%) PDTC/ATC but not in any other tumors analyzed (Table 1; Figure S1A). Besides known TERT promoter mutations, we identified two novel mutations: c.‐332C > T and c.‐104_‐83dup (Table 1; Figure 1A; Figure S1B). The former is a substitution of cytosine-to-thymine at a −332 bp position (Chr 5:1 295 436 hg19 position) from the ATG initiation site. The tumor harboring this mutation was diagnosed as MTC and had no known hotspot TERT mutations. Similar to the hotspot mutations, the c.‐332C > T mutation generates the “ATCC” sequence, which is a de novo putative ETS-transcription factor binding motif (Figure 1A).\(^\text{15,20}\) The other mutation was found in one PTC and was a duplication of the 22-bp TERT promoter region located −104 bp to −83 bp (Chr 5:1 295 208 to Chr 5:1 295 187 hg19 position) from ATG site. This tumor was also WT for hotspot TERT mutations. The duplication contained the sequences of the ETS-195 and ETS-200 motifs, resulting in the generation of two additional de novo “TTCC” ETS-binding motifs (Figure 1B).\(^\text{19,20}\) Therefore, in comparison to WT sequence, which harbors three native ETS motifs (ETS-195 bp, ETS-200 bp, and ETS-294 bp), the mutant sequence was found to harbor five adjacent ETS-binding sites, which include three native and two de novo sites (Figure 1B).

In addition to somatic mutations, we found several single-nucleotide polymorphisms (SNP) in analyzed TERT promoter region in different thyroid tumor types. Specifically, we detected a common polymorphism rs2853669 in 50% of all thyroid tumors analyzed. We also identified four rare polymorphisms, rs35161420 and rs35226131, together in 5.6% of cases, and rs34233268 and rs347646448 each in 0.3% of cases studied (Table S2).

3.2 | Functional characterization of novel TERT promoter mutations

Next, we determined whether the newly identified TERT mutations are functional and lead to the increase in TERT promoter activity. To test this, the WT and all TERT promoter mutations found in this study (c.‐124C > T, c.‐146C > T, c.‐124C > A, c.‐332C > T, c.‐104_‐83dup) were assayed using a luciferase reporter assay in several thyroid cell lines. First, we genotyped several human thyroid cell lines for TERT promoter mutations and the majority of them were positive for mutations (Table S3). For luciferase assay, we used TTA1 (ATC) and HTori-3 (normal human thyroid) cell lines with WT TERT promoter sequence, and FTC‐133 (follicular thyroid cancer) cell line carrying the c.‐124C > T mutation. As compared to the WT TERT promoter, all mutations conferred a significant up to twofold increase in transcriptional activity.

| Thyroid tumor types | Number of cases studies | c.‐124C > T (C228T) n/N (%) | c.‐146C > T (C250T) n/N (%) | Other TERT mutations n/N (%) |
|---------------------|-------------------------|-----------------------------|-----------------------------|-----------------------------|
| PTC                 | 198                     | 10/198 (5%)                 | 4/198 (2%)                  | c.‐104_‐83dup 1/198 (0.5%) | c.‐124C > A 1/198 (0.5%) |
| FTC                 | 34                      | 6/34 (17.6%)                | 0/34 (0%)                   | —                           |
| HCC                 | 40                      | 10/40 (25%)                 | 0/40 (0%)                   | —                           |
| PDTC/ATC            | 14                      | 8/14 (57.1%)                | 4/14 (28.6%)                | —                           |
| MTC                 | 15                      | 0/15 (0%)                   | 0/15 (0%)                   | c.‐332C > T 1/15 (6.7%)     |

**TABLE 1**: Frequency of TERT promoter mutations in thyroid tumors

Abbreviations: FTC, follicular thyroid carcinoma; HCC, Hürthle cell carcinoma; MTC, medullary thyroid carcinoma; PDTC/ATC, poorly differentiated/anaplastic thyroid carcinoma; PTC, papillary thyroid carcinoma.
in all three thyroid cell lines examined, whereas the most common rs2853669 polymorphism did not alter the promoter activity as compared to the WT TERT sequence (Figure 2A). Among the mutated TERT promoters, the promoters with c.-124C > T, c.-124C > A, and c.-104_‐83dup had the strongest transcriptional activities (Figure 2A).

Next, we explored whether the increased transcriptional activity of TERT promoter harboring the c.-104_‐83dup is related to the generation of two de novo ETS-binding motifs. To test this, we eliminated the core of both motifs by site-directed mutagenesis (Figure 2B). This significantly reduced the promoter transcriptional activity compared to the intact c.-104_‐83dup mutation (Figure 2C). Taken together, these results suggest that all novel somatic mutations in the TERT promoter region are functional and lead to the increase in its transcriptional activity.

3.3 Copy number variations of TERT in thyroid cancer

In order to test whether CNV may represent another mechanism of TERT activation in thyroid cancer, DNA samples from 184 thyroid tumors were screened for CNV using a TaqMan copy number assay based on quantitative real-time PCR (qPCR) (Table 2). Our analysis identified nine thyroid tumor samples with increased TERT locus copy number. Specifically, 1/107 PTC showed four copies of the TERT region, and 2/22 FTC, 4/29 HCC, and 2/4 PDTC/ATC showed three copies. None of the 22 MTCs analyzed showed numerical changes in the TERT locus (Table 2). Among the thyroid tumors with increased TERT gene copy numbers, 2/4 HCC samples and 1/2 PDTC/ATC samples were positive for c.-124C > T mutation (Table 2). Several thyroid cell lines, including FTC133, K1, HTori-3, T241, and TPC1, were also tested for TERT CNV and have not shown any alterations in the TERT copy number. To validate the qPCR CNV results, FISH was performed on available frozen samples from eight tumors with increased TERT locus copy number (a PTC with normal copy number of TERT was used as a diploid control). FISH analysis confirmed the qPCR results and showed that increased copies of TERT were represented by the numerical change of the entire chromosome 5 or by amplification of the TERT locus (Figure 3). Only limited number of these samples had mRNA available, precluded the informative analysis of correlation between the copy number alterations, and expression levels of the TERT gene in these tumors.
Reactivation of telomerase activity in cancer cells has been recognized as an important feature of cancer cells, although its mechanisms are not fully understood. A significant advance in this area was achieved after the discovery of recurrent TERT promoter mutations first in melanoma and subsequently in many other human cancers including thyroid cancer. In thyroid cancer, multiple studies have found c.−124C > T (C228T) and c.−146C > T (C250T) TERT mutations in follicular cell-derived thyroid cancers and particularly in PDTC and ATC. No TERT promoter mutations were previously documented in MTCs.

The prevalence of the hotspot mutations, c.−124C > T and c.−146C > T, in our study is consistent with previous reports as it shows the lowest prevalence in PTC, followed by FTC and HCC, with the highest prevalence in PDC/ATC, whereas none of these mutations were seen in MTC. An additional mutation, c.−124C > A, was also detected in one PTC. This mutation was already reported with low frequency in different cancer types and it was previously reported to occur in 0.3% of PTC.
However, in this study, we report two novel mutations found using the analysis of an extended region of TERT promoter. The first mutation, c.‐332C > T, was detected in a single tumor case and was only one TERT promoter mutation in MTC group of tumors (1/15). The second novel mutation, c.‐104_‐83dup, was identified in one PTC case and was presented as duplication of 22 base pairs within the TERT promoter. Both c.‐332C > T and c.‐104_‐83dup mutations showed a stimulatory effect on transcriptional activity of TERT promoter in normal and cancer thyroid cells as we showed using luciferase reporter assay approach. The increased activity, if TERT promoter affected by these mutations, as well as other mutations detected in this study, is likely associated with the generation of de novo ETS‐transcription factor binding sites. Genetic alterations that create additional ETS sites in the vicinity of native sites are a known mechanism of aberrant TERT activation in cancer. In fact, Bell et al showed that, in addition to point mutation creating de novo ETS sites, a duplication event of 41‐bp generates one de novo ETS motif in one tumor. Similarly, the novel c.‐104_‐83dup creates two additional ETS motifs adjacent to three native sites. In addition, we showed that this is in a significant degree due to the two de novo ETS sites generated by the duplication. Indeed, elimination of the ETS sites in the duplicated region significantly reduced the promoter activity, although it did not bring it back to the WT level. Such residual activity is likely associated with the presence of binding motifs for other transcriptional factors and changes in the DNA helical turns. Due to the lack of RNA material from the tumor cases positive for new mutations, we have not tested whether the stimulatory effect of this mutation results in increased TERT mRNA level.

Reactivation of telomerase in human malignancies has also been linked to TERT amplification. In fact, the TERT gene locus was found to be amplified in various cancers including lung, breast, cervical carcinomas, and FTC. prompting us to examine the TERT gene for copy number alterations in different types of thyroid cancer. Interestingly,
we observed that the increase in TERT locus copy number was more common in FTC, HCC, and PDTC, as compared to PTC. Moreover, TERT copy number gain co-occurred with TERT hotspot mutations in some HCC and PDTC, suggesting that it may cooperate with hotspot mutations in TERT reactivation and cancer progression. Due to the unavailability of mRNA samples, we could not study directly the effects of CNV on TERT mRNA expression. However, other studies have shown the association between TERT copy number gain and mRNA expression in several cancer types, including FTC.26,28,48 Nevertheless, larger studies, particularly those focusing on advanced thyroid cancers, are required to provide definitive confirmation of this mechanism.

In summary, we report herein two novel TERT promoter alterations in thyroid tumors and show that these mutations are functional and lead to increase in the transcriptional activity of TERT promoter in thyroid cancer cells by creating new consensus motifs for transcriptional regulators. This suggests that when the promoter region of TERT is analyzed for mutations, it may need to include a more extended region, and otherwise some functionally relevant mutations may be missed. Our study also shows a higher prevalence of TERT copy number gains in aggressive thyroid cancer types, particularly in HCC and PDTC. Thus, novel functional mutations uncovered in this study and increased copy number of TERT may represent additional mechanisms of TERT activation in thyroid cancer.

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CONFLICT OF INTEREST

All authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

FP: Conceptualization, investigation, methodology, data analysis. AN: Investigation, methodology, data analysis. MN: Conceptualization, investigation. YN: Conceptualization, data analysis, project administration. All authors: Drafting of manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SIGNIFICANCE

Novel functional mutations in TERT promoter, including single-nucleotide variants and duplications, lead to increased TERT promoter activity, highlighting additional mechanisms of TERT activation in thyroid cancer.

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REFERENCES

1. Gomez DE, Armando RG, Farina HG, et al. Telomere structure and telomerase in health and disease (review). Int J Oncol. 2012;41(5):1561-1569.
2. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet. 2005;6(8):611-622.
3. Shay JW, Wright WE. Telomeres and telomerase in normal and cancer stem cells. FEBS Lett. 2010;584(17):3819-3825.
4. Kim N, Piatyszek M, Prowse K, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266(5193):2011-2015.
5. Hahn WC, Stewart SA, Brooks MW, et al. Inhibition of telomerase limits the growth of human cancer cells. Nat Med. 1999;5(10):1164-1170.
6. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33(5):787-791.
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-674.
8. Akincilar SC, Unal B, Tergaonkar V. Reactivation of telomerase in cancer. Cell Mol Life Sci. 2016;73(8):1659-1670.
9. Li Y, Tergaonkar V. Telomerase reactivation in cancers: Mechanisms that govern transcriptional activation of the wild-type vs. mutant TERT promoters. Transcription. 2016;7(2):44-49.
10. Stern JL, Theodorescu D, Vogelstein B, Papadopoulos N, Cech TR. Mutation of the TERT promoter, switch to active chromatin, and monoallelic TERT expression in multiple cancers. Genes Dev. 2015;29(21):2219-2224.
11. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339(6122):957-959.
12. Liu X, Wu G, Shan Y, Hartmann C, von Deimling A, Xing M. Highly prevalent TERT promoter mutations in bladder cancer and glioblastoma. Cell Cycle. 2013;12(10):1637-1638.
13. Killela PJ, Reitman ZJ, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci USA. 2013;110(15):6021-6026.
14. Liu X, Bishop J, Shan Y, et al. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer. 2013;20(4):603-610.
15. Li Y, Zhou QL, Sun W, et al. Non-canonical NF-kappaB signalling and ETS1/2 cooperatively drive C250T mutant TERT promoter activation. Nat Cell Biol. 2015;17(10):1327-1338.
16. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013;339(6122):959-961.
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17. Rao VN, Reddy ES. elk-1 proteins interact with MAP kinases. Oncogene. 1994;9(7):1855-1860.

18. Bell R, Rube HT, Xavier-Magalhaes A, et al. Understanding TERT promoter mutations: a common path to immortality. Mol Cancer Res. 2016;14(4):315-323.

19. Bell R, Rube HT, Kreig A, et al. The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. Science. 2015;348(6238):1036-1039.

20. Huang D-S, Wang Z, He X-J, et al. Recurrent TERT promoter mutations identified in a large-scale study of multiple tumour types are associated with increased TERT expression and telomerase activation. Eur J Cancer. 2015;51(8):969-976.

21. Park CK, Lee SH, Kim JY, et al. Expression level of hTERT is regulated by somatic mutation and common single nucleotide polymorphism at promoter region in glioblastoma. Oncotarget. 2014;5(10):3399-3407.

22. Piscuoglio S, Ng CK, Murray M, et al. Massively parallel sequencing of phyllodes tumours of the breast reveals actionable mutations, and TERT promoter hotspot mutations and TERT gene amplification as likely drivers of progression. J Pathol. 2016;238(4):508-518.

23. Borah S, Xi L, Zaug AJ, et al. TERT promoter mutations and telomerase reactivation in urothelial cancer. Science. 2015;347(6225):1006-1010.

24. Vinagre J, Almeida A, Pópulo H, et al. Frequency of TERT promoter mutations in human cancers. Nat Commun. 2013;4:2185.

25. Wang N, Liu T, Sofiadis A, et al. TERT promoter mutation as an early genetic event activating telomerase in follicular thyroid adenoma (FTA) and atypical FTA. Cancer. 2014;120(19):2965-2979.

26. Xie H, Liu T, Wang N, et al. TERT promoter mutations and gene amplification: promoting TERT expression in Merkel cell carcinoma. Oncotarget. 2014;5(20):10048-10057.

27. Cao Y, Huschtscha LI, Nouwens AS, et al. Amplification of telomerase reverse transcriptase gene in human mammary epithelial cells with limiting telomerase RNA expression levels. Can Res. 2008;68(9):3115-3123.

28. Paulsson JO, Mu N, Shabo I, et al. TERT aberrancies: a screening tool for malignancy in follicular thyroid tumours. Endocr Relat Cancer. 2018;25(7):723-733.

29. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review. 1975–2010. Bethesda, MD: National Cancer Institute. http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data delivery. Bethesda, MD: National Cancer Institute. http://seer.cancer.gov/csr/1975_2012, based on November 2013 SEER data submission, posted to the SEER web site, April 2013.

30. Nikiforov YE. Chapter 7, Thyroid Tumors: Classification, Staging, and General Considerations. In: Nikiforov YE, Biddingger PW, Thompson L, eds. Diagnostic Pathology and Molecular Genetics of the Thyroid. Baltimore, MD: Lippincott Williams & Wilkins; 2009.

31. Penna GC, Vaisman F, Vaisman M, Sobrinho-Simoes M, Soares P. Molecular markers involved in tumorigenesis of thyroid carcinoma: focus on aggressive histotypes. Cytogenet Genome Res. 2016;150(3-4):194-207.

32. Brehar AC, Brehar FM, Bulgar AC, Dumitrache C. Genetic and epigenetic alterations in differentiated thyroid carcinoma. J Med Life. 2013;6(4):403-408.

33. Nikiforova MN, Nikiforov YE. Molecular genetics of thyroid cancer: implications for diagnosis, treatment and prognosis. Expert Rev Mol Diagn. 2008;8(1):83-95.

34. Liu R, Xing M. TERT promoter mutations in thyroid cancer. Endocr Relat Cancer. 2016;23(3):R143-R155.

35. Xu B, Ghassema R. Genomic landscape of poorly differentiated and anaplastic thyroid carcinoma. Endocr Pathol. 2016;27(3):205-212.

36. Melo M, da Rocha AG, Vinagre J, et al. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. J Clin Endocrinol Metab. 2014;99(5):E754-E765.

37. Nikiforov YE. Role of molecular markers in thyroid nodule management: then and now. Endocr Pract. 2017;23(8):979-988.

38. Landa I, Ganly I, Chan TA, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab. 2013;98(9):E1562-E1566.

39. Liu T, Wang N, Cao J, et al. The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. Oncogene. 2014;33(42):4978-4984.

40. Evdokimova VN, Gandhi M, Nikitski AV, Bakkenist CJ, Nikiforov YE. Nuclear myosin/actin-motored contact between homologous chromosomes is initiated by ATM kinase and homology-directed repair proteins at double-strand DNA breaks to suppress chromosome rearrangements. Oncotarget. 2018;9(17):13612-13622.

41. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell. 2014;159(3):676-690.

42. Piscuoglio S, Ng CK, Murray M, et al. Massively parallel sequencing of phyllodes tumours of the breast reveals actionable mutations, and TERT promoter hotspot mutations and TERT gene amplification as likely drivers of progression. J Pathol. 2016;238(4):508-518.

43. Kyo S, Takakura M, Taira T, et al. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). Nucleic Acids Res. 2000;28(3):669-677.

44. Zhang A, Zheng C, Lindvall C, et al. Frequent amplification of the telomerase reverse transcriptase gene in human tumors. Can Res. 2000;60(22):6230-6235.

45. Cao Y, Bryan TM, Reddel RR. Increased copy number of the TERT and TERC telomerase subunits in cancer cells. Cancer Sci. 2008;99(6):1092-1099.

46. Zhu C-Q, Cutz J-C, Liu N, et al. Amplification of telomerase (hTERT) gene is a poor prognostic marker in non-small-cell lung cancer. Br J Cancer. 2006;94(10):1452-1459.

47. Zhang A, Zheng C, Hou M, et al. Amplification of the telomerase reverse transcriptase (hTERT) gene in cervical carcinomas. Genes Chromosom Cancer. 2002;34(3):269-275.

48. Gay-Bellile M, Veronese L, Combes P, et al. TERT promoter status and gene copy number gains: effect on TERT expression and association with prognosis in breast cancer. Oncotarget. 2017;8(44):77540-77551.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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